Late Abstracts

The American Society for Cell Biology

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SAMPLE CITATION:

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**SUNDAY, DECEMBER 10**

**GROWTH FACTORS AND RECEPTORS**

L1 *Drosophila Protein Tyrosine Phosphatase dPTP61F, an Ortholog of Human PTP1B and T Cell PTP, Modulates Insulin Signaling through Dock Engagement*

C. Wu, H. Lee, T. Meng; Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

Mammalian insulin regulates cell growth and metabolism through its specific insulin receptor (InR). It is now clear that the insulin signaling is evolutionarily conserved. In *Drosophila melanogaster*, several kinases controlling this signaling cascade have been characterized as the orthologs of their mammalian counterparts. Despite the appreciated role of kinases, it is not known how phosphatases participate in the regulation of *Drosophila* insulin receptor (dInR)-mediated signaling. In the current study, we have investigated the function of a nontransmembrane protein phosphatase dPTP61F, the ortholog of human PTP1B and T Cell-PTP, both of which have been identified as negative regulators for human InR. In vitro studies demonstrated that dPTP61F effectively dephosphorylates dInR and reduces its dbr as its potential substrate. The ablation of endogenous dPTP61F by RNA interference leads to the enhanced tyrosine phosphorylation of dInR in S2 cells stimulated with insulin, suggesting that dPTP61F may regulate insulin signaling through direct dephosphorylation of dInR. We further pursued that the mechanistic details on how dPTP61F gains an access to dInR for tyrosine dephosphorylation in vivo. We were further pursuing that the mechanistic details on how dPTP61F gains an access to dInR for tyrosine dephosphorylation in vivo. We were particularly interested in the role of an adaptor DOCK, which is a dInR and dPTP61F-associated protein. When ectopically expressed in S2 cells, DOCK promotes dPTP61F-mediated tyrosine dephosphorylation of dInR. The C-terminal proline-rich motifs of dPTP61F are essential for binding to DOCK, and the presence of this region is important for the binding of *Drosophila* insulin receptor (dInR)-mediated signaling. In the current study, we have investigated the function of a nontransmembrane protein phosphatase dPTP61F, the ortholog of human PTP1B and T Cell-PTP, both of which have been identified as negative regulators for human InR. In vitro studies demonstrated that dPTP61F effectively dephosphorylates dInR and reduces its dbr as its potential substrate. The ablation of endogenous dPTP61F by RNA interference leads to the enhanced tyrosine phosphorylation of dInR in S2 cells stimulated with insulin, suggesting that dPTP61F may regulate insulin signaling through direct dephosphorylation of dInR. We further pursued that the mechanistic details on how dPTP61F gains an access to dInR for tyrosine dephosphorylation in vivo. We were particularly interested in the role of a dock, which is a dInR and dPTP61F-associated protein. When ectopically expressed in S2 cells, DOCK promotes dPTP61F-mediated tyrosine dephosphorylation of dInR. The C-terminal proline-rich motifs of dPTP61F are essential for binding to DOCK, and the presence of this region is important for the binding of dInR. Interestingly, we found that NCK, the mammalian ortholog of DOCK, which forms a stable complex with PTP1B, plays a key role for effective tyrosine dephosphorylation of human InR mediated by PTP1B. Our findings not only identify a *Drosophila* PTP that controls the activity of dInR, but also provide novel insights into a regulatory mechanism through which an adaptor protein acts coordinately with a kinase and a phosphatase in fine tuning the insulin signaling.

L2 *Simultaneous Monitoring of RTKs by Multiplex Bead Immunoassay*

C. Fountain, J. DeSimone, W. Zheng, J. Wang, K. Reagan; Invitrogen, Camarillo, CA

Activation of receptor tyrosine kinases (RTKs) triggers signaling cascades leading to a number of cellular responses such as proliferation, apoptosis, differentiation, and metabolic regulation. Alterations in the cellular expression of these types of receptors have been associated with inflammatory diseases, diabetes, and various types of cancer, making RTKs potential drug targets. In this study, the activity of key RTK family members (IR, IGF-1R, EGFR, c-kit, and c-met) was simultaneously monitored by measuring the phosphorylation of activation-specific tyrosine sites using a multiplex bead immunoassay. Specificity of the assay was confirmed by examining various cell lines stimulated with the corresponding growth factors. Commercially available inhibitory compounds of RTKs were tested in cell-based models as a means of investigating compound specificity. Our results demonstrate that simultaneous monitoring of receptor phosphorylation and consequential activity can serve as a valuable tool for research and drug discovery.

L3 *3-Ethylpyridine, a Double Agent: Death Factor for Endothelial Cells and Growth Factor for Fibroblasts*

V. Tran, S. Lin, K. Riveles, R. Yu, N. Xu, P. Talbot; Cell Biology and Neuroscience, UC Riverside, Riverside, CA

Pyridines in cigarette smoke inhibit chick chorioallantoic membrane growth (Tox. Sci. 69, 217, 2002), blood vessel development, and vessel migration (Tox. Sci. 68, 237, 2002) at picomolar doses. Since these studies were done only with the chick, our purpose was to test the hypothesis that 3-ethylpyridine inhibits growth of cultured mammalian cells. Two endothelial cell lines (human umbilical vein endothelial cells [HUVEC] and human microvascular endothelial cells [HMVEC]) and two fibroblast cell lines (NIH 3T3 cells and mouse embryonic fibroblasts [MEF]) were compared. Cells were cultured in proliferation or survival medium containing various doses (10^{-14} M to 10^{-3} M) of 3-ethylpyridine, and after 48 hours, total cell number was counted using a hemacytometer, and the lowest observed adverse effect levels (LOAELs) were determined. 3-Ethylpyridine inhibited endothelial cell growth (HUVEC LOAEL = 10^{-6} M; HMVEC LOAEL = 10^{-4} M) in proliferation medium and decreased total cell number in survival medium (HUVEC LOAEL = 10^{-12} M; HMVEC LOAEL = 10^{-11} M), supporting our hypothesis and prior studies with this chemical. In contrast, 3-ethylpyridine stimulated fibroblast growth at doses as low as 10^{-14} M (NIH 3T3 cells) and 10^{-15} M (MEF) in both media. To determine if cigarette smoke contained significant levels of 3-ethylpyridine, its concentration was measured using gas chromatography-mass spectrometry in solutions of mainstream and sidestream smoke made from research, traditional commercial, and harm reduction cigarettes. The concentration of 3-ethylpyridine averaged 0.16 M (range 3.7 x 10^{-12} to 1.23 M) in mainstream and 0.1 M (range 4.4 x 10^{-12} to 6.0 M) in sidestream. In contrast, 3-ethylpyridine acted as a death factor for endothelial cells and a growth factor for fibroblasts at low doses. Additionally, the concentration of 3-ethylpyridine measured in smoke solutions from all commercial cigarettes tested was significantly higher than the lowest doses that affected cultured cells.

L4 *Separation of Dual Affinity of Betacellulin to ErbB Receptors*

T. Nagoaka, H. Tada, H. Yamada, M. Seno; Department of Medical and Bioengineering Science, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan

Betacellulin (BTC), a member of epidermal growth factor (EGF) family, not only induces mitosis of fibroblasts, epithelial and smooth muscle cells but also promotes differentiation of mesenchymal and pancreatic beta-cells. BTC has preferential affinity to both ErbB1 and ErbB4 receptors. However, it is not clear the stimulation of either cell growth or differentiation depends on the receptor binding specificity. If an amino acid residue responsible for the affinity to each receptor is found, the mutation may show the differentiation activity without mitogenic activity and vice versa. It should be greatly useful for analyzing the signal transduction mechanism essential for cellular differentiation of islet beta-cells. In this study, we fabricated a library of BTC mutants of which EGF domain was altered by error-prone PCR mutagenesis. Since this procedure is carried out based on the expression vector, every mutation is designed to be ready for expression just after the transformation of bacteria. The pool of mutant genes were used to transform *E. coli* BL21-Gold (DE3)/pLyS5 and the mutants were expressed. Then the cells from independent colonies were lysed and the crude extracts were collected. The affinity of BTC mutants in the crude extracts for the receptors were assessed with soluble ErbB1 and soluble ErbB4 receptors in EIA system. As the result, six mutants with less binding ability to one of the two receptors were screened from the library. DNA sequence revealed independent mutations of two amino acids showed significant loss of affinity to ErbB1. Meanwhile three amino acid residues responsible for the affinity to ErbB4 were revealed. These findings suggest the amino acid residues or the primary structure of BTC essential for the affinity to either ErbB1 or ErbB4. Further investigation on the phosphorylation of ErbB receptors and downstream factors stimulated by these mutants are now undergoing.

L5 *The Classic Xenopus laevis Progesterone Receptor Associates to the Plasma Membrane through Its Ligand Binding Domain*

M. V. Hinrichs, S. P. Martinez, M. A. Montecino, J. A. Olate; Biochemistry and Molecular Biology, Universidad de Concepcion, Concepcion, Chile

During the last decade, considerably evidence is accumulating that supports the view that the classic progesterone receptor (xPR-1) is modifying *Xenopus laevis* oocyte maturation through a nongenomic mechanism. Overexpression and depletion of oocyte xPR-1, have been
shown to accelerate and to block progesterone-induced oocyte maturation respectively. In addition, rapid inhibition of plasma membrane adenyl cyclase by the steroid hormone, supports the idea that xPR-1 should be localized at the oocyte plasma membrane. To test this hypothesis, we transiently transfected xPR-1 cDNA into Cos-7 cells and analysed its subcellular distribution. Through western blot and immunofluorescence analysis, we were able to detect xPR-1 associated to the plasma membrane of transfected Cos-7 cells. In addition, we also incubated xPR-1 expressing Cos cells with Progesterone-BSA-FITC and found specific steroid binding sites at the plasma membrane, that were completely displaced by unlabelled progestrone. Finally, we analyzed which domain of the receptor was conferring it the capacity to associate to the plasma membrane by expressing its N-terminal and ligand binding domain (LBD) and found that only the LBD displayed membrane association. The N-terminal domain was expressed in equal amounts, but remained cytosolic. Therefore we conclude that a fraction of xPR-1 expressed in cos-7 cells, has the capacity to associate to the plasma membrane through its LBD. Sponsored by: CONICYT, Ring of Research in Advances Studies in Cell Signalling and Gene Regulation, ACT-44.

**SIGNAL TRANSDUCTION**

L6

Gαt6 Binds to RhoA-specific Guanine Nucleotide Exchange Factor p63RhoGEF and Competes with the Canonical PLC/PKC Pathway

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Heterotrimeric G proteins regulate diverse physiological processes by modulating the activities of intracellular effectors. The α subunit of Gt6 family members couples G protein-coupled receptor (GPCR) activation to phospholipase C-β (PLCβ) activity and subsequently triggers intracellular calcium signaling cascades. However, they differ markedly in biochemical properties as well as tissue distribution. Recent findings also showed that some of the cellular activities of Gt6 family members are independent of PLCβ activation. Novel binding partners of Gt6 subunits were also reported. A guanine nucleotide exchange factor, p63RhoGEF, has been shown to interact with Gt6α proteins and thus provides linkage to RhoA activation. However, it is unknown if p63RhoGEF can associate with other Gt6 family members such as Gαt4. In the present study, we used cellular co-immunoprecipitation studies to demonstrate that p63RhoGEF can form a stable complex with the constitutively active mutant of Gαt6 (Gαt6(Q61L)). The C-terminal binding site of p63RhoGEF was shown to be responsible for the association with Gαt6. By using cellular fractionation, the intracellular localization of p63RhoGEF was also examined. It was found that p63RhoGEF mostly localized in membrane fraction of transfected HEK293 cells. The N-terminal half of p63RhoGEF was sufficient and important for membrane targeting. Interestingly, overexpression of p63RhoGEF inhibited Gαt6-QL-induced IP3 production. The inhibitory effect was further characterized by dose-dependent relationship between the amount of p63RhoGEF and Gαt6-QL-induced IP3 formation. This suggested that p63RhoGEF may compete with PLCβ isoforms for activated Gαt6 proteins and thereby modulate the fidelity of Gαt6/PLCβ signaling. Supported by RGC (HKUST 6120/04M) and UGC (AoE/B-15/01) of Hong Kong.

L7

The p38 MAPK Is Critical to c-Fos and NFATc1 Induction by RANKL during Osteoclastogenesis

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Receptor-activator of nuclear factor kappaB (NF-κB) ligand (RANKL) is an essential factor for osteoclast differentiation from bone marrow hematopoietic progenitors. The critical role of nuclear factor of activated T cells c1 (NFATc1) in osteoclastogenesis has recently been demonstrated. It has also been reported that p38 MAPK activity is important for osteoclast differentiation induced by RANKL. In addition, NFATc1 induction has been shown to be mediated by c-Fos in osteoclast precursors. In this study we aimed to determine the specific contribution of the signal transduction by p38 MAPK to the RANKL-stimulated induction of c-Fos and NFATc1 in osteoclast precursors. We found that SB203580 inhibited the elevation of mRNA and protein levels of NFATc1 by RANKL in bone marrow-derived macrophages (BMMs). Next, we examined effects of p38 MAPK in RANKL induced c-Fos expression. The results indicated that p38 MAPK is critical for c-Fos induction by RANKL in BMMs. Retroviral transduction of dominant-negative (DN) forms of p38 MAPK upstream proteins MKK3 and MKK6 also inhibited c-Fos and NFATc1 induction by RANKL. Furthermore, overexpression of c-Fos rescued the inhibitory effects of SB203580 on NFATc1 induction and osteoclast differentiation. These results indicate that the p38 MAPK signaling pathway plays an important role in c-Fos and NFATc1 induction by RANKL for osteoclast differentiation from BMMs.

L8

Casein Kinase I and PP2A Phosphatase Regulate the SPS Amino Acid Sensing Pathway by Mediating the Phosphorylation State of Prt3p

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Cells of the budding yeast, Saccharomyces cerevisiae, sense extracellular amino acids and activate expression of amino acid permeases through the SPS sensing pathway, which consists of Syl1p, an amino acid sensor on the plasma membrane, and Prt3p and Ssy5p, two factors that function downstream of Syl1p. Upon activation of the SPS sensing pathway, two transcription factors, Stp1p and Stp2p, upregulate Ssy5p, Prt3p, and several other genes that encode events that remove an N-terminal fragment, which enables these factors to translocate into the nucleus to activate target gene expression. Here we show that Prt3p is a phosphoprotein whose hyperphosphorylation is induced by external amino acids and is dependent on Syl1p, but not on Ssy5p or Grt1p, a component of the SFC1+ E3 ubiquitin ligase. We found that two casein kinase I (CKI) proteins, Yck1p and Yck2p, are required for hyperphosphorylation of Prt3p, with threonine residue 525 as a candidate phosphorylation site. Mutations in threonine 525 inactivate Prt3p and block its hyperphosphorylation. We found that defects in PP2A phosphatase activity due to an rts1Δ mutation lead to hyperphosphorylation of Prt3p and constitutive activation of the SPS sensing pathway. Our findings reveal that CKI and PP2A phosphatase play antagonistic roles in SPS sensing by regulating Prt3p phosphorylation.

L9

Caffeic Acid Phenethyl Ester Inhibits Osteoclastogenesis through Suppression of NF-kappaB, NFATc1, and c-Fos

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Osteoclasts are multi-nuclear cells derived from myeloid lineage cells and are responsible for bone resorption. Caffeic acid phenethyl ester (CAPE), which is derived from the propolis of honey bee hives, has been revealed to have anti-inflammatory properties. Regulation of differentiation and activation of osteoclast cells has a close correlation with inflammation. In this study, we investigated the possible protective effect of CAPE on osteoclastogenesis. CAPE completely blocks osteoclastogenesis from bone marrow derived precursor cells. Specifically NF-kappaB DNA binding and their transcriptional activity but not AP-1 was inhibited by CAPE. MAPK (ERK, JNK, and p38) pathways, and the canonical NF-kappaB pathway, phosphorylation and degradation of IkB alpha and phosphorylation of p65 (Ser536 and Ser276), were not affected by CAPE. Since NFATc1 and c-Fos are potent transcriptional factors in osteoclastogenesis, we evaluated the effect of CAPE on the induction of these transcription factors. We found that that CAPE completely suppressed NFATc1 and c-Fos induction by RANKL. Retroviral transfer of c-Fos completely rescue the effect of CAPE on osteoclastogenesis. In summary, CAPE, a potent inhibitor of osteoclastogenesis, blocks differentiation into mature osteoclasts through suppression of NFATc1 and c-Fos expression and NF-kappaB DNA binding and their transcriptional activity. It is possible to have a wide application of CAPE to many bone loss-associated diseases involving an increase in osteoclast number.
L10 Sprouty2 Inhibits Ras/ERK Signaling by Binding to the SH3 Domain of Grb2
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Since the Sprouty (Spry) proteins were shown to be inhibitors of the mainstream Ras/ERK pathway, there has been considerable interest in ascertaining their mechanism of action especially since a possible role as tumor suppressors for these inhibitory proteins has been suggested. We compared the ability of the mammalian Spry isoforms to inhibit the Ras/ERK pathway in the context of FGFR signaling. Spry2 is considerably more inhibitory than Spry1 or Spry4 and this correlates with its binding to Grb2 via a C-terminal proline-rich sequence that is found exclusively on Spry2. This PxxPXR motif binds directly to the N-SH3 domain of Grb2 and when added onto the C-terminus of Spry4 the resultant chimera inhibits the Ras/ERK pathway. The ability to inhibit neurite outgrowth in PC12 cells correlates with the propensity of Spry isoforms and engineered constructs to inhibit the phosphorylation of ERK1/2. The PxxPXR motif is cryptic in unstimulated cells and is revealed upon stimulation. The activation of Spry2 appears to be linked to sequences in the N-terminal half of the protein and correlates with a bandshifting seen on SDS-PAGE. The bandshifting was caused by changes in the phosphorylation status of key serine/threonine residues following receptor stimulation. Dephosphorylation of at least two serine residues within a conserved serine/threonine rich sequence is accomplished by the Ser/Thr phosphatase PP2A that binds to Spry2 around residues 50-60. PP2A thus competes with c-Cbl which binds to phosphorylated Y55 within this region. The exposed PxxPXR motif competes with SOS1 for Grb2 binding and derails the ERK pathway. The results indicate that Spry2 is regulated in the cell by a novel, carefully orchestrated activation mechanism that is likely offset by a c-Cbl/ubiquitin-directed destruction of the protein.

L11 EGF Receptor Is Involved in WNT3a-mediated Proliferation and Motility of NH3T3 Cells via ERK Pathway Activation
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WNT3a stimulates proliferation of NH3T3 cells via activation of the extracellular signal-regulated kinase (ERK) pathway. The RAF-1→MEK→ERK cascade was immediately increased by WNT3a treatment, however, the upstream event triggering ERK pathway activation by WNT3a is not clear. WNT3a activated RAS and WNT3a-induced ERK activation was blocked by dominant-negative RAS, indicating that WNT3a might act upstream of RAS. WNT3a-induced ERK pathway activations were blocked by AG1478, the epidermal growth factor receptor (EGFR) inhibitor, and EGFR siRNA. The WNT3a-induced ERK pathway activation was not observed in fibroblasts retaining defective EGFR, but the WNT3a effect was restored by EGFR reconstitution. These results indicate involvement of EGFR in the WNT3a-induced ERK pathway activation. WNT3a-induced motility of osteosarcoma cells is also regulated by EGFR and as proliferative activities of NH3T3 cells were blocked by AG1478 and EGFR siRNA or abolished in EGFR knock out fibroblasts, indicating involvement of EGFR in those cellular processes. WNT3a-induced ERK pathway activation was not affected by Dikkof-1 (DKK-1) although WNT3a-induced activations of the WNT/β-catenin pathway and proliferation were reduced by DKK-1. EGFR is involved in WNT-3a-induced proliferation via both routes dependent on and independent of the WNT/β-catenin pathway. These results indicate that WNT3a stimulates proliferation and motility of NH3T3 fibroblasts via EGFR-mediated ERK pathway activation.

L12 Identification of Proteins Interacting with the Catalytic Subunit of PP2A with Proteomics
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The protein phosphatase 2A (PP2A) is a serine/threonine phosphatase involved in the regulation of multiple signaling pathways including the Wnt/β-catenin and the ERK pathways. To understand the complex signaling network associated with PP2A, we searched proteins interacting with the catalytic subunit of PP2A (PP2A2) by a pull-down approach followed by two-dimensional gel electrophoresis (2-DE) and mass spectrometric analyses. The probability of identification of the proteins interacting with PP2A was increased by searching proteins differently interacting with PP2A according to stimulation of Wnt3a, which regulates both the Wnt/β-catenin and the ERK pathways. Around 100 proteins, pulled-down by His-PP2A2, were identified in 2-D gels stained with Coomassie blue, respectively. By matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analyses of 45 protein spots, we identified several proteins that were previously known to interact with PP2A, such as Axin and CaMK IV. In addition, we also identified many proteins that potentially interact with PP2A. The interactions of several candidate proteins, such as TSC2, RhoB, R-Ras, and Nm23H2, with PP2A2, were confirmed by in vitro binding analyses.

L13 Role of AMP-activated Protein Kinase in T Cell Receptor-induced IL-2 Production in Jurkat T Cells
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AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis and its activation during T cell receptor stimulation has recently been reported. In this study, we examined the role and signaling mechanisms of AMPK in T cell receptor induced IL-2 production. AMPK was activated in T cells. AMPK activity decreased upon stimulation of LTβR and when added onto the C-terminus of Spry4 the resultant chimera inhibits the Ras/ERK pathway. The ability to inhibit neurite outgrowth in PC12 cells correlates with the propensity of Spry isoforms and engineered constructs to inhibit the phosphorylation of ERK1/2. The PxxPXR motif is cryptic in unstimulated cells and is revealed upon stimulation. The activation of Spry2 appears to be linked to sequences in the N-terminal half of the protein and correlates with a bandshifting seen on SDS-PAGE. The bandshifting was caused by changes in the phosphorylation status of key serine/threonine residues following receptor stimulation. Dephosphorylation of at least two serine residues within a conserved serine/threonine rich sequence is accomplished by the Ser/Thr phosphatase PP2A that binds to Spry2 around residues 50-60. PP2A thus competes with c-Cbl which binds to phosphorylated Y55 within this region. The exposed PxxPXR motif competes with SOS1 for Grb2 binding and derails the ERK pathway. The results indicate that Spry2 is regulated in the cell by a novel, carefully orchestrated activation mechanism that is likely offset by a c-Cbl/ubiquitin-directed destruction of the protein.
exhibiting increased expression included IL-8, TNF-α, and MIP-1α, among others. Our experiments clearly demonstrate the utility of antibody microarray analysis of cell culture supernatants for the profiling of cellular inflammatory mediator release.

L15
Rab35 Regulates an Endocytic Recycling Pathway Essential for the Terminal Steps of Cytokinesis
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Cytokinesis is the final step of cell division that leads to the physical separation of the daughter cells. Following the ingression of a cleavage membrane furrow that pinches the mother cell, future daughter cells spend much of the cytokinesis phase connected by an intercellular bridge. Rab proteins are major regulators of intracellular transport in eukaryotes and here, we report an essential role for Rab35 in both the stability of the bridge and its final abscission. We find that Rab35, whose function in membrane traffic was unknown, is localised to the plasma membrane and endocytic compartments, and controls a fast endocytic recycling pathway. Consistent with a key requirement for Rab35-regulated recycling during cell division, inhibition of Rab35 function leads to the accumulation of endocytic markers on numerous cytokinetic vakuoles in cells that failed cytokinesis. Moreover, Rab35 is involved in the intercellular bridge localisation of two molecules essential for the post-furrowing steps of cytokinesis: the phosphatidylinositol 4,5-bisphosphate (PIP2) lipid, and the septins, L19. We propose that the Rab35-regulated pathway plays an essential role during the terminal steps of cytokinesis by controlling septin and PIP2 subcellular distribution during cell division. We are currently investigating how the Rab35 pathway contributes to the establishment of lipid domains essential for the completion of cytokinesis.

L16
AKAP12/Gravin Is Required for the Completion of Cytokinesis
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A Kinase Anchoring Protein 12 (AKAP12/Gravin) acts as a scaffold that binds protein kinase A, protein kinase C and is known that associates with G-protein coupled receptor, the β₂-adrenergic receptor. It has been reported that AKAP12 shows growth suppressor activity, and is down-regulated in several cancers, including gastric, breast and prostate cancers. SSekCSs, a rodent homologue of human AKAP12, also shows tumor suppressor activity, and have the ability to control actin-based cytoskeletal architecture. However, the biological role of AKAP12 in cancer development is not clear. Aneuploidy, one of the hallmark features in cancer cells, often caused by abnormal cell division. In this study, we found that AKAP12 is related in cell division, especially cytokinesis. Like many other cytokinesis related molecules, AKAP12 located to contractile ring during cytokinesis with actin and myosin II, and AKAP12 knock-down using siRNA resulted in increasing of multinucleated cells. Moreover, we studied the requirement for AKAP12 during the process of cytokinesis and found that the depletion of AKAP12 disrupts the completion of cytokinesis. This result demonstrates that AKAP12 regulates the final stage of cytokinesis. Myosin light chain binds to the AKAP12 chain domain and its phosphorylation plays an important structural role in acto-myosin contractile ring. Myosin Light Chain Kinase (MLCK) inhibitor, ML-9 and Rho-Kinase (ROCK) inhibitor, Y-27632 inhibit the phosphorylation of myosin light chain. Interestingly, ML-9, not Y-27632 dramatically changed AKAP12 localization from plasma membrane to cytosol during interphase. Also, during cytokinesis, ML-9 treatment removed AKAP12 in contractile ring. These results suggest that AKAP12/Gravin regulates cytokinesis through MLCK-dependent pathway.

L17
Checkpoint1: A Chromosomal Passenger Protein Necessary for Completing Mitosis to Prevent Genomic Instability
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The spatial-temporal regulation of proteins during mitosis is critical for the completion of normal cell division. In mammals, Checkpoint1 (Chk1) is a serine/threonine kinase that regulates mitotic entry by phosphorylating and suppressing the activity of Cdc25 phosphatases, thus enabling cell cycle arrest for DNA repair, and the induction of apoptosis. Here, we show that Chk1 is a chromosomal passenger protein and its abrogation during mitosis leads to binucleated progeny. Impairment of Chk1 function in mitotic cells was demonstrated by a novel methodology using antibody transfection reagent to efficiently deliver protein-specific antibodies into mammalian cells. Additionally, based on both in vitro and in vivo studies, we present evidence of functional interaction between Chk1 and Cdc14- a dual-specificity serine/threonine phosphatase. To correlate this functional interaction to its structure, in silico analysis was carried out revealing conserved putative Chk1 phosphorylation sites in Cdc14. The consequence of this functional interaction in mammalian cells was analyzed after ectopically expressing Cdc14 mutants. The mutants act dominant negatively and fail to bundle digitigrating microtubules during cytokinesis leading to binucleation. In addition, disruption of Chk1 in mitotic cells results in mis-localized Cdc14 leading to binucleation. Thus, we have identified that Chk1-Cdc14 complex regulates microtubule dynamics necessary for completing cytokinesis to prevent binucleation. Furthermore, using Chk1 mouse models we demonstrate that normal mitotic primary embryonic cells result in the formation of chromosome mis-alignment held by multipolar spindles and chromosome mis-segregation leading to binucleation. Therefore, our results provide evidence for an evolutionary conserved signaling network, made up of DNA damage-recognizing and mitotic exit components necessary for completing cytokinesis. Levels of these cell cycle regulators are critical to prevent failure of cytokinesis leading to binucleation, initiation of genomic instability and cancer.

L18
ARF6 Controls Completion of Cytokinesis through JIP Proteins
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ARF6 (ADP-Ribosylation Factor 6) is a small G protein implicated in cortical actin rearrangement and polarized membrane recycling. A yeast two-hybrid screen performed in collaboration with Hybrigenics (Paris, France) identified the conserved JIP proteins (JIPs) as potential partners of the constitutively activated mutant form of ARF6. JIPs are scaffolding proteins in the MAP Kinase signalling pathway. Moreover, JIPs interact with the Kinesin Light Chain (KLC) and the dynein complex and control axonal movement of vesicles along microtubules. Our finding suggests the interesting possibility that ARF6 may control polarized movement of cargos - membranes and/or MAPKs - along microtubules through JIPs. We first confirmed that ARF6 interacts with JIPs, and that the interaction is direct and strictly dependent on the nucleotide (GTP) state of ARF6. Furthermore, we mapped the binding sites of KLC and the dynactin subunits p50 and p150054 on JIPs and analyzed the regulation of these associations by ARF6.In Hela cells, overexpression of the amino-terminal region of JIP induced a redistribution of transferrin-loaded endosomes to the cell periphery as compared to control cells. Cells knocked-down for JIP also showed delocalized endosomes suggesting that JIP controls endosome positioning through microtubule motors. In addition, we observed an elevated number of JIP-N-expressing cells connected by a midbody as compared to control cells. These cells did not display the accumulation of the endosome and endosome related molecules necessary for completing cytokinesis probably by controlling the positioning of endosomes at the midbody, allowing for membrane delivery and further abscission of the daughter cells. ARF6 has been shown to localize to the midbody and is involved in completion of cytokinesis. Our results provide a molecular mechanism for ARF6’s control of membrane recruitment at the midbody through JIP-microtubule to allow completion of cytokinesis.

MITOSIS AND MEIOSIS
L19
Expression of Nek2-interacting Protein (nip2) Was Correlated With the Proliferation Activity of the In Vivo Tissues
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Nek2 is a mitotic kinase whose activity is critical for centrosome splitting. Involvement of Nek2 on spindle assembly and mitotic checkpoint was also known. We recently identified a novel substrate of Nek2 and named Nek2-interacting protein 2 (Nip2). The identical protein was previously reported as Centrinobin that is required for centrosome duplication. In the present study, we determined Nip2 expression in various mouse tissues. Nip2 was expressed in all tissues tested but most abundantly in testis, spleen and thymus. Immunohistochemical analysis revealed that Nip2 was abundant in cells with proliferation activities. Furthermore, reduction in the Nip2 levels was observed in NIH3T3 cells cultured in a serum-free medium. These results suggest that the biological functions of Nip2 are linked to cell division.

L20 Analysis of the Spindle Checkpoint Pathway
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The spindle checkpoint prevents anaphase onset until all the kinetochores of the chromosomes have successfully attached to the spindle microtubules. The major components of this surveillance mechanism, originally identified in budding yeast, include Mad1-3, Bub1-3 and Mps1 as well as CENP-E, Rod, ZW10, Aurora B and MAP kinase. Despite the large number of studies performed to investigate this checkpoint, the exact role of each of these proteins as well as the place that they occupy in this pathway is poorly understood. Moreover, the current data does not allow us to explain how this pathway senses the microtubule attachment to kinetochore, how the stimulus is transmitted and how it is transformed to an inhibitory “wait anaphase” signal. We have developed a biochemical procedure to characterize new proteins involved in this checkpoint in order to obtain the complete picture of this pathway. We have subsequently analyzed the role and place that the new proteins occupy in this pathway. A second aspect of our study concerns the role of some of these checkpoint proteins on mitotic divisions of Xenopus oocytes. We are currently investigating a putative involvement of some of these proteins on the regulation of mitosis.

L21 Regulation of Microtubule Stability at Metaphase to Anaphase Transition by Calcium Dependent Activation of Protein Phosphatase 2A
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The primary task of dividing eukaryotic cells is the replication of genetic information and the segregation of the chromosomes among daughter cells. These processes are regulated by the cell cycle control network, the central components of which are cyclin dependent kinases (Cdks). Cdk1/CyclinB is the key regulator of mitosis. Its activation initiates cell entry into mitosis whereas the degradation of CyclinB marks the beginning exit of mitosis. Recent research mainly focused on Cdk1 and its substrates and their impact on mitotic progress, while the role of phosphatases in keeping a well-balanced mitotic phosphorylation pattern is still poorly understood. One key parameter regulated in mitosis is microtubule stability. Microtubules get more dynamic at mitotic onset, which allows formation of the spindle, and their stability increases again in interphase. Moreover, microtubule dynamics need to be regulated at metaphase to anaphase transition. The regulation of microtubule dynamics during the cell cycle can be recapitulated in Xenopus egg extracts. Using the Xenopus system, we show that a calcium-dependent activation of protein phosphatase 2A (PP2A) is essential for the regulation of microtubule stability in anaphase. Surprisingly, the activation of PP2A is not dependent on the activation of the APC. Knock-down of the catalytic as well as a regulatory subunit of PP2A in HeLa cells via RNAs resulted in cells arrested in mitosis with aberrant spindles and hyper-stable microtubules, which do not allow successful completion of cell division. These findings demonstrate an essential role of PP2A in regulating microtubule dynamics in mitosis.

L22 High-throughput Screen for Compounds Causing a Forced Mitotic Exit: Study of Two Flavonoids

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Cancer treatment is increasingly facing the problem of cancer cells becoming resistant to treatment. Another problem is the damage cancer drugs cause to normal tissue as side-effects. We have conducted high-throughput screens of chemical compound libraries to identify new lead compounds for anti-cancer drug development. We screened for compounds that can override chemically activated spindle checkpoint and induce a forced mitotic exit in human cancer cells. By using Spectrum library from Microsource, we have screened 2000 small molecules including natural compounds, newly synthesized compounds, and known drugs. In the screens we identified two dietary flavonoids that turn off the hyperactivated spindle checkpoint and induce abnormal mitotic exit. The cells decondensed chromosomes and reformed nuclear envelope without cytokinesis. Several studies suggest that flavonoids have anti-cancer properties and inhibitory effects on cell proliferation. Moreover, certain flavonoids have been reported to inhibit cyclin-dependent kinases (Cdks) but the effects on the spindle checkpoint signalling have not been explored before. Cdk1 is still unknownly demarcated though, including treatments, induce a more active, cell division phase. This was confirmed by live cell microscopy. Co-treatment with MG132 (a proteasome inhibitor) and either flavonoid arrests the cells at pseudometaphase indicating that the compound induced forced exit is a proteasome-dependent process. No difference was observed in the override of the checkpoint control between nocodazole and taxol treated cells. We are currently investigating how these flavonoids modulate spindle checkpoint by analysis of kinetochore localization of several key checkpoint proteins in the compound treated cells. In addition, we are testing effects of the compounds on cell cycle kinetics such as Cdks and members of the Polo kinase family using in vitro activity assays. Finally, we are studying possible synergistic anti-proliferative effects between these flavonoids and known clinical cancer drugs such as doxorubicin using both normal human epithelial cells and various cancer cell lines.

ACTIN

L23 Direct Measurement of Force Generation by Actin Filament Polymerization Using an Optical Trap
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Actin filament polymerization generates force for protrusion of the leading edge in motile cells. In protrusive structures, multiple actin filaments are arranged in crosslinked webs (as in lamellipodia or pseudopodia) or in parallel bundles (as in filopodia). We have used an optical trap to directly measure the forces generated by elongation of a few parallel growing actin filaments brought into apposition with a rigid barrier, mimicking the geometry of filopodial protrusion. We find that the growth of ~8 actin parallel growing filaments can be stalled by relatively small applied load forces on the order of 1 pN, consistent with the theoretical load required to stall the elongation of a single filament under our conditions. Indeed, large length fluctuations during the stall phase indicate that only the longest actin filament in the bundle is in contact with the barrier at any given time. These results suggest that force generation by small actin bundles is limited by a dynamic instability of single actin filaments, and therefore that living cells must use actin-associated factors to suppress this instability in order to generate substantial forces by elongation of parallel bundles of actin filaments.

L24 A Novel Actin-based Plasma Membrane GLUT4 Retention Model as a Basis for Impaired Insulin Responsiveness in Insulin-resistant Cells
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Hyperinsulinemia defines early stages of insulin resistance and exacerbates the progression of this metabolic derangement. Recent in vitro analyses reveal cortical filamentous actin (F-actin) losses in adipocytes and skeletal muscle cells rendered insulin resistant by sustained insulin exposure. Correction of this cytoskeletal defect restored insulin sensitivity. Here, we set out to investigate the ramifications of this structural perturbation on the kinetics of glucose transporter GLUT4 movement. Utilizing L6 myotubes stably expressing GLUT4 that carries an exofacial myo-epitope tag, acute insulin stimulation (100 nM) elicited a plasma membrane GLUT4myc in steady state level 2.1-fold above basal conditions. Steady-state levels of plasma membrane GLUT4myc were achieved at 20 min of acute insulin stimulation. Inclusion of 5 nM insulin in the media for 12 h prior to the acute insulin challenge significantly reduced steady-state plasma membrane GLUT4myc levels. Time course studies revealed that this reduced level of insulin-stimulated GLUT4 was achieved at 10 min where it leveled off and remained 15% lower than that observed in control cells throughout the 30 min time course. Similar plasma membrane GLUT4 steady-state trends were observed in control and chronic insulin-treated 3T3-L1 adipocytes. Furthermore, basal and insulin-stimulated 2 deoxy-D-glucose cellular uptake paralleled these translocation results in both cell types. As GLUT4 exocytosis and endocytosis rates ultimately define plasma membrane steady-state levels, we next evaluated these parameters. Remarkably, GLUT4 efflux rates appeared comparable between control and chronic insulin-treated cells. In contrast, hyperinsulinemic conditions tended to elevate GLUT4 endocytosis rates. In summary, chronic insulin-induced F-actin losses do not appear to preclude GLUT4 translocation to and fusion with the plasma membrane. Rather, this structural defect seems to impair the retention of GLUT4 at the plasma membrane, thus leading to the insulin-resistant phenotype. These observations are consistent with the idea that the cytoskeletal actin meshwork functions to maintain plasma membrane-localized GLUT4.

L25 Molecular Evolution of Bacterial Actin-like Proteins
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The possession of a cytoskeleton is a property once thought to be limited to eukaryotic cells. However, it has recently become clear that bona fide homologs of eukaryotic actin and tubulin exist in many prokaryotic organisms. Less clear has been the variety of these proteins, their evolutionary relationships, or their distribution among prokaryotic groups. To address these questions, we have probed all fully sequenced eubacterial and archaeabacterial genomes for proteins detectably related to structurally characterized members of the actin superfamily. It has generally been assumed that prokaryotic actin relatives fall into three subfamilies: MreB, FtsA, and ParM, with the greater actin superfamily including HSP70 and, more distantly, the sugar kinases. Phylogenetic analysis of the full set of bacterial actin-like proteins (>1200 sequences) shows that the diversity of the actin superfamily in prokaryotes is in fact much larger, consisting of at least 12 subfamilies as well as a great number of proteins with more limited phylogenetic distribution. These include the recently characterized archaeal Tau583 protein, as well as the type IV pilus forming protein (PilM, found in organisms such as Myxobacteria), and the ethanolamine utilization protein (EutJ, found in a small set of diverse bacteria). Most of the remaining prokaryotic actin superfamily members are uncharacterized. Actin-related proteins appear to be ubiquitous, but in eukaryotes, the evolutionary conservation of actin has enabled us to study its function. In prokaryotes, the actin-related proteins can be divided into two major categories, MreB-related proteins and Par-related proteins. MreB-related proteins are involved in cell division, while Par-related proteins are involved in cell motility. The study of the mechanisms underlying cell motility is an important field in basic cell biology. Single cell motility assays allow scientists to study the mechanisms underlying cell motility at a molecular level in the context of whole cell behavior, specifically movement. Despite the large and broad need for individual cell motility assays, and the improving performance and availability of automated live cell imaging systems, research efforts in individual cell motility analysis continue to be a tedious, largely manual and inexact process. This is due primarily to a lack of accurate and robust kinetic recognition tool, particularly for cells in phase contrast images. We developed a kinetic recognition algorithm that uses the slow changing background and strong correlation between cells in consecutive frames to automatically recognize and track individual cells in time-lapse phase contrast images. It maintains a running background reference for highly sensitive cell detection and performs automatic next frame prediction, matching, and refinement. We validated the performance of the kinetic recognition algorithm using 30 time-lapse phase contrast movies recorded from crawling cells. A segmented cell tracking accuracy study are conducted. The truth are created manually and validated by independent review and update. The kinetic recognition algorithm results are compared with the truth using Average Cell Segmentation Error and Average Cell Tracking Error Ratio as test metrics. The tests show about 80% cell segmentation accuracy and promising tracking results.

L27 Automated Kinetic Analysis in Individual Cell Motility Assays
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Cell motility is a fundamental process central to embryonic development, immune response, wound healing, angiogenesis, tissue engineering and various disease processes, including cancer metastasis. The study of the mechanisms underlying cell motility is an important field in basic cell biology. Single cell motility assays allow scientists to put findings from a molecular / subcomponent level in the context of whole cell behavior, specifically movement. Despite the large and broad need for individual cell motility assays, and the improving performance and availability of automated live cell imaging systems, research efforts in individual cell motility analysis continue to be a tedious, largely manual and inexact process. This is due primarily to a lack of accurate and robust kinetic recognition tool, particularly for cells in phase contrast images. We developed a kinetic recognition algorithm that uses the slow changing background and strong correlation between cells in consecutive frames to automatically recognize and track individual cells in time-lapse phase contrast images. It maintains a running background reference for highly sensitive cell detection and performs automatic next frame prediction, matching, and refinement. We validated the performance of the kinetic recognition algorithm using 30 time-lapse phase contrast movies recorded from crawling cells. A segmented cell tracking accuracy study are conducted. The truth are created manually and validated by independent review and update. The kinetic recognition algorithm results are compared with the truth using Average Cell Segmentation Error and Average Cell Tracking Error Ratio as test metrics. The tests show about 80% cell segmentation accuracy and promising tracking results.

CELL MOTILITY–REGULATION

L28 Coordinate Regulation of Gia, RGS1, and CCRX4 in B Lymphocyte Chemotaxis
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Regulators of G-protein signaling (RGS) are a family of proteins that accelerate the intrinsic GTPase activity of Gia subunits. The analysis of B cells prepared from Rgs1−/−, Gna12−/−, and Rgs1−/−Gna12−/− mice has shown that the RGS1/Gia2 ratio directly affects CXCL12/CXCR4
Fer-cortactin Signaling Axis Promotes Fibroblast Migration

L29

Background/Objective: Elevated levels of reactive oxygen species (ROS) have been correlated with tumor progression and metastasis and are present at high levels in tumor cell lines. ROS are produced in response to growth factor (GF) and adhesion receptor engagement and have been shown to be required for the propagation of downstream signaling. Elevated ROS could therefore promote GF and adhesion signaling in cancer. Recent studies in fibroblasts, suggests the existence of a novel signaling axis downstream of GF and adhesion receptors. This signaling axis consists of the ROS H₂O₂, the tyrosine kinase Fer, and cortactin (Ctn) - a scaffolding protein strongly implicated in breast cancer metastases. Our objective was to characterize the impact of this signaling-axis in migration.

Methods/Results: Stimulation of murine embryonic fibroblasts (MEF) with H₂O₂ produced marked tyrosine phosphorylation (pY) of Fer and cortactin (Ctn). In fer-deficient MEF, H₂O₂ induced trace levels of Ctn pY signal which could be completely ablated by the Src-family kinase (SKK) inhibitor, PP2. In srcyeayfin (sy) fer-deficient MEFs, Ctn pY strongly correlated with Fer expression levels suggesting an SKK-independent role for this kinase in mediating Ctn pY in oxidative pathways. We also found that Fer and Ctn pY are inducible by cell adhesion however, Ctn pY is dramatically reduced in adhering fer-deficient MEF suggesting a link between upstream adhesion and oxidative signaling mechanisms. Consistent with this, Fer pY in adhering MEF was attenuated by H₂O₂ scavengers, and by an inhibitor of NADPH membrane oxidases involved in oxidative species (ROS) generation. Importantly, impaired migration of fer-deficient MEF correlated with defects in adhesion- and H₂O₂-induced Ctn pY in these cells. Conclusions: These data suggest that a novel oxidative Fer-Ctn signaling axis regulates fibroblast migration.

L30

Pak1 and Pak2 Mediate Heregulin Stimulated T47D Invasion through Distinct Pathways

Sunday

Phosphatidylinositol 3-Kinase Activity and Membrane Ruffling in Macrophages

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The leading edge of leukocytes is characterized by Rac1 and phosphatidylinositol 3-kinase (Pl3K) activity and dynamic turnover of filamentous actin (F-actin), which maintains the leading edge membrane ruffles. Moreover, maintenance of an actively ruffling leading edge is thought to involve positive feedback signaling between Rac1, Pl3K, and F-actin. RAW264.7 cells (RAW cells), a mouse macrophage cell line, display all these characteristics with the Pl3K activity and F-actin turnover in RAW cells sensitive to both the Pl3K inhibitor wortmannin and to the actin sequestering agent latrunculin B. Here, we investigated the role of Ca²⁺ in the maintenance of leading edge in RAW cells using fluorescent protein (FP)-tagged protein kinase Ca (FP-PKCa), which also localizes to the ruffling edge of RAW cells, as a read-out of intracellular Ca²⁺, FP-Akt1PH domain as a readout of PI3K activity, and FP-actin to monitor leading edge actin polymerization. Lowering the extracellular Ca²⁺ concentration with EGTA or blocking Ca²⁺ influx with La⁺ resulted in the rapid loss of FP-PKCa from the leading edge. This was quickly followed by a similar loss of FP-Akt1PH and FP-actin from the leading edge, and an immediate cessation of membrane ruffling, similar to the effect seen by treating RAW cells with wortmannin or latrunculin B. Addition the Ca²⁺ mobilizing agonist ATP stimulated a transient increase in FP-Akt1PH at leading edge membranes and a transient, actin-driven expansion of the leading edge membranes. Wortmannin treatment also resulted in the loss of FP-PKCa from the leading edge. These results point to a clear, and previously unrecognized, role for the Pl3K-sensitive, extracellular Ca²⁺ influx in the maintenance of leading edge, and suggest that Ca²⁺ is intimately involved in the Rac1/Pl3K/actin feedback loops that maintain a leading edge in leukocytes.

L32

Activated Androgen Receptor (AR) Down-regulates E-cadherin Gene Expression and Promotes Tumor Metastasis

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Metastasis is a multistep process during which cancer cells migrate from the primary sites to the secondary sites in distant organs. In cancer cells, the loss of E-cadherin gene expression causes dysfunction of the cell-cell junction triggering cancer invasion using constitutively active and dominant negative mutants have implicated a central role for these proteins in cell migration and invasion. However, there is very little evidence available for specific functions of different Pak isoforms. The purpose of this study is to determine the contribution of Pak1 and Pak2 to Heregulin (HRG)-stimulated invasion signaling in the human breast carcinoma cell line T47D, using small interfering RNA (siRNA) directed specifically against Pak1 or Pak2. Silencing of either Pak1 or Pak2 blocks HRG-stimulated invasion in a Matrigel invasion assay. However silencing Pak1 has a more robust inhibitory effect on lamellipodial protrusion than Pak2. Inhibition of Pak1 significantly interferes with HRG signaling to Cofilin and JNK (effectors required for T47D invasion), whereas Pak2 has little or no effect on these pathways. Interestingly, Pak1 and Pak2 play opposite roles in regulating the phosphorylation of the Myosin Light Chain subunit of Myosin II, a critical regulator of cell contractility and focal adhesion dynamics. We also found that depletion of Pak2 enhances RhoA activity. Inhibition of RhoA/ROCK/Myosin II signaling axis using siRNA against RhoA, Y-27632 (a ROCK inhibitor) or blebbistatin (an inhibitor of Myosin II) enhances HRG mediated invasion, demonstrating that the Rho pathway acts as brake for cell invasion. In summary, we propose a model whereby HRG stimulates cell invasion via both Pak1 and Pak2. Pak1 is required for the activation of Cofilin and JNK and lamellipodia formation, while Pak2 is required for deactivation of the RhoA/ROCK/Myosin II pathway and turnover of focal adhesions/stress fibers.
metastasis. To understand the effect of the loss of E-cadherin function on tumor metastasis we studied how E-cadherin gene expression is regulated in metastatic and non-metastatic cancer cells. Previously we had identified several enhancing and suppressing regulatory transcription factors that affect the expression of human E-cadherin gene. In addition we detected a new regulatory element that could exert suppressive effect on the human E-cadherin gene expression. The detected element is different from E-box that is the binding site for Snail or Twist. In the present study we characterized the new repressive element and carried out promoter functional assays. The results indicated the novel repressor binding element is the binding site for androgen receptor (AR), a member of the steroid receptor superfamily of ligand-dependent transcription factors. EMSA and ChIP assays confirmed the binding of AR on the E-cadherin regulatory sequence in vitro and in vivo. Co-transfection assay also showed the ligand bound AR and HDAC1 have synergistic effect to down-regulate the E-cadherin expression. Ligated activated AR resulted in the translocation of AR to the nucleus, suppression of E-cadherin and activation of mesenchymal markers, vimentin. The ligand stimulated cells was found to be able to change the morphology from epithelial to mesenchymal type; also the AR siRNA can reverse this change. Furthermore, in human breast cancer, high level expression of AR in the nucleus is correlated with invasive ductal carcinoma. These results suggested that AR, besides Snail or Twist, can down regulate E-cadherin expression in a ligand dependent manner to activate epithelial-mesenchymal transition and thus promote metastasis.

L33

EMT-inducible Protein, Erythrocyte Protein Band4.1 Like-5, Plays an Essential Role for the EMT Progression

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EMT-inducible Protein, Erythrocyte Protein Band4.1 Like-5, Plays an Essential Role in the Progression of EMT. We are currently analyzing molecular mechanisms as to how Ebl5 is involved in the onset of EMT.

L34

Matrix Metalloproteinase Inhibitor Suppresses Skeletal Muscle Cell Migration In Vitro

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Skeletal muscle satellite cells are quiescent stem cells that localized between plasmalemma and basal lamina of muscle fibers. When muscle is injured, satellite cells are activated, migrate to the injured site and contribute to the regeneration of muscle. However, little is known about the mechanism that satellite cells migrate beneath or within basal lamina in muscle. Skeletal muscle satellite cells synthesize some matrix metalloproteinases (MMPs), such as MMP-1, -2, -3 and -9 (Guerin & Holland, 1995). In this study, to clarify the role of MMPs that synthesized in muscle cells, we investigated the effect of MMP inhibition on the migration of muscle cells in vitro. First, to confirm the synthesis of MMPs in muscle cells, we examined the localization of MMPs in the primarily cultured muscle cells from adult rat skeletal muscle. These MMPs were also located in C2C12 cells, a murine myogenic cell line. Next, to investigate the effect of MMP inhibition on muscle cell migration, C2C12 cells were cultured in 10% FBS-DMEM containing MMP inhibitor reagents, N-acetyl cysteine (NAC), Chlorhexidine, MMP inhibitorII and GM 6001. The migration of the cells was monitored by time-lapsed imaging. The migration rate of cells cultured with MMP inhibitorII or GM 6001 was significantly lower (P<0.01) than the control cultured without inhibitor reagents. The persistency index was significantly higher (P<0.01) in cells cultured with these inhibitors than those without inhibitors. Cells cultured with NAC did not migrate during 24 hours after addition of the inhibitor. These results suggest that MMPs participate in skeletal muscle cell migration.

L35

IQGAP1 Regulates Cell Motility by Linking Growth Factor Signaling to Actin Assembly

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IQGAP1 has been implicated as a regulator of cell motility because its overexpression or underexpression stimulates or retards cell migration, respectively, but the underlying mechanisms are not well understood. Here we present evidence that IQGAP1 stimulates branched actin filament assembly, which provides the force for lamellipodial protrusion, and that this function of IQGAP1 is regulated by binding of type 2 fibroblast growth factor (FGF2) to a cognate receptor, FGFR1. Stimulation of serum-starved MDBK cells with FGF2 promoted IQGAP1-dependent filamentous actin (F-actin) polymerization and formation of intracellular associations of IQGAP1 with FGFR1, and two other factors, the Arp 2/3 complex and its activator, N-WASP, that coordinate promote formation of branched actin filament network; and recruitment of IQGAP1, FGFR1, N-WASP and Arp2/3 complex to lamellipodia. N-WASP was also required for FGF2-stimulated migration of MDBK cells. In vitro, IQGAP1 bound directly to the cofilin/polymerization tail of FGFR1 and not to N-WASP. IQGAP1 enhanced FGF2-stimulated cell migration, which was inhibited by antibodies against IQGAP1, FGFR1 and N-WASP. IQGAP1 was able to cause reorganization of F-actin at cell periphery in the presence of N-WASP and Arp2/3 complex. Based on these observations, we conclude that IQGAP1 links FGF2 signaling to Arp2/3 complex-dependent actin assembly by serving as a binding partner for FGFR1 and as an activator of N-WASP.

L36

Filamin A Is Not Essential for Cell Motility

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Filamins are large actin-binding proteins that cross-link the actin cytoskeleton and link it to plasma membrane. Based on several lines of evidence Filamin A (FLNa), the most abundant and widely expressed of the human filamins, was believed to be necessary for cell migration. Firstly, several cell lines generated from human melanomas showed loss of FLNa expression and impaired motility while efficient migration was restored in a line stably re-expressing FLNa. More recently loss-of-function mutations in the human FLNa gene were shown to be the cause of periventricular nodular heterotopia (PVH), an X-linked disease characterized by nodules of neurons adjacent to the lateral ventricle wall. Since this is the localization of neuron precursors in the embry, it was hypothesized that in the absence of FLNa neurons are unable to migrate to their final localization in the cortex. To investigate the role of FLNa in cell migration we selectively silenced FLNa expression in three different cell lines (HT1080, NIH 3T3 and CHO) by stable shRNA transfection, and assayed those cells for motility. Satellite cells in FLNa and N-WASP, and stimulated branched actin filament nucleation in the presence of N-WASP and Arp2/3 complex. Based on these observations, we conclude that IQGAP1 links FGFR2 signaling to Arp2/3 complex-dependent actin assembly by serving as a binding partner for FGFR1 and as an activator of N-WASP. More recently loss-of-function mutations in the human FLNa gene were shown to be the cause of periventricular nodular heterotopia (PVH), an X-linked disease characterized by nodules of neurons adjacent to the lateral ventricle wall. Since this is the localization of neuron precursors in the embry, it was hypothesized that in the absence of FLNa neurons are unable to migrate to their final localization in the cortex. To investigate the role of FLNa in cell migration we selectively silenced FLNa expression in three different cell lines (HT1080, NIH 3T3 and CHO) by stable shRNA transfection, and assayed those cells for motility. Satellite cells in FLNa and N-WASP, and stimulated branched actin filament nucleation in the presence of N-WASP and Arp2/3 complex. Based on these observations, we conclude that IQGAP1 links FGFR2 signaling to Arp2/3 complex-dependent actin assembly by serving as a binding partner for FGFR1 and as an activator of N-WASP.
effect of FLNa expression on cell migration. Thus our data indicate that FLNa expression is not essential for cell motility. Instead, FLNa may modulate cell migration in a cell-type or context specific fashion.

L37 Developmental Regulation of Distal Tip Cell Migration in C. elegans
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Cell migration is essential during embryonic development and tissue morphogenesis. During gonadogenesis in the nematode Caenorhabditis elegans, migration of the distal tip cells (DTC) forms two U-shaped gonad arms. Malformation results if the distal tip cells stop prematurely or follow an aberrant path and abnormalities are easily visualized in living nematodes. In a comprehensive, in vivo RNA interference screen for genes required for cell migration, we identified 99 genes required for distal tip cell migration. We used genetic and physical interaction data to connect the genes we identified and DTC regulatory genes from the published literature into a network model for DTC migration. Three of the genes (HLH-2, GON-1 and PPN-1) isolated in the screen had very similar phenotypes with early termination of DTC migration and poorly extended gonad arms. HLH-2 and GON-1 are known early regulators of gonad formation. We demonstrate that the ECM protein papilin PPN-1 is expressed by the DTC precursor cells in the primordial gonad and is required for initiation of gonad migration. In addition, we found that expression of PPN-1 and the matrix metalloprotease GON-1 requires the transcription factor HLH-2. We are using the gene network predictions and detailed phenotypic analysis to begin to construct a model for the developmental control of cell migration in vivo.

CELL MOTILITY–STRUCTURAL BASIS

L38 Age-related Changes in Fish Keratocyte Adhesion, Morphology, and Locomotion
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Fish keratocytes are useful in the study of locomotion because they move rapidly (up to 1 micron/second) with an almost uniform speed and direction while maintaining a fan-shape morphology. This rapid gliding motion requires balancing the intricate interrelationship between adhesion strength, cell shape and speed which is disrupted as cells age. Young cells move rapidly, form labile adhesions, and adopt the fan-shape characteristic of keratocytes. Older cells move more slowly, form stronger adhesions, and have a larger spread area. These morphological and adhesion strength changes are associated with changes in cytoskeletal organization that correlate with different locomotory behaviors. Keratocytes of middle age switch rapidly from a young-cell to an older-cell morphology when the formation of new adhesions is inhibited by the addition of RGD peptide to the media. We hypothesized that rapid changes in locomotory behavior involve reorganization of existing cytoskeletal and adhesion structures, but that the age-related changes described here require changes in gene expression.

L39 Dissection of the Role of Centrin Proteins in Vorticella convallaria
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The stalked ciliate, Vorticella convallaria, is a good model system to study mechanoochemical motility because its contractile organelles (spasmoneme and myonemes) use a mode of contraction that differs from most other eukaryotic motile systems. Vorticella’s contractile cytoskeleton consists of a longitudinal lattice of filaments in the cell body (myonemes) that are bundled together to form the single contractile organelle (spasmoneme) of the stalk. Calcium triggers the cytoskeletal contraction. Upon binding calcium, the spasmoneme coils to 10% of its extended length in a few milliseconds. Since calcium triggers this contraction, we have undertaken the molecular characterization of the calcium-binding proteins associated with these organelles. We have identified a multi-gene family of calcium-binding proteins using a degenerate PCR-based cloning method to the conserved calcium-binding protein, centrin. Many organisms have evolved centrin multi-gene families with the prediction of an increased range of functions within these organisms. Therefore, the V. convallaria centrin multi-gene family is an attractive system to ascertain the various function of each centrin in the cell. We have isolated and identified seven unique centrin-like cDNAs from V. convallaria. We predict each centrin has a distinct function within the cell. To define these functions, we have initiated immunolocalization studies at the light and the ultra-structural level utilizing various anti-centrin antibodies and a collection of mono-clonal antibodies raised against stalk protein which contains the contractile spasmoneme. Western analysis indicates that each antibody recognizes a distinct subset of proteins in Vorticella. We have localized these antibodies to various contractile cytoskeletal structures within the cell. Additionally, data indicate a further restriction in the localization of these antibodies within these organelles. By using mass spec analysis, we are characterizing the proteins recognized by these antibodies. This analysis allows us to begin to dissect the function of this multi-gene family in Vorticella.

CENTROSOMES

L40 Dynein and Hsp90 Are Required for Duplication of Functional Centrosomes but Not Pre-centriole Assembly in S-phase Arrested Cells
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Centrosome duplication has been extensively characterised at the morphological level, but its regulation remains poorly understood. Previous studies report that microtubules are essential for centrosome duplication (Balczon et al., 1999 Cell Motil. Cytoskel. 42:60-72), but the motor protein dynein is not (Quintyne and Schroer, 2002 J. Cell Biol. 159:245-54). We have examined centrosome overduplication in S-phase arrested CHO cells, CHO cells expressing a centrin-1 GFP protein and U2OS cells. CHO cells stably expressing centrin-1 GFP overduplicate centrosomes more rapidly than wild-type cells, consistent with a role for centrin isoforms in centrosome duplication. We confirmed that microtubules are required for duplication as measured by γ-tubulin staining. However, in the absence of microtubules small centrin foci continue to accumulate. These centrin foci were stable as measured by FRAP analysis and contained modified tubulin and a subset of other centrosomal proteins, including pericentrin. We postulate that these centrin-containing foci represent ‘pre-centriole’ structures. Centrin-containing pre-centrioles form during de novo centrosome duplication following laser ablation of existing centrosomes (La Terra et al., 2005 J. Cell Biol. 168:713-22). However, in our experiments centrin foci formed in the presence of a pre-existing centrosome indicating that centrosomes do not limit pre-centriole formation. Inhibition of dynein, using drugs, dynamin overexpression, or dynein antibody microinjection, also prevents the duplication of γ-tubulin containing centrosomes, but not pre-centriole formation. We propose that centrosome duplication in S-phase arrested cells occurs in two steps: firstly, formation of centrin-containing pre-centrioles and, secondly, recruitment of microtubule-nucleating components such as γ-tubulin to these pre-centrioles. The second, but not first, step is dependent on dynein and microtubules and thus most likely requires minus-end directed microtubule transport. Finally, we found that Hsp90 inhibitors also prevent the second step in this pathway, suggesting a role for chaperones in assembly or transport of PCM components but not pre-centriole formation.

L41 The Interplay of hNinein and Astrin- hNinein Is Required for Targeting Spindle-associated Protein Astrin to the Centrosome
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Cortical Microtubule Contacts Regulate Spindle Positioning in C. elegans Embryos

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We studied microtubules dynamics in anaphase during the first division of the C. elegans embryo. Imaging EBP-2:GFP and α-tubulin::YFP strains showed that microtubules catastrophize soon after cortical contact. Observing the embryo from its posterior end indicated that the frequency of microtubules reaching the cortex is determined by the direction of the pole motion. Based on these observations, we propose a model in which microtubule depolymerization forces are transduced into pulling by a cortical adaptor, and pole speed is regulated by microtubule arrays in the large epidermal cells is exceptionally sensitive to developmental and environmental regulation. Silencing PLDB and γ by 22 and 25 %, respectively, promoted transverse orientation of microtubules and reduced random and longitudinal orientations. Silencing PLDC by 53 % also reduced random and increased transverse microtubule orientation, although it did not affect significantly the proportion of cells with transverse microtubules. Apparently, these four isotypes are all involved in microtubule organization, with the interaction between microtubules and PLD being unique for each isotype and corresponding to their distinct evolutionary relationships.

Loss and Disintegration of the Yeast Actin Cytoskeleton during Inositolless Death in Saccharomyces cerevisiae

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When an inositol-requiring mutant of Saccharomyces is starved for inositol, there is a rapid loss of viability known as inositolless death and cell membranes disintegrate because of the depletion of phosphatidylinositol, a major membrane phospholipid. Another possible cause of membrane disintegration is the decrease in ACT1 transcription during inositolless death. Gene expression in yeast is regulated by a combination of two mechanisms of transcription: (1) transcription of genes encoding actin, actin-like proteins, and actin-binding proteins results in an increase in actin mRNA and a decrease in translation of actin mRNA, and (2) transcription of genes encoding actin, actin-like proteins, and actin-binding proteins results in a decrease in actin expression. The ACT1 gene encodes for a protein that is involved in the actin cytoskeleton. When ACT1 is silenced, the yeast cells undergo inositolless death and the actin cytoskeleton disintegrates. This suggests that ACT1 plays a crucial role in the actin cytoskeleton and in the inositolless death of yeasts. However, the exact mechanism by which ACT1 regulates actin expression is not yet clear. Further studies are needed to understand the role of ACT1 in actin expression and inositolless death in Saccharomyces cerevisiae.
contractile rings, however, the cells undergoing inositolless death lost the cables and patches in the budding cells. Cells also appeared not to finish budding. The proportion of budding cells increased from 5% to 35% but the total number of cells decreased. The losses of actin cables and patches would affect budding and cell membrane and wall integrity by preventing their proper synthesis and localization and would contribute to the membrane disintegration observed during inositolless death.

L47

The C-terminus of Myelin/Oligodendrocyte Glycoprotein Interacts with Stathmin, a Microtubule Destabilizing Protein

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Myelin/oligodendrocyte glycoprotein (MOG) is an immunoglobulin (Ig)-like transmembrane protein specific to oligodendrocytes, glial cells of the central nervous system that myelinate large diameter neurons. In multiple sclerosis (MS), myelin sheaths are targeted for immune-mediated degradation resulting in demyelination, axonal scarring, and eventual axonal loss. As a primary antigen targeted during immune-mediated demyelination, MOG plays an important role in this process, and immunization with MOG peptides can elicit an MS-like disease in rodents and primates. As an Ig-like protein, MOG may function as a receptor or cell adhesion molecule. The cytoplasmic tail of MOG’s cytoplasmic tail was used as “bait” in a yeast-two-hybrid screen. A member of the stathmin family of microtubule destabilizing proteins was identified as a putative interaction partner. Stathmin sequesters tubulin heterodimers, and this event is inhibited by hyperphosphorylation of stathmin. When MOG mAb is used as a ligand mimic for MOG, a dramatic reorganization of microtubule networks occurs in oligodendrocytes. MOG-stathmin interaction was confirmed through immunocytochemistry and reciprocal co-immunoprecipitations. Colocalization studies by confocal immunofluorescence reveals merged staining just below the plasma membrane. MOG and stathmin Abs can each reciprocally pull down the interacting protein complexes. Several C-terminal mutant constructs of MOG were tested to pinpoint the interaction domain. Truncation of the C-terminal tail decreased MOG's ability to form a complex with smgGDS and Rac1; down-regulation of smgGDS expression by siRNA causing significant inhibition of βPIX-mediated Rac1 activation and neurite outgrowth. These results provide evidence for a new and unexpected mechanism whereby βPIX can regulate Rac1 activity. This work was supported, in part, by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-205-E00010) and Grants R01-2005-000-00386-0 (2006) through the Basic Research Program from the Korean Science & Engineering Foundation (KOSEF).

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L49

Human Primary Ligament Fibroblasts Produce Nitric Oxide in Response to an Altered Fibronectin Molecule in a JNK and p38 MAPK Dependent Pathway with Concomitant Decreases in p53 Levels

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Nitric oxide is a ubiquitous gaseous molecule with a very short half-life and is associated with a multitude of physiological functions. It can function either as a pro-apoptotic or anti-apoptotic molecule depending on its concentration inside the cell and on the neighboring environment. We have previously reported the identification of a novel apoptotic mechanism triggered by an altered fibronectin molecule where there is a down-regulation of p53. In our present study, we show that there is a dose and time dependent increase in nitric oxide production when the primary human ligament fibroblasts are treated with the altered fibronectin molecule. Usually increased nitric oxide production is associated with increased p53 accumulation. However, here we report the novel finding that p53 levels decrease with increases in nitric oxide production. We show by western blot analysis that this increase in nitric oxide production is mediated by an increase in inducible nitric oxide synthase (iNOS) expression. Furthermore, there was a significant reduction in nitric oxide generation when the cells were treated with iNOS specific inhibitors, 2-ethyl-2-thioisopseudouridine hydrobromide or 2-methyl-2-thioisopseudouracil. Also, transfection of cells with iNOS siRNA resulted in a marked decrease in nitric oxide generation when the cells were treated with the altered fibronectin fragment. Treatment of cells with SB203580, an inhibitor of JNK and p38 MAPK, led to reduced nitric oxide generation. Treatment with the altered fibronectin fragment. Taken together, our results provide evidence that altered fibronectin matrix triggers nitric oxide generation in human fibroblasts by inducing iNOS through a pathway that involves activation of JNK and p38 MAPK with a concomitant decrease in p53 levels. (This work was supported by NIH RO1 grant DE013725 to YLK.)

CADDHERSINS

L50

VEGF Controls Endothelial Cell Permeability by Promoting the Rac-dependent Endocytosis of VE-cadherin

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Vascular Endothelial Growth Factor (VEGF) was first described as a potent vascular permeability factor (VPF) secreted by tumor cells that stimulates the rapid and reversible increase in microvascular permeability. Significant progress has been made towards the elucidation of the intracellular signaling pathways by which VEGF promotes endothelial cell growth and survival. In contrast, the mechanism by which VEGF leads to endothelial cell permeability and vascular leakage is still elusive. Previous reports suggest that the endothelial-specific cell-cell adhesion molecule VE-cadherin plays a key role in the maintenance of the integrity of blood vessels. Thus, we hypothesized that removing VE-cadherin from the plasma membrane, even transiently, could contribute to the enhanced endothelial permeability in response to VEGF. We have recently obtained evidence of the existence of a novel signaling pathway linking the activation of VEGFR-2 by VEGF to the rapid internalization of VE-cadherin thereby disrupting the endothelial barrier. Indeed, in response to VEGF, we observed that VE-cadherin is rapidly internalized through a clathrin-dependent mechanism and sequestered in endosomes, following the microtubule trafficking and sorting. This process is initiated by the stimulation of VEGFR-2 by VEGF, and the activation of the small GTPase Rac through the Src-dependent phosphorylation of Vav2, a guanine-nucleotide exchange factor. Indeed, knock-down and inhibition of Src, Vav2 and Rac prevent both VE-cadherin endocytosis and enhanced endothelial cell permeability in response to VEGF. Rac

EXTRACELLULAR MATRIX AND CELL SIGNALING

L48

Involvement of smgGDS in βPIX-mediated Rac1 Activation

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Neuropathogenesis requires active actin cytoskeleton rearrangement in which Rho GTPases play a pivotal role. We demonstrated that βPIX guanine nucleotide exchange factor (GEF) mediates basic fibroblast growth factor (bFGF)-stimulated Rac1 activation through the Basic Research Program from the Korean Science & Engineering Foundation (KOSEF).
activation, in turn, can promote the PAK-mediated phosphorylation of a 
conserved highly motif within the intracellular tail of VE-cadherin. 
This results in the recruitment of serine-phosphorylated VE-cadherin 
into clathrin-coated endosomes and the consequent disassembly of 
endothelial adherens junctions. Ultimately, this novel biochemical route 
by which VEGF promotes endothelial permeability through the 
endocytosis of VE-cadherin may help identify new therapeutic targets 
for the treatment of many human diseases that are characterized by 
pathological vascular leakage.

L51
Reactive Oxygen Species Enhance Tumor Cell Invasion in 
Hepatocellular Carcinoma Cell Lines

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Reactive oxygen species (ROS) correlated with tumor cell invasion and 
metastasis in several cancers, but little is known about the correlation 
of ROS and invasion in hepatocellular carcinoma (HCC). Moreover, 
the underlying mechanisms are poorly understood. In our previous data, 
we demonstrated that ROS stress correlated with tumor cell invasion in 
HCC tissues. To probe the molecular basis of invasion via ROS in 
HCC, we have investigated the relation between ROS stress and 
invasion in HCC cell lines. We treated H2O2 to HCC cell lines and 
analyzed E-cadherin expression and morphological changes by using 
immunoblot, real-time PCR, and immunofluorescence analyses. Also, 
we studied correlation between ROS stress and invasiveness with 
Matrigel invasion assay. In response to ROS, E-cadherin expression 
was decreased and the ability of invasion was increased. In addition, 
NAC treatment or overexpression of antioxidant enzymes could inhibit 
ROS induced EMT and invasion in HCC cell lines. In conclusion, ROS 
enhance tumor cell invasion via down-regulation of E-cadherin in 
HCC cell lines.

L52
Overexpression of Cadherin Extracellular Domains Interferes 
with Early Vertebrate Development

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The release of the cadherin extracellular domain by proteolytic 
cleavage, a process called shedding, occurs both in cancer and during 
development. Although cadherin shedding has been implicated in the 
regulation of cell-cell adhesion, migration, and invasion, and β-catenin 
signaling, it is still unclear if this is a consequence of downregulation of 
full-length cadherin at the cell surface or a direct effect of the released 
eXenopus laevis. Whereas the cadherin extracellular domain did not affect adhesion, migration or growth under 
2D-conditions in different cell lines, we found that expression of this 
domain interfered with gastrulation in Xenopus by blocking convergent 
extension movements. Further experiments indicated that the 
eXenopus laevis domain directly affects endogenous cadherin function. 
Interestingly, we found that overexpression of N-cadherin ECD and a type II or protocadherin ECD, also interfered with gastrulation, 
suggestion no specificity within the type I classical cadherins. 
Overexpression of dominant active Rac was able to rescue the 
gastrulation defect caused by the ECD, suggesting that regulation of 
cadherin–cytoskeletal interactions is crucial for convergent extension.

MEMBRANE RECEPTORS

L53
Characterization of the 18-Methoxycoronaridine Binding Site on the 
Nicotinic Acetylcholine Receptor

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We characterized the 18-methoxycoronaridine (18-MC) binding site on 
the nicotinic acetylcholine receptor (AChR) in the resting and desensitized 
states. In this regard, Scatchard-plots using [3H]18-MC, 
competition binding experiments using the noncompetitive antagonist 
[3H]TCP, and modulatory binding experiments using the agonist 
[3H]cytisine, were performed. These experiments yielded the following 
results: (1) there is one (0.86 ± 0.13) high-affinity (Kd = 0.23 ± 0.04 
μM) binding site for [3H]18-MC in the desensitized AChR; (2) the 
affinity (in μM) of each 18-MC congener for the [3H]TCP locus in the 
desensitized state follows the sequence: 18-MC (0.19 ± 0.01) > methoxyethylcoronaridine (0.24 ± 0.04) > (+)coronaridine (3.2 ± 0.4) > alibifloranine (3.7 ± 0.4) > ibogaine (5.7 ± 0.5). Whereas, the affinity 
sequence in the resting state is: methoxyethylcoronaridine (3.3 ± 0.4) > 18-MC (10 ± 1) > ibogaine (22 ± 6) > alibifloranine (31 ± 4) > (+)coronaridine (78 ± 7). (3) Scatchard-type analysis suggests that 18-MC 
sterically interacts with the TCP site; and (4) [3H]cytisine binding is 
enhanced by the 18-MC congeners when the AChR is in the resting but 
avactivatable state, but not in the desensitized state. Considering these 
results we concluded that the 18-MC congeners: (a) bind to a site that 
overlaps the TCP locus; (b) bind with higher affinity to the desensitized 
than to the resting AChR; and (c) may inhibit the AChR by inducing the desensitization process.

L54
WITHDRAWN

L55
G_{\alpha} Protein Mediates Rhodopsin Downregulation, Leading to 
Reduced Light Sensitivity of Fly Photoreceptors

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Fly visual transduction is a model pathway in genetic dissection of 
G-protein coupled receptor signalling. Timely termination of the visual 
response depends on rapid deactivation of the G_{\alpha}-coupled light receptor 
rhodopsin, which relays on both a visual arrestin Arr2 and a pathway 
that involves a transcription factor dCAMTA and a F-box protein 
dFbxl4. Here we report that rapid deactivation of rhodopsin is also 
important for the light sensitivity of photoreceptors. In arr2 and 
dCAMTA4 mutant flies or wild-type flies that had accumulated excessive 
active rhodopsin (via blue light stimulation), rhodopsin was 
downregulated due to increased endocytosis and degradation. As a 
consequence, the light sensitivity in arr2 and dCAMTA4 flies deceased 
about 25 folds. With various mutant combinations, we demonstrated 
that Arr1, another visual arrestin that had been implicated in rhodopsin 
edendysdispensability, was dispensable for the observed downregulation. Instead, 
the increased rhodopsin endocytosis depended on the very G_{\alpha} protein 
that mediates the visual transduction. Nonetheless, the effector enzyme 
PCL did not mediate this new function of G_{\alpha}. The data indicate that 
prolonged rhodopsin activation of G_{\alpha}, but not PLC or downstream 
signalling molecules, leads to rhodopsin endocytosis and downregulation. This mechanism may represent an important, arrestin- 
independent endocytic pathway for other G_{\alpha}-coupled receptors, and 
could underlie several drug tolerances occurred in mammalian brain.

L56
Angiopoietin-1 Induces Adhesive Trans-association of Tie2 at the 
Cell-Cell Contacts and Specifies Its Downstream Signaling 
Pathways

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Angiopoietin-1 (Ang1) is a ligand for the receptor tyrosine kinase Tie2, 
which is specifically expressed on endothelial cells. Ang1/Tie2 signal 
regulates not only developmental angiogenesis, but also endothelial 
integrity and survival to maintain quiescence of adult vasculature. 
However, molecular mechanism by which Ang1/Tie2 controls the 
vascular quiescence is still largely unknown. We monitored the 
dynamics of Tie2 during its activation by time-lapse imaging of Tie2- 
GFP fusion protein (Tie2-GFP) expressed in endothelial cells. Tie2- 
GFP was diffusely expressed on plasma membrane in unstimulated 
cells. However, it was rapidly oligomerized and recruited to the cell-cell 
contacts upon stimulation with COMP-Ang1, a potent activator for

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Tie2. Endogenous Tie2 was also accumulated at the cell-cell contacts when stimulated with Ang1 or COMP-Ang1. Furthermore, we analyzed dynamics of Tie2-GFP or its mutant lacking cytoplasmic domain of Tie2 (Tie2Δcyto-GFP) expressed in Chinese hamster ovary cells. Upon COMP-Ang1 stimulation, Tie2-GFP and Tie2Δcyto-GFP was translocated to the cell contacts only between adjacent cells expressing Tie2-EGFP and Tie2Δcyto-GFP, respectively. COMP-Ang1 was also co-localized with Tie2-GFP at cell-cell contacts. These findings suggest that Ang1 induces trans-association of Tie2 at the cell-cell contacts without intracellular signaling. To understand biological significance of trans-associated Tie2, endothelial cells were stimulated with COMP-Ang1 under either sparse or confluent condition. Although cell density did not affect COMP-Ang1-induced Tie2 phosphorylation, the presence of endothelial cell-cell contacts significantly enhanced COMP-Ang1-induced Akt activation, but down-regulated COMP-Ang1-induced Erk activation compared to the absence of cell-cell contacts. We furthermore found that Ang1-dependent Tie2 trans-association exhibited potential adhesive function by examining the aggregation of 293F cells expressing Tie2-GFP or Tie2Δcyto-GFP, but not GFP. Collectively, Ang1 induces trans-association of Tie2 at the cell-cell contacts, which results in preferential activation of Akt and may contribute to the enhancement of endothelial cell integrity.

L57 Differential Modulation of Toll-like Receptor Signaling Pathways by Phytochemicals

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Toll-like receptors (TLRs) are pattern-recognition receptors that detect conserved molecular patterns of invading microorganisms to initiate innate and inflammatory responses. Toll-like receptors have two distinct branches of downstream signaling pathways, MyD88- and TRIF-dependent pathways leading to the expression of pro-inflammatory cytokines and type I interferon genes. Many phytochemicals are known to exert anti-inflammatory effects; however, in many cases the detailed mechanisms are not fully understood or controversial. Here, we investigated the molecular target of phytochemicals, resveratrol, EGCG, and curcumin, in TLR-mediated signaling pathways. We found that depletion of membrane cholesterol, not the type of lipid raft, wa

L60 Proteasome-regulated Cleavage of the GABA-B1 Receptor in Glia

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The GABA-B1 receptor functions as a pentameric complex formed by the association of two subtypes, GABA-B1 and GABA-B2, and is a key mediator of GABAergic neurotransmission in the CNS. The GABA-B1 receptor is an important target for many drugs such as antiepileptics and addictive substances. However, the molecular mechanisms underlying GABA-B1 receptor cleavage in response to different stimuli are not fully understood.

Our studies revealed that GABA-B1 receptor cleavage is controlled by the ubiquitin-proteasome system (UPS). We observed that proteasome activity is significantly increased in GABA-B1 expressing cells treated with proteasome inhibitors. Moreover, we found that the proteasome inhibitors impaired the localization of the GABA-B1 receptor in the cell membrane.

These results suggest that proteasome inhibitors could be used as a potential therapeutic strategy to increase GABA-B1 receptor expression and function.
using confocal microscopy and by Western blotting of nuclear extracts. Altogether, our observations suggest the existence of proteasome-regulated intranuclear GABA-B1 fragments in DI-TNC1 cells. Such fragments could, in association with ATF-4, directly regulate nuclear events such as transcription and/or splicing.

L61 LDLR Related Protein 9 (LRP9), the First Receptor Shown to Interact with Calnuc
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Calnuc is an ubiquitous, EF-hand Ca2+ binding protein found 1) in the cytoplasm where it binds different Galpha subunits, 2) in the Golgi lumen where it constitutes an important Ca2+ storage pool, and 3) secreted outside the cell. To further understand Calnuc's function, a yeast-two-hybrid approach has been used to identify new interacting partners. LRP9 (LDLR related protein 9), a poorly characterized member of the LDLR superfamily, has been identified as a potential Calnuc-binding protein. The aim of this study was to further characterize this receptor and its interaction with Calnuc. Yeast one-on-one interaction, in vitro and in vivo pull-down and co-immunoprecipitation assays have confirmed the interaction between the N-terminus of Calnuc and the cytosolic C-tail of LRP9. LRP9 is the only identified transmembrane protein to date that has been shown to bind Calnuc and it has a putative function in trafficking and signaling. By confocal microscopy, exogenously expressed LRP9 was shown to colocalize with various markers of the Trans-Golgi Network (TGN) and endosomes. Moreover, immunoprecipitation and pull-down assays indicated that LRP9 interacts with GGAs (Golgi-localized, gamma ear-containing, ADP ribosylation factor binding proteins), clathrin adaptors involved in sorting at the TGN and in TGN-endosome transport. Furthermore, mutagenesis analysis has shown that the DXXLL motif in the C-terminal tail of LRP is critical for this interaction. These results suggest that LRP9 traffics between TGN and endosomes and that Calnuc could be involved in the trafficking and signaling of this receptor.

EXOCYTOSIS: REGULATED SECRETION

L62 Conditional Knockout Mice to Analyze Munc Proteins Regulating Airway Mucous Secretion
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Airway mucus is a component of the lung's innate immune defense that forms a protective barrier against inhaled particles and pathogens. Upon inflammatory stimulation, the airway epithelium undergoes a significant change in structure and function, due to increased mucin production by Clara cells. However, excessive mucous secretion or a failure of mucinous clearance causes airway obstruction and promotes airway infections. The precise roles and mechanisms of mucin secretion and clearance in these protective and pathologic processes are not well understood at molecular, cellular, or tissue levels. To better understand these roles, we have engineered mouse conditional knockouts of key genes that regulate secretion, including Munc13-4 and Munc18b. These will be crossed to mice with the Cre conditional knockouts of key genes that regulate secretion, including Munc13-4 and Munc18b. These will be crossed to mice with the Cre conditional knocked out and 4 in vivo. Of the four Munc13-4 isoforms, three of these contain Diacylglycerol (DAG) binding sites, including the two Munc13-2 splice variants. The ubiquitous Munc13-2 splice variant is expressed in airway secretory cells, though lower than then brain and hindbrain levels. Munc13-4 does not contain a DAG binding site. Using novel polyclonal antibodies we show that Munc13-4 is expressed at higher levels in lung and hematopoietic tissues then in the brain. Our experiments with mutant mice lacking specific Munc proteins allow us to analyze the molecular complexities of regulated airway epithelial cell exocytotic secretion at the level of gene expression, and to test directly whether the presence of mucous on airway luminal surfaces is essential for lung clearance and host defense.

L63 Airway Zone Material Orient Synaptic Vesicles Prior to Docking So That a Specific Fusion Domain on Each Vesicle Interacts with the Presynaptic Membrane
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Synaptic vesicles (SVs) docked on (in direct contact with) the presynaptic membrane of axon terminals are connected to an aggregate of macromolecules attached to the presynaptic membrane called active zone material (AZM). We have been using electron tomography on tissue sections from aldehyde fixed, heavy metal stained, plastic embedded frog neuromuscular junctions to obtain insights to the mechanisms involved in movement of undocked vesicles to replace docked ones after they have fused with the presynaptic membrane to release their neurotransmitters during synaptic transmission. We have found that the lumened of docked SVs contains a network of filamentous macromolecules connected to the vesicle membrane and composed of luminal portions of integral vesicle membrane proteins. The arrangement of the network and its sites of contact with the vesicle membrane indicate that the integral membrane proteins contributing to the filaments are stereotypically localized to specific domains in the membrane and that these domains are stereotypically oriented with respect to the AZM and to the presynaptic membrane. Some of the protein domains partially or completely in contact with presynaptic membrane, the fusion domain. In the experiment presented here we found that the arrangement of connections of luminal filaments to the membrane of undocked vesicles in contact with and away from AZM is similar to that for docked vesicles, which leads to the conclusion that the fusion domain and integral protein domains are specified in undocked as well as docked vesicles. By using the arrangement of protein domains on each undocked vesicle to analyze its orientation with respect to the presynaptic membrane and AZM, we conclude that movement of undocked vesicles to the active zone involves a stepwise rotation, and that connection to the AZM directs the orientation of the vesicles so that the fusion domain contacts the presynaptic membrane.
BAIAP3, Partner in Regulated Exocytosis

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BAIAP3 belongs to the Munc13 family which is defined by the presence of a Munc Homology Domain (MHD) and two calcium dependent phospholipid binding (C2) domains. Its members function in late stages of regulated exocytosis in various cell types. Recently it was shown for Munc13-4, the closest homologue of BAIAP3, that it plays a role in release of secretory lysosomes in hematopoietic cells. BAIAP3 is mainly expressed in brain and it has been reported that overexpression induces metastatic transformation, likely due to increased release of growth factors. We aimed to define the role of BAIAP3 in regulated exocytosis. First we tested if BAIAP3 could interact with members of the rab family of small GTPases in analogy with Munc13-4. To our surprise we found no such interaction. To study the function of BAIAP3 in vivo we evaluated BAIAP3 expression in a variety of rat cell lines and found that it is highly expressed in the rat insulinoma cell line INS1, both at mRNA and protein level. In initial functional experiments we found increased glucose-stimulated insulin secretion upon knockdown of BAIAP3 expression by siRNA. These data suggest an inhibitory role for BAIAP3 in secretion. To clarify the mode of its action mechanism, we searched for interaction partners and found that the second C2 domain of BAIAP3 bound directly and specifically with the μ2 subunit of the AP-2 complex. In addition, we show that this interaction required a WLSL motif similar to that of the neonatal Fc receptor.

CAVEOLAE

L66

Anti-fibrotic and Anti-inflammatory Roles of Caveolin-1 in Scleroderma

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Scleroderma is a complex autoimmune disease characterized by inflammation and fibrosis of the skin, lungs and other organs. Our studies have identified caveolin-1 as a potential therapeutic target for this disease. We found that a PKC/MEK/ERK/caveolin-1 signaling cascade regulates collagen accumulation in normal lung fibroblasts (NLF). Caveolin-1 depletion leads to increased collagen levels in lung fibroblasts. When full-length caveolin-1 was overexpressed using an adenosine construct and its function increased using the caveolin-1 scaffolding domain peptide (CSD) MEK/ERK activation and collagen expression were decreased in NLF and in lung fibroblasts derived from patients with scleroderma lung disease (SLF). Caveolin-1 also regulates the differentiation of fibroblasts to myofibroblasts and plays a direct role in inflammation by regulating signaling in monocytes. Less caveolin-1 is present in peripheral blood monocytes isolated from scleroderma patients than in these cells isolated from healthy volunteers, and the expression/activity of several signaling molecules regulated by caveolin-1 is also altered. Upregulating caveolin-1 function in TNF-alpha or TGF-beta-activated normal fibrocytes reversed the altered expression of caveolin-1-regulated kinases, possibly regulating pathogenic immune functions. In vivo studies have shown that systemic CSD delivery inhibits bleomycin-induced lung fibrosis and inflammation in mice. Thus caveolin-1 may provide protection against the progression of lung fibrosis by blocking collagen accumulation and differentiation of myofibroblasts, and by blocking inflammation by restoring the normal signaling that regulates immune cell functions.

PROTEIN FOLDING AND ASSEMBLY IN THE ENDOPLASMIC RETICULUM

L67 Novel Robinow Syndrome Causing Mutations in the Frizzled-like Domain of the Orphan Receptor Tyrosine Kinase ROR2 Are Retained in the Endoplasmic Reticulum

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ROR2 is a member of the orphan receptor tyrosine kinase (RTKs) family of proteins and is involved in the developmental morphogenesis of the skeletal, cardiovascular and genital systems. Mutations in ROR2 have been shown to cause two distinct human disorders, Robinow syndrome and Brachydaetly type B. Robinow syndrome is an autosomal recessive disorder caused by loss-of-function mutations whereas Brachydaetly type B is a dominant disease and is presumably caused by gain-of-function mutations in the same gene. We have previously established that all the missense mutations causing Robinow syndrome in ROR2 are retained in the endoplasmic reticulum and therefore concluded that their loss of function is due to defect in their cellular trafficking. Here we report the identification of two novel mutations in the frizzled-like domain of ROR2 causing Robinow syndrome. We also established their retention in the endoplasmic reticulum of HeLa cells and therefore failure to reach the plasma membrane. The clustering of Robinow-causing mutations in the frizzled-like domain of ROR2 suggests a stringent requirement for the correct folding of this domain before export of ROR2 from the endoplasmic reticulum to the plasma membrane.

MECHANISMS OF NUCLEAR TRANSCRIPTION

L69

Oxidative Stress Induces Premature Senescence by Stimulating Caveolin-1 Gene Transcription through p38 MAPK/Sp 1-mediated Activation of Two GC-rich Promoter Elements

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Cellular senescence is believed to represent a natural tumor suppressor mechanism. We have previously shown that up-regulation of caveolin-1 was required for oxidative stress-induced premature senescence (SIPS) in fibroblasts. However, the molecular mechanisms underlying caveolin-1 up-regulation in senescent cells remains unknown. Here, we demonstrate that sacytoxic oxidative stress generated by hydrogen peroxide application promotes premature senescence and stimulates the activity of a (1296) caveolin-1 promoter reporter gene construct in fibroblasts. Functional deletion analysis mapped the oxidative stress response elements of the mouse caveolin-1 promoter to the sequences -244/-222 and -124/-101. The hydrogen peroxide-mediated activation of
both Cav-1 (-244/-222) and Cav-1 (-124/-101) was prevented by the anti-oxidant quercetin. Combination of EMSA studies, ChIP analysis, Sp1 over-expression experiments, as well as promoter mutagenesis, identify enhanced Sp1 binding to two GC-boxes at -238/-231 and -118/-116 as the core mechanism of oxidative stress-triggered caveolin-1 transactivation. In addition, signaling studies demonstrate p38 MAPK as the upstream regulator of Sp1-mediated activation of the caveolin-1 promoter following oxidative stress. Inhibition of p38 MAPK prevents the oxidant-induced Sp1-mediated up-regulation of caveolin-1 protein expression and development of premature senescence. Finally, we show that oxidative stress induces p38-mediated up-regulation of caveolin-1 and premature senescence in normal human mammary epithelial cells but not MCF-7 breast cancer cells, which do not express caveolin-1 and undergo apoptosis. This study delineates for the first time the molecular mechanisms that modulate caveolin-1 gene transcription upon oxidative stress and brings new insights into the redox control of cellular senescence in both normal and cancer cells.

**L70**

**Androgen and Glucocorticoid Receptors Regulating NRIP Gene Expression**

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Previously, we found a novel gene and named nuclear receptor interaction protein (NRIP) that functions as a transcription cofactor (GenBankTM database with accession numbers AY766164 and AAQ09330, JBC 2005), and NRIP role either by enhancing androgen receptor-driven prostate specific antigen expression in prostate cancer or in human papillomavirus-causing cervical cancer by enhancing glucocorticoid receptor (GR)-driven HPV-16 and -18 promoter activity. It indicates that NRIP likely plays an important role in the development of prostate and cervical cancer. Here, we investigated the NRIP gene regulation. We have cloned 5'-flanking fragments of the human NRIP gene and assess the promoter activity of these fragments in cells. The 5'-flanking fragments (2538 bps, 415 bps, and 99 bps) of NRIP gene were cloned into pGL3-Basic vector for determination of the promoter activity. Our results showed that 5'-flanking 2538 bps fragment (NRIP-P-2538) showed high transcriptional activity compared with other NRIP fragments (NRIP-P-415→93) in its proximal regions presented stronger promoter activity. We then analyzed this 5'-flanking 415 bps fragment of NRIP promoter. Based on DNA sequence, this 5'-flanking 415 bps fragment promoter is TATA-less and contains one AR and two GR-binding sites. We then found that AR and GR can activate NRIP-promoter activity in the presence of its ligand. In addition, we successfully generated NRIP antibody and used it to monitor NRIP protein expression in prostate and cervical lesions. The results demonstrated that NRIP protein was expressed in pre-cancerous and cervical glandular intraepithelial neoplasia (cGIN), but not in both normal and invasive cervical cancer regions. Therefore, NRIP also as a useful biomarker of cervical intraepithelial neoplasia (CIN), shows increased NRIP expression with worsening grades of CIN. Taken together, NRIP is likely to regulate a subset of GR-responsive genes important to cervical cancer growth and progression.

**L71**

**FoxO Upregulates Gadd45 Gene through a Novel Interaction with KLF6 upon DNA Damage**

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Eukaryotic cells express the genes related to anti-stress in response to genotoxic stress. Among them, Gadd45 is transcriptionally upregulated by forkhead box O (FoxO) transcription factors, which directly bind to the FoxO-binding motif in the Gadd45 promoter. FoxO families are known to regulate the transcription of genes involved in stress response, cell cycle and glucose metabolism. By investigating the sequence of Gadd45 promoter, we found GC-box which is a potential binding motif for Sp1/Krüppel-like transcription factor (KLF). Sp1/KLF family members are known to bind to GC-box through their carboxy-terminal three zinc-finger domains. We confirmed that both Sp1 and KLF6 belonging to KLF family bound to this region by using ABCD assay. The effect of KLF6 and FoxO1 on the promoter activity of Gadd45 was examined by luciferase reporter assay. Ectopic expression of KLF6 and constitutive-active form of FoxO1 induced 2- and 5-times increased promoter activity than control, respectively. Instead, Sp1 did not activate Gadd45 promoter. Furthermore both showed the synergistic effect for transcription. By using a series of truncated promoter regions, we found that GC-box region lacking FoxO-binding motif was enough for synergistic effect of FoxO1 and KLF6, indicating that Gadd45 transcription could be upregulated by KLF6 without the direct binding of FoxO1 to the Gadd45 promoter. Furthermore, we found the association of FoxO1 with KLF6 using pull-down assay. We further investigated how KLF6 and FoxO1 cooperatively regulate the transcription in living cells. The endothelial cells responded to MMS (methyl methane sulfonate) stimulation and expressed Gadd45 three hours after stimulation. In these cells, FoxO1 were localized in the nucleus. Thus, we examined the dynamics of GFP-tagged KLF6 and found the accumulation of GFP-tagged KLF6 into the nucleus after the MMS stimulation. Collectively, our data demonstrate the novel FoxO-regulated transcription of Gadd45 through its association with KLF6.

**L72**

**Smad3 Gene Expression Is Up-regulated by Smad3 Protein in Response to TGF-β1**

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Members of the transforming growth factor-β (TGF-β) superfamily mediate a broad range of biological activities by regulating the expression of target genes. Smad proteins play a critical role in this process by binding directly to the promoter elements and/or associating with other transcription factors. We previously showed that TGF-β1 increased the expression of Smad3. In this study, we investigated the molecular mechanism of TGF-β1-mediated Smad3 up-regulation in hepatocellular carcinomas (HCC). Real-time RT-PCR and Western blot analysis revealed that TGF-β1 significantly induced Smad3 expression in Hep3B and HepG2 cells, however, knockdown of Smad3 by RNA interference (RNAi) blocked the Smad3 promoter activity in response to TGF-β1. These findings suggest that Smad3 protein and Smad-binding element in the Smad3 promoter are required for the up-regulation of Smad3 expression by TGF-β1.

**L73**

**PHD Domain-mediated E3 Ligase Activity Directs Intramolecular Sumoylation of an Adjacent Bromodomain Which Is Required for Gene Silencing**

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Pharyngocutaneous Tandem PHD and Bromodomains are often found in chromatin-associated proteins and have been shown to cooperate in gene silencing. While both domains can bind specifically modified histone tails, the specific mechanisms of cooperation between the domains are unknown. Here we show that the PHD domain of the KAP1 corepressor functions as an obligate, intramolecular E3 ligase for sumoylation of the adjacent Bromodomain. The RING-finger-like structure of the PHD domain is required for both Ub9 binding and sumoylation and directs modification to specific lysine residues in the Bromodomain. Sumoylation is required for KAP1 mediated gene silencing in vivo and functions by directly recruiting the SETDB1 histone methyltransferase and the CHD3/Mi2 alpha component of the NuRD complex via highly conserved SUMO interacting motifs (SIMs), and sumoylated KAP1 stimulates this histone methyltransferase activity of SETDB1. Our data provide a novel mechanistic explanation for
cooperation of PHD and Bromodomains in gene regulation and describe a new function of the PHD as an E3 SUMO ligase.

**DEVELOPMENTAL CONTROL OF GENE EXPRESSION**

**L74**

Differential Changes of Lysophosphatidic Acid-induced Gene Expression Profiles in Senescent Human Diploid Fibroblasts

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In order to clarify the molecular mechanism of altered responsiveness of senescent human diploid fibroblasts (HDFs) to lysophosphatidic acid (LPA), the time-dependent gene expression profile after LPA treatment were examined by cDNA microarray. Among 7834 genes in the microarray, the expressional changes of gene groups involved in signal transduction, transcriptional regulation, cell cycle, cell growth and maintenance, immune response, stress response, and cell death/apoptosis were compared. The genes with the global M value above +1.8 were considered as up-regulated and those below -1.8 as down-regulated. Many genes including DUSP6 (dual specificity phosphatase 6), IER3 (immediate early response-3, an antiapototic factor), CYR61 (a cysteine-rich angiogenic inducer), CTGF (connective tissue growth factor), F3 (coagulation factor) are up-regulated at 1 hr then decreased at 8 hrs in LPA-treated young HDFs. However, these genes are still up-regulated at the later time in LPA-treated senescent HDFs. Some genes including EGR1/3 (early growth response-1/3, zinc finger transcription factors) are up-regulated at 1 hr and decreased at 8 hrs in both young and senescent cells. The time-dependent differential gene expression profile could provide a clue for the molecular mechanism of altered responsiveness of senescent cells to growth factors.

**L75**

Fine Distribution of Polycomb and Trithorax Group Gene Regulation Proteins

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In order to determine the relationship between gene activity and epigenetic gene silencing on one hand and chromatin structure organization on the other hand, we have analyzed the subnuclear localization of Polycomb (PcG) and Trithorax group (TrxG) proteins by means of immunofluorescence and post-embedding immunogold electron microscopy approach. These protein groups are involved in epigenetic gene regulation in higher eukaryotes. PcG/TrxG localization experiments were performed on bovine embryos in different stages of early preimplantation development where the exceptional de- and re-programming of genome expression takes place. Mammalian cell lines were also used. Antibodies against YY1 and Bmi-1 (PcG) and against ML1 and TRX2 (TrxG) were applied on permeabilized cells or were also used. Antibodies against YY1 and Bmi-1 (PcG) and against ML1 and TRX2 (TrxG) were applied on permeabilized cells or

**L76**

Identifying a Transcriptional Target of Nkx2.2 Important in Pancreatic Islet Cell Type Specification

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Previous studies show that the homeodomain transcription factor Nkx2.2 is essential for pancreatic islet development and islet cell type differentiation. Mice disrupted for Nkx2.2 function die postnatally with severe hyperglycemia. During embryogenesis, insulin-producing β cells fail to differentiate, glucagon-producing α cells numbers are decreased, while ghrelin-producing ε cells numbers are largely increased. To understand the molecular role of Nkx2.2 in islet cell specification, it will be important to identify direct transcriptional targets. In this study we have identified the basic helix-loop-helix transcription factor, NeuroD1, as a possible target of Nkx2.2. NeuroD1 plays a role in islet cell development and β cell maintenance. We have determined that NeuroD1 mRNA and protein levels are reduced in β cell-like islets of Nkx2.2 mutant mice after e12.5, when increased cell type specification normally occurs. Interestingly, reporter assays in several pancreatic cell lines indicate distinct Nkx2.2 regulation of a 768bp NeuroD1 promoter region, which is dependent on cell type and lineage. Chromatin immunoprecipitations on wildtype e13.5 pancreata demonstrated Nkx2.2 occupies the NeuroD1 enhancer. However, electrophoretic mobility shift assays indicate that Nkx2.2 does not bind potential Nkx2.2 consensus binding sites within this region. This would suggest that Nkx2.2 binds to a novel sequence in this promoter or influences the binding of unidentified protein complexes. We are currently performing in vivo footprint analyses of the 768bp NeuroD1 promoter. Occupied sequence fragments will be used in genetic bead-oligonucleotide purifications to isolate and identify bound proteins by mass spectrometry. In addition, we are using chromatin immunoprecipitation technologies combined with the pancreas promoter chip developed by K. Kaestner (UPenn) to identify novel targets of Nkx2.2. Determination of Nkx2.2 targets and its molecular interactions will enhance our understanding of the transcriptional network and regulatory mechanisms necessary for proper islet cell type specification.

**L77**

Cytokine Modulation of the Angiogenic Transcription Factor HOXD3 in Human Microvascular Endothelial Cells

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Wound healing is a complex multistep process and is impaired in diabetes, in part due to dysregulation of cytokine expression. Previous work from our lab has shown that expression of the transcription factor HOXD3 is significantly decreased in diabetic wounds of mice. Constitutive expression of HOXD3 in vivo improves diabetic wound healing. In this study, we have evaluated cytokines that are important during the inflammatory and proliferative/granulation phases of wound healing to determine whether they are upstream regulators of HOXD3 expression. We evaluated HOXD3 mRNA expression in human microvascular endothelial cells (HMEC) using real-time RT-PCR. Total RNA was isolated from HMECs treated with transforming growth factor-beta (TGF-β) and tumor necrosis factor-alpha (TNF-α) after 4 hours. RNA was also isolated from HMECs treated with insulin-like growth factor-1 (IGF-1) and basic fibroblast growth factor (bFGF) after 4 and 8 hours. After 4 hours of treatment with cytokines TGF-β and TNF-α, HOXD3 mRNA levels were decreased by 44.0% and 50.0%, respectively. After 4 hours of treatment with IGF-1 and bFGF, there were minimal changes in HOXD3 mRNA levels. However, after 8 hours of IGF-1 and bFGF treatment, HOXD3 mRNA levels were increased by 83.3% and 21.2%, respectively. Our findings support that sequential cytokine expression after wounding plays an important role in the wound healing process. We found that TGF-β and TNF-α, integral to the initial inflammatory response, inhibit HOXD3 mRNA expression; however, angiogenic cytokines bFGF and IGF-1 are prevalent during the proliferative/granulation phases of wound healing.
stimulated HOXD3 mRNA expression. These results indicate that HOXD3 expression is differentially regulated by local cytokine expression.

**CHROMATIN AND CHROMOSOMES**

L78 Nuclear Functions for the Spindle Pole Body Component and SUN Protein Mps3 in Budding Yeast J. Bupp,1 A. Martin,1 K. Zueckert-Gaudenz,1 R. Brost,2 C. Boone,2 C. Seidel,1 S. L. Jaspersen1; 1Stowers Institute, Kansas City, MO, 2C.H. Best Institute, University of Toronto, Toronto, ON, Canada

SUN proteins (for Sad1-LINC-84 homology) are a family of structurally conserved inner nuclear envelope proteins that play a role in tethering the nucleus to components of the cytoskeleton. To better understand additional functions of SUN proteins in the cell, we performed systematic synthetic genetic interaction analysis using three mutants in *Saccharomyces cerevisiae* MPS3, which encodes the sole SUN protein in budding yeast. We individually crossed each mps3 mutant to each of the viable yeast deletion mutants so that we could determine if the double mutant was viable, lethal or showed slow growth compared to the single mutants parents. In addition to the anticipated phenotypes for genes involved in microtubule-related processes, we found that several classes of chromosome binding proteins, such as histones and histone acetyltransferases/deacetylases, certain kinetochore and nuclear pore complex subunits, transcriptional regulators and telomere binding proteins, showed genetic interactions with mps3 mutants. This suggests that Mps3 may interact with chromosomes and play a role in regulating chromatin structure, transcription and/or DNA segregation in the nucleus. Consistent with a possible nuclear function, DNA microarray experiments show that Mps3 binds to specific regions on all 16 yeast chromosomes, and mps3 mutants are defective in a number chromosome-related functions, including establishment of sister chromatid cohesion, chromosome positioning within the nucleus and transcriptional regulation. Interestingly, the growth defect of mps3 mutants can be rescued by overexpression of the histone H2 variant, Htz1, and several lines of biochemical evidence indicate that Mps3 and Htz1 interact in vivo. Because Htz1 is thought to prevent the spread of silent heterochromatin into adjacent regions of euchromatin, it is tempting to speculate that Mps3 and other SUN proteins have a conserved function in establishing and/or maintaining boundaries between different chromosome domains at the nuclear periphery.

L79 Decondensation of Chromosomes by Topoisomerase II Reveals That Mitotic Chromatin Is Constrained by DNA Entanglements R. Kawamura,1 M. O. Christensen,2 J. F. Marko2; 1Physics, University of Illinois at Chicago, Chicago, IL, 2Institut für Klinische Chemie und Laboratoriansdiagnostik, Universitätsklinikum Düsseldorf, Duesseldorf, Germany, 3BMBCB, Northwestern University, Evanston, IL

During eukaryote cell division, chromosomes undergo a condensation process which converts them from an open interphase conformation into a compact cylindrical mitotic form. While molecular details of this process remain unclear, it is well established that non histone SMC proteins play an important role. However other mechanisms also contributing to constrain mitotic chromosomes are expected, since extensive proteolysis is observed to only partially decondense mitotic chromosomes. Here we show that topoisomerase II, an enzyme responsible for passing one DNA double helix through another so as to resolve DNA entanglements during mitosis, is able to decondense proteolyzed mitotic chromosomes. This decondensation effect indicates that DNA entanglements contribute in concert with protein-mediated compaction in the folding of chromatin into mitotic chromosomes.

L80 Tandem Repeats Recruit siRNA-directed DNA Methylation S. W. L. Chan,1 X. Zhang,1 J. Yazaki,2 S. Cokus,3 M. Pellegrini,2 J. R. Ecker,1 S. E. Jacobsen1; 1Section of Plant Biology, UC Davis, Davis, CA, 2University of California, Los Angeles, Los Angeles, CA, 3Salk Institute, La Jolla, CA, 4HHMI/University of California, Los Angeles, Los Angeles, CA

DNA methylation protects the genome by silencing transposons and other harmful DNAs. The *Arabidopsis FWA* gene is silenced by DNA methylation on two tandem repeats, and a new copy of FWA transformed into plants is an efficient target for de novo DNA methylation. We have used to study transformation of DNA methylation, and have found that de novo DNA methylation is guided by siRNAs produced by an RNA interference (RNAi) pathway. DNA methylation of transformed FWA requires tandem repeats, and we have shown that recruitment of RNAi to tandem repeats is separable from the activity of downstream DNA methylation factors. Tandem repeats throughout the *Arabidopsis* genome produce siRNAs, suggesting that repeat acquisition may be a general mechanism for the evolution of gene silencing. We have recently used tiling microarrays to characterize DNA methylation throughout the Arabidopsis genome. CG DNA methylation primarily silences transposons, but a surprising number of endogenous genes are regulated by non-CG methylation (which is often targeted by siRNA). Future experiments will focus on the role of epigenetic mechanisms in chromosome segregation and genome stability.

L81 Pif1p Levels Affect Telomere End Protection and Suggest a Role for Telomerase in End Protection L. R. Vega,1,2 J. A. Phillips,3 B. R. Thornton,3 M. T. Onigbanjo,2 L. M. Arencibia,3 D. P. Tosczysz,3 V. A. Zakian; 1School of Natural and Health Sciences, Barry University, Miami Shores, FL, 2Molecular Biology Department, Princeton University, Princeton, NJ, 3Department of Biochemistry and Biophysics, University of California, San Francisco, CA

Telomerase, a specialized reverse transcriptase, preserves genome integrity by maintaining telomeres at the ends of linear eukaryotic chromosomes. In *Saccharomyces cerevisiae*, telomerase is telomere-associated in both the G1 and S/G2 phases and in the late G2 phase of the cell cycle. However, telomerase normally acts to lengthen telomeres only in late S/G2. The G1 association of telomerase requires a specific interaction between Ku and telomerase RNA. We have previously shown that the Pif1p helicase is a negative regulator of telomerase that acts by removing telomerase from chromosome ends in *S. cerevisiae*. In this study, we used genetic epistasis analysis to show that essential Pif1p can interact directly with S/G2 phase of the cell cycle. We examined endogenous Pif1p expression levels and found that they were very low in G1 phase cells due to APC (anaphase promoting complex) mediated degradation. Moreover, we found that over-expression of Pif1p from a non-cell cycle regulated promoter dramatically reduced viability in strains with impaired end protection such as cdc13-1 and yklc. This reduced viability was suppressed by deleting the EXO1 gene, which encodes a nuclease that acts at compromised telomeres, suggesting that the removal of telomerase by Pif1p exposed telomeres to degradation. Consistent with this interpretation, depleting cells of Pif1p suppressed the temperature sensitivity of yklc70A and cdc13-1 cells. Furthermore, eliminating G1 bound telomerase in a cdc13-1 strain also reduced viability. These data are consistent with a model in which yeast telomerase plays a structural role at telomeres that contributes to telomere protection from degradation. L.M.A. supported by NIH NIGMS MARC ST34 GM050021 and NIH-NIGMS MBRSRSE 5R25 GM059244, Barry University.

L82 Effects of hTERT on Genomic Instability Caused by Either Metal or Radiation or Combined Exposure A. Glaviano,1 F. Lyng,2 C. Mothersill,3 M. A. Rubio,4 C. P. Case; 1Department of Orthopaedic Surgery, University of Bristol, Bristol, United Kingdom, 2School of Physics, Dublin Institute of Technology, Dublin, Ireland, 3Dublin Institute of Technology, School of Physics, Dublin, Ireland, 4Lawrence Berkeley National Laboratory, University of Berkeley, Berkeley, CA

Genomic instability (GI), which is considered to be an important component in carcinogenesis, can be caused by low-dose exposure to agents which appear to act through induction of stress-response pathways related to oxidative stress. These agents have been studied mostly in the radiation field but evidence is accumulating that chemicals, especially heavy metals such as Chromium(VI), can also act in the same manner. Therefore, either metal or radiation can initiate long-term GI in generations of daughter cells from parent human primary fibroblasts. This phenomenon is regulated by telomerase. In this study, we have examined the clongenic survival and the cytogenetic damage of normal human fibroblasts (hTERT- cells) and...
engineered human fibroblasts, infected with a retrovirus carrying a cDNA encoding hTERT, which rendered these cells telomerase positive and replicatively immortal (hTERT⁺ cells). Metal induced GI in hTERT⁺ cells but not in hTERT⁻ cells, whereas radiation induced GI in hTERT⁺ cells and in less extent in hTERT⁻ cells. Combined exposure caused GI in both types of cells. This GI was more pronounced in hTERT⁻ cells after Radiation Followed by Metal, and more pronounced in hTERT⁺ cells after Metal Followed by Radiation. Moreover, with or without either metal treatment or radiation exposure or combined exposure, there was a higher level of tetraploidy in hTERT⁺ cells compared to hTERT⁻ cells. Besides, the biological effects provoked by combined exposure of metal and radiation also led to a synergistic action in both types of cells, compared to metal treatment only or radiation exposure only. This study suggests that telomerase does prevent GI caused by metal, but not radiation. Furthermore, GI induced by combined exposure of Radiation Followed by Metal may be prevented by telomerase, but it does not appear to be prevented after combined exposure of Metal Followed by Radiation.

NUECLEAR IMPORT AND EXPORT SIGNALS

L85 Human CMV Polymerase Holoenzyme Nuclear Import: Regulation by PKC Phosphorylation of Processivity Factor ppUL44

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Replication of the human cytomegalovirus (HCMV) genome takes place in the nuclei of infected cells and is mediated by a viral-encoded DNA polymerase complex, formed by the catalytic subunit ppUL54 and the processivity factor ppUL44, which tethers the catalytic subunit to DNA. ppUL44 dimerizes in the cytoplasm of infected cells before being translocated to the nucleus and has been proposed to act as a scaffold promoting the assembly of several HCMV replication fork proteins, such as ppUL54 and the DNA uracil glycosylase ppUL14. Although both ppUL44 and ppUL54 contain importin α/β recognized nuclear localization signals (NLSs) they are also capable of being imported into the nucleus as a complex. Whereas ppUL44 nuclear import is a CK2-phosphorylation enhanced process, ppUL54’s is not. Here we show that ppUL44 nuclear import is likely to be also negatively regulated by protein kinase C-mediated phosphorylation of residue T427. As shown by quantitative confocal laser scanning microscopic analysis of ppUL44, D77 cells expressing ppUL44 GFP and DsRed2 fusion proteins, Phorbol 12-myristate 13-acetate (PMA)-induced PKC activation resulted in reduction of the nuclear accumulation of GFP-UL44 but not of the non phosphorylatable A427 derivative mutant. In the absence of PMA, the phosphomimetic D427 derivative mutant exhibited reduced nuclear import when compared to the wild-type and the A427 mutant. Since ppUL114 nuclear accumulation seems to be dependent on ppUL44, we suggest that the phosphorylation state of ppUL44 could regulate the nuclear import rate of the HCMV DNA polymerase holoenzyme, and other viral proteins such as ppUL114.

L86 Nucleolar Binding of Human Ribosomal Protein S6 Is Independent of Its Three Authentic Nuclear Import Signals

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Over the past fifty years, pioneering research has demonstrated that the nucleolus functions as the site of ribosome biogenesis. Concurrent with nuclear transcription, the ribosomal RNA precursor is modified and associates with ribosomal proteins. The availability of complete genomes from various organisms has revealed that the evolutionary conservation of the rRNAs of all species is more apparent in the comparisons of secondary structure than of nucleotide sequences. Despite sequence deviations in the rRNA molecules, the overall structure of the ribosomal subunits and their function in translation have been conserved. In recent years, proteomic analyses of the nucleolus have revealed almost 700 functionally diverse proteins implicated in ribosome biogenesis, nucleolar assembly, and regulation of various vital cellular processes. This nucleolar inventory has not unveiled a specific nucleolar targeting sequence or consensus motif necessary for nucleolar binding. However, the ribosomal protein family, characterized by their basic nature, should exhibit a distinct set of binding sequences that enable interactions with the rRNA precursor molecules, ultimately leading to subunit assembly. We delineated two minimal nucleolar binding sequences of human ribosomal protein S6 by fusing S6 cDNA fragments to the 5’ end of the LacZ gene and subsequently detecting the intracellular localization of the beta-
galactosidase fusion proteins. One nuclear binding sequence is located in the central region of S6 and functions independently of the three authentic NLSs. Both nuclear binding domains are relatively long suggesting an extensive interaction between ribosomal protein S6 and the 18S rRNA. Remarkably, although the amino acid sequences of the S6e family members have changed during evolution, both nuclear binding domains and the overall S6 structure have been conserved over 1 billion years probably due to co-evolution with the corresponding rRNAs.

L87 Substrate Recognition by Karyopherin 104
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The transport of proteins and nucleic acids between the nucleus and cytoplasm is a fundamental activity of all eukaryotic cells. This active process is mediated by Karyopherin βs, a family of nucleocytoplasmic transport proteins. Import Karyopherin βs recognize and bind substrates containing nuclear localization sequences (NLSs) in the cytoplasm, translocate them through the nuclear pore complex, and finally release substrates in the nucleus upon binding Ran-GTP. A new class of NLS known as the PY-NLS recognized by Karyopherin β2/Transportin in humans, has recently been identified. A set of rules describe recognition of PY-NLSs by Karyopherin β2. Specifically, we examine nuclear import into the nucleus of three authentic NLSs. Both nucleolar binding domains are relatively long suggesting an extensive interaction between ribosomal protein S6 and the 18S rRNA. Remarkably, although the amino acid sequences of the S6e family members have changed during evolution, both nuclear binding domains and the overall S6 structure have been conserved over 1 billion years probably due to co-evolution with the corresponding rRNAs.

GERM CELLS AND FERTILIZATION

L88 Molecular Cloning of cDNA Encoding Phospholipase C Beta Isoform from Chaetopterus Eggs
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Intracellular calcium release is a ubiquitous event regulating egg activation at fertilization. This calcium release results from hydrolysis of phosphatidylinositol (4,5)-bisphosphate (PIP2) to generate inositol 1,4,5-triphosphate (IP3) by phospholipase C (PLC). Identification of phospholipase C (PLC) from the eggs and sperm is a key step in investigating the signal transduction pathways of egg activation. To date, six PLC isoforms (β, γ, δ, ε, ζ and η) have been identified in mammals, one of which is sperm-specific, but few PLC isoforms have been studied in invertebrate fertilization. In the present study, we used degenerate oligonucleotide primed PCR (DOP-PCR) combining with 3' and 5' rapid amplification of cDNA end PCR (RACE PCR) to get the full-length cDNA encoding a novel PLC isoform from Chaetopterus eggs, which we have named cp-PLCβ. The full-length cDNA is 3537 base pairs and contains an open reading frame encoding 1178 amino acids. The deduced amino acid sequence of cp-PLCβ exhibits the phospholipase C-specific pleckstrin homology (PH) domain, X, Y catalytic domains, C2 domain, and shares 63% and 62% identities with human PLCβ4 and sea urchin PLCβ3, respectively. It is more distantly related to other mammalian PLCβ isoforms. This newly-identified cp-PLCβ will have significance for further investigation into the roles of the PLC signal transduction pathways in Chaetopterus egg activation.

L89 Expression of BT-IgSF during Spermatogenesis and Its Role in Fertilization
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We have cloned and characterized a novel gene from rat, mouse and human encoding a new member of immunoglobulin superfamily (IgSF). Since the gene contained two immunoglobulin domains in the extracellular region, and northern blot analysis showed the gene was exclusively expressed in both brain, especially in corpus callosum and hippocampus, and testis, the gene was termed BT-IgSF (brain- and testis-specific immunoglobulin superfamily). We examined in detail the expression of BT-IgSF in spermatogenic cells by in situ hybridization. BT-IgSF gene was expressed in the round spermatid at stages corresponding to the period of acrosomal formation. Other cells such as spermatogonial cells and Sertoli cells did not express BT-IgSF. In contrast, Basigin, another type of IgSF gene existing in testis, was expressed extensively more in spermatogenic cells including primary spermatocytes and round spermatid. An antibody specific for BT-IgSF was raised against extracellular domain of BT-IgSF, and immunohistochemistry was performed to observe the expression in rat testis and spermatocyte obtained from testis and epididymis, respectively. We also examined whether or not the molecule concerns fertilization. In rat in vivo fertilization (IVF), addition of the antibody into the IVF medium resulted in lowering the fertilization rate. These results suggest that BT-IgSF plays an important role in fertilization.

L90 The Role of Phosphatidylinositol-4-Kinase IIIα in Drosophila Development
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Phosphatidylinositol (PI) and its phosphorylated derivatives (PIPs) are essential for a broad array of cellular processes. Two PIPs, PI4P and PI5P, are conserved between invertebrates and vertebrates and are involved in cell adhesion, membrane trafficking, actin remodeling, cell polarity and regulation of the cytoskeleton. In Drosophila, PI4KIIIα catalyzes one of the first steps in PI modification to produce PI4-phosphate (PI4P). Stt4p is a PI 4-kinase type IIα (PI4KIIα) that catalyzes one of the first steps in PI modification to produce PI4-phosphate (PI4P). Stt4p is required for actin organization, vacuole morphology and cell wall integrity. Given its importance in single-celled yeast, PI4KIIα likely carries out crucial cellular functions during the development of multicellular organisms such as Drosophila melanogaster. Indeed, another PI4K, PI4KIIIβ-Fwd is required only for cytokinesis in the male germline, indicating that the remaining two PI4Ks, PI4KIIα and PI4KIIβ perform essential or overlapping functions during development. By imprecise excision of a P element, we generated a deletion in PI4KIIα that results in lethality at the first larval instar stage. Female germline clones were induced using the ovo FLP-FRT system to address maternal contribution of PI4KIIα to embryogenesis. However, no maternal null PI4KIIα eggs were laid, and mutant oocytes arrest development at early embryonic stages. Further experiments addressing cell polarity of clones and cell-autonomy of PI4KIIα are required to determine PI4KIIα function in oogenesis. Creating somatic clones as well as tissue-specific RNAi will identify other cell types that require PI4KIIα. Examining PI4KIIα mutant cells for aberrant cellular features will provide the first step in understanding the biological role of PI4KIIα and PI4P during development.

L91 Novelities in the Anatomical and Cellular Morphology in the Reproductive Tract of the Poeciliid, Poeciliopsis elongata
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Multiple clades in the teleost genus Poeciliopsis and its out-group Aulophallus show independent evolution of matrotrophy. Even though these live-bearing genera are used in life history studies, their basic cellular reproductive biology is poorly characterized. Microscopic analyses were used in order to understand the morphological characteristics of Aulophallus’ reproductive structures. Reproductive tissue from several Poeciliopsis elongata females were prepared for scanning electron (SEM) or light microscopy using standard methods. The results indicate that there are novel features in the reproductive tissue of this species, possibly playing roles in both pre and post

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fertilization development. The anatomical characteristics of the reproductive tract varied along the anterior-posterior axis. Oocytes were found only on the lateral and ventral sides of the ovary, with the germinal tracts lying closest to the ovarian lumen. Mature oocytes lay deeper within the ovarian stroma and were connected to the ovarian lumen via ducts formed by luminal epithelium. In SEM micrographs, these ducts were visualized as cords and directly attached to granulosa cells lacking tight junctions and appearing to have secretory activity. To our knowledge, this is the first report of these novel characters that polarize the granulosa cell layer. The epithelium lining the oviduct and ovarian lumen also showed regions of cells with secretory activity that lacked tight junctions, and these regions were also observed to be associated with sperm. Only the luminal epithelium of the anterior ovary lacked secretory activity; however, a large, unusual radiating secretory structure was observed in the anterior ovarian lumen. Models for the morphology of the ovary, the cellular anatomy of the oocyte-granulosa complex, and fertilization have been developed. Currently, members of the genus Poeciliopsis are being assayed in order compare the different species with the intent of understanding the cellular basis for the evolution of matrotrophy.

L92 Phosphatidylinositol 4-kinase Type II Is Required for Spermatogenesis in Drosophila
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The lipid phosphatidylinositol 4-phosphate (PtdIns(4)P) is synthesized from phosphatidylinositol (PI) by PI 4-kinases (PI4Ks). PI4Ks is a key regulator of trafficking through the secretory pathway and is also a precursor of PI 4,5-bisphosphate, which regulates the cytoskeleton, endocytosis/exocytosis, and signal transduction. The PI4KII beta is required for male germ cell cytokinesis, encodes a type III PI 4-kinase (Fwd/P4KIIIBeta). Surprisingly, fwd is not essential, although P4KIIIBeta activity is required for growth and secretion in budding yeast. BLAST analysis revealed that Drosophila contains two additional PI4Ks enzymes, another type III PI4 kinase (PI4KIIAlpIpha) and a single type II PI4 kinase (PI4KII). We predict that fwd is redundant with one (or both) of these PI4 kinases. To further investigate the role of P4K during development, I generated a series of deletions that remove the entire CG2929 (P4KII) gene, as well as an upstream gene CG14671. All deletions result in larval lethality and an antibody specific to somatically expressed P4KII protein indicates these deletions are protein null. A transgene containing both CG14671 and the somatic cDNA of P4KII restores viability. We have made new transgenic rescue constructs, which contain either CG14671 or CG2929 (P4KII) alone, to test which gene is specifically responsible for the lethality. Additionally, somatically rescued P4KII mutants are male sterile and have a defect in maintaining attachment of nuclei to elongated sperm tails at late stages of development. This indicates that both Fwd/P4KIIIBeta and P4KII is necessary for male fertility.

SIGNAL TRANSDUCTION IN DEVELOPMENT

L93 The Effect of γ-glutamylcysteine Synthetase and Glutathione Reductase on the Muscle Differentiation via Transcriptional Activation of NF-E2-related Factor 2
Y. Ding, W. Choe, S. Kim; Department of Biochemistry and Molecular Biology, Kyung Hee University, Seoul, Republic of Korea
Muscle differentiation is a complex process regulated at multiple levels. This study addressed the effect of γ-glutamylcysteine synthetase (GCS) and Glutathione Reductase (GSR) induction via transcriptional activation of NF-E2-related factor 2 (Nrf2) on the differentiation of rat cardiac muscle H9c2 cell and skeletal muscle C2C12 cell. We found both cellular GCS and GSH levels increased within 48 hours on myogenic stimulation of myoblasts. We also revealed the nuclear Nrf2 levels increased within 12 hours on myogenic stimulation of myoblasts. Nrf2-small interfering RNA study showed muscle differentiation was partially blocked and the myogenesis-induced GCS and GSR expression were again abolished in such cells. And then we also found that pharmacological inhibition of PI3-kinase resulted in effectively attenuated Nrf2 nuclear accumulation, myogenesis-induced GCS and GSR expression and subsequent Myogenin expression. Taken all together, our findings suggest that GSH contributes to the formation of myotubes from satellite myoblasts through activation of PI3-kinase, Nrf2, and GCS signal pathways.

L94 The In Vivo Role of the MKK4/JNK Signalling Pathway in Neuron Migration
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The c-Jun N-terminal protein kinase (JNK) is a mitogen-activated protein kinase (MAPK) involved in the regulation of various physiological processes. Its activity is increased upon phosphorylation by MAPK kinases, such as MKK4. MKK4 is widely expressed in the mouse brain during development, but little is known about the functional importance of the MKK4/JNK signaling pathway during this process. Here, we have generated a mutant mouse model carrying a specific deletion of the mkk4 gene in the brain. The mutant mice die around post-natal day 20 due to multiple brain abnormalities. These include the delayed migration of neurons in the cortex and irregular alignment of Purkinje cells in the cerebellum. Further analysis showed that the phosphorylation levels of the microtubule-associated protein 1b (MAP1b) and the neurofilament heavy chain (NF-H) were dramatically decreased in the mutant mice, which may account for the defect in neuron migration. We conclude that the MKK4/JNK signaling pathway is critical for brain development.

L95 Rho Kinase (ROK-α) Associates with Insulin Receptor Substrate and Negatively Regulates Muscle Cell Differentiation
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Myogenesis is an important process for the terminal differentiation of skeletal muscle. Previous studies suggested that Rho kinase regulates skeletal myogenic differentiation, but little is known about its related signaling pathways. So we examined the RhoA/ROK-α and IRS-1/PI3-kinase signal pathway of myoblast fusion in myocardin H9c2 and skeletal muscle C2C12 cells. We found that activity of RhoA and Rho kinase and serine phosphorylation of IRS-1 and 2 are increased in proliferating myoblasts, while tyrosine phosphorylation of IRS-1, 2 and PI3-kinase activity are increased during myogenesis. Treatment with Y27632, a Rho kinase inhibitor, not only increased IRS-1 tyrosine phosphorylation but also led to association with p85 PI3-kinase activity in myoblasts. In addition, FGF-induced inhibition of myogenesis stimulated activity of RhoA and Rho kinase and serine phosphorylation of IRS-1, 2 and 3 and activation of PI3-kinase activity. Furthermore, FGF-induced inhibition of myogenesis was reversed by Rho kinase inhibitor. Activation of Rho kinase also inhibited myogenesis through increasing serine phosphorylation but decreasing tyrosine phosphorylation of IRS-1 and 2 as well as inactivation of PI3-kinase. Therefore, we conclude that inactivation of Rho kinase signaling enhanced the myogenesis through tyrosine phosphorylation of IRS and activation of PI3-kinase signal pathways between both H9c2 and C2C12 cells.

L96 Inhibition of Cdk5 Sustains Maph (Erk1/2) Activation in Cortical Neurons and Induces Apoptosis
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Cdk5, a cyclin-dependent kinase, is critical for neuronal development, neuronal migration, cortical lamination and survival. Its survival role is functional importance of the MKK4/JNK signaling pathway during this process. Here, we have generated a mutant mouse model carrying a specific deletion of the mkk4 gene in the brain. The mutant mice die around post-natal day 20 due to multiple brain abnormalities. These include the delayed migration of neurons in the cortex and irregular alignment of Purkinje cells in the cerebellum. Further analysis showed that the phosphorylation levels of the microtubule-associated protein 1b (MAP1b) and the neurofilament heavy chain (NF-H) were dramatically decreased in the mutant mice, which may account for the defect in neuron migration. We conclude that the MKK4/JNK signaling pathway is critical for brain development.

L97 Activation of NF-E2-related Factor 2 (Nrf2) on the Differentiation of Rat Cardiac Muscle H9c2 Cell and Skeletal Muscle C2C12 Cell
Y. Ding, W. Choe, S. Kim; Department of Biochemistry and Molecular Biology, Kyung Hee University, Seoul, Republic of Korea
Muscle differentiation is a complex process regulated at multiple levels. This study addressed the effect of γ-glutamylcysteine synthetase (GCS) and Glutathione Reductase (GSR) induction via transcriptional activation of NF-E2-related factor 2 (Nrf2) on the differentiation of rat cardiac muscle H9c2 cell and skeletal muscle C2C12 cell. We found both cellular GCS and GSH levels increased within 48 hours on myogenic stimulation of myoblasts. We also revealed the nuclear Nrf2 levels increased within 12 hours on myogenic stimulation of myoblasts. Nrf2-small interfering RNA study showed muscle differentiation was partially blocked and the myogenesis-induced GCS and GSR expression were again abolished in such cells. And then we also found that pharmacological inhibition of PI3-kinase resulted in effectively attenuated Nrf2 nuclear accumulation, myogenesis-induced GCS and GSR expression and subsequent Myogenin expression. Taken all together, our findings suggest that GSH contributes to the formation of myotubes from satellite myoblasts through activation of PI3-kinase, Nrf2, and GCS signal pathways.
Apoptosis was correlated with a significant shift of phosphorylated tau and neurofilaments from axons to neuronal cell bodies. These results suggest that survival of cortical neurons is also dependent on tight Cdk5 modulation of the MAPK signaling pathway.

**L97**

**Hedgehog Signaling in Endoderm and Mesoderm Development in the Sea Urchin, Lytechinus variegatus**

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Hedgehog (Hh) is a morphogen that has been shown to be critical in cell-cell communication between tissue compartments during development. All main members of the Hedgehog signaling pathway have been identified in the sea urchin genome. The expression profile for Hh mRNA in sea urchin is consistent with a role in skeletal patterning and morphogenetic movements of gastrulation including subdivision of endomesoderm into gut and secondary mesenchyme cells (SMCs). Preliminary data indicates that overexpression of Hh increases endomesoderm (data not shown) while blocking Hh signaling disrupts skeletal and gut patterning. Ptch and Smo mRNA expression in the coelomic pouch area indicate a possible role for Hh signaling in the development of this SMC subtype. Current studies include distinguishing the role of Hh in skeletal patterning, defining the exact alternative roles in endomesodermal differentiation, and determining the possible role of Hh signaling in SMC subdivision, and identifying upstream modulators and downstream targets of Hh signaling in an effort to place this pathway within the sea urchin gene regulatory network.

**L98**

**The Involvement of NADPH Oxidases in Insulin-induced Chondrogenesis of the Mouse Embryonal Carcinoma-derived Cell Line ATDC5**

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Chondrogenesis is the process by which ectodermal mesenchymal cells differentiate into chondrocytes. Mouse embryonal carcinoma-derived cell line ATDC5 is a model of chondrogenesis in the early stages of endochondral bone development in response to insulin stimulation. Insulin stimulation of target cells elicits a burst of H2O2 that enhances tyrosine phosphorylation of the insulin receptor and its cellular substrate proteins. During differentiation of ATDC5 cells, ROS generation was increased. And the treatment of antioxidant N-acetylcysteine (NAC) suppressed the differentiation. Therefore we investigated whether ROS generated by NADPH oxidases is involved in differentiation. Among NOX family, gp91phox (NOX2) and NOX4 were highly expressed during differentiation of ATDC5 cells. Treatment of DPI, inhibitor of NADPH oxidase, and suppression of NOX2 and NOX4 using siRNA, inhibited chondrogenic differentiation and affected the organization of the actin cytoskeleton in ATDC5 cells. These data suggest ROS generated by NADPH oxidases is involved in chondrogenic differentiation of ATDC5 cells in response to insulin.

**DEVELOPMENT**

**L99**

**Sonic Hedgehog Stimulates Coronary Vasculogenesis from Progenitors in the Proepicardium**

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Fate maps show that the proepicardium (PE) contains progenitors that give rise to coronary vessels. The purpose of this study was to test whether or not sonic hedgehog (Shh) can stimulate vascular progenitors in the PE to differentiate into endothelial cells (ECs) and undergo tubular morphogenesis in explant cultures. Methods: PEAs were isolated from E9.5 mouse embryos, explanted onto 3-dimensional collagen gels and cultured in growth medium with or without Shh (1-2ug/ml) for 7-26 days. Expression of vascular cell type markers and Shh target genes was examined by RT-PCR and immunostaining. Results: PE exposed to Shh exhibited strong upregulation of all EC marker genes examined. In addition, formation of vessel-like tubular structures was greatly enhanced by Shh, indicating that coronary angioblasts respond to Shh signaling. Expression of smooth muscle cell (SMC) markers was also increased, although to a lesser extent than EC markers. Moreover, ECs differentiated from preformed angioblasts in the PE, whereas SMCs differentiated from epicardial cells that had undergone epithelial to mesenchymal transformation. Conclusions: Preliminary results in ATDC5 cells suggest that Shh upregulated the expression of secreted factors including VEGF-A, Fgfr16 and CTGF (connective tissue growth factor). Conditioned medium from Shh-stimulated PE cultures was considerably more active at promoting coronary vasculogenesis than Shh alone. Co-culture of PE in close proximity to E10 mouse hearts produced accelerated tube formation that was directed toward the explanted heart. Conclusions: Our data suggest that Shh initiates a hierarchy of signaling responses that coordinates tube formation by ECs and differentiation of SMCs from PE. This unique property of Shh to orchestrate the development of both principle coronary cell types may be useful in novel approaches to augment neovascularization of ischemic myocardium. Supported by HL-19242.

**L100**

**PRL-3 Initiates Tumor Angiogenesis by Recruiting Endothelial Cells In Vitro and In Vivo**

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Cancer metastasis is a complex process. The molecular governing these various events of metastasis remains elusive. Previous data implicates that PRL-3 (PL-3) is a tumor suppressor gene that is selectively enhanced in liver metastasis of colorectal cancers. Overexpression of PRL-3 can transform cells and enhance cell motility and invasion in vitro, and facilitate cancer metastasis in experimental metastasis mouse model. However, the molecular mechanism of PRL-3 action in cancer metastasis remains largely unknown. In this study, we provide evidences to show that PRL-3 plays an important role in tumor angiogenesis. Using in vitro cell co-culture assay, human endothelial cells and fibroblasts were co-grown in matrix-coated culture dishes followed by overlaying Chinese hamster ovary (CHO) cancer cells or DLD-1 colon cancer cells engineered to express PRL-3 or its PTP domain-dead mutant, respectively. After 12 days of co-culture, cells expressing PRL-3 (but not the mutant) can recruit endothelial cells to form blood vessel-like network. To further confirm the results in vivo, cells expressing PRL-3 or its PTP domain-dead mutant were mixed respectively with matrigel and injected into nude mice. Cells expressing PRL-3 (but not PTP domain-dead mutant) exhibited increased recruitment of host endothelial cells, enhanced tumor angiogenesis, and more robust tumor growth. Cytokine arrays of culture medium derived from cells expressing PRL-3 vs the mutant revealed that PRL-3 expressing cells secreted less amounts of Interleukin 4 (IL-4). In vitro angiogenesis assay showed that IL-4 inhibited the formation of mesh-like network of endothelial cells. Furthermore, expression of PRL-3 was specifically detected in the early developing stage of cardiovascular system. Taken together, these results suggest that PRL-3 plays an important role in tumor angiogenesis, and it may serve as an attractive therapeutic target.

**L101**

**Expression of Heat Shock Protein 60 under Dehydrated Condition**

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**BACKGROUND AND PURPOSE:** Heat shock proteins (HSPs) is abundantly expressed protein in almost living cellular organisms. Synthesis of HSPs is increased under stress, such as heat shock, inflammation, and infection. HSPs are believed that they are important in protection of cells from harmful stimuli. HSP60, one of the major families of HSPs, is also generated in response to tissue injury, and/or stress. In the mammalian cell, HSP60 may play key roles including chaperone activity, immunophilin, and antiapoptosis. In our previous study, HSP60 expressed different in cancer tissue and cell. Dehydration is one of the common symptoms of cancer patients, due to their cancer or its treatment. In this study, we investigated different expression patterns of HSP60 under various dehydration conditions. METHODS: A 549 and HEL 299 cell lines were cultured in RPMI 1640 medium. To make diverse dehydration conditions, 50mM, 100mM, and 200mM sodium chloride was added in medium, respectively. Osmotic shock was taken to cell lines in 2h, 4h, 8h, 16h, and 48h and harvested. RNAs and proteins were extracted from each well. Purified RNAs were analyzed by RT-PCR and gel electrophoresis. Western blotting was used for protein analyzing. To compare and confirm previous, we used...
cervix cancer and gastric cancer tissue. Purified RNAs and proteins from cancer tissue were analyzed same way. RESULT AND CONCLUSION: Expression of HSP 60 was similar in cancer tissue and cultured cell under dehydration condition.

L102
Beta-Catenin and Progesterone Signaling in Alveolar Development and Cancer
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Beta-catenin is necessary for alveologenesis during mammary development. Overexpression of beta-catenin leads to alveolar hyperplasia resulting in adenocarcinomas in virgin and breeding females. This phenotype is also seen in males, suggesting that beta-catenin is a probe de novo alveologenesis in the absence of hormonal stimuli provided by pregnancy. In order to test this hypothesis, we crossed MTV-AN89β-catenin mice to PR-LacZ knockout mice. PR+/-, PR-/-, PR-/-AN89β-catenin and PR-/-AN89β-catenin mice generated from this cross, were analyzed for precocious alveolar development in virgin females. These mice were also analyzed for pregnancy-induced alveolar development using Rag1-/- transplanted mammary glands. Virgin females and males were also analyzed for to determine if beta-catenin development using Rag1-/- transplanted mammary glands.

L103
Gene Expression Profiles on Mouse Liver Regeneration and Development
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BACKGROUND AND PURPOSE: Liver has a strong ability of regeneration after hepatic injury or resection. It has been reported that growth factor(EGF), transforming growth factor(TGF) and cytokotins including interleukins -1(IL-1) and -6(IL-6), tumor necrosis factor-alpha(TNF-α) participate in liver regeneration. However, the mechanism of liver regeneration is not fully understood. To understand liver regeneration process, we compared the gene expression involved in liver regeneration and development. METHOD: We used ICR mice and divided into 4 groups; fetus, adult mice operated hepatectomy, adult mice treated carbon tetrachloride (CC4), and normal adult mice. Developing livers were obtained from E10.5, E12.5, and E14.5. Regenerating livers were acquired from operated adult mice and CC4 treated mice. Hepatocytosis was 68% resection of adult liver and liver cirrhosis was induced by intra peritoneal (IP) injection of CC4. After hepatocytosis and carbon tetrachloride treatment, gene expressions of 4 groups were analyzed by microarray. RESULT AND CONCLUSION: The expression data of regenerating liver revealed that genes associated with metabolism were somewhat down-regulated. In the expression profiles of developing liver, the data suggested that some genes associated with regeneration were also expressed in the mouse fetal liver. Our study suggests that these genes may have crucial roles in the liver growth.

L104
Lipid-droplet Cholesterol Ester Profiling: Prediction of Adrenal Expression of Retinoid-X-Receptor Proteins
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Retinoid-X-Receptor (RXR) is an orphan receptor implicated in regulation of lipid metabolism, cell differentiation and apoptosis. New research findings have shown that certain polyunsaturated fatty acids (PUFA): corkic acid, arachidonic acid, and arachidonic acid can serve as ligands for RXR. Interestingly, rat adrenal lipid-droplets store cholesterol esters (CE) as steroidogenic reserves, in whose composition these fatty acids predominate. Upon ACTH stimulation, the CE is hydrolyzed to free cholesterol for steroidogenesis, but role of the liberated PUFA remains unknown. Since growth of the rat is accompanied by increased PUFA-linked CE contents, we predicted that the rat adrenal tissue should express RXR proteins, and the protein levels should be influenced by animal growth and by ACTH stimulation. Methods: Adrenals were collected from foetus, neonate and adult rats, and from the adult rats stimulated with ACTH (30 µg/100 g) for four consecutive days. RXR protein levels were determined by Western blotting, using polyclonal anti-RXRα, anti-RXRβ and anti-RXRγ antibodies and HRP-conjugated IgG as the secondary antibody. CE profiles were analyzed by reversed-phase HPLC. Results: The antibodies employed detected RXRα-like proteins and RXRβ-like proteins in rat adrenals. Lipid droplet-CE levels were low in foetal and neonatal rats, but increased remarkably in adult animals. This change was accompanied by elevated expressions of the RXR-like proteins. ACTH-stimulation of adult rats resulted in an 89%-increase of RXRα-like proteins and a 21%-decrease of RXRβ-like proteins. In contrast, RXRγ protein was not present at measurable levels in these studies. Conclusion: Rat adrenals express two types of RXR-like proteins. Levels of these proteins appear to be correlated with animal growth, and altered by ACTH stimulation (but each in a different manner). These expression patterns suggest a possible involvement of the proteins in regulation of CE storage and utilization in lipid droplets for steroidogenesis.

L105
Peroxisiredoxin III Is One of Negative Regulator during the Late Erythropoiesis of K562 Cells
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Recent studies have shown that ROS act as oxidative signaling molecules in cellular differentiations, resulting in various gene expressions. Peroxisiredoxin III (Pxr III) is antioxidant protein that reduces peroxide levels by using reducing agents such as thioreredoxin. This protein was characterized to have a number of cellular functions, including proliferation, differentiation and protection of specific organelles. In erythropoiesis of human CD34 + , Prx III-overexpressed cells decreased ROS level and then reduced ROS induction by Ara-C or hemin. But, Prx III is increased at day 4, that is to reduce expression of β-globin or γ-globin . To confirm the effect of Pxr III in late erythropoiesis of K562 cells, we prepared Pxr III- and dominant negative (DN)-overexpressed cells. Although ROS was increased after Ara-C and hemin treatment, K562 cells were more sensitive by Ara-C treatment. Pxr III-overexpressed cells had reduced ROS level than mock and DN cells. At that time, Pxr III-overexpressed cells had reduction of GPA+ population and β-globin or γ-globin expression than mock and DN cells. Overall the results suggest that Pxr III-overexpressed cells decreased ROS level and then reduced ROS level delayed late erythropoiesis of K562 cells. This work was supported by the Korea Research Foundation Grant funded by Korea Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-070-E00238). 1, 2) These authors are supported by the second stage of brain Korea 21.

L106
Histone H3 Variants in Mouse Preimplantation Embryos
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Mammalian cells contain three histone H3 variants that differ at a few amino acids: H3.1, H3.2, and H3.3. H3.1 and H3.2 are exclusively expressed throughout preimplantation development. The localization of three H3 variants in mouse preimplantation embryos, which undergo dynamic changes in histone modifications and gene expression processes, is not known. In this study, we analyzed H3.1, H3.2, and H3.3 expression by RT-PCR analysis and immunohistochemistry. Our results showed that H3.1 is expressed in trophoblast giant cells whereas H3.2 and 3.3 are expressed in inner cell mass and trophectoderm layer. H3.3 expression is observed in trophoblast giant cells and small cells at blastocyst stage. These results suggest that these H3 variants have discrete biological functions. Further study is needed to understand the role of H3 variants in mouse preimplantation development.
H3 variant proteins was examined by microinjecting oocytes with mRNA encoding tagged versions of the histone variants. H3.3 accumulated at the perinuclear region, but not the female pronucleus of early one-cell stage embryos and localized in both pronuclei at the late one-cell stage. After the first cleavage, H3.3 was detected in all nuclei throughout preimplantation development. H3.2 was not detected in either pronucleus at the early one-cell stage. However, it was detected in both pronuclei at the late one-cell stage; thereafter, it was always observed up to the blastocyst stage. H3.1 was not detected in the nuclei during the one- or two-cell stages, but was deposited in the nuclei at the morula/blastocyst stages. These results suggest that H3.2 and H3.3, but not H3.1, predominantly compose the chromatin in early preimplantation embryos and may be involved in the dynamic changes in chromatin structure and gene expression patterns.

L107
Interplay between Nuclear Receptor and β-Catenin Signaling during a Cell Fate Decision in C. elegans
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Nuclear receptor and Wnt/β-catenin signaling pathways intersect during important processes such as cell differentiation, proliferation or malignant growth. In the somatic gonad precursors (SGPs) of Caenorhabditis elegans, a β-catenin pathway plays a crucial role in the proximal-distal cell fate decision. The Wnt nuclear effector TCF/POP-1 and β-catenins WRM-1 and SYS-1 are the key molecules for this decision. Impaired function of all of the respective genes leads to a Sys-1(0(q645)) mutant phenotype, when all SGPs adopt the same, proximal fate. Thus, no distal tip cells (DTCs) that would lead germ-line differentiation and elongation of the gonadal arms are formed in hermaphrodites. Here, we show that loss of the nuclear receptor NHR-25 (a homolog of SF-1/LRH-1 and Ftz-F1) causes an extra DTC density of the spines of hippocampal pyramidal neurons. Therefore, Cas is critical for maintaining proper spine morphology and its function can be regulated by EphA4 receptor. We also found that ephrin-A3 decreases β1 integrin activity in neuronal cells. Taken together, our data suggest that EphA4 receptor controls hippocampal dendritic spine morphology by inhibiting the activity of β1 integrin and its downstream targets, such as the adapter protein Cas.

L109
EphA4 Receptor Controls Dendritic Spine Morphology by Regulating the Assembly of Cas Signaling Complexes
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Dendritic spines are dynamic protrusions localized on neuronal dendrites. Morphological reorganization of spines is likely important for synaptic transmission and plasticity as a number of cognitive disorders are associated with spine malformations. In mature mouse hippocampus, stimulation of the EphA4 receptor with exogenous ephrin-A3 Fc ligand promotes spine retraction. However, the signaling pathways that are involved remain to be characterized.

Involvement of the Aryl Hydrocarbon Receptor in Netrin-1-mediated Signal Transduction
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Netrin-1 plays an important role in neuronal guidance during nervous system formation and is integral to the survival of developing neurons. Netrin-1 directs neurons and axons to their targets with netrin-1 acting either as an attractant or a repellent depending on receptor expression levels. The two vertebrate families of netrin-1 receptors, Deleted in Colorectal Cancer (DCC) and Unc5, mediate axonal guidance through interactions with netrin-1 and each other. The DCC family mediates attraction to netrin-1 while the UNC5 family forms a netrin-1-dependent complex with DCC to mediate repulsion. Recently, the aryl hydrocarbon receptor, AHR-1, has been genetically linked to netrin-1-mediated axon guidance in C. elegans. This finding is significant as AHR mediates the toxicological effects of aryl hydrocarbons (AHRs) in vertebrates. In the developing human nervous system, exposure to AHRs due to both an increase in their life-time and a higher insertion rate. These results are consistent with the hypothesis that the role of AHR in vivo might be to stabilize and enhance synapses.
immonostaining that AHR and DCC proteins are expressed in developing rat hippocampal neurons. Costaining verifies these proteins colocalize to the same neurons. Since AHR is a known transcription factor, we sought to determine if AHR localizes to the nucleus following netrin-1 stimulation. We find that upon netrin-1 stimulation, levels of AHR expression in the nucleus increase within forty-five minutes of exposure. Together, these data support the hypothesis that AHR is involved in netrin-1-stimulated signaling and indicate that AHR may mediate netrin-1-stimulated transcription. (Funded by R1-INBRE Grant # P20RR016457 from NCRR, NIH).

L111
Protein Tyrosine Phosphatase MEG2 Regulates NSF Activity in the Brain
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Neurotransmission is a highly regulated process requiring the participation of soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNAREs), as well as accessory proteins such as soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP), and N-ethylmaleimide-sensitive factor (NSF). Extensive studies have led to the identification of NSF as a positive regulator of fusion machinery through the activation of SNAREs. Moreover, studies in lymphocytes suggested the importance of tyrosine phosphorylation in modulating NSF function. Specifically, tyrosine phosphatase PTP-MEG2 has been implicated in promoting homotypic membrane fusion by dephosphorylating NSF, rendering it active for SNAP binding and subsequent SNARE uncoupling. However, it is unclear whether tyrosine phosphorylation of NSF is important in the context of controlling neurotransmission. In this report, we demonstrate that neuronal NSF is tyrosine phosphorylated and associates with PTP-MEG2. Furthermore, brains of MEG2-deficient mice harbor hyperphosphorylated NSF while neurotransmitter release from synaptosomes is impaired. In addition, overexpression of wild-type PTP-MEG2 in B35 cells resulted in elevated secretion of a reporter molecule, whereas dominant negative PTP-MEG2 mutants suppressed release. Taken together, our data suggests conserved means of regulating NSF activity in the brain through tyrosine phosphorylation.

ENDOSOMES AND LYSOSOMES

L112
Alpha Factor Stimulates Multivesicular Body Formation in Yeast
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Stimulation of EGF receptors by EGF causes downregulation of the receptors. A bolus of stimulated receptors is internalised and directed to lysosomes, where the receptors are degraded. Recent work shows that, in order to accommodate this influx of cargo, the endocytic pathway is upregulated. Internalisation machinery is recruited to the cell surface and formation of late endosomes (multivesicular bodies, MVBs) increases. We sought to determine whether similar mechanisms occur in the experimentally tractable Saccharomyces cerevisiae. Stimulation of yeast mating type α cells with alpha factor pheromone stimulates a MAPK pathway via the alpha factor receptor, Ste2. It also induces downregulation of Ste2, in a manner similar to EGFRs. Preliminary results suggest that there is an increase in the number of MVBs in cells stimulated with alpha factor, and that this is restricted to mating type α cells. Results from analysis of MVB numbers in MAPK pathway mutants will also be discussed.

L113
Effect of Tyrosine Kinase Inhibitor AG1478 on Internalized EGF Receptor in A431 Cells
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Cell responses to EGF are mediated through its transmembrane receptor with intrinsic tyrosine kinase (TK) activity. EGF binding to EGF activates TK and stimulates both signaling and endocytosis of EGF. Upon internalization EGF travels from early to late endosomes and finally is degraded in lysosomes. For many years it was believed that endocytosis serves as attenuator of signals stimulated by EGF at plasma membrane. However, new data were accumulated suggesting independent role of internalized receptor in signaling. But they are rather controversial. The main problem in the field is the separation of signals starting at plasma membrane and those originating from endosomes. Among others, the approach with inhibiting of endosomal EGF receptor TK by AG1478 followed by its washout was used to reveal the role of internalized receptor, but inhibitor’s effect was not analyzed in detail. We report here that tyrosine phosphorylation of EGF receptor was inhibited for more than 90% by AG1478 at 0.5 mM in A431 cells. The inhibitory effect was fully developed in 5 min upon addition and both surface and endosomal EGF receptors were inhibited. When added at different times upon endocytosis stimulation, AG1478 caused the recycling of a portion of internalized EGF-EGFR complexes but this portion significantly decreased with time. Recycled EGF did not dissociate from EGF receptor on the cell surface possibly due to high degree of the receptor oligomerization produced by AG1478. TK inhibition of endosomal EGF receptor results in c-Cbl dissociation from its complex with EGF and EGF receptor deubiquitination. Wash out of AG1478 during 30 min partially restored tyrosine phosphorylation of EGF receptor, but not its association with c-Cbl. We conclude that protocols involving AG1478 washout deal with impaired EGF receptor and are unacceptable for evaluation of endosomal EGF receptor role in signal transduction.

L114
WITHDRAWN

L115
Myosin II Regulates MHC Class II Trafficking and B Cell Receptor-driven Antigen Presentation
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Antigen presentation onto MHC class II molecules by B lymphocytes is mediated by their surface antigen receptor (BCR). Trafficking of vesicles that carry MHC class II- and BCR-Ag complexes must therefore be coordinated for them to converge for processing. Here, we identify the actin-associated motor protein Myosin II as being essential for this process. Myosin II is activated upon BCR engagement and associates to MHC class II-Invariant chain complexes. Inhibition of Myosin II activity compromises the convergence and concentration of MHC class II and BCR-Ag complexes into lysosomes devoted to Ag processing. Accordingly, formation of MHC class II-peptides and subsequent CD4 T cell activation are impaired in cells lacking Myosin II activity. Myosin II therefore emerges as a key motor protein in BCR-driven Ag processing and presentation.

L116
Are Urothelial Fusiform Vesicles Lysosome-related Organelles?
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The urinary bladder is lined by a stratified epithelium that is covered by highly differentiated umbrella cells. Their apical plasma membranes consist of an asymmetric unit membrane (AUM) structure that is composed of four major uroplakins (UPs) Ia, Ib, II, and III. These four membrane proteins are assembled into crystalline hexagonal arrays that are stored in fusiform vesicles (FVs). In response to stretching of the bladder FVs are inserted, probably through a Rab27b-mediated pathway into the apical plasma membrane of umbrella cells, thus increasing its surface area. Rab27b is thought to be involved in the maturation and targeting of lysosome-related organelles, including melanosomes. We found that Rab27b is rather selectively expressed in bladder epithelium and is FV-associated. We, therefore, considered the possibility that FVs belong to the class of lysosome-related organelles. Immunolocalization showed that FVs do not contain the lysosomal markers LAMP-1 or LAMP-2, but they accumulate DAPM, indicating that their lumen is acidified. The Buff mouse, one of the models for Hermansky-Pudlak Syndrome, carries a point mutation in Vps33a resulting in defective targeting of melanosomes and platelet dense granules. We found that the Vps33a mutation also affects the morphology of urothelial umbrella cells, reducing drastically the number of FVs but amplifying that of multivesicular body (MVB)-like vacuoles. These MVBs contain AUMs, but are LAMP-1/2 positive, and do not associate with Rab27b. It, therefore, seems likely that they are
derived from modified FVs, which acquire morphological features and markers of late endosomes. The Vps33a mutation in the Buff mouse may interfere with the maturation of MVBS into mature lysosomes resulting in their accumulation in umbrella cells. The association of FVs with Rab27b, their acidic character, and their biogenetic relationship to lysosomal organelles suggest that they are a subtype of lysosome-related organelles.

L117
Phosphatidylinositol 3-phosphate Mediates Phagocytosis and Phagosome Maturation in the Protozoan Parasite Entamoeba histolytica
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Entamoeba histolytica is a causative agent of amebiasis. Since phagocytosis is essential for the in vitro and in vivo virulence of E. histolytica, we have been trying to understand molecular basis of phagocytosis and phagosome biogenesis. We previously demonstrated that Rab GTPases and their effectors play a central role in the regulation of phagosome maturation (Saito-Nakano JBC 2004; Nakada-Tsukui MBC 2005). It was also shown by other groups that phosphatidylinositol (PI) metabolism plays an indispensable role in phagocytosis and endocytosis because they were abolished by wortmannin. Here we investigated molecules that are involved in PI-3-phosphate (PI3P) signaling in phagocytosis and phagosome maturation. Green fluorescent protein (GFP) fused with 2x FYVE domain, which binds to PI3P, expressed in E. histolytica, revealed localization on internal vesicles/vacuoles in quiescent state. When the amoeba started engulfment of a CHO cell, PI3P was immediately accumulated on both the phagocytic cup and newly-formed phagosomes. After 10, 30 and 60 min of engulfment, PI3P was positive on 65, 55 and 20% of phagosomes, respectively. These data suggest that PI3P participates in the initial phase of phagocytosis and the formation and further metabolism of PI3P occurs along with the phagosome maturation. Among 11 E. histolytica FYVE domain-containing proteins (EFGs) present in the E. histolytica genome, 10 showed a similar overall structure consisted of RhoGEF, PH, and FYVE domains. Two out of four FYVE-domain-containing proteins were localized on the phagocytic cup but not on phagosomes, suggesting temporary association of these EFG proteins only in the initial phase of engulfment. This localization pattern was similar to that of F-actin during CHO cell ingestion. We are currently investigating downstream signaling transduced via not-yet-characterized Rho likely activated by RhoGEF domain of these EFG proteins, which should lead to rearrangement of cytoskeleton close to the phagocytic cup.

L118
Role of the Eye-color Gene, Light, in Endosomal Trafficking
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“Granule group” of eye color genes in Drosophila are homologues of the Vps (Vacular Protein Sorting) genes of yeast implicated in membrane trafficking and organelle biogenesis. Products of two of these genes, deep orange (dor) and carnation (car), have been shown to localize to Rab7– positive late endosomes that contain golgi-derived degradation enzymes. Endosomal degradation is impaired in primary hemocytes derived from mutants of deep orange, due in part to a failure of fusion of golgi-derived vesicles carrying proteases with the late endosomes. The Sec1p homologue, Carnation, has an independent role in the fusion of late endosomes with tubular lysosomes. Another eye color gene, light, interacts genetically with deep orange and with carnation. Homologues of Light, Deep orange and Carnation in yeast, namely, Vps41, Vps18 and Vps33 are a part of a multi-protein subunit complex known as the “HOPS” complex, required for fusion of pre-vacuolar late endosomes with the vacuole. Here, we have investigated the role of Light in endosomal biogenesis in cells from Drosophila. Using high-resolution imaging and immunofluorescence microscopy, we see that Light also localizes to Rab7-positive late endosome compartments similar to those marked by Dor and Car. Unlike Dor and Car, we find that it also marks tubular lysosomes. Using RNAi-based depletion in Schneider cells and primary hemocytes derived from light mutants, we show that Light is necessary for endosomal degradation. However, unlike mutants of deep orange, Golgi-derived enzymes appear to be delivered to late endosomes, and defect in degradation appears to be due to a failure in acidification of late endosomes. We are currently studying the basis for this acidification defect.

L119
Mechanisms of Myelin Biogenesis: Regulation of Membrane Transport in Oligodendrocytes
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During vertebrate brain development, axons are enwrapped by myelin, an insulating membrane produced by oligodendrocytes. Our aim is to elucidate the cellular machinery that is required for the formation of this membrane. Since the development of myelin depends on these axonal cues, we analyze how neuronal signals regulate the generation of myelin membrane. We have investigated the regulation of myelin membrane trafficking in oligodendrocytes by neurons. In the absence of neurons, the major myelin membrane protein, the proteolipid protein (PLP), is internalized and stored in late endosomes/lysosomes (LE/Ls) by a cholesterol-dependent and clathrin-independent endocytosis pathway. Upon maturation, the rate of endocytosis is reduced, and a neuronal signal triggers the transport of PLP from LE/Ls to the plasma membrane. By total internal reflection fluorescence microscopy fusion of these vesicles with the plasma membrane was shown. Furthermore, we demonstrate that the myelin membrane protein, ASBP, which is coordinately regulated by Rho GTPases and the tyrosine kinase, c-src. Rho GTPases and c-src do not only regulate the transport of cargo to LE/L, but also control the dynamics of LE/L membrane. These findings reveal a fundamental and novel role of LE/Ls in oligodendrocytes: to store and release PLP in a regulated fashion. We propose that this mechanism ensures the proper timing and controls the growth of the myelin membrane.

L120
Localization and Functional Characterization of LvsB, a Beige/CHS Related Protein from Dictyostelium
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The Beige/CHS related protein, LvsB, is a poorly understood genetic disorder. Impaired lysosomal function in these patients results in many physiological problems, including immunodeficiency, albinism and neurological problems. The gene affected in humans with CHS encodes a 430KDa protein named LYST (lysosomal trafficking regulator). Dictyostelium discoideum LvsB protein is the ortholog of mammalian LYST and is also important for lysosomal function. Our studies focus on characterizing the LvsB localization and function to help understand the regulation of lysosome biogenesis and the Chediak-Higashi syndrome. We present here a knock-in approach to tag the Dictyostelium LvsB with GFP. This construct allowed the recombination of the GFP coding sequence upstream of the lvsB gene leading to the production of a fusion protein. Imaging of tagged-LvsB revealed that while a fraction of soluble GFP-LvsB was observed in the cytoplasm, it was also associated with vesicles of various sizes. LvsB labeled vesicles were of endocytic nature and colocalized with Rab7 and vacuolins, known markers of the endocytic pathway. In fact, the majority of that GFP-LvsB colocalized with vacuolins, which is a marker specific for post-lysosomes. This result indicated that LvsB must exert its function primarily on the post-lysosomal compartment. Indeed we found that LvsB mutants showed abnormally enlarged post-lysosomes that frequently exhibited altered actin markers. Utilizing fluid phase markers, we showed that in LvsB mutants, the earlier endosomes fuse inappropriately with late post-lysosomes. In agreement with the fusion assays, LvsB mutants exhibited frequent intermixing of lysosomal proton pumps with vacuolins. In conclusion, we have demonstrated that LvsB localizes on late endocytic compartments and provides an additional level of specificity by controlling fusion events between late and early endosomes.

L121
Genome-wide RNAi Screen for Polarised Membrane Trafficking in C. elegans
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The suitability of C. elegans as a model system to identify genes functioning in endocytosis has been demonstrated by previous genetic
screens (Grant and Hirsh 1999; Fares and Greenwald, 2001), encouraging us to complement these efforts by devising a visual screening method which aims at identifying novel components required for apical transport and recycling. Offering excellent morphological resolution, the nematode intestine was chosen to study the sorting and trafficking of an apical transmembrane marker. Both breeding of worms as well as gene-silencing using Julie Ahirngers’ RNAi feeding library is achieved in a largely automated process. Following image acquisition using a 96-well format epifluorescence microscope, data is analyzed using a custom-built software solution allowing objective phenotypic analysis. An overview of the C. elegans RNAi screening assay as well as of the automated image analysis will be presented.

L122
Rnf13 Is an Endosomal Ubiquitin Ligase Expressed in Neurons
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The PA-RING family of transmembrane proteins contains a protease-associated (PA) luminal domain and a cytoplasmic RING finger domain. Previously described family members include GRAIL (Immunity 18:535-547, 2003), upregulated in the T-cell anergic response, and RMR, a receptor mediating targeting to plant protein storage bodies (J. Cell Biol. 150:735-70, 2000). Although chicken Rnf13 was predicted to function as a transmembrane protein localized to the nucleus (Proc. Natl. Acad. Sci. 93:3105-3109, 1996), we have utilized confocal immunofluorescence microscopy to show that in mammalian epithelial cells over-expressed mouse Rnf13 is localized to the early and late endosomes, lysosomes, and multivesicular bodies, as demonstrated by colocalization with LAMP2, CD63, and M6PR.

Our data establish that the RING domain of Rnf13 is capable of catalyzing polyubiquitination with several E2 enzymes. Mutation of a critical cysteine residue in the RING finger (C266) causes loss of E3 enzyme activity and stabilizes the protein. This point mutant localizes to the plasma membrane, as does a mutant having an altered dileucine endocytosis motif. Deletion of either the PA domain or the cytoplasmic tail also results in targeting to the plasma membrane. RT-PCR data indicate that RNF13 is expressed at low levels in neurons and a recent array analysis study indicated that it induces neuronal phenotype (Neuron 22:8-13 (2005)). We are using a lentiviral expression system to study the physiological effects of over-expression of the above mutants of this protein in neurons.

L123
Mvb12 Is a Novel Member of ESCRT-I Involved in Cargo Selection by the MVB Pathway
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Degradation of activated cell surface receptors is an important process for cellular function. Sorting of receptors into the multivesicular body (MVB) pathway allows for their complete degradation in the lumen of the lysosome/vacuole. Entry into this degradative pathway is tightly regulated and is dependent on a group of trans-acting factors referred to as the Endosomal Sorting Complexes Required for Transport (ESCRTs). The ESCRTs recognize, bind, and sort cargo proteins into the MVB pathway. In order to identify novel factors involved in cargo selection by the MVB pathway, we performed a genetic screen for mutants that displayed differential defects in the sorting of cargo into this pathway. This screen identified loss of MVB12 as defective for the delivery of a subset of cargoes into this pathway. Mvb12 executes this function as a sub-component of the ESCRT-I complex, suggesting that it modulates the cargo-recognition activity of ESCRT-I.

EPITHELIA

L124
The Shroom Family of Actin-binding Proteins as Evolutionarily Conserved Determinants of Cellular Architecture and Epithelial Morphogenesis
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In mammals, the Shroom family of proteins consists of Shroom2, Shroom3, and Shroom4. In mice deficient for Shroom3, epithelia expressing the neural ectoderm fail to undergo the requisite changes in cell shape necessary for morphogenesis of the neural tube. Consequently, mutant embryos exhibit defects in neural tube closure or morphology along the entirety of the anterior-posterior axis. These phenotypes stem from the inability of cells to assemble a contractile acto-myosin network in the apical junctional complex (AJC). In the neural epithelium, Shroom3, myosin II, and F-actin co-distribute in the AJC, while expression of Shroom3 in MDCK cells induces the formation of a contractile acto-myosin ring and causes apical constriction. Similar to Shroom3, both Shroom2 and Shroom4 also control specific aspects of cytoskeletal organization and do so via direct interactions with F-actin and the regulation of myosin II distribution. Importantly, each family member exhibits differential specificity in actin association in vivo that directs their subcellular localization to distinct subcellular compartments. Based on homology, we have identified and characterized the Drosophila ortholog of Shroom, dShrm (formally CG8603). Drosophila Shrm is expressed in polarized epithelial cells in the embryo and has the capacity to trigger changes in epithelial cell shape in a myosin II dependent fashion. Finally, we have used genetics to assess the role of Shroom3 in other aspects of vertebrate neural tube morphogenesis. Through this approach we have determined there is a genetic interaction between the Shroom3 pathway and the planar cell polarity pathway in neural tube closure and that Shroom3 is also important for regulating secondary neurulation, a tubulogenic process achieved through canalization. Our data suggest that Shroom proteins comprise a conserved family of actin-associated proteins that function to regulate epithelial morphology during numerous developmental processes.

L125
Potent Protective Effect of α-Tocopherol and Fish Oil on In Vivo Paraquat Induced Oxidative Damage in Rats
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The potential protective roles of α-tocopherol and fish oil against oxidative damage induced by paraquat were investigated. Forty male albino rats with average body weight of 100-120 gm. They were housed in groups of 5 each and divided randomly into 8 groups. The first group served as control, groups 2 and 3 were administered orally with fish oil (20 mg/kg) and vitamin E (100 mg/kg) respectively. Group 4 was injected with a single dose of paraquat (10 mg/kg, intraperitoneally), 24 h. prior decapitation, while groups 5, 6 and 7 were injected with paraquat-vitamin E, paraquat-fish oil and paraquat-vitamin E - fish oil respectively; Group 8 received fish oil and vitamin E. The content of microsomal proteins, drug metabolizing enzymes and thioarbituric acid reactive substances (TBARS) were determined in liver microsome after treatment. Vitamin E together with fish oil significantly decreased the content of cytochrome b5 (p<0.001), cytochrome P-450 (p<0.01), glutathione-S-transferase (p<0.01) and cytochrome C-reductase (p<0.001) when given before paraquat injection. Meanwhile, this combination (vitamin E - fish oil) has no significant effect on amiodopyrine N-demethylation. On the other hand vitamin E and fish oil alleviated the paraquat induced increase in TBARS. In conclusion, oral administration of vitamin E and fish oil are effective in reducing the activity of selected drug metabolizing enzymes and are also effective in reducing lipid peroxidation process caused by paraquat. So, this combination provide a potent protection against paraquat-induced oxidative toxicity in rats liver.

L126
WITHDRAWN

L127
Efficient Electroporation of DNA and Protein into Fully Polarized Epithelial Monolayers In Vitro
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To date, the only available procedure to deliver macromolecules efficiently into fully differentiated epithelial cells requires viral vectors, e.g., adenoviruses, with the consequent dependence on the possession of the required viral receptors in the apical surface of the cells under study and the inconvenient requirement to build the viral expression vector. Electroporation or cationic-lipid mediated transfection are not efficient procedures to deliver macromolecules into fully polarized epithelial cells grown on plastic, glass or filters, although they operate efficiently for the introduction of macromolecules into cells in suspension culture, and even into cells in their native tissue.
environment, e.g. retina and embryonic tissues. We report here an electroporation protocol that operates at high efficiency in polarized, differentiated epithelial monolayers in culture. The procedure uses commercially available equipment and cheap electroporation buffers and is effective for protein expression into a variety of epithelial cell lines, including MDCK, Caco-2 and ARPE-19, even after six weeks in culture. The electroporated monolayers preserve their integrity for a minimum of 24h, depending on the concentration of DNA and express exogenous markers with the correct polarity. The electroporation procedure is successful for both DNA and proteins. Supported by NIH grants NEI 08538 and GM34107 and awards from RPB and Dyson Foundation to ERB.

L128

A High Potassium Diet on the Expression of Voltage Sensitive Potassium Channel (Kv1) in Kidney

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Potassium (K) homeostasis is vital for the function of the cells: A high intracellular K concentration is critical for cell growth, whereas maintenance of low intracellular K in a non-symmetrical function of neurons, cardiac myocytes, and skeletal muscle. The voltage gated potassium channels (Kvs) belong to one of the most widely expressed and diverse family of ionic channels in excitable cells. An increase in a K body diet stimulates insulin secretion in response to an elevated glucose uptake. Insulin activates Na-K-ATPase in skeletal muscle and transfers K from extracellular to intracellular fluid. This shift favors the rise of intracellular K stored in skeletal muscle and liver is eventually released into the extracellular fluid, filtered, and also secreted into the urine by the kidney. The final urinary regulation of the Na and K ions occurs in the collecting duct. The goal of this work is to determine the impact of a high K diet on the Kv1 subfamily expression and distribution in the epithelial cells of the rat kidney. We demonstrate the presence of Kv1.1, Kv1.2, Kv1.4, Kv1.5 and Kv1.6 in the collecting ducts across the different kidney sections. Most of these channels are observed mainly in the cytoplasm; however, their expression level responded to a high K diet, and particularly, the polarity of Kv1.3 changed from basolateral to apical. Urinary K excretion and aldosterone levels in plasma increased with high K uptake, but the blood K levels were kept constant. The physiological role of the Kv1 channels could be associated with K recycling (Na reabsorption) besides the membrane potential maintenance in the IMCD. Furthermore, our results also suggest that Kv1.3 participates in K secretion due to an excess in the K intake.

L129

E-cadherin Trafficking in Drosophila Epithelium

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The E-Cadherin-catenin complex plays a critical role in epithelial cell-cell adhesion, polarization and morphogenesis. Here, we have analyzed the mechanism of Drosophila E-Cadherin (DE-Cad) localization. Loss of function of the Drosophila exocyst components sec5, sec6 and sec15 in epithelial cells results in DE-Cad accumulation in an enlarged Rab11 recycling endosomal compartment and inhibits DE-Cad delivery to the membrane. Furthermore, Rab11 and Armadillo interact with the exocyst components Sec15 and Sec10 and positively. Our results suggest a model whereby the exocyst regulates DE-Cadherin trafficking, from recycling endosomes to sites on the epithelial cell membrane where Armadillo is located.

L130

Isolation and Characterization of Potential Stem Cells from the Colonic Crypts and Identification of Preferentially Expressed Functional RNAs in These Cells That Regulate Cell Growth

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The gastrointestinal epithelium is one of the most rapidly renewing tissues in the body turning over approximately 7 tons of cells in average human lifetime. The nature of the stem cells that supply this epithelium is a mystery and the genes that control this process are still not well understood. To identify critical genes that regulate colonic renewal, gene expression at the bottom of crypts (where the stem cells reside) were compared to that in the top of the crypts by microarray and a small subset of genes that are preferentially expressed in the stem cell compartment were found. Some of these genes code for potential cell surface markers, which we have subsequently used to enrich for clonogenic cells using cell sorting. Clonogenic cells grown in culture and derived from the crypt epithelium resemble (by gene expression criteria) neither the bottom nor top crypt cells. These results imply that outside of their crypt niche, progenitor cells have very different genes expressed and/or that there are very few precursor cells that make up the total transient amplifying and differentiated cell population of crypts. Surprisingly many of the genes found to be preferentially expressed in the bottom of crypts were found to be functional RNAs that affect the growth of intestinal cells without actually coding for protein. These genes expressed in the progenitor compartment of the colonic crypt have been cloned into lentivirus and have been used to transfect cell lines derived from immortomouse colonos that are conditionally immortalized with the temperature sensitive T-antigen. In one such cell line, YAMC cells, transfection of such a functional RNA lead to altered growth characteristics. By overexpressing these genes found in the progenitor compartment we hope to define their role in regulating cellular differentiation and growth of epithelial progenitors.

L131

Sta1/3 Is Critical for Regulating MDCK Tubulogenesis

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Tubulogenesis is fundamental to the development of the kidney and many other epithelial organs. In vivo kidney tubulogenesis is a very complex process, involving many cell types and growth factors, and it is difficult to analyze the cellular and molecular mechanisms by which cells rearrange themselves to form tubules. Here we use a simplified model system in which MDCK cells are cultured in 3D. Single MDCK cells grow up in ~7 days to form hollow, spherical cysts, lined by a monolayer of polarized epithelial cells and form tubules over ~3 days in response to HGF. Our lab previously demonstrated that a transient, 24 hr pulse of MAPK activation was sufficient to produce a partial Epithelial-Mesenchymal Transition (p-EMT) but to complete tubulogenesis subsequent inactivation of MAPK signaling was required. To dissect the cellular mechanisms of tubulogenesis we studied the role of STAT1/3 using either MDCK cells treated with HGF or expressing an inducible form of Raf-1. STAT1 is required. To dissect the cellular mechanisms of tubulogenesis subsequent inactivation of MAPK signaling was required. To dissect the cellular mechanisms of tubulogenesis we studied the role of STAT1/3 using either MDCK cells treated with HGF or expressing an inducible form of Raf-1. STAT1 is required.

CELL CULTURE

L132

Microfabricated Embryonic Stem Cell Divider for Large-scale Propagation of Human Embryonic Stem Cells

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Human embryonic stem (ES) cells have emerged in recent years as a precious asset in developmental biology, regenerative medicine, and drug discovery. Such applications will require rigorous quality control of in vitro ES cell culture. However, conventional enzymatic and non-enzymatic (chemical and mechanical) methods for ES cell dissociation are still needed to improve in many aspects such as loss of genetic integrity and uncontrolled ES clump size. Thus, we developed an advanced simple micro-stamping tool “embryonic stem cell divider” (“ESC divider”) constructed from the poly(dimethylsiloxane) (PDMS) replica with square or hexagonal pattern. The aspect ratio of device over 2 was perfectly replicated in cutting line and stamped onto the pre-vascularized ES cells by homemade stamping apparatus. Once ES cell colonies were quickly pressed by either square- or hexagonal-shaped ESC divider, uniform, square- (or hexagonal-) shaped pattern was
Asiasarum Heterotropoide conditions. In addition we detected a significant increase in the rate of transformation into insulin cells than was seen in mice under the same culture period (E110). Pig duct cells evidenced a higher rate of transformation were obtained from fetal mice. In mice, duct cells isolated during the mid-gestational age. Based on the data regarding mice fetal duct cells, it is suggested that the most differentiation into insulin cells when compared to those of the earlier stages (E12.5) and later stages (E18.5). This suggests that the most advantageous of both embryonic and adult stem cells: namely, higher rates of differentiation into insulin-secreting cells and a variety of stem and precursor cells. In the present study, we isolated stem cells from murine and porcine pancreatic tissues at various developmental stages, and monitored the features of these stem cells. Dorsal pancreatic buds were obtained from fetal mice. In mice, duct cells isolated during the mid-gestational stage (E15.5) evidenced increased expression of nestin, a marker of pancreatic stem cell as well as a higher rate of differentiation into insulin cells when compared to those of the earlier (E110) and later (E18.5). This suggests that the most advantageous stem cells might be acquired from the fetal pancreas at mid-gestational age. Based on the data regarding mice fetal duct cells, we isolated duct cells from pig fetal tissue during the mid-gestational period (E110). Pig duct cells evidenced a higher rate of transformation into insulin cells than was seen in mice under the same culture conditions. In addition we detected a significant increase in the transformation of duct cells into insulin-secreting cells, as well as self-renewal, in the presence of nicotinamide. Taken together, porcine fetal pancreas may be considered a useful source of insulin-secreting beta cells for use in cell replacement therapy for the treatment of diabetes.

Isolation of Fetal Pancreatic Stem Cells for the Generation of Insulin-producing Cells
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Diabetes is caused by a dysfunction of the insulin-producing β-cells in the pancreatic islets. Stem cells have been considered as a source of cell replacement for the treatment of diabetes. Researchers have encountered problems, not only in the relatively low rate of differentiation of the embryonic stem cells, but also in terms of the paucity of stem or progenitor cells in adult pancreatic tissues. We hypothesized that stem cells in fetal pancreatic tissues may harbor the advantages of both embryonic and adult stem cells: namely, higher rates of differentiation into insulin-secreting cells and a variety of stem and precursor cells. In the present study, we isolated stem cells from murine and porcine pancreatic tissues at various developmental stages, and monitored the features of these stem cells. Dorsal pancreatic buds were obtained from fetal mice. In mice, duct cells isolated during the mid-gestational stage (E15.5) evidenced increased expression of nestin, a marker of pancreatic stem cell as well as a higher rate of differentiation into insulin cells when compared to those of the earlier (E110) and later (E18.5). This suggests that the most advantageous stem cells might be acquired from the fetal pancreas at mid-gestational age. Based on the data regarding mice fetal duct cells, we isolated duct cells from pig fetal tissue during the mid-gestational period (E110). Pig duct cells evidenced a higher rate of transformation into insulin cells than was seen in mice under the same culture conditions. In addition we detected a significant increase in the transformation of duct cells into insulin-secreting cells, as well as self-renewal, in the presence of nicotinamide. Taken together, porcine fetal pancreas may be considered a useful source of insulin-secreting beta cells for use in cell replacement therapy for the treatment of diabetes.

Effects of Asiasarum Heterotropoide on LPS-induced NO Production by BV2 Microglial Cells
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Excessive proinflammatory cytokine and NO production by activated microglia may be a possible etiologic factor of neurodegenerative disorders. The current study examined the effect of Asiasarum Heterotropoide on nitric oxide (NO) induction by lipopolysaccharides (LPS) activated BV2 murine microglial cell line. The results showed that Asiasarum Heterotropoide suppressed LPS-induced NO release, inducible nitric oxide synthase (iNOS) expression in BV2 cells. In addition, Asiasarum Heterotropoide prominently diminished LPS-induced production of proinflammatory cytokines such as TNF-α. MTT assay revealed that Asiasarum Heterotropoide exerted no significant cytotoxicity in the BV2 cells. Through methods such as NO assay, ELISA, and western blotting it proved to inhibit such factors that are mentioned above and was shown to suppress those factors in a dose-dependent manner. Conclusions: It is very possible that Asiasarum Heterotropoide can offer a valuable mode of therapy for the treatment of brain inflammatory and various neurodegenerative diseases including ischemic cerebral disease. Acknowledgement: This study is supported by Brain Korea 21 project 2006.
translation of mRNAs. This study examines whether miR-206, previously shown to be elevated in ERα-negative BrCa, regulates the expression of ERα. RNAhybrid and miRanda software revealed two putative miR-206 binding sites, one conserved and one non-conserved, within the 3’-untranslated region (3’-UTR) of the human ERα mRNA sequence. Reporter constructs were made with 70 basepair fragments, spanning each of the two ERα miR-206 binding sites, and inserted into the 3’- UTR of the luciferase gene. The conserved and non-conserved miR-206 sites conferred 38% and 55% repression in luciferase activity, respectively. Both miR-206 binding sites were validated as bona fide sites, using hsa-miR-206, 2’-O-methyl antagoniR-206, and reporter constructs with 5’ and 3’ mutations in each binding site. A rare C/T single nucleotide polymorphism exists in the conserved site, which increased repression of luciferase activity to ~70%. Transfection of MCF-7 breast cancer cells with 100nM miR-206 decreased endogenous ERα mRNA levels as much as two fold, as measured by real-time RT-PCR and Northern blot hybridization. The efficiency of ERα mRNA degradation due to miR-206 treatment was greater in rat GH3/B6 cells due to the T residue of the SNP, rebuilding the conserved site. Interestingly, MCF-7 cells treated with estrogen (E2) decreased the endogenous pool of miR-206, while IC182,780 treatments had an inverse effect of the miR-206 population. These results show that miR-206 can target and regulate ERα expression, but conversely, be regulated by steroid hormones such as estrogen. These findings identify a new level of posttranslational regulation of ERα which may aide in the etiology of ER+ and ER-brest tumors.

L138 Expression and Analysis of RhoA and Plk1 during the Mammalian Cell Cycle
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Both RhoA (Rhо1) and Plk1 are implicated in the regulation of cytokinesis, a cellular process that marks the division of the cytoplasm of a parent cell into daughter cells after nuclear division. Cytokinesis failure is often accompanied by the generation of cells with unstable tetraploidy contents, which predisposes the cell to chromosomal instability and oncogenic transformation. RhoA belongs to a family of small GTPases that oscillate between the inactive GDP-bound form and the active GTP-bound form. Several studies using low eukaryotic systems demonstrate that RhoA plays a critical role in mitotic exit. Plk1, or its orthologue Cdc5 in budding yeast, is essential for mitotic progression and mitotic exit. A recent study shows that Cdc5 regulates the activation of Rhо1 at the division site during late mitosis, thus promoting cytokinesis in the budding yeast. To study whether there exists a physical and functional interaction between RhoA and Plk1, we have analyzed subcellular localization of RhoA and Plk1 in HeLa cells. Plk1 localizes to kinetochores as well as spindle poles during prophase and metaphase; it translocates to the midbody during telophase. RhoA is also enriched at the midbody region during telophase. Recombinant RhoA, expressed as a GFP fusion protein, is enriched in the nucleus of HeLa cells. Ectopically expressed GFP-RhoA does not cause significant cell death, though a fraction of cells appears to exhibit a delay in mitotic exit or impaired cytokinesis. On the other hand, extended inhibition of RhoA through treatment with C3-exoenzyme adversely affects cell proliferation. Co-immunoprecipitation reveals an extended inhibition of RhoA through treatment with C3-exoenzyme and that RhoA and Plk1 physically interact and that their interaction adversely affects cell proliferation. Western blot analysis determined increased expression of Bcl-2 protein in HePα,β integrin expressing cells in comparison to HeP2 cells. In addition, we found increased level of glutathione (GSH) in HeP2α,β integrin expressing cells as compared to HeP2 cells. However, there was no significant difference in the extent of plating between HeP2 cells and HeP2α,β integrin expressing cells. Pretreatment of HeP2α,β integrin expressing cells, with specific inhibitor of glutathione synthesis, buthionine sulfoximine (BSO), decreased level of GSH and abrogated integrin-mediated cisplatin-resistance to the level observed for HeP2 cells. However, the BSO treatment did not influence the expression of Bcl-2. To determine the involvement of Bcl-2 in resistance we established several clones from HeP2 cells with decreased expression of Bcl-2. Increased expression of Bcl-2 did not confer cisplatin resistance nor increase increased level of GSH. Therefore, our results suggest that α,β integrin mediated cisplatin resistance in HeP2 cells is dependent on increased GSH level, which contributes to cell survival by mechanisms independent of cisplatin inactivation or inhibition of DNA adduct formation. Since the upregulation of α,β integrin has been found in cisplatin-resistant cells, obtained by repeated cisplatin treatment of HeP2 cells, this phenomenon may be one of the mechanisms of resistance development.

L140 Phorbol-12-myristate acetate [PMA] Mediated Regulation of Spermidine/Spermine N2-acytetyltransferase Expression in H-ras Transformed Fibroblasts
E. J. Francis, R. A. R. Hirtta; Biology, University of Prince Edward Island, Charlottetown, PE, Canada
Altered cellular growth and regulation are important in the progression of tumors. Cell growth regulation by tumor promoters can be very complex. This present study demonstrates a novel link between phorbol ester tumor promoter mediated alterations in the expression of SSAT, a key rate limiting enzyme in the polyamine degradation pathway, and H-ras mediated cellular transformation. Treatment of parental, H-ras transformed, benign tumor forming cells with PMA resulted in a dose and time dependent induction of SSAT expression. SSAT expression increased dramatically in NR3 cells exposed to PMA for 8 hours, whereas SSAT expression was apparently unaffected in parental nontransformed 10T1/2 cells. Studies investigating the possible mechanisms whereby PMA mediated alterations in SSAT expression occurs in NR3 cells suggest a possible role for PKC, MAP kinase, and P-I-3 kinase mediated events playing regulatory roles. Altered expression and regulation of SSAT by tumor promoters represents an aspect of the altered cellular growth program inherent to H-ras transformed cells. [ N.S.E.R.C / Canadian Cancer Society [ P.E.I. Division ] funded ]

L141 Overexpressed Cyclophilin A in Cancer Cells Renders Resistance to Hypoxia- and Cisplatin-induced Cell Death
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Cyclophilin A (CypA) has been reported to be overexpressed in cancer cells, especially in solid tumors. In order to determine the role of CypA in tumorigenesis, we investigated the induction of CypA as well as the role it plays in cancer cells. Here, we have demonstrated that induction of CypA is associated with hypoxia in a variety of cells including DU145 human prostate cancer cell line. Our analysis of the CypA promoter clearly showed that CypA upregulation is mediated by HIF-1α transcription factor. Interestingly, overexpression of CypA prevented hypoxia-induced apoptosis, as well as cisplatin-induced cell death, by suppression of Reactive Oxygen Species (ROS) generation and depolarization of mitochondrial membrane potential, whereas siRNA-based CypA knockdown aggravated these factors. These results suggest that CypA is important in tumorigenesis, especially in tumor apoptosis.

L142 Scintillation Proximity Assay (SPA) to Detect DNA Binding of P53 and the P53 Protein from Human Cell Extracts
deterioration. A hallmark of the disease is the presence of insoluble Lafora bodies (LBs) that result from abnormally branched glycogen. LD results from loss of function mutations in either of the genes that encode the E3 ubiquitin ligase laforin or the dual specific phosphatase laforin. Laforin is composed of a carbohydrate-binding module followed by a dual specificity phosphatase domain. It is reported as only being conserved among vertebrates; however, we have identified and biochemically characterized laforin orthologs in five unicellular eukaryotes. These organisms all synthesize an insoluble carbohydrate called floridean starch, and, surprisingly, the biochemical composition of floridean starch closely resembles that of LBs. Furthermore, members of Kingdom Plantae store complex carbohydrates as starch, a mixture of amylose and amylopectin. The composition of mylopectin closely resembles both LBs and floridean starch. Although Kingdom Plantae lacks a laforin ortholog, a protein called SEX4 was recently described that contains similar domains as laforin. Loss of function mutations in SEX4 in Arabidopsis results in a starch excess phenotype that is very reminiscent of LBs in LD patients. We biochemically characterized SEX4 and found that it shares multiple characteristics with laforin that no other phosphatase possesses. Therefore, we entertained the notion that laforin and SEX4 could be functional orthologs and carry out similar reactions in Kingdom Animalia and Plantae, respectively. To test this hypothesis, we stably expressed laforin in SEX4-deficient plants and laforin fully complemented the SEX phenotype. Thus, laforin and SEX4 are functional orthologs that perform an essential and unstudied aspect of carbohydrate metabolism. We will present mechanistic data that generate a unifying theme of how this previously overlooked aspect of carbohydrate metabolism is shared amongst branches of Kingdom Animalia and Kingdom Plantae.

L143
Ionizing Radiation Induces Changes Associated with Epithelial-mesenchymal Transdifferentiation and Increased Cell Motility of A549 Lung Epithelial Cells
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Radiotherapy is a major therapeutic option for patients with advanced lung cancer. Nevertheless, the effects of irradiation on malignant biological behaviors (e.g., migration and transcription of cancer cells) have yet to be clarified. We conducted an in vitro study to investigate the radiation-induced alterations including morphology, adhesion, and cell motility of A549 human lung cancer cells. These changes, which are associated with epithelial-mesenchymal transdifferentiation (EMT), seem to be linked to radiation-induced fibrosis, which represents one of the most common long-term adverse effects of curative radiotherapy. In addition, loss of intercellular adhesion and increased cell motility may be involved in post-radiotherapy-associated metastasis. We showed that stress fibers and focal adhesions are increased and that cell-cell junctions are decreased in response to ionizing radiation. Radiation also significantly increased cell motility. The p38-specific inhibitor, SB203580, reduced the radiation-promoted migration of A549 cells, whereas SP600125, a JNK MAPK-specific inhibitor, inhibited both inherent and radiation-mediated cell motility. Consistent with this observation, radiation up-regulated the phosphorylation of p38 MAPK. Current approaches to cancer treatment involving more intensive radiotherapy regimens have been suggested to be associated with a higher incidence of local or distant metastasis. Therefore, a subset of patients may benefit from combination of radiotherapy with inhibitors of EMT or cell migration.

METABOLIC DISEASES

L144
An Unexpected Link between Neuronal Degeneration in Lafora Disease Patients and Starch Metabolism in Unicellular Eukaryotes and Kingdom Plantae
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Lafora disease (LD) is a fatal progressive myoclonus epilepsy, characterized by neuronal degeneration and progressive neurological deterioration. A hallmark of the disease is the presence of insoluble polyglucosan inclusion bodies called Lafora bodies (LBs) that result from abnormally branched glycogen. LD results from loss of function mutations in either of the genes that encode the E3 ubiquitin ligase malin or the dual specific phosphatase laforin. Laforin is composed of a carbohydrate-binding module followed by a dual specificity phosphatase domain. It is reported as only being conserved among vertebrates; however, we have identified and biochemically characterized laforin orthologs in five unicellular eukaryotes. These organisms all synthesize an insoluble carbohydrate called floridean starch, and, surprisingly, the biochemical composition of floridean starch closely resembles that of LBs. Furthermore, members of Kingdom Plantae store complex carbohydrates as starch, a mixture of amylose and amylopectin. The composition of mylopectin closely resembles both LBs and floridean starch. Although Kingdom Plantae lacks a laforin ortholog, a protein called SEX4 was recently described that contains similar domains as laforin. Loss of function mutations in SEX4 in Arabidopsis results in a starch excess phenotype that is very reminiscent of LBs in LD patients. We biochemically characterized SEX4 and found that it shares multiple characteristics with laforin that no other phosphatase possesses. Therefore, we entertained the notion that laforin and SEX4 could be functional orthologs and carry out similar reactions in Kingdom Animalia and Plantae, respectively. To test this hypothesis, we stably expressed laforin in SEX4-deficient plants and laforin fully complemented the SEX phenotype. Thus, laforin and SEX4 are functional orthologs that perform an essential and unstudied aspect of carbohydrate metabolism. We will present mechanistic data that generate a unifying theme of how this previously overlooked aspect of carbohydrate metabolism is shared amongst branches of Kingdom Animalia and Kingdom Plantae.

L145
WITHDRAWN

L146
Cytohesin Inhibition by SecinH3 Leads to Hepatic Insulin Resistance
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Cytohesins are a highly conserved family of guanine nucleotide exchange factors (GEFs) which, by catalyzing the GDP to GTP exchange, activate ADP-ribosylation factors (ARFs), a class of Ras-like G-proteins. It has been shown that cytohesins regulate such diverse cellular functions as cytoskeleton organization, integrin activation and signaling. Unfortunately, cytohesins are insensitive to the only known small-molecule inhibitor of GEFs, Brefeldin A (BFA). However, inhibitors for this class of GEFs would be valuable for further elucidating the roles of these proteins and their effectors in disease-related G protein-controlled signaling networks and for eventual therapeutic intervention. We have developed an aptamer displacement screen based on fluorescence polarization to find specific antagonists for cytohesins and applied it to a library of 5.000 drug-like small molecules. SecinH3, the most potent inhibitor out of several hits, bound to the catalytic sec7-domain of cytohesins with a Kd of about 200 nM and inhibited in vitro the GDP to GTP exchange on ARF1 with an IC50 of about 5 µM. Application of SecinH3 in the human liver cell line HepG2 revealed that cytohesin inhibition resulted in the suppression of insulin signaling as shown by reduced insulin-stimulated phosphorylation of protein kinase B / Akt and of FoxO transcription factors and by impaired insulin-dependent regulation of gene expression. Mice treated with SecinH3 showed increased hepatic expression of gluconeogenic genes, reduced expression of glycolytic, fatty acid and ketone body metabolism genes, reduced liver glycogen levels and an increase in plasma insulin, which all are characteristic features of hepatic insulin resistance. As insulin resistance is a hallmark of developing type 2 diabetes and present before the disease is clinically recognized our chemical genetics approach might have the potential of helping understand the complex molecular pathogenesis of this disease.

L147
Reduction of Placental Thioredoxin Expression in Diabetic Pregnancies
Gestational diabetes mellitus (GDM) is defined as glucose intolerance with its first onset recognized during pregnancy. Patients from GDM are subjected to increase oxidative stress and reduced activity of the placental antioxidant enzymes, all of which contribute to the increase in tissues damages and is associated with adverse perinatal outcome. Thioredoxin (Trx) is a small multifunctional protein ubiquitously expressed in many different tissues. In normal human placenta, it is localized in the trophoblastic region and plays a protective role against oxidative stress in the placenta. Here, a prospective study was carried out in which high-risk women with singleton pregnancies were recruited from the clinic for placental studies. Following delivery, placental specimens were collected and placental protein expression of Trx and TrxR was measured by western blot analysis. A significant reduction of placental Trx and TrxR protein expression was found in diabetic pregnancies in comparison to normal pregnancies. In addition, the reduction of the expression on the two proteins was negatively correlated with the severity of glucose intolerance. In line with the placental Trx protein expression level, we also demonstrated a decrease in placental Trx mRNA expression level in GDM patients, suggesting a possible transcription level down-regulatory mechanism of Trx in GDM patients. Furthermore, using immunohistochemistry techniques, we showed that this reduction of Trx expression was mainly localized in the stromal region of the placental villi. Taking together, we have demonstrated a reduction in placental Trx expression in GDM patients, and further investigation is required to elucidate the role of Trx in GDM placenta.

**L148**

**Trans Fatty Acids Induce Pro-inflammatory Responses and Endothelial Dysfunction**

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Diets high in *trans* fatty acids (TFAs) have been associated with an increased risk of developing cardiovascular disease. Increased intake of TFA causes elevated ratios of LDL/HDL cholesterol, and leads to atherosclerosis. Furthermore, epidemiological data indicate that there is a strong correlation between intake of C18:2 TFA and sudden cardiac death. There is apparently very little known about the mechanisms by which TFAs exert harmful effects on cardiovascular system. Our in vitro study is the first to demonstrate the effects of incorporated C18:2 TFA on human arterial endothelial cell (HAEC) function. Flow cytometry analysis indicated that HAECs treated with C18:2 TFA significantly increased the expression of endothelial adhesion molecules, including intercellular adhesion molecule-1 (CD54) and vitronectin receptor (CD51/CD61). TFA incorporation increased HAECs adhesion to fibronectin-coated plates by approximately 40%. Neutrophil adhesion to HAECs monolayers was nearly proportional to CD54 expression, which confirms the physiological relevance of elevated expression of CD54 on HAECs. TFA treatment also induced the release of Monocyte Chemoattractant Protein-1 (MCP-1) by two fold in unstimulated HAECs. Furthermore, we examined the role of TFA on HAECs angiogenesis, a process that involves cell migration and differentiation. Chemotactic migration of TFA-treated HAECs toward sphingosine-1-phosphate (SPP) was significantly increased over 50% compared to controls. Conversely, capillary morphogenesis of TFA treated HAECs was significantly inhibited in response to VEGF, suggesting that 18:2 TFA suppresses endothelial cell differentiation. In conclusion, these in vitro studies demonstrated that TFAs play a role in the induction of pro-inflammatory responses and endothelial dysfunction. These effects of TFAs on HAECs may explain the role of TFAs in the development of atherosclerosis.

**BIOINFORMATICS/BIOLOGICAL COMPUTING**

**L149**

**Toward Automated TEM Screening and Analysis of Chromatographic Fractions of Virus Particles**

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We have developed a prototype automated system for analyzing size exclusion chromatography (SEC) fractions using transmission electron microscopy (TEM). The system has been developed for assessing the purification of virus particles such as cowpea mosaic virus (CPMV) that are used as platforms for a variety of nanoparticle applications. UV irradiation of these particles can be used to produce non-infectious yet functional particles. SEC is used to purify the irradiated particles by separating RNA filled vs. empty capsids and broken or aggregated particles. 20 to 100 chromatographic fractions are taken from each sample and negative-stain TEM is used to assess the particles in each fraction. Although the tasks of imaging and qualitative classification of the various particles are relatively straightforward, it is a labor intensive task for such large scale screening. We will present the details of a prototype application for automatically imaging and analyzing these SEC fractions using automated data collection and a robotic grid loader coupled with automated analysis. Our preliminary analysis of the images showed that the automated particle selection and classification agreed well with the manual classification on well-defined virus particles. Disagreement between the two methods was more apparent for the broken fragments. Nevertheless, the ratios of various types of particles obtained from chromatographic fractions displayed similar trends in different samples. For example, our analysis showed that the ratio of RNA-filled particles was higher in the second of the two main chromatogram peaks. In addition, the UV absorption values, being a measurement of total protein or RNA concentration, did not always correspond to the particle concentration found on the EM grids.

**L150**

**A Systematic Search Strategy for the Study of Stem Cell Differentiation**

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Stem cell differentiation and fate control are complex events likely to be regulated by many factors including (1) internal factors such as those related to cell cycle progress, transcription factors, and chromatin modifiers; (2) external factors such as growth factors, extracellular matrix (ECM) proteins and (3) cell-cell connection and communication. Only when these factors come together properly, cells will be instructed to produce a specific read-out. To effectively determine the optimal combinations for desired cell activity, one possibility is large scale, high-throughput screening. However this approach is often limited by the available resources. Here we apply engineering control principles to address this question. The essence of our strategy is to use feedback control: based on the information obtained from the systems, through a stochastic search algorithm, iteratively determines the inputs to achieve a desired phenotype. This method will dramatically reduce the amount of tests required to reach the optimal point, thus enable us to manipulate many variables at the same time. In previous work, we have successfully demonstrated this method in controlling NF-kB expression in 293T cell (Wong 2005). For the current research, we manipulated Brychaury knock-in GFP mES cell line (Fehling et al., 2003) to determine the optimal conditions for meseoderm differentiation. A battery of six factors, each at ten different concentration levels, is tested under the guidance of a search algorithm. Out of 1 million possible combinations, this method takes about 20 tests to reach an optimal condition. This strategy is very generic and can be applied to many different systems.

**L151**

**Protein Interaction Prediction Using the Structural Domain Information**

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**Background:** Understanding of biological processes necessitates knowing not only which proteins exist in a certain organism or cell type but also how these proteins interact with each other. The determination of the protein-protein interaction (PPI) networks is a daunting task and it has been the subject of extensive research. Despite the development of reasonably successful methods, serious technical difficulties still exist and is evident from the small overlap between the high-throughput experimental approaches. **Results:** Here we present DomainGA, a
quantitative computational approach that uses the information about the
domain-domain interactions to predict the interaction between proteins.
We show the robustness and insensitivity of the DomainGA method to
the selection of the parameter sets, score ranges, and detection rules
using the Yeast PPI data. Our DomainGA method achieves very high
explanation ratios for the positive and negative PPIs in yeast. Based on
our cross-verification tests on Human PPI, comparison of the optimized
scores with the structurally observed domain interactions obtained from
the iPFAM database, and sensitivity & specificity analysis; we
conclude that our DomainGA method shows great promise to be
applicable across multiple organisms. Obtained explanation ratios
during the reported test case studies clearly show that the false
prediction rates of the obtained templates would be reasonable low,
which can be lowered even further with additional secondary tests.
Conclusions: We envision the DomainGA as a first step of a multiple
tier approach to constructing organism specific PPIs. As it is based on
the fundamental structural information, DomainGA approach can be
used to create the potential PPIs and the accuracy of the constructed
interaction template can be further improved using complementary
methods such as literature search or other prediction methods.
MONDAY, DECEMBER 11

SIGNAL TRANSDUCTION

L1 Lipopolysaccharide Induces Hypoxia Inducible Factor-1 Alpha mRNA and Hypoxic Gene Expressions via NADPH Oxidase, PI 3-kinase, Protein Kinase C Pathways in Microglia

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Microglia are activated during cerebral ischemia/hypoxia and inflammation. Although these conditions are known to stimulate hypoxia-inducible factor-1 (HIF-1), the key player of hypoxia-induced gene expression, little is known about the microglial HIF-1 activation. In this study, we thus examined effects of lipopolysaccharide (LPS) on HIF-1α and hypoxic gene induction in BV-2 microglial cells. LPS increased expression of hypoxia responsive genes such as vascular endothelial growth factor (VEGF), glucose transporter-1 (Glut-1), and nitric oxide synthase-2 (NOS-2). LPS induced HIF-1α mRNA and protein expression as well as HIF-1-dependent luciferase reporter activity. LPS-induced expressions of hypoxia proteins were blocked by pharmacological inhibitors of HIF-1α YC-1, Topotecan, and by small interfering RNA (siRNA) of HIF-1α. We then found that an antioxidant NAC or inhibitions of NADPH oxidase by pharmacological inhibitors DPI, apocynin, or siRNA of gp91phox, a subunit of NADPH oxidase, blocked LPS-induced HIF-1α mRNA and VEGF expression. Inhibition of PI 3-kinase and protein kinase C by pharmacological inhibitors or siRNAs also blocked LPS-stimulated HIF-1α mRNA and VEGF induction. Consistently with BV-2 cell lines, in rat primary microglial cells, LPS increased HIF-1α and VEGF mRNA via NADPH oxidase/ROS-dependent pathway. Taken together, the results suggest that LPS induces HIF-1 activation and hypoxic gene expression via NADPH oxidase, ROS, PI 3-kinase, and PKC dependent pathways in microglial cells.

L2 Regulation of Dual Specificity Phosphatase hYVH1 (dusp12) by Phosphorylation

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Human YVH1 (dusp12) is a dual specificity protein phosphatase able to dephosphorylate phospho-serine/threonine and tyrosine residues. The dephosphorylation mechanism for dual specificity phosphatases proceeds through the dual-phosphate enzyme intermediate. Several lines of evidence suggest a role for YVH1 in cellular growth and proliferation, however the specific function and regulation remains elusive. In order to address the mechanism of hYVH1 regulation, we utilized an in vivo labelling approach along with phosphoamino acid and two-dimensional phosphopeptide mapping analysis to demonstrate that hYVH1 is phosphorylated on multiple serine residues. The combination of immobilized metal affinity chromatography and MALDI/TOF mass spectrometry has allowed us to identify novel phosphorylation sites within the N-terminal and C-terminal regions of hYVH1. In order to further characterize these phosphorylation sites, a site-directed mutagenesis approach was utilized to generate point mutants that mimic either unphosphorylated (mutation to Ala) or constitutively phosphorylated amino acid residues (mutation to Gln) within hYVH1. These phosphorylation events may potentially act as regulators of hYVH1 activity and studies are ongoing to further elucidate their functional significance.

L3 Inhibition of Cdc28 Activity after G1 DNA Damage in Budding Yeast

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In budding yeast, G1 DNA damage induces a checkpoint arrest that slows progression through G1 and delays both onset of budding and initiation of DNA replication. Cdc28, a cyclin-dependent kinase (Cdk) and homolog of mammalian CDK2 and CDK1, is the master regulator of the yeast cell cycle. By forming complexes with Clns, paralogs of the D- and E-type cyclins, and a later, G1–type cyclin, Clns, which correspond to the A and B-type cyclins, Cdc28 promotes cell cycle progression. The Cic/Kip family Cdk inhibitor Sic1 binds Cdc28-Cib to sequester kinase activity. Sic1 does not inhibit Cdc28-Cln, as shown by biochemical assays and molecular modeling, but can be phosphorylated by Cdc28-Cln and destabilized, releasing Cdc28-Cib to promote replication and mitosis. We have shown that Sic1 is transiently stabilized during G1 DNA damage checkpoint arrest, blocking S phase onset. In turn, cells lacking Sic1 display attenuated G1 delay. DNA damage exposes a role for the redundant cyclin-dependent kinase Pho85, a homolog of CDK5, in G1 progression. Pho85 inhibition prolongs Sic1 stabilization and G1 delay, suggesting the hypothesis that G1 DNA damage targets Cdc28-Cln to slow budding and block Sic1 degradation. Consistent with this model, Cdc28-Cln specific activity, as detected by in vitro assays with a truncated Sic1 substrate, is delayed in G1 cells after DNA damage. The slow accumulation of Cln2 protein in G1 after DNA damage may underlie the lower Cdc28-Cln activity. Our data are consistent with a pathway directly linking DNA damage signaling to Cln expression, as proposed by Breeden and colleagues.

L4 Involvement of CIP4 in Epidermal Growth Factor Signaling and Cytoskeletal Regulation

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Epidermal Growth Factor (EGF) treatment of A431 Epidermoid carcinoma cells promotes Epidermal Growth Factor Receptor (EGFR) activation, cell proliferation, cytoskeletal reorganization and cell migration. EGF-induced activation of Cdc42 and its effecter N-WASP cause reorganization of the actin cytoskeleton to regulate endocytosis and migration of A431 cells. Cdc42-interacting protein-4 (CIP4) is an adaptor protein that interacts with both activated Cdc42 and WASP, and is thought to play a role in endocytosis and cell migration. Here, we describe that CIP4 is localized to F-actin-rich projections of A431 cells. Also, CIP4 is phosphorylated by Src downstream of EGFR, and associates with N-WASP and dynamin. Interestingly, CIP4 knock-down by RNA interference causes EGFR upregulation, and increased activation of Erk Mitogen-Activated Protein Kinase. Taken together, these results suggest that CIP4 is a substrate of Src downstream of EGFR, and that CIP4 regulates cell growth signals in A431 cells treated with EGF.

L5 Interaction between Heterotrimeric Gialpha Proteins and Their Regulator, RGS14 in the Mammalian Centrosomes

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Heterotrimeric G proteins are signal transducers acting downstream of seven transmembrane receptors. However, recent studies in C. elegans and Drosophila have revealed another essential function of these proteins in regulation of positioning and pulling force of the mitotic spindle during asymmetrical cell division. We previously reported surprising localization of a member of regulators of G protein signaling (RGS) family, RGS14 in the centrosomes of mammalian cells. In this study, we report localization of Gialpha1, Gialpha2, and Gialpha3 of heterotrimeric G protein in the mammalian centrosomes and at the midbody. Using acceptor photobleaching fluorescence energy transfer (FRET) analysis, we demonstrated that RGS14 binds to Gialpha1 and Gialpha1 interact in the centrosomes of HeLa cells expressing Gialpha-yellow fluorescent protein (YFP) and RGS14-cyan fluorescent protein (CFP). No positive FRET signals are detected when RGS14-CFP deletion mutant lacking the RGS and GoLoco domains is co-expressed in the centrosomes with Gialpha1-YFP. To further understand the mechanism of interaction between RGS14 and Gialpha in the centrosomes, various mutants of Gialpha1-YFP and RGS14-CFP were tested by acceptor photobleaching. Our results demonstrate for the first time the interaction between a Gialpha and a RGS protein in the centrosomes of mammalian cells and open a new chapter for further studying the role of heterotrimeric G and RGS proteins in centrosome biology.
L6 APC Is a Chaperone of Phosphorylated β-catenin
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The beta-catenin transcription factor is inactivated in unstimulated cells by ubiquitin-proteolysis pathway. A critical step in this regulatory pathway is phosphorylation of beta-catenin. Here we show that the NH2-terminal phosphorylated serine/threonine residues of beta-catenin are instantaneously protected by the adenomatus polyposis coli (APC) tumor suppressor protein. Importantly, this protection is essential for targeting beta-catenin to its downstream ubiquitination cascade. In vivo, only APC-protected phospho-beta-catenin is recruited to the ubiquitin-proteasome pathway and degraded. In contrast, without such protection, as occurring in APC mutant cancer cells, phosphorylated beta-catenin is rapidly dephosphorylated by protein phosphatase 2A. The dephosphorylation prevents beta-catenin from entering into the downstream ubiquitination cascade, resulting in its stabilization and rapid accumulation in the absence of Wnt stimulation. Thus, by acting as a chaperone of phosphorylated beta-catenin, APC provides a novel regulatory mechanism that ensures beta-catenin culminated in its degradation pathway. Similar mechanism likely exists in other phosphorylation-regulated cellular events.

L7 Genetic Analysis of the Cyc8-Tup1-Sko1 Corepressor Complex in Response to Osmostress in Yeast
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In the yeast Saccharomycyes cerevisiae, Tup1, in association with Cyc8 (Ssn6), functions as a general repressor of transcription. Tup1 and Cyc8 are required for repression of diverse families of genes. Here we present evidence that Tup1 and Cyc8 are controlled by osmotic stress and other stress signals through the Cdc25c checkpoint. Under conditions of decreased osmosis, Tup1 is prevented from interacting with Sko1, while it is recruited to the Sko1 complex. This repression is mediated by recruitment of the Cyc8-Tup1 complex to target promoters by sequence-specific DNA-binding proteins. Of these, Sko1 is a repressor that mediates HOG pathway-dependent regulation by binding to cyclic AMP response elements (CRE) in target promoters. However, detailed molecular mechanisms underlying how cyc8 and sko1 are regulated by osmotic stress are poorly understood. Recently we showed that Hog1, the yeast orthologue of p38 (MAPK), and calcineurin perform antagonistic roles in an early step of bud formation. We took a genetic approach to identify the components of the signaling pathways by screening for mutations that suppress calcium sensitivity of hog1Δ strain and the mutants were classified to 6 complementation groups (designated as sgh1 to 6). Our genetic screening identified the mutant alleles of CYC8, TUP1 and SKO1 genes, whose products form a general transcriptional repression complex, as the suppressors of calcium/osmotic stress also sensitivity of hog1Δ strain. We found that the mutant protein Tup1 (G356S) and Cyc8 (389 STOP) were unable to repress the expression of GRE2, which is regulated by Sko1 upon osmotic stress. However, the expression of the glucose repressed regulation or florescence phenotype was remained unchanged. Our data suggested that Tup1 (G356S) and Cyc8 (389 STOP) are specifically defective in the repression of Sko1 dependent gene expression.

L8 The Role of Signaling from cAMP to MAPK in the Regulation of Bone Marrow Hematopoietic Stem Cells Growth by α-Fetoprotein
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We have previously identified that α-fetoprotein (AFP) display direct growth regulatory activity on hematopoietic stem cells (HSCs) in vivo and in vitro. However, molecular mechanism of AFP growth regulatory signal transmission in HSCs is not investigated. We examined the possible molecular mechanism of AFP regulating activity regarding growth of mice bone marrow HSCs. We found that AFP action on three different HSCs subpopulations led to intensive cAMP synthesis. Downstream, cAMP directly phosphorylated of PKA as well as indirectly phosphorylated Rap1 via Epac1 and PKA activation. In turn, activated PKA and Rap1 were absolutely required for activation B-Raf but not Ras, Src or PKC. In addition, activated Rap1 had not effects neither Ras nor Raf-1. Treatment of all HSCs subpopulation with selective B-Raf inhibitor prevented as well as Raf/MAPK2 activation as well ERK1/2 phosphorylation. In order to downstream targets of ERK1/2 in investigated HSCs subpopulations were different. In CD34+CD133+ HSC, activated ERK1/2 directly regulated activity of MNK1/2 and p70S6 kinase in the cytosol and also translocated into the nucleus where it phosphorylated MSK1/2 and a number of transcription factors such as STAT3, Erg-1, SRC, C-Jun and Sap1. Upon phosphorylation, ERK1/2 directly phosphorylated of RSK1, MNK1/2 and p70S6 kinase in the cytosol and STAT3-, Erg-1-, SRC-, C-Jun-, C-Fos- and Elk-1 transcription factors in CD34+CD135+ HSC nucleus. In contrast of both this HSCs types, direct targets of ERK were only STAT3-, SRC-, C-Jun-, C-Fos- and Sap1 transcription factors in CD34+CD117+ HSC nucleolus. Nevertheless, usages of B-Raf or MEK1/2 inhibitors led to significant reduction of proliferative-, protein-synthetic- and metabolic potential of all AFP stimulated HSCs types. On the other hand, usage of adenylylcylose inhibitor brought to significant suppression as MAPK activity as well as presence cell activities. Therefore, B-Raf/ERK1/2 pathway plays a crucial role in cAMP-mediated regulation of HSCs growth activity which initiated by AFP.

L9 p53 Independent Regulation of p21 Expression by Sphingosine Kinase 2
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The sphingolipid metabolite sphingosine-1-phosphate (S1P) is the ligand for a family of five specific G protein-coupled receptors (S1P1-5) that regulate a wide variety of important cellular functions, including growth, survival, cytoskeletal rearrangements, and cell motility. However, whether it also has an intracellular action is still a matter of debate. S1P is formed by the ATP-dependent phosphorylation of sphingosine catalyzed by types 1 and 2 sphingosine kinase (SphK1 and SphK2). In this study, we investigated the role of SphK2 in human breast carcinoma MCF7 cells in response to the DNA damaging agent doxorubicin. Overexpression of SphK2 increased basal expression of p21 in MCF7 cells in a p53-independent manner. Correspondingly, downregulation of endogenous SphK2 with siRNA decreased both basal and doxorubicin-induced expression of p21 without affecting p53. Downregulation of SphK2 resulted in decreased expression of genes encoding G2/M and S phase populations and an increased G0/G1 population upon induction by doxorubicin. In addition, decreasing SphK2 expression markedly enhanced apoptosis induced by doxorubicin. Our results suggest that endogenous SphK2 is important for p53-independent induction of p21 expression by doxorubicin and that it may influence the balance between cytostasis and apoptosis of human breast cancer cells.

CELL CYCLE CONTROLS

L10 NIRF Constitutes a Nodal Point in Cell-Cycle Regulation
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NIRF is a nuclear ubiquitin ligase capable of inducing cell-cycle arrest in the G1 phase. Through searching for NIRF-interacting factors, NIRF was found to form complexes with many cell-cycle regulators, suggesting that NIRF co-operates with these molecules. When we tested whether NIRF ubiquitinated these interacting molecules by overexpression, NIRF ubiquitinated them for destruction. Further experiments using RNAi, a series of dominant negative mutants, and in vitro ubiquitination assay confirmed that the NIRF ubiquitin ligase participates in ubiquitination-dependent degradation of the interacting molecules. In addition, NIRF forms complexes with a number of other regulatory factors controlling a broad range of cell functions, and altered expression of NIRF variously affected the amount of those regulatory factors. Thus, NIRF appears to be the master component of cell-cycle machinery. In conclusion, NIRF directs progression of the cell-cycle via ubiquitination, and occupies a central position within the information processing network in the cells.

L11 Regulation of Cdc25c by Erk-MAP Kinases during the G2/M Transition
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Cancer Center, Houston, TX, 2Program in Genes and Development, The University of Texas Graduate School of Biomedical Sciences, Houston, TX, 2Department of Biochemistry and Molecular Genetics, The University of Virginia Medical School, Charlottesville, VA, 3Department of Systems Biology, Harvard Medical School, Boston, MA

One of the rate-limiting steps of G2/M phase transition in eukaryotic cell cycle is the activation of Cdc25, a protein phosphatase regulating the activation of Cdc2/cdc2-cyclin B. Cdc25 is activated by phosphorylation and this process involves two steps, initiation and amplification. Accumulating evidence indicates that Cdc2 kinase and polo-like kinase 1 (Pik1) are involved in the amplification step of Cdc25 activation. However, other kinase(s) either that involve in the amplification step or that activate Cdc25 at the initiation step remain to be identified. Through sequential chromatographies, we identified p42 MAPK, the Xenopus ortholog of mammalian Erk2, as one of the major Cdc25 phosphorylating activities in M phase-arrested Xenopus egg extracts. We demonstrated that p42 MAPK interacts with hypophosphorylated Cdc25 before mitotic induction and that p42 MAPK phosphorylates Cdc25 at T48, T138 and S205, increasing Cdc25’s phosphatase activity, which in turn promotes mitotic induction. In a mammalian cell line, we further showed that Erk1/2 interacts with Cdc25C in interphase cells and phosphorylates Cdc25C at T48 in mitotic cells and T45 inhibition of Erk activation partially inhibits the T48 phosphorylation, activation of Cdc25C and mitotic induction. These findings demonstrate that Erk MAP kinases are directly involved in activating Cdc25 during G2/M phase transition in both the xenopus oocytes and mammalian cells.

L12 Molecular Characterization of Centrosome Cohesion
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The centrosome acts as the microtubule organizing center of an animal cell contributing to cellular architecture and polarity, and participating in cell adhesion, migration and division. During G1 phase of the cell cycle, it is composed of two centrioles, the mother and daughter, connected by a collar defined structurally by the intercentriolar linkage. This linkage is maintained through S and G2 as centrosomes undergo duplication, but is then disassembled before mitotic entry to allow separation of duplicated centrosomes to opposite poles of the emerging spindle. Recent studies have identified two potential components of the intercentriolar linkage: C-Nap1 and rootletin (Mayor et al. 2000 JCB 151, 837-846; Bahe et al. 2005 JCB 171, 27-33; Yang et al. 2006 MCB 17, 1033-1040). These proteins are conserved in sequence and localize to centrosomes during interphase but are absent from mitotic spindle poles. Importantly, both C-Nap1 and rootletin can be phosphorylated by the Nek2 protein kinase that has previously been implicated in regulating centrosome cohesion at the G2/M transition (Faragher and Fry 2003 MCB 14, 2876-2889). Our current goal is to extend the molecular understanding of centrosome cohesion and its regulation through further studies on the role of C-Nap1 and/or rootletin in the assembly of the linker. RNAi depletion studies are also being undertaken to examine the importance of these linker proteins for microtubule organization and cell cycle progression.

L13 Inhibition of iPLA2 Induces Phosphorylation of p53 by Activating ATR Kinase
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Group VIA Ca2+-independent phospholipase A2 (iPLA2) plays a key role in regulation of G1-phase phospholipid turnover. We previously reported that inhibition of iPLA2 arrests cells in G1 by activating the p53-p21 checkpoint pathway. Here we further investigate the mechanisms by which the perturbation of phospholipid homeostasis activates p53. We found that inhibition of iPLA2 by the specific inhibitor bromoeno lactone (BEL) induces the rapid phosphorylation of p53 at serine-15 in the human colon cancer cell line HCT116 and in rat insulinoma INS-1 cells. This phosphorylation was caffeine sensitive, suggesting the involvement of the ataxia telangiectasia-mutated (ATM) kinase/ataxia telangiectasia and Rad-3-related (ATR) kinase. Since it is thought that genotoxic stress activates ATM/ATR, we investigated whether BEL causes DNA damage. We were unable to detect either double-stranded or single-stranded DNA breaks by Western blots, immunofluorescence microscopy and TUNNEL staining following iPLA2 inhibition. We next used ATM-deficient cells (GM01526) and U2OS-derived osteosarcoma cells with doxycycline-inducible expression of a kinase-dead ATR to further differentiate the roles of ATM and ATR in p53 activation. We found that BEL treatment induced similar levels of phosphorylation of p53 at serine-15 in both ATM+ and ATM− cells as caffeine-sensitive ATR inactivation. In contrast, BEL-induced phosphorylation of p53 at serine-15 in U2OS cells was enhanced by expression of ATR and inhibited by the expression of ATR-kinase dead mutants. Our data demonstrate that iPLA2 inhibition induces rapid phosphorylation of p53 at serine-15 by ATR but not ATM in a DNA damage-independent manner. We propose that cells monitor membrane phospholipid homeostasis and respond to the disruption of phospholipid turnover by activating the ATR-p53-p21 pathway.

L14 Genetic Suppression of the Rapamycin Hypersensitivity of Acl6b Mutant Cells Suggests Cooperation between Clb5-Cdc28, Phosphatase 2A, and Kinesin Family Motor Proteins in Mitotic Spindle Assembly and Nuclear Positioning in Budding Yeast
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The drug rapamycin is currently under investigation as an anti-cancer agent because of its potent inhibition of the Target of Rapamycin (TOR) kinase, a central controller of eukaryotic cell growth. Budding yeast (Saccharomyces cerevisiae) that lack the S-phase cyclin gene CLB5 (acl6b) exhibit rapamycin hypersensitivity (rap+). Despite the functional redundancy of yeast cyclins, this phenotype is not shared by any other cyclin deletion mutant. We sought to elucidate the functional basis for acl6b rap+ by screening for genes that could suppress this phenotype when overexpressed. We identified PHH22, one of two yeast genes that encode the catalytic subunit of phosphatase 2A (PP2A), as a strong rap+ suppressor and KIP3 and KAR3, which encode kinesin related microtubule motor proteins involved in mitotic spindle assembly and nuclear positioning, as weaker suppressors. Wildtype yeast exposed to a low dose of rapamycin exhibit microtubule defects that lead to the accumulation of binucleate cells; our preliminary microscopy studies indicate that these defects are more severe in rapamycin-treated acl6b cells. Together with our genetic results, these data suggest functional cooperation between the Clb5-Cdc28 cyclin-dependent kinase, PP2A, and kinesin family motor proteins in mitotic spindle assembly and nuclear positioning during mitosis. Increased levels of Rpap+ specifically implicate Clb5-Cdc28-mediated phosphorylation in the mitotic function of these motor proteins in vivo. Increased levels of Kip3 or Kar3 may enable their phosphorylation by Clb2-Cdc28 in vivo, thus suppressing acl6b rap+.

L15 Regulation of hWW45 Protein Stability by MST1 In Vivo
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Establishing and maintaining homeostasis can be determined by the balance of cell proliferation and death. Two genes that are involved in regulation of growth, proliferation, and apoptosis in Droso phila are hippo (Hpo), encoding a serine.threonine kinase, and Salvador (Sav), encoding a coiled coil domain. Human counterparts of these genes, MST1 and hWW45 respectively, have been found to be frequently down-regulated or deleted in cancers, but their molecular implications in tumorigenesis are still obscure. In the present study, we demonstrate...
that hWW45 interacts with MST1 (mammalian sterile20-like kinase), and that MST1 is able to phosphorylate hWW45 C-terminal region including SARAH domain. In addition to binding and phosphorylation, we show that the stability of hWW45 is increased by co-expression MST1, and that the level of endogenous hWW45 protein is also significantly decreased in MST1-depleted cell. However, unlike the case of fruit fly, the MST1 dependent phosphorylation of hWW45 doesn’t have any effect on its stability. Based on our observations, we propose that the stability of hWW45 protein might be regulated by MST1 in vivo.

L16 Identification and Sequence of Checkpoint Kinase 1 (SpChk1) from Purple Sea Urchins, Stronglylocentrotus purpuratus
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Exposure to ultraviolet radiation (UVR) causes Developmental delays, abnormalities and decreased survivorship in the sea urchin Stronglylocentrotus purpuratus. Mammalian cells maintain internal checkpoints that arrest the cell cycle at both the G1/S and G2/M transitions if mutations are detected. Although these checkpoints have not previously been characterized in sea urchins, studies by our laboratory demonstrate a Cdc2-dependent developmental delay in embryos upon exposure to UVR. Our main objective has been to identify effects of UVR on these proteins that control the cell cycle. An important first step to reaching this goal is to determine which components of mammalian cell cycle machinery are present in sea urchin eggs and embryos. We are studying members of the protein signal transduction pathway that regulate the cell cycle via Cdc2, most specifically, Checkpoint Kinase 1 (Chk1). It is predicted that UVR will activate sea urchin Chk1, as in other model organisms, through upstream regulators such as ATM/ATR. This increased Chk1 activity would result in the inhibition of Cdc25, a phosphatase responsible for driving the G2/M transition via activation of Cdc2. Recently, the S. purpuratus genome was sequenced and is being annotated through the Baylor College Human Genome Sequencing Center. Based upon the characterization of Chk1 orthologs in humans and several other organisms, a predicted sea urchin Chk1 sequence was identified. This sequence was used to design primers that successfully amplified Chk1 cDNA derived from sea urchin egg mRNAs. Overlapping sequences were obtained from several cloned isolates and the resulting consensus was found to contain an additional exon that had not been included in the original predicted sequence. A synthetic peptide was created based upon this sequence and is currently being used to raise urchin-specific anti-Chk1 antibodies for further analysis of the role that UVR plays in Chk1 activation.

L17 A Dual Role of Clusterin in Astrocytic Cell Growth: Proliferation and Growth Arrest
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Clusterin, a secretory glycoprotein, has been shown to be up-regulated in the reactive astocytes in response to brain injury and neurodegenerative diseases, but its function has not been clearly elucidated. Here, we report a dual role of clusterin on primary astrocytic cell growth. Clusterin induced both proliferation and growth arrest in constant astrocytes, depending on the concentration. Treatment with low doses of clusterin (1-10ng/ml) resulted in an increase in cell proliferation, whereas cells underwent growth arrest at high concentrations of clusterin (1-100ng/ml). The proliferation by clusterin was mediated through the activation of ERK, and it was abrogated by addition of the either neutralizing antibody (1g8) against clusterin or specific inhibitor of ERK (PD98059). On the other hand, clusterin at high doses caused induction of p53 protein to restrain cell growth, in spite of the augmentation in the activation of ERK. Cells lacking functional p53 were not arrested but enhanced in proliferation continuously by clusterin even at higher doses. Thus, secretory clusterin plays an important role in controlling cell proliferation and mechanisms of the biphasic effect were linked with ERK signaling and p53 induction. [This study was supported by a grant of the Korea Research Foundation (E000361)].

L18 Knock-down of Human DNA Glycosylase MutY Homologue (hMYH) Decreases the Phosphorylation of Chk1 Induced by Hydroxyurea and UV Light Treatment in Hek293 Cells
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Human DNA glycosylase MutY homologue (hMYH) plays a major role in the DNA repair process known as base excise repair, which is necessary for the repair of DNA damage caused by reactive oxygen species. hMYH is responsible for removing misincorporated opposite DNA strands containing guanine or 7,8-dihydro-8-oxoguanine thereby preventing G:C to T:A mutations. Human Rad9, Rad1 and Hus1 are members of the Rad family that are required for both DNA replication and DNA damage checkpoints. Rad9/Rad1/Hus1 (9-1-1) complex has been known to function as sensor in the DNA integrity checkpoint control and to play a role in DNA repair by interacting with BER pathway related proteins. Also, it has a critical role as a mediator of checkpoint kinase 1 (Chk1) phosphorylation by ATR in response to DNA damage and replication disruption. In this work, we investigated the interaction and functional analysis of hMYH and human 9-1-1 complex. The physical interaction of hMYH and 9-1-1 complex was confirmed by immunoprecipitation assay using transiently transfected Hek293 cell lysates. In immunoprecipitation assay using N-terminal or C-terminal deleted hMYH, hRad9 and hRad1 interacted with N-terminal region of hMYH and hHus1 interacted with an internal region of hMYH containing catalytic domain. To investigate the effect of hMYH on Chk1 phosphorylation mediated by 9-1-1 complex, hMYH was knock-downed by RNA interference technique using siRNA. The phosphorylation of Chk1 induced by DNA damage such as hydroxyurea and UV light was decreased in hMYH knock-downed Hek293 cells. The degradation of Cdc25A, which has been known to be degraded by Chk1 phosphorylation, was reduced in hMYH knock-downed Hek293 cells. The phosphorylation of Chk2 was
also enhanced in hydroxyurea or UV light treated cells. However, it was not affected by hMYH knock-down. These data strongly suggest that hMYH may function as a mediator of Chk1 phosphorylation with 9-1-1 complex.

L21

The Acetyltransferase Activity of San Stabilizes the Centromeric Cohesion in HeLa Cells

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Genetic instability as the result of chromosome missegregation during cell division has been found in cancers and various genetic diseases. Faithful chromosome segregation is facilitated by sister chromatid cohesion tethering sister chromatids together. Sister chromatid cohesion is established immediately after DNA replication and maintained until anaphase. The mechanisms that regulate the dynamics of sister chromatid cohesion remain elusive in higher eukaryotic cells. San is a putative acetyltransferase important for sister chromatid cohesion in Drosophila. However, its budding yeast homologue is completely dispensable. In this report, loss-of-function studies show that the human orthologue of San is also critical for sister chromatid cohesion, suggesting that this regulation may be conserved in mammals. Furthermore, although a small fraction of San interacts with the NatA complex, San appears to mediate sister chromatid cohesion independently. San exhibits acetyltransferase activity in vitro and the rescue experiment shows that the activity is required for sister chromatid cohesion in vivo. Depletion of Polo-like kinase 1 rescues the cohesion defects along the chromosome arms but not at the centromeres in the San-depleted cells. This result indicates that San is necessary for the centromeric sister chromatid cohesion in HeLa cells.

MITOSIS AND MEIOSIS

L22

Structural/Ligand and Cell-based High-throughput Screens for Identification of Allosteric PLK1 Inhibitors

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The activity and cellular concentrations of polo-like kinase 1 (PLK1) are crucial for the ordered progression of cell division. PLK1 overexpression can cause transformation of NIH3T3 cells as well as induce tumours in nude mice. High PLK1 overexpression in clinical tumours correlates with poor patient survival. Together this suggests that tumour cells may depend on elevated PLK1 levels for their growth and survival. Furthermore, although a small fraction of San interacts with the NatA complex, San appears to mediate sister chromatid cohesion independently. San exhibits acetyltransferase activity in vitro and the rescue experiment shows that the activity is required for sister chromatid cohesion in vivo. Depletion of Polo-like kinase 1 rescues the cohesion defects along the chromosome arms but not at the centromeres in the San-depleted cells. This result indicates that San is necessary for the centromeric sister chromatid cohesion in HeLa cells.

L23

The siRNA-directed Depletion of the Nuclear Protein RED Can Overcome the Mitotic Arrest Induced by the Spindle Damage

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By yeast two-hybrid screening, we identified that RED, a nuclear protein of 557 amino acids, interacted with the human spindle checkpoint Mad1. The protein levels of RED were found to be the same in S and M phase cells. Fluorescence microscopic analysis revealed that RED was not a kinetochore-associated protein. Also, RED was within the nucleus and did not associate with hMad1 in the nuclear envelope in interphase whereas RED and hMad1 colocalized to the centrosomes in both metaphase and anaphase. Ectopically overexpressed RED proteins may form granules and lose its ability to properly localize to the centrosomes. Overexpression of RED did not affect the localization of hMad1 to kinetochores. We also applied RNAi technique to explore the function of RED in mitosis. The siRNA-directed depletion of RED resulted in a change in mitosis progression. Furthermore, misaligned chromosomes may be observed in RED-depleted metaphase cells. Nevertheless, flow cytometric analyses showed that the histogram profiles of the number of cells at different stages of cell cycle were similar between control and RED-depleted cells. We also analysed chromosomal and spindle abnormalities. Altogether 12 lead compounds that yielded high mitotic indices accompanied by cell death were identiﬁed. Some compounds induced severe structural spindle defects and hampered normal chromosome movements. The best lead compound appears to have cancer cell speciﬁc effects. The leads are currently under further characterization that aims to determine their target signalling cascade and mechanisms, in vitro assay and in vivo assays. Furthermore, in vitro assays are being performed to test the effects of the lead compounds on PLK1 substrate binding and on the activities of key mitotic kinases.
L25 Both Actin and Myosin Inhibitors Block or Slow Chromosome Motion in Mitotic PtK-1 Cells: Does an Actomyosin System Control Tension and Compression in the Mitotic Spindle? J. A. Snyder, C. Olsofsk; Biological Sciences, University of Denver, Denver, CO

We have used the myosin inhibitor 2, 3-butanediol (BDM) to test its ability to block or slow chromosome motion in PtK-1 cells during mitosis. Following cells with light microscopy, 80% of cells treated with 8 to 16 mM BDM in prometaphase or metaphase failed to enter anaphase within 40 min, compared with the normal 25-15 min, respectively. Chromosomes also failed to congress to the equatorial region of the cell and were dislocated to the periphery of the spindle. Using antibodies to tubulin, myosin, actin, and a DNA stain, we demonstrate dislocation of chromosomes to the periphery of the spindle, chromosome detachment, and the spaying of interpolar microtubules from the spindle domain. Have we previously shown that both actin and myosin were co-localized in the mitotic PtK-1 spindle (Robinson and Snyder, (2005). Protoplasma: 225: 113-122). Actin poisons, particularly cytochalasin D, slow or blocked prometaphase chromosome congression, detached several to many chromosomes from the spindle, and changed microtubule architecture in the spindle. Microtubules lost compression and spayed from the spindle domain and ultrastructural analysis revealed loss of 1-2 kinetochore laminae. (Wrench and Snyder. Cell Motil. Cytoskel. 36:112-124). These results suggest that both myosin and actin proteins interact with both interpolar and kinetochore fibers, and tension, created along kinetochore fibers, may be responsible for the shortening of the spindle during prometaphase. Immunocytochemical analysis shows that BDM has the same effect on the progression of mitosis, and changes in spindle architecture, as do actin inhibitors. We conclude from this study that an actomyosin system is present in the spindle and may act to create tension and compression in the spindle and, through checkpoints, assure fidelity in chromosome segregation during mitosis. (Supported by the Welller Endowment to JAS.)

CYTOKINESIS

L26 Characterization of the Interaction Domain between Mammalian SEPT2 and SEPT6 M. Kim, C. Froese; Hospital for Sick Children, Toronto, ON, Canada

Septins are filamentous GTPases that have a conserved role in cytokinesis. There are at least 13 mammalian septins which can be grouped into 4 categories. In Hela tissue culture cells, Septin 2 (SEPT2) is found in an immunoprecipitated complex with septins 6/7/9. Understanding how septins associate into large heterotetrameric complexes and how these multimeric complexes are able to form filaments remain largely unknown. Using the SEPT2/6/7/9 complex as a model for studying septin association, we used the LexA yeast hybrid system to map septin-septin interactions. SEPT6 and SEPT7 formed the core of the complex, with SEPT2 and SEPT9 interacting with both SEPT6 and 7 but not one another. Fine mapping analysis of the SEPT2/6 interaction using deletion constructs of SEPT2 determined a small 6kDa region that was necessary and sufficient for interaction with SEPT6. Nine charged residues and five hydrophobic residues were individually mutated to either lysine or aspartic acid, and the interaction was abrogated with 4 mutated hydrophobic residues (F273/L276/L280/L287). In particular, the L276R mutant is not able to bind to any other septin except for SEPT2. Generally, this region is predicted to be alpha-helix using secondary prediction software and the hydrophobic residues important for interaction with SEPT6 lie along one side of the helix. Consistent with the yeast two-hybrid data, GFP-SEPT6 readily immunoprecipitates FLAG-SEPT2, but fails to immunoprecipitate FLAG-SEPT2(L276R). In addition, coexpressing SEPT2/6 in Hela cells form short exogenous filaments, whereas such filaments are not present when either overexpressing SEPT2 or coexpressing SEPT2(L276R)/SEPT6. We are currently characterizing the SEPT2 mutant to determine whether it is bound to nucleotide and whether the FPLC elution profile is similar to wide type bacterially-expressed SEPT2. We have also constructed Tet-inducible, siRNA-resistant Hela cell lines of GFP-SEPT2 and GFP-SEPT2(L276R) to determine if SEPT2/SEPT6 interactions are important for cytokinesis.

L27 The Mammalian Septin Sept2 Is Required for Scaffolding Non-muscle Myosin II and Citron-kinase during Cytokinesis E. Joo, M. C. Surka, W. S. Trimble; 1Hospital for Sick Children, Toronto, ON, Canada, 2The Scripps Research Institute, La Jolla, CA

During the cell cycle, division of the cytokplasmic material is achieved in a process termed cytokinesis, and inhibition of this process is linked to genomic instability and tumorigenesis. Animal cytokinesis begins during anaphase with the accumulation of cleavage furrow proteins such as actin, non-muscle myosin II, molecules involved in the rho signaling pathway, septins, anillin and other molecules, to the division site. The cleavage furrow ingresses by the contractile force produced by non-muscle myosin II on the actin ring. Ingession of the cleavage furrow leads to the formation of a compact and stable structure called the midbody, which is required for the final abscission. In order to understand how midbody stability is achieved, we have investigated protein complexes associated with this structure. We found that a mammalian septin Sept2 binds directly to non-muscle myosin IIA and that this association is important for the full activation of the non-muscle myosin II, which is needed for stabilization of the midbody. Moreover, we found that inhibition of the septin-myosin II interaction results in regression of the cleavage furrow and dissociation of citron-kinase and possibly rho-kinase from non-muscle myosin II. We propose that septins function as a scaffold, bringing together non-muscle myosin II and citron-kinase and possibly rho-kinase to ensure the complete activation of non-muscle myosin II required for the final stages of cytokinesis.

L28 Probing the Spatial Regulation of Cytokinesis by Micromanipulation of Cellular Geometry and Spindle Bipolarity D. F. Rodriguez, C. B. Shuster; 1Molecular Biology, New Mexico State University, Las Cruces, NM, 2Biology, New Mexico State University, Las Cruces, NM

Cleavage plane determination involves an interaction between microtubules of the mitotic apparatus and the actomyosin cortical cytokleton. Two models have been proposed to explain the role of microtubules during cytokinesis: a polar relaxation model, which proposes an inhibitory signal from the two spindle poles restricts the furrow to the region where microtubule density is lowest; and a second equatorial stimulation model proposing that at the midpoint between two spindle poles, a region of microtubule overlap delivers a stimulatory signal to the cortex. A recent report suggested that cleavage furrows could be induced by monopolar spindles in cultured mammalian cells, raising the possibility that microtubule formation can occur without pole formation. We have investigated cytokinesis in the absence of spindle bipolarity. In an effort to further understand the role of spindle bipolarity in cleavage plane determination in large embryonic cells, we first generated monopolar spindles in sea urchin eggs by inhibiting the microtubule motor Eg5. Eggs treated in this manner formed monopolar spindles, and following anaphase onset, underwent random cell surface contractions resembling unorganized furrows. To confine the area of cell surface influenced by the monopolar spindle, eggs were manipulated into capillary pipettes. Cells manipulated in this manner underwent pseudofurrow formation in the polar regions not influenced by the monopolar spindle, whereas a furrow formed adjacent to (but not over) the monaster. In a second set of experiments, we eliminated the central portion of the mitotic spindle using dominant-negative (T24N) RanGTPase, which inhibited polarized microtubule nucleation while retaining astral microtubules. Preliminary studies indicate that while T24N Ran-injected cells failed to form a central spindle, they were capable of anaphase-B like pole separation and cytokinesis. Together, these experiments implicate both stimulatory and inhibitory roles for microtubules in cleavage plane determination, and provide evidence against specific populations of microtubules directing furrow formation.

L29 Evaluating the Role of the Chromosomal Passenger Complex in Directing the Final Events of Cell Division in Sea Urchin Embryos H. Argiros, J. Sterling, C. B. Shuster; 1New Mexico State University, Las Cruces, NM, 2Molecular Biology and Biology, New Mexico State University, Las Cruces, NM

The spatial regulation of cytokinesis has been a topic of active investigation for over a century, and classic micromanipulation...
experiments demonstrated that overlapping arrays of astral microtubules were sufficient for furrow induction in eukaryotic eggs. Other model systems have recently implicated the chromosomal passenger complex (CPC) in the assembly of the spindle midzone following anaphase onset. This complex, which includes, INCENP, survivin and aurora B kinase, is ultimately responsible for the local activation of Rho GTPase and assembly of the contractile ring. In contrast to somatic cells, post-anaphase sea urchin eggs contain only a small bundle of central spindle microtubules, as well as overlapping microtubules that accompany the ingressing furrow. Additionally, MKLP-1 a kinesin-like protein and central spindle marker, can be found along the cell equator following anaphase onset, but is not enriched along astral microtubules. Because experiments performed by Rappaport and others have demonstrated that the cleavage plane may be established in the absence of a central spindle, our efforts are focused on evaluating the CPC during cytokinesis in the early sea urchin embryos. To ask whether aurora B was required for cytokinesis, sea urchin eggs were treated with the aurora B inhibitors, Hesperadin and ZM447439, resulting in dose-dependent defects in cytokinesis. Inhibition of Aurora B did not, however, affect the activation of myosin II at the metaphase-anaphase transition, nor did it appear to affect astral microtubule elongation. However, comparison of ZM447439-treated cells with cytochalasin-treated cells revealed that in the absence of Aurora B activity there was a general defect in microtubule overlap at the cell equator. Ongoing efforts are focused on determining whether Aurora B is required for the local activation of RhoA, as well as disrupting the CPC with dominant-negative mutants of sea urchin INCENP and Survivin.

L30 Structural Characterization of Eps8 Proteins
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Eps8, is the founding member of a recently identified family of actin binding proteins involved in signaling to actin remodelling. Eps8 possesses two distinct activities on actin dynamics: firstly, it acts as a cross-linking protein that organizes actin into highly dynamic and arch-like, intracellularly diverse subcellular scaffolds, thus orchestrating a variety of fundamental mechanical functions in motile cells. Additionally, Eps8 acts as a barbed ends capping proteins, blocking actin filaments elongation and thus regulating actin-based motility. The holo Eps8 full-length protein is autoinhibited as a capper, whereas it displays constitutively bundling activity. The structural and molecular mechanisms underlining these activities are unknown. Here, we show that the isolated C-terminal capping region of Eps8 (residues 648-821) contains a putative SAM-PNT domain, whose structure has been solved and shown to be composed of 5 amphipatic helices (H1 to H5). NMR analysis revealed that a complete change of folding occurs upon actin binding, suggesting that extensive surfaces if interaction. Consistently, Eps8 648-821 forms a high affinity (50 nM) and stable complex with monomeric actin. Chemical cross-linking and sedimentation assays revealed the existence of two high affinities binding surface (kd=50 nM and 2 μM, respectively) corresponding to the H1-H2 pair and H5 helices. Single point mutations disrupting the hydrophobic faces of these amphipatic helices significantly reduced capping activity, suggesting that multiple surfaces of interaction are required for barbed end capping. A model of how Eps8 caps actin filament will be discussed.

ACTIN-ASSOCIATED PROTEINS

L31 Receptor Mediated Endocytosis Involves Tyrosine Phosphorylation of Cortactin
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Efficient internalization of ligand occupied receptors requires actin polymerization mediated by Arp2/3 complex and cortactin, a protein that binds to dynamin and serves as a prominent substrate of protein tyrosine kinase Src. However, the physiological role of tyrosine phosphorylation of cortactin is unknown. Inhibition of cortactin tyrosine phosphorylation by PP2A, a Src selective inhibitor, or overexpression of a cortactin mutant deficient in tyrosine phosphorylation resulted in decrease in receptor mediated endocytosis of transferrin. Likewise, the phosphorylation null mutant failed to restore the formation of clathrin coated vesicles in a cell extract with depletion of cortactin. Suppression of cortactin expression by RNA interference significantly impaired transferrin uptake, which was effectively rescued by overexpressing wild type cortactin but not the cortactin mutant deficient in tyrosine phosphorylation or a mutant with deletion of the SH3 domain. In vitro analysis of binding of cortactin to dynamin 2 demonstrated that Src mediated phosphorylation enhanced the interaction between cortactin SH3 domain and dynamin proline rich domain by approximately 6 folds. In contrast, Src had little effect on the interaction between phosphorylation null cortactin mutant and dynamin 2 either in vitro or within cells. Therefore, our data evidence that clathrin mediated endocytosis is subjected to a regulation via Src mediated cortactin phosphorylation.

L32 CAP2, Cyclase Associated Protein 2, Is a Dual Compartment Protein
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CAP2 are evolutionary conserved proteins that have roles in regulating the actin cytoskeleton and in signal transduction. Whereas most organisms have a single CAP gene, mammals have two CAP genes that code for related proteins, CAP1 and CAP2. We have generated specific antibodies for both proteins and studied their distribution and subcellular localisation. CAP1 shows a broad tissue distribution, whereas CAP2 is significantly expressed only in brain, heart and skeletal muscle, and in skin. CAP2 is found in the nucleus in undifferentiated myoblasts and at the M-line of differentiated myotubes. In PAM212, a mouse keratinocyte cell line, CAP2 is enriched in the nucleus, the cytosol was only weakly stained. CAP1 in contrast localises to stress fibers and F-actin rich regions such as lamellipodia in PAM212 cells. In human skin CAP2 is present in all living layers of the epidermis where it localises to the nuclei and to cell-cell junctions.

L33 Exploring Drug Stabilized F-actin as a Live Cell Model for Hirano Body Formation
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The number of people suffering from neurodegenerative diseases is substantial and growing. Neurodegenerative diseases are characterized by the formation of abnormal protein aggregates, and one of the least studied of these are Hirano bodies. The structures are large aggregates of F-actin and other proteins that affect the cytoskeletal structure and function of cells. Using actin stabilizing drugs we are able to observe the formation of F-actin aggregates in Dictostelium discoideum. Here we report titration experiments which establish the dosage of jasplakinolide necessary to initiate aggregates. The treated cells expressed a GFP probe which localizes to F-actin filaments (Pang, 1999). This allows us to observe the formation of aggregates in living cells. A 3gM concentration of drug induced large aggregates in all cells. We have examined the ultrastructure of drug initiated actin inclusions and compared them to traditional Hirano Bodies and to aggregates formed by expression of an aberrant actin binding protein (Maselli et al.2002). Although the actin aggregates are morphologically similar by fluorescence microscopy, in the EM there are striking differences. Jasplakinolide induced aggregates appeared to be amorphous clouds of filaments, while Hirano Bodies traditionally show a highly ordered structure (Maselli, 2002). Like the classic Hirano Bodies, the drug induced structures are not membrane bound and exclude other organelles. Experiments were carried out to evaluate the temporal stability of jasplakinolide induced aggregates. Surprisingly, a significant number of cells retain aggregates 24 hours after drug removal. We also see evidence that cells lose aggregates into the media. Further investigation of drug initiated aggregates will allow us to gain a

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better understanding of the kinetics of aggregate formation and disassembly using a live cell model system.

L34 Villin Interacts with F-actin in a Manner Distinct from Gelsolin
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The polar actin bundles of the microvilli are tightly cross-linked by two proteins, villin and fimbrin. Villin is an F-actin nucleating, cross-linking, severs, and capping protein within the gelsolin superfamily. Villin is unique in this group by its ability to cross-link filaments, which is due to an additional small headpiece domain. Villin shares high sequence homology to gelsolin, which has no known cross-linking ability. In order to define villin’s cross-linking structure we have used electron tomography of 2-D rafts of F-actin cross-linked with villin on a lipid monolayer to generate 3-D volumes of F-actin: villin cross-links. These rafts are ≥ 1 µm across and consist of polar arrays of F-actin spaced ~126 Å apart with villin cross-links occurring approximately once per actin crossover. More than 6,000 pairs F-actin crossover repeats with villin protein bound between them were selected as single particles, aligned, and classified by correspondence analysis to produce class averages. The majority of villin repeat volumes fell into a single class while two minor classes showed villin truncation, while others showed minor differences. Docking of the homologous gelsolin domain structures plus the villin head piece structure into the average density reveals the invariant localization on actin’s N-terminus which is quite distinct from that of other actin-binding proteins, such as cofilin, profilin, DNase I, gelsolin domain G1 and G2. This is the first glimpse of the entire structure of villin in a cross-linking role. Up until now it has been assumed that villin interacts with F-actin in a similar fashion to its close homolog, gelsolin. This study shows this assumption to be wrong and instead lends concrete evidence to the notion that there can be different modes of interaction with actin among highly homologous actin-binding proteins.

L35 WASP Suppresses the Growth Defect of S. cerevisiae las17Δ in the Presence of WIP
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Wiskott-Aldrich Syndrome (WAS) is an X-linked disease caused by abnormal interactions of WASP with other proteins to form the WASP-Wiskott-Aldrich Syndrome Protein (WASP-WIP) and several of these mutations affect WASP’s interaction with WIP (WASP Interacting Protein) suggesting that loss of interaction between WASP and WIP is causal to the disease. However, the role of WIP in regulating WASP’s activity is still not clear. Las17p is the yeast homologue of human WASP and yeast cells deficient for Las17p are unable to grow at the restrictive temperature of 37°C. We have found that suppression of human WASP suppression of the growth defect of las17Δ strain by expressing human WIP in S. cerevisiae las17Δ strain, only in the presence of WIP. WIP mediates localization of WIP to cortical patches as well as to stabilize WASP in yeast cells. WASP’s ability to suppress the growth defect of las17Δ strain is dependent on the presence of a functional Arp2/3 activating domain at the C-terminus of WASP as well as the presence of Verprolin domain (V) at the N-terminus of WIP. We have used the S. cerevisiae Las17p to demonstrate that WASP and WIP form a complex, WIP in the complex is active and that the V domain of WIP is essential for the activity of WASP perhaps by providing an additional actin binding domain to the complex. Amongst the 53 mutations causing WAS, 40 are located in the WASP-WH1 domain. We analyzed five (E31K, L46P, G70W, T111P and A134T) of these mutations in the WH1 domain, implicated in causing classic Wiskott Aldrich Syndrome. When these mutations were engineered in WASP, WASP-WIP interaction was abolished and WASP’s ability to suppress the las17Δ strain was abrogated, probably due to inefficient localization of WIP to cortical patches and/or lack of stabilization of WIP. We would use this yeast model system for functional characterization of the remaining mutations causing WAS.

L36 Functional Analysis of Wiskott Aldrich Syndrome, Interacting Protein (WIP) and Its Homologues Using S. cerevisiae

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Wiskott Aldrich Syndrome (WAS) is an immunodeficiency disorder characterized by recurrent bacterial infection, thrombocytopenia and eczema. The WAS protein (WASP) is an adaptor protein involved in the regulation of actin cytoskeleton and it interacts with several proteins including WIP (WASP Interacting Protein), WH1/WIRE (WIP- and CR16-homologous protein / WIP-related) and CR16 (glucocorticoid-regulated gene). Vrp1 is a S. cerevisiae actin-associated protein related to WIP, WIRE and CR16. Vrp1-deficient (vrp1Δ) cells are inviable at high temperature and have partially depolarized cortical actin patches as well as defects in fluid phase endocytosis. In order to characterise the functional domains of WIP, WIRE and CR16 we have expressed these three proteins in vrp1Δ strains of S. cerevisiae using Vrp1 promoter. All three mammalian proteins suppressed both the growth defect and endocytosis defect of vrp1Δ strain. However none of the three mammalian proteins were able to rescue the actin patches polarization defect of the vrp1Δ strain. All three mammalian proteins are able to localise to cortical patches in a pattern similar to that of Vrp1p localization. This cortical patch localization does not require the WH2/V domain which mediates interaction with actin. Site directed mutagenesis on WH2/V domain, abrogated the ability of WIP and WASP to suppress the growth defect of the vrp1Δ strain while CR16 was still able to suppress the growth defect of vrp1Δ strain. We will further characterize the function of Human Verprolin family of proteins by examining the effects of the deletions of their known domains, on their ability to suppress the growth defects of vrp1Δ strain.

UNCONVENTIONAL MYOSIN

L37 Phenotypic Characterization of a Myosin 1a/Villin Null Mouse
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The enterocyte brush border (BB) has been a model system to study the actin cytoskeleton for over 30 years. Recently, mice mutant for BB proteins such as villin and myosin 1a (myo1a) have been described. Villin and myo1a, via calmodulin light chains, are the major calcium binding proteins within the BB. To test the hypothesis that myo1a and villin are essential for calcium homestatis within the enterocyte, a double mutant mouse was generated. The myo1a/villin double mutant mice (M-V KO) are viable and exhibit no growth defects or other overt phenotype when fed an ad libitum diet as compared to wild type and myo1a mutant mice. However, ultrastructural analysis of duodenal tissue reveals significant cellular and tissue perturbations. Myo1a/villin KO BBs do not exhibit uniform microvilli and there is microvillar blebbing, loss of microvilli, dilation of the mitochondria and luminal debris. There are a striking number of vacuoles just below the BB, which is exacerbated in fasting/refeeding experiments. However, no microvillar vesiculation reminiscent of the myo1a KO mouse was observed, presumably due to the loss of villin. Sections of the epithelial monolayer appear to have become detached from the basement membrane in the double mutant. The vacuolarization and detachment from the basement membrane may indicate an increase in the apoptotic index of the mucosa, a result consistent with TUNEL staining. In contrast to the myo1a KO, myosin 1e is not mislocalized from the BB in the M-V KO mouse, but rather retains robust BB localization. However, myosin 6 is mislocalized, as observed in the myo1a KO mouse. Taken together, these data indicate that the M-V KO mouse retains some of the phenotype of the myo1a single mutant, but also has several unique cellular perturbations.

KINESIN

L38 A Kinesin 4 Family Member Is Essential for Cytokinesis in the Early C. elegans Embryo
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Cytokinesis is the physical division of one cell into two daughter cells at the end of the cell cycle. The early *C. elegans* embryo is a powerful model system for identifying genes required for cytokinesis. Many genes required for cytokinesis in *C. elegans*, however, are also required for germ-line development and gonad function. Therefore disruption of these genes results in sterility- precluding analysis of any potential gene function in the early embryo. To overcome this pitfall, we have developed a forward genetics approach to isolate conditional alleles of genes involved both in gonad function as well as cell division in the early embryo. We first isolated mutants that could make viable offspring at the permissive temperature (15°C) but became sterile if shifted to the restrictive temperature (26°C) at the L4 larval stage (after the majority of gonad development has occurred). We then took advantage of the conditional nature of these mutations to determine which mutants are defective specifically in cytokinesis. To do this, we shifted adult worms from the permissive to the restrictive temperature after they began producing embryos, and then imaged the first two embryonic cell cycles using DIC microscopy. Using this approach, we have identified an allele of *klp-19* (or57ts) a member of the kinesin 4 family of microtubule motor proteins. Embryos produced at the restrictive temperature show late defects in the first cytokinesis resulting in bi-nucleate embryos. We are currently characterizing the cell biological nature of the cytokinesis failure in this conditional allele of *klp-19* (or57ts) mutant in vivo using GFP-tagged markers combined with immunofluorescence analysis of key mechanical, regulatory, and signaling molecules essential for cytokinesis.

**MICROTUBULE DYNAMICS AND ASSEMBLY**

**L39**

A Goi-kinesin Interaction Regulates Microtubule Positioning and Dynamics Essential for Nuclear Migration during Mating in Yeast

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The regulation of the actin and microtubule cytoskeletons by external stimuli is essential to many fundamental processes in eukaryotic cells, including chemotaxis, differentiation, morphogenesis, and secretion. The mating reaction of the budding yeast, *Saccharomyces cerevisiae*, is an excellent model with which to study signal-induced changes in cytoskeletal polarity and organelle function. As a yeast cell elongates toward its mating partner, astral microtubules connect the nucleus to the cell cortex at the growth tip. A Myo2-Kar9-Bim1 complex transports microtubule plus ends along polarized actin cables into the mating projection where polymerizing microtubules are anchored to the cortex by Bim1 and Kar9. The Kar3 kinesin-like motor protein is then thought to stimulate plus-end depolymerization of these microtubules, thus drawing the nucleus closer to the site where cell fusion and karyogamy will occur. It is not known how Kar3-tipped microtubule plus ends are recruited to the growth site and anchored at the cortex as they shrink, nor how the pheromone signal regulates Kar3 localization and function. Here we show that the pheromone-responsive Gs protein, Gpa1, positions the plus ends of astral microtubules at the cortex of the growth tip via direct interaction with Kar3, thereby affecting both microtubule orientation and dynamics. Our data reveal a novel mechanism for communicating external signals to the microtubule cytoskeleton. In addition to the expected influence of Gpa1 (Gα) on microtubule positioning due to its known involvement in klp-polarization of actin cables, Gpa1 directly affects microtubule dynamics by interacting with Kar3-tipped microtubule plus ends. Moreover, Gpa1 appears to be the hypothesized cortical anchor for Kar3. We infer that the signal-induced accumulation of Gpa1 at the growth tip during mating helps to regulate Kar3/microtubule dynamics in space and time. Gpa1 thus serves as an externally-regulated positional determinant, a previously unappreciated role for Gα proteins.

**L40**

Microtubule Organization: Targeting the Nucleator

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The gamma-tubulin ring complex (gammaTuRC) is a large multi-protein complex required for microtubule nucleation from the centrosome. Here we show that the GCP-WD protein (originally named NEDD1) is the ortholog of the *Drosophila* Dpp17WD protein, and is a subunit of the human gammaTuRC. GCP-WD has the properties of an attachment factor for the gammaTuRC: depletion or inhibition of GCP-WD results in loss of the gammaTuRC from the centrosome, abolishing centrosomal microtubule nucleation, although the gammaTuRC is intact and able to bind to microtubules. GCP-WD depletion also blocks mitotic chromatin-mediated microtubule nucleation, resulting in spindle assembly failure. Mitotic phosphorylation of GCP-WD is required for association of gamma-tubulin with the spindle, separately from association with the centrosome. Our results indicate that GCP-WD broadly mediates targeting of the gammaTuRC to sites of microtubule nucleation and to the mitotic spindle, which is essential for spindle formation.

**Monday**

**L41**

EGF Stimulates Reorganization of Microtubules

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Stimulation of cells with EGF results in formation of EGF receptor-containing peripheral endosomes, which tend with time to concentrate in juxtranuclear region localized near MT-organization center. This translocation was shown to be dependent on MT and dynein. It is generally considered that MT remains intact during this process. However, anti-tubulin staining has revealed significant remodeling of MT organization during EGF endocytosis in cells of epithelial origin, A431 and HeLa. During first 15 min after endocytosis stimulation tubulin cytoskeleton demonstrated typical radial organization. In 30-60 min MT retraction from cell periphery was seen with the intensity of tubulin fluorescence growing in the juxtanuclear region. Then, long individual MTs have disappeared and cytoplasm show diffuse staining in combination with a meshwork of short MT fragments. At this time EGF-containing endosomes were localized in juxtranuclear region. Later on, disappearance of EGFR staining due to EGFR lysosomal degradation went in parallel to reestablishment of radial MT system. The observed remodeling was much evident when the cells were allowed to prebind EGF on ice before stimulation of endocytosis by raising the temperature to 37 °C. Depolymerized at these conditions MTs were rapidly reestablished in 5 min upon warming the cells. In absence of EGF MTs have restored after cooling but did not demonstrate any further remodeling. Despite of MT organization specificity in A431 and HeLa both cell lines behaved similarly.

**L42**

Effect on Microtubules and Antitumor Activity by Newly Synthesized 1,8-Naphtyridine Derivatives

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Microtubules show highly dynamic instability and play an important role in mitosis. Inhibition of microtubule dynamics appears to be the mechanistic basis underling antitumor effects of most anticancer compounds. Vinca alkaloids and taxoids are well-know examples of antimitotic agents that are widely used clinically in cancer treatment. However, classical approaches involving the discovery of cytotoxic agents interfering with DNA have led to the identification of new promising anticancer agents. Quinolinones and their isosteric counterpart, naphthyridonones are known for their antimicrobial and antitumor activity. These molecules were also shown to inhibit GTP- binding polymerization resulting in the disruption or suppression of both microtubule structure and normal functions of cells with consequent arrest of mitosis. On the basis of these considerations a large series of substituted 1,8-naphthyridine derivatives were synthesized and tested for a potential anticancer screening assay. METHODS: Synthesis of 1,8-naphthyridine derivatives variously substituted. Identification growth assay: a blue color assay was performed to determine human lymphoblastoid CEM cell viability and cytotoxic effect of each compound (IC50 values ± SD). Apoptosis assays: DNA fragmentation was studied by propidium iodide staining and flow cytometric analysis, nuclear morphological modification by DNA-binding fluorescent dye Hoechst 33258. Microtubule polymerization was induced by taxol, in the presence of different 1,8-naphthyridine derivatives and analyzed by Western blotting and immunofluorescence. RESULTS AND CONCLUSIONS: Some compounds induced high cytotoxicity after 72h of exposure.
Several tested 1,8-naphthyridine derivatives elicited antiproliferative effects with IC50 values comparable with those of the reference drug Paclitaxel (21 ± 0.3 nM). Apoptosis evaluation indicated some compounds as marked programmed cell death inducers. Moreover, when correlation between cytotoxicity and inhibition of tubulin polymerization was evaluated, we found a strict structure-activity relationship. Three of the analyzed series of compounds showed a potent antimitotic and antitumor activity in the nanomolar range, indicating these as drugs suitable for further development.

L43 Automated Microtubule Tracking and Analysis
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Innumerable cell biology studies make extensive use of imaging technologies. Owing to the high variability of analytical needs we have to develop our efforts to advance our ability to utilize imaging in a rigorously quantitative manner, our recent work focuses on computer-assisted, image enhancement and automated tracking of individual microtubules in time lapse images, both in vitro and live cell imaging experimental paradigms. Our tracking methods can manage high levels of noise and variations in image quality with excellent efficiency. Further, our methods can track significantly more microtubules per video, with increased objectivity and accuracy when compared with previous studies. We have employed manual tracking methods. Given the increased analytical efficiency made possible by our methods, we can now more readily acquire sufficient data to employ statistical methods to assess and model microtubule behavior. Our methods automatically determine growth and shortening rates for each experimental condition, as well as the normalized transition frequencies between these events. Additionally, we now look beyond the traditional growth and shortening characteristics. For example, we define and quantify a new quantitative measure of microtubule curviness or bending and calculate this parameter from sample microtubule videos. This new statistical parameter may reveal novel biological phenomena and allow investigators to pose new questions. For example, a quantitative analysis of microtubule curvature may provide novel insights into cell shape changes or changes in the direction of cellular growth and migration. Funding for this work provided by NSF ITR grant # 0331697 and NIH RO1 grants NS35010 (SCF) and NS13570 (LW).

L44 Microtubule Catastrophe Induction at Focal Adhesion Sites
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Microtubules play an essential role in polarized cell motility. Directionally growing microtubules specifically target focal adhesions (Kaverina et al., 1998). At adhesions, microtubules can switch from growth to depolymerization (undergo catastrophe), decreasing local microtubule density. We aimed to identify if catastrophes at adhesions occur more frequently than elsewhere, suggestive of a local up-regulation of microtubule shortening. Live cell TIRF microscopy was used to record fluorescently tagged microtubules and adhesions at the ventral cell layer. The recordings were used to analyze microtubule dynamics. As catastrophes occur only in the context of growing microtubule tips, catastrophe numbers were normalized by the length of microtubule extension. We found that microtubule catastrophe frequency per μm of extension is 8 times higher at adhesions than elsewhere indicating that adhesions dramatically increase catastrophe probability. We have further questioned if the ability of adhesions to cause catastrophes depends on adhesion maturation stage. Early adhesions can be distinguished from mature ones since they contain Paxillin but not Zyxin. In addition, Paxillin extended more distantly than Zyxin within mature adhesion sites. We observed that up to 17% of adhesion-associated catastrophes at the leading edge occur at early focal contacts. Adhesions that catastrophes repetitively occur at distal (zyxin-free) ends of long adhesions. A critical question is whether catastrophes at adhesions are triggered by a specific biochemical mechanism or by the mechanically rigid adhesion structure. Our data show that microtubules can grow continuously and form a loop while their EB3-marked polymerizing tips are prevented from moving forward by an obstacle. Such loops are often observed at adhesions. Therefore, microtubule tips slow down at adhesions but resume the normal elongation rates after passing through the adhesion. Combined, these data suggest that microtubule catastrophes at adhesions do not require a mature adhesion status and are caused by a local regulatory event, not by a mechanical obstacle.

L45 Effects of Stathmin, a Microtubule Destabilizing Factor, on Intracellular Microtubule Dynamics and Taxol Resistance
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Stathmin is a major cellular phosphoprotein that appears to play an important role in the regulation of microtubule dynamics, by sequestering tubulin and increasing the catastrophe frequency at microtubule ends in purified microtubule systems in vitro. Stathmin is expressed at high levels in a wide variety of human cancers, and attempts to target the microtubule assembly/disassembly regulation mechanism. Live cell TIRF microscopy was used to record BT549 human breast cancer cells in which we have stably knocked down stathmin (KD) by shRNA or stably overexpressed stathmin (OE). Taxol sensitivity was increased in KD and decreased in OE. The intracellular function of stathmin is regulated by phosphorylation by many kinases, but the phosphorylation regulatory system has not been solved yet. Therefore, measurement of microtubule dynamic instability in interphase cells was carried out in KD cells +/- taxol. Without taxol, no difference was found in dynamics parameters between control and KD cells. At a low concentration of taxol (2 nM for 1d), growth and shortening rates were increased in both control and KD cell lines, but no difference between the cell lines. Microtubule transition frequency was suppressed, and dynamics (overall visually detectable growth and shortening) was enhanced by taxol in both cell lines, more dramatically in KD cells than in control cells. Microtubule transitions (catastrophe and rescue) are important for regulation of cell proliferation and migration. These results suggest that stathmin might be involved in microtubule transition regulation in cells. Moreover, the modification of microtubule transition frequencies may be related to stathmin-induced drug resistance. Supported by NIH CA57291.

CILIA AND FLAGELLA

L46 Functional Genomics in Trypanosoma brucei Identifies Evolutionarily-conserved Components of Motile Flagella
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family of novel TbCMF proteins that function to maintain connections between outer doublet microtubules, suggesting that they are the first identified components of nexin links. Overall, our results provide insights into the workings of the eukaryotic flagellum, identify several novel human disease gene candidates, reveal unique aspects of the trypanosome flagellum and underscore the value of \textit{T. brucei} as an experimental system for studying flagellar biology.

L47 Knockdown of IFT27 Lead to Severe Cell Cycle Defect in \textit{Chlamydomonas}

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In many cell types, the presence of cilia is correlated with the cell cycle: the cilium, which is present in stationary phase, disappears as the cell enters S phase and reappears following the completion of mitosis. The reassembly of a cilium requires the transport of ciliary precursors in the nascent cilium by a process known as intrflagellar transport (IFT). The IFT machinery consists of the microtubule motors kinesin-2 and cytoplasmic dynein 1b and IFT particles comprised of two components, A and B, containing at least 16 proteins. Mutations affecting these motors or IFT proteins generally result in defects in ciliary assembly, but not in cell division. However, in \textit{Chlamydomonas} the knockdown of IFT27, a small Rab-like G-protein that is part of the IFT complex B, causes defects in cell division. A severe knockdown of IFT27 is lethal to the cell. In those clones with a partial knockdown of IFT27, cells had difficulty forming a cleavage furrow and completing division, resulting in cells that grew very slowly, became large, often with multiple nuclei and sets of basal bodies. In addition, knockdown of IFT27 caused extensive flagellar defects. Only half of the cells grew flagella. Among the flagellated cells, 40% had short flagella of variable length, and many cells had only one flagellum, had unequal length flagella, or flagella were abnormally located. The reduced level of IFT27 in cells also correlated with a reduction of other IFT complex proteins expression, suggesting that IFT27 may be involved in regulating the level of other IFT proteins. (Supported by NIH 4GM 14642)

L48 HEF1-dependent Aurora A Activation Induces Ciliary Disassembly

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The mammalian cilium protrudes from the apical/luminal surface of polarized cells, and acts as a sensor of environmental cues, including physical stimuli such as directional fluid flow, and diffusible growth factors that bind to cilia-localized receptors. Despite mounting evidence that cilia are essential sites for coordination of cell signaling, almost nothing is known about the cellular mechanisms controlling their formation and disassembly. Here we define a novel signaling pathway in which interactions between the pro-metastatic scaffolding protein HEF1/Cas-L/NEEDD9 and the oncogenic Aurora A (AurA) kinase at the basal body of cilia causes phosphorylation and activation of HDAC6, a tubulin deacetylase, promoting disassembly of cilia. We show that this pathway is both necessary and sufficient for ciliary reabsorption. Moreover, we demonstrate that small molecule inhibitors of AurA and HDAC6 selectively stabilize cilia from normally regulated resorption, suggesting a novel mode of action for these clinical agents.

L49 Game-Specific Chemical Sensing in \textit{Chlamydomonas}

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The behavior in the green flagellate alga \textit{Chlamydomonas reinhardtii} is regulated by light and chemical stimuli. Phototaxis in \textit{C. reinhardtii} involves generation of a cascade of rapid electrical currents across the cell membrane, whereas mechanisms of chemosensory transduction in this model microorganism are not yet known. Tryptone (a product of casein hydrolysis) acts as a strong chemotaxant in mature gametes, but not in vegetative cells of \textit{C. reinhardtii}. We have shown that the presence of tryptone induces a temporary inhibition of photoreceptor currents in gametes of both mating types, which reveals the existence of a common link between the photo- and chemosensory cascades. Measurement of this inhibition provides an approach to probing early stages of chemosensory transduction in \textit{C. reinhardtii}, direct methods for which are not yet available. The sensitivity of photoreceptor currents to the chemotaxant tryptone rapidly decreases upon gamete mating, which indicates inactivation of the gamete-specific chemosensory system that mediates chemotaxis to tryptone. This inactivation is brought about by flagellar adhesion between the gametes of the opposite mating types and/or a subsequent increase in the intracelluar cAMP concentration, as shows analysis of impotent mutants blocked at different stages of the mating process and the influence of dibutyryl-cAMP and IBMX on the sensitivity of the photoreceptors to tryptone. Partial block of this inactivation at the restrictive temperature in the \textit{fla10} mutant with a temperature-sensitive lesion in the kinesin-II motor subunit suggests the functional importance of the intraflagellar transport for switching off the gamete-specific chemosensory system upon fertilization in the wild type of \textit{C. reinhardtii}.

L50 Syndecan-2 Functions as a Cell Surface Docking Receptor for Pro-matrix Metalloproteinase-7 at Early Stage of Colon Carcinogenesis

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Although elevated expression of syndecan-2 a cell surface heparan sulfate proteoglycan is known to be crucial for the tumorigenic activity in colon carcinoma cells, the mechanism by which syndecan-2 regulates colon carcinogenesis is unclear. Syndecan-2 mRNA expression was observed in 72.7% of human colon adenocarcinoma tissue, whereas 18.2% of neighboring normal tissues. Interestingly, increased syndecan-2 expression was detected in 65% of the primary tumor from the patients who did not undergo metastasis, but only 15% of the metastatic tumor. Besides, syndecan-2 expression was increased in the colon of AOM-induced adenoma and carcinoma in situ, suggesting the importance of syndecan-2 during early colon tumorigenesis. Consistently, syndecan-2-overexpressing HT-29 cells showed enhanced migration/invasion, anchorage-independent growth and primary tumor formation in SCID mice, paralleled with the morphological changes into highly invasive cells. Syndecan-2 enhanced secretion of matrix metalloproteinase-7 (MMP-7), and directly interacted with MMP-7 in the plasma membrane. Furthermore, syndecan-2 potentiated the enzymatic activity of pro-MMP-7 through accelerating its processing into the active form, but not through affecting its proteolytic activity. Taken together, these data strongly suggest that syndecan-2 functions as a docking receptor for pro-MMP-7 at early stage of colon carcinogenesis.

L51 N-WASP Inhibits mDia1 Actin Nucleation Activity in Carcinoma Cells

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Three members of the WASP family, N-WASP, WAVE1 and WAVE2, which promote branched actin filament assembly via Arp2/3, are expressed in MTLn3 carcinoma cells. In this study, the role of N-WASP, WAVE2 and WAVE1 in carcinoma cell motility was examined following siRNA-mediated knockdown. WAVE1 siRNA knock down had no function on lamellipod, filopod and ruffle formation. However, WAVE2 knockdown suppressed EGF-induced formation of the branched actin network at the leading edge and inhibited lamellipod formation. In contrast, N-WASP knockdown had no significant effect on lamellipod formation. Interestingly, simultaneous knockdown of both WAVE2 and N-WASP caused cells to form jagged protrusions which lead to the increased formation of filopods upon EGF stimulation. These jagged protrusions resulted from an increase in
banded end formation. The results suggest that another actin assembly activity might be at work but otherwise suppressed by WAVE2 and/or WASP. Mammalian Diaphanous-related (mDia), known to nucleate and elongate non-branched actin filaments, might have a role in EGF-induced protrusions. We looked at endogenous mDia1 and found it to localize to the thick actin bundles in the jagged protrusions formed by the WAVE2/N-WASP double knockdown. Also, the effect of WAVE2/N-WASP knockdown was recapitated by expression of constitutively active mDia2. DN-mDia1 expression confirmed that the protrusions formed by the WAVE2/N-WASP knockdown cells were due to mDia1 activation. DN-mDia1 inhibited the formation of filopods and barbed ends in WAVE2/N-WASP knockdown cells. Therefore, mDia1 activity is increased in the absence of N-WASP and WAVE2, which leads to the production of jagged protrusions and an increase in filopod formation. We believe that N-WASP acts as an inhibitor of mDia1 while WAVE2 masks mDia1 activity in carcinoma cells.

L52
Role of Syndecan-4 in Fibronectin Remodelling and Directionally-persistent Migration
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The transmembrane proteoglycan syndecan-4 has an important role in wound healing; a morphogenetic process that relies critically on matrix remodelling and directional cellular migration. Synergistic signalling between α5β1 integrin and syndecan-4 is required to initiate prototypical cytoskeletal reorganisation and focal adhesion formation upon cell binding to fibronectin. Through the use of recombinant fibronectin fragments and genetic ablation we have demonstrated an absolute requirement for syndecan-4 in the regulation of Rac GTP-loading during cell spreading, and in the maintenance of Rac-mediated directionally-persistent migration on cell-derived matrices. Furthermore, through expression of syndecan-4 cytoplasmic domain mutants we have elucidated novel roles for syndecan-4 in matrix reorganisation and cellular migration. Syndecan-4 null fibroblasts, retrovirally-transduced with Syn4Y180L, a construct unable to be phosphorylated at tyrosine180, exhibited enhanced fibronectin secretion but were deficient in their ability to remodel fluorophore-conjugated cellular fibronectin. In contrast, reorganisation and remodelling of fibronectin was promoted in cells expressing the phosphomimetic cytoplasmic mutant, Syn4Y180E. Moreover, expression of Syn4Y180E conferred directional persistence to cells even when non-migratory, with Syn4Y180L, a construct unable to be phosphorylated at tyrosine180, exhibited enhanced fibronectin secretion but were deficient in their ability to remodel fluorophore-conjugated cellular fibronectin. In contrast, reorganisation and remodelling of fibronectin was promoted in cells expressing the phosphomimetic cytoplasmic mutant, Syn4Y180E. Moreover, expression of Syn4Y180E conferred directional persistence to cells even when non-migratory, with Syn4Y180L, a construct unable to be phosphorylated at tyrosine180, exhibited enhanced fibronectin secretion but were deficient in their ability to remodel fluorophore-conjugated cellular fibronectin.

L53
μ-Calpain Activation and Re-distribution in Myeloid Cells Induced by Elevated Cytosolic Free Ca2+ and Phagocytosis
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Phagocytosis of zymosan by neutrophils is accelerated by the interaction of an opsonin (iC3b ) with its ligand β2-integrin. We have previously suggested that the mechanism of this acceleration involves the Ca2+-dependent activation of calpain. In order to test this, novel technologies have been developed. YFP-calpain 4, was expressed in NB4 neutrophil-like myeloid cells. As this protein has neither proteolytic activity nor the membrane targeting C2-like domain, it provided a marker of the cellular location of μ-calpain when bound to its endogenous partner (calpain 1). There was a rapid translocation of cytosolic YFP-calpain 4 to areas of the plasma membrane when cytosolic free Ca2+ was raised by thapsigargin and ionomycin. This translocation was found to be dependent on the association of calpain 4 with its substrate which reacts with expressed cytosolic luciferase to generate luminescence. However, when cytosolic free Ca2+ was elevated to cause calpain translocation in these cells, calpain activation was observed as a transient luminescent signal. Thus an elevation of cytosolic free Ca2+ caused both the activation and translocation of μ-calpain in myeloid cells. During phagocytosis of iC3b opsonised zymosan by neutrophils, there was also evidence for calpain activation using a fluorogenic calpain substrate (t-boc-leu-net-CMCA). Furthermore, immunohistochemistry of calpain in fixed cells suggested that translocation of calpain to the plasma membrane also occurred during phagocytosis. This latter conclusion was confirmed by monitoring the movement of fluorescently labelled calpain microinjected in living neutrophils. It was therefore shown that both translocation and activation of μ-calpain occurs during elevation of cytosolic free Ca2+ induced either experimentally with ionomycin or during iC3b-mediated phagocytosis. This was consistent with a role for the Ca2+-dependent activation of calpain in the acceleration of phagocytosis by neutrophils.

L54
Syndecan-4 Maintains Directionally-persistent Migration and Determines Direction through the PKCα-binding Domain
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Adhesion to the extracellular matrix is essential for establishing cell polarity and directing migration during development and disease. While integrin-mediated adhesion is known to regulate the Rho-GTPases and thereby control cell movement, the heparin sulphate proteoglycan, syndecan-4, is vital for Rac1 regulation. Here, the biological role of Rac1 regulation by syndecan-4 was demonstrated using pre-assembled extracellular matrices. Wild type fibroblasts were found to migrate persistently along fibronectin fibres, whereas syndecan-4 null fibroblasts, which display elevated levels of GTP-Rac, migrated randomly. FRET analysis suggested that the absence of syndecan-4 reduced directionally-persistent migration by promoting the formation of myosin II-coated fibres. Moreover, syndecan-4 co-expression with mDia2, which is found to interact with Rac1, reversed this phenotype. The results demonstrate that syndecan-4-directed dependent regulation of Rac1 mediates off-axial lamella formation and that this determines directionally-persistent migration. Furthermore, syndecan-4 detection of the extracellular matrix is required for efficient changes in the direction of migration.

L55
Cofilin Knockdown Triggers Arp2/3 Relocalization and Changes the Motility Behavior of Metastatic Cancer Cells
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Cofilin F-actin severing ability is important for the regulation of actin dynamics and hence cell motility. We studied the effect of cofilin knockdown in rat mammary adenocarcinoma cells and found that it resulted in an elongated phenotype. Analysis of the constitutive motility of cofilin knock down cells showed that the cells lost their motility ability resulting in directional and persistent motility when compared to the frequent-turning and random-walking control cells. A wild type human cofilin plasmid, WT(ΔH)COF, was able to rescue the phenotype, and directional motility of cofilin knockdown cells. However, a mutated form of this plasmid, D122K(ΔH)COF, which has increased binding to polyphosphoinositide phosphatidylinositol-4,5-bisphosphate (P(1,4,5-P)) was not able to rescue the motility and phenotype of cofilin knockdown cells. A pipette-introduced EGF source showed that the response of serum-starved cofilin knock down cells is dependent on the location and shape in the activated cells, while control cells show equal protrusive response regardless of the location of the pipette. Interestingly, we found that the Arp2/3 levels...
in colfin knockdown cells showed re-localization to the front of the cells and that double knockdown of Arp2/3 and colfin show reversion from the low-turning and directional walking behavior into the random walking motility typical of control cells. These results reveal that colfin has to be released from its PI-4,5-P2 binding as a prerequisite for its initiation of stimulated protrusion and for the direction of cell movement. In addition, the failure of proper localization of the Arp2/3 complex in colfin knockdown cells results in the inability of these cells to initiate multiple protrusions causing the cells to proceed in one direction, which supports previous findings that the synergistic interaction between Arp2/3 and colfin is important for the stimulated lamellipod extension.

L56
Decreased PTP-PEST Expression Is Associated with Increased Motility and Invasion in Colon Carcinoma Cells
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PTP-PEST is a protein tyrosine phosphatase that regulates cell motility through its action on Rho GTPases. Previous studies suggest that PTP-PEST is aberrant in breast cancer, however the role of this phosphatase in colon carcinoma invasion has not been addressed. In this study, we examined the expression of PTP-PEST in colon cancer tissues and cell lines. We find that compared to normal tissue samples, PTP-PEST expression is reduced in colon tumors. Likewise, western analysis shows that PTP-PEST expression is weak or undetectable in several colon carcinoma cell lines including Caco-2, DLD-1, Clone A and KM20 relative to less aggressive KM12C cells or normal intestinal (IEC) and kidney epithelial cells (MDCK). SiRNA-mediated knockdown of PTP-PEST in KM12C cells leads to increased migration towards collagen I as well as increased chemotaxis towards HGF. In contrast, ectopic expression of wild type PTP-PEST, but not a catalytically inactive mutant, in KM20 or DLD-1 cells impairs motility. Finally, we show that PTP-PEST localizes in adherens junctions in KM12C cells. SiRNA ablation of PTP-PEST enhances activation of Rac1 and decreases activation of RhoA in response to cadherin engagement. Furthermore, decreased PTP-PEST expression enhances Rac1 activity and suppresses RhoA activity in response to plating on collagen I. Taken together, these findings suggest that PTP-PEST is required to modulate Rho GTPase activity at sites of cadherin and integrin engagement to control colon carcinoma motility. These results are the first to show that PTP-PEST plays a functional role in colon carcinoma motility and suggest that loss of PTP-PEST expression during colon cancer progression may influence invasive potential.

L57
Prostaglandin E2 Modulates Cytoskeletal Dynamics and Migration of Podocytes
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Prostaglandin E2 (PGE2) regulates cell motility in diverse cell types. For instance, PGE2 promotes chemotaxis of human hepatocytes, monocyte-derived dendritic cells, and trophoblasts; conversely, in human lung fibroblasts, this prostanoid suppresses migration. We found that PGE2 strongly suppressed epidermal growth factor (EGF)-induced serum-induced lamellipodia formation, respectively, indicating that EGF-induced phosphorylation in the cells. The inhibition of MLCP activity by 14-3-3 γ inhibited the phosphorylation of MYPT1 was achieved via its interaction with MYPT1, and 14-3-3 failed to phosphorylate in the cells. The inhibition of MLCP activity by 14-3-3 γ inhibited the phosphorylation of MYPT1 and dissociate MYPT1 from the holoenzyme, suggesting that the inhibition of MLCP activity is not due to the dissociation of MYPT1 from the holoenzyme. We found that the phosphorylation of MYPT1 was critical for the binding of 14-3-3 γ to MYPT1. The binding of 14-3-3 γ to MYPT1 was confirmed by the direct binding assay with the isolated 14-3-3 and MYPT1 and by immunoprecipitation using cell lysates. A critical finding is that the binding of 14-3-3 γ to MYPT1 diminished the binding between MYPT1 and myosin II. Conversely, the over-expression of 14-3-3 WT in COS7 cells abolished the localization of MYPT1 at the stress fiber. 14-3-3 K49E, lacking the dimer forming ability of 14-3-3, failed to hamper the stress fiber localization of MYPT1. The result suggests that the dimer formation of 14-3-3 is important for the binding to MYPT1. Interestingly, 14-3-3 γ inhibited MLCP holoenzyme activity. Consistently, the over-expression of 14-3-3 γ WT but not K49E mutant resulted in the increase in myosin II phosphorylation in the cells. The inhibition of MLCP activity by 14-3-3 γ was achieved via its interaction with MYPT1, and 14-3-3 γ failed to inhibit the activity of PP1δ catalytic subunit. 14-3-3 γ did not dissociate MYPT1 from holoenzyme, suggesting that the inhibition of MLCP activity is not due to the dissociation of MYPT1 from the holoenzyme. We found that the phosphorylation of MYPT1 was critical for the binding of 14-3-3 γ to MYPT1. The binding of 14-3-3 γ to MYPT1 was confirmed by the direct binding assay with the isolated 14-3-3 and MYPT1 and by immunoprecipitation using cell lysates. A critical finding is that the binding of 14-3-3 γ to MYPT1 diminished the binding between MYPT1 and myosin II. Conversely, the over-expression of 14-3-3 WT in COS7 cells abolished the localization of MYPT1 at the stress fiber. 14-3-3 K49E, lacking the dimer forming ability of 14-3-3, failed to hamper the stress fiber localization of MYPT1. The result suggests that the dimer formation of 14-3-3 is important for the binding to MYPT1. Interestingly, 14-3-3 γ inhibited MLCP holoenzyme activity. Consistently, the over-expression of 14-3-3 γ WT but not K49E mutant resulted in the increase in myosin II phosphorylation in the cells. The inhibition of MLCP activity by 14-3-3 γ was achieved via its interaction with MYPT1, and 14-3-3 γ failed to inhibit the activity of PP1δ catalytic subunit. 14-3-3 γ did not dissociate MYPT1 from holoenzyme, suggesting that the inhibition of MLCP activity is not due to the dissociation of MYPT1 from the holoenzyme.
Monday

Phospholipase C-gamma 1 (PLC-gamma 1) has been implicated in the pathway responsible for the reorganization of the cytoskeleton. Alternatively, EGFR activation leads to membrane ruffling and reorganization of focal adhesions through activation of member of the Rho subfamily of GTP-binding proteins. Given that both PLC-gamma 1 and Rho GTPases regulates cell motility induced by EGF, it would be interesting to examine whether there is a direct functional linkage between PLC-gamma 1 and Rho GTPase in EGF-induced cell movement. Recently, it was shown that PLC-gamma 1 SH3 domain is a GEF for GTPases including PIKfyve and dynamin. Thus, it is possible that PLC-gamma 1 may regulate cell motility and cancer metastasis by regulating Rho GTPase activity through its SH3 domain GEF activity. We tested this possibility in this research. We showed that in response to EGF PLC-gamma 1 and Rac1 co-localize to the plasma membrane and interact with each other. The interaction between PLC-gamma 1 and Rac1 is mediated by PLC-gamma 1 SH3 domain and Rac1 Proline rich motif 109. We further showed that EGF-induced PLC-gamma 1 and Rac1 interaction resulted in the activation of Rac1, which suggest that PLC-gamma 1 is a GEF for Rac1 in vivo. Moreover, we demonstrated by in vitro GEF assay that PLC-gamma 1 SH3 domain is a strong and specific GEF for Rac1. Finally, we showed that the preparation of extracellular physiological Ca2+-concentration.

GAP JUNCTIONS

L61
Opening Gap Junction Hemichannels in GFSHR-17 Granulosa Cells under Physiological Conditions

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Application of ATP onto GFSHR-17 granulosa cells under perforated patch-clamp induced depolarization of the cell membrane by about 10 mV. The ATP related depolarisation was not suppressed if Na+ in the extracellular solution was substituted by NMDG⁺ or Choline⁺, indicating that ATP stimulated opening of channels permeable to large ions. Gap junction hemichannels are known to be permeable to large molecules such as the fluorescent molecule Lucifer yellow. Using fluorescence microscope, it could be shown that ATP stimulated uptake of Lucifer yellow. These results demonstrate that gap junction hemichannels of GFSHR-17 granulosa cells can be opened in presence of extracellular physiological Ca²⁺-concentration.

GOLGI TO CELL SURFACE TRANSPORT

L62
The Clathrin Adaptor Gga2p Is a Phosphatidylinositol 4-Phosphate Effector at the Golgi Exit

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Phosphoinositides are key regulators of membrane transport. In Saccharomyces cerevisiae, phosphatidylinositol 4-phosphate synthesized by the essential P4 kinase Pkl1p is required for maintenance of Golgi morphology, transport of cargo for exocytosis as well as for vacuolar delivery. It however remains elusive how P4(4)P mediates its effects on Golgi secretory function. In a search for P4(4)P effectors, we performed a genetic screen for synthetic lethal interactions of a conditional pkl1 (pkl1-101) mutant. The screen revealed a synthetic genetic interaction between pkl1-101 and a deletion mutant of GGA2. Gga2 proteins are monomeric clathrin adaptors that regulate Golgi-to-endosome transport. As previous work suggested a role for Pkl1p in the control of vesicle formation at the TGN, we investigated a possible common function of Pkl1p and Gga2p. Here we show that P4(4)P generated by Pkl1p is required for recruitment of Gga2p to the Golgi. Pkl1p-P4(4)P binding is mediated by the VHS domain of Gga2p. Liposome recruitment assays demonstrate a cooperative function of P4(4)P and the small GTPase Arf1p in Gga2p recruitment to the TGN. We further show that P4(4)P and Gga2p are not only involved in transport of cargo from the TGN to late endosomes but are also required for Golgi-to-surface transport of a specific subset of exocytic cargo, suggesting a common regulatory mechanism for exit of vacuolar and secretory cargo from the TGN.

Endocytosis

L64
Role of Palmitoylation in IFN Receptor Trafficking and Signaling

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Type I (alpha/beta) and type II (gamma) IFNs bind to distinct receptors but activate the same signal transducer and activator of transcription (STAT1), raising the question of how signal specificity is maintained. We have recently shown that both IFN-alpha (IFNAR) and IFN-gamma (IFNGR) receptors are palmitoylated by a cysteine rich motif 109PNTP109. We further showed that palmitoylation is required both for IFNAR and IFNGR signaling. Palmitoylation plays a role in the trafficking of IFNAR. Accordingly, we could show that IFNAR palmitoylation prevents IFNAR endocytosis, together with the activation of Stat1 and nuclear translocation, suggesting that palmitoylation plays a role in the trafficking of IFNAR. Moreover, we found that palmitoylation inhibitor, 2-bromopalmitate (BP), prevents IFNAR endocytosis, together with the activation of Stat1 and its nuclear translocation, suggesting that palmitoylation plays a role in the trafficking of IFNAR. Accordingly, we could show that IFNAR1 is palmitoylated. We have also designed IFNAR chains mutated for the two cysteines present in their cytoplasmic domains that could be palmitoylated, we investigated the potential role of palmitoylation in IFN-R trafficking and signaling. We found that the palmitoylation inhibitor, 2-bromopalmitate (BP), prevents IFNAR endocytosis, together with the activation of Stat1 and its nuclear translocation, suggesting that palmitoylation plays a role in the trafficking of IFNAR. We further show that PI(4)P and Gga2p are not only involved in transport of cargo from the TGN to late endosomes but are also required for Golgi-to-surface transport of a specific subset of exocytic cargo, suggesting a common regulatory mechanism for exit of vacuolar and secretory cargo from the TGN.
L65 Cell Selection Mechanism of Homologous Amphiphilic Basic Polypeptides as Revealed by Heparan Sulfate-mediated Cell Surface Retention of Three-fingered Cobra Cardiotoxins
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Animal venoms usually consist of homologous toxins to act on cells with similar targets in different species and/or different cells with distinct targets in the en-venomated victim. It is not known whether they reach their target mainly by mass action or there are other mechanisms to facilitate the cell selection of specific toxins, although cell surface retention of biologically active ligands through heparan sulfate binding is known to play an important role in cell development, ligand internalization by cells and certain disease states. In this study, we performed surface plasmon resonance (SPR) studies on the binding of 10 Cardiotoxin(CTX) homologues from Taiwan and African cobra venoms to immobilized heparin and demonstrated that three different groups of CTXs were distinguishable based on their retention behavior on heparin surfaces. The different retention characteristics were correlated with the structural diversity at the functionally important loop II region and the presence or absence of hydrophobic residues, which promote contact between two adjacent CTXs. Since this was corroborated by the retention behavior of CTX homologues on Chinese hamster ovary (CHO) cells and glycosaminoglycan biosynthesis deficiency mutant CHO pgsA745 cells, we suggest that HS-induced oligomerization of amphiphilic basic CTXs through their hydrophobic domains may also be responsible for the localization of different CTX homologues in different tissues.

L66 Activation of Elk-1 by Glucose and EGF Requires Ras Interference
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Elk-1 transcriptional activity is regulated by phosphorylation in a cell-specific manner in response to growth factors and other agents. Ras Interference 1 (Rin1) is a multifunctional protein that had been shown to regulate growth factor signaling and endocytosis, including EGF and insulin. In this study, we examine the role of Rin1 in the activation of Elk-1 by glucose, KCl-induced depolarization and EGF treatments by expressing Rin1 and deletion mutants in NR6 and Min6 cells using a retrovirus system. Our results demonstrated that expression of Rin1 blocked the stimulatory effect of EGF on the phosphorylation of Elk-1 in both NR6 and Min6 cells. However, expression of Rin1 partially blocked the activation of Elk-1 by the addition of either glucose or KCl-induced depolarization in Min6 cells. Furthermore, we found that the expression of Rin1 decreased the thymidine incorporation induced by glucose, KCl-induced depolarization and EGF treatments by insulin. In this study, we examine the role of Rin1 in the activation of Elk-1 by glucose, KCl-induced depolarization and EGF treatments by expressing Rin1 and deletion mutants in NR6 and Min6 cells using a retrovirus system. Our results demonstrated that expression of Rin1 blocked the stimulatory effect of EGF on the phosphorylation of Elk-1 in both NR6 and Min6 cells. However, expression of Rin1 partially blocked the activation of Elk-1 by the addition of either glucose or KCl-induced depolarization in Min6 cells. Furthermore, we found that the expression of Rin1 decreased the thymidine incorporation induced by either glucose or EGF in Min6 cells. In addition, we also found that both Elk-1 and INK4A were not involved in the regulation of Elk-1 activity in both cell lines. These results demonstrate that Rin1 is required for the nutrient- and growth factor-mediated signaling pathways on Elk-1 stimulation through activation of Ras/Rin1 signaling complex.

PROTEIN TARGETING TO THE CELL SURFACE
L67 ARL4D Recruits Cytohesin-2/ARNO to Modulate ARF6 Activation and Actin Remodeling
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Developmentally regulated ARL4D and ARL4A are members of ARF/ARL family of Ras-related GTases. Although the primary structure of ARL4D and ARL4A are very similar to that of ARF/ARL, their function remains unclear. Cytohesin-2/ARNO is a guanine nucleotide exchange factor (GEF) for ARF and at plasma membrane it can activate ARF6 to regulate actin reorganization and membrane ruffling. We show here that ARL4D interacts with the C-terminal pleckstrin homology (PH) domain of cytohesin-2/ARNO in a nucleotide-dependent manner and localization of ARL4D to plasma membrane is GTP-dependent. GTP-restricted ARL4D induces translocation of cytohesin-2/ARNO to plasma membrane. Consistent with a known role of cytohesin-2/ARNO, GTP-restricted ARL4D increases the GTP-bound ARF6 in vivo and induces the disassembly of actin stress fibers. Expression of catalytic inactive cytohesin-2/ARNO(E156K) or siRNA knockdown of cytohesin-2/ARNO blocks ARL4D-induced disassembly of actin stress fibers. Furthermore, ARL4D induces translocation of cytohesin-2/ARNO to plasma membrane is independent of phosphoinositide 3-kinase signaling. Together, these data demonstrate that ARL4D acts as a novel upstream regulator of cytohesin-2/ARNO to modulate ARF6 activation and actin remodeling.

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L68 Quantifying the Dynamics of the γ-aminobutyric Acid Transporter, GAT1, at the Membrane Surface and the GAT1 Vesicle with Confocal Microscopy and Total Internal Reflection Fluorescence Microscopy
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We seek to understand the trafficking dynamics of the neurotransmitter transporter GAT1 as it is translocated onto the neuronal plasma membrane. Quantifying transporter trafficking and membrane mobility is fundamental to understanding how these molecules efficiently clear the synapse of neurotransmitter to terminate its signal. The vesicle that contains GAT1 is approximately 50 nm in diameter, lending itself to high resolution observation via TIRFM. An algorithm was developed to track and characterize puncta as either vesicles or filopodia. To determine the dynamics of transporter exchange at the cell membrane we utilize fluorescence recovery after photobleaching (FRAP). Disrupting microtubules with nocodazole has no effect on GAT1 mobility; however, disruption of G-actin filaments with latrunculin B increases the mobility of GAT1 by 10-30%, while reducing the time constant for recovery by up to 60%. Altogether, we present kinetic characterization of the GAT1 vesicle and a possible GAT1 interaction with actin filaments. Support: DA-09121.

L69 Clathrin Cooperates with AP1B to Maintain the Polarity of Transferrin Receptor in MDCK Cells
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Clathrin-coated pits are present on nearly all intracellular organelles including the various endosomes. The role of clathrin in receptor mediated endocytosis has been extensively characterized. However its role in recycling is more controversial where only circumstantial evidence are suggesting it. To investigate this potential function of clathrin, we depleted clathrin heavy chain in MDCK cells using siRNA and analyzed how the various endocytic and recycling steps were affected using biochemical assays. As expected, the internalization of the transferrin receptor (TfR) was compromised. Strikingly, we measured that the kinetic of the basolateral recycling of this same receptor was significantly delayed. Even more surprising we noticed that the absence of clathrin leads to an increase in the level of transferrin receptor from the basolateral to the apical plasma membrane, at a level similar to the one found in clathrin adaptor AP1B depleted cells. The polarity of the TfR was therefore affected. These data suggested that clathrin together with AP1B work as a check up point in recycling endosomes to transport mistargetted receptor back to the appropriate plasma membrane. Currently, we are investigating if clathrin and AP1B also participate in the traffic step between sorting endosomes and recycling endosomes in order to better understand this ‘guardian’ function.

L70 Clathrin Controls Selectively Basolateral Protein Sorting in MDCK Cells
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Clathrin plays a key role in the endocytosis of plasma membrane receptors, either directly or indirectly via adaptor proteins. By contrast, the role of clathrin in exocytosis, particularly in polarized cells, remains practically unknown. Here, we used biochemical polarity assays, confocal microscopy and time lapse microscopy assays to investigate the role of clathrin in the maintenance and generation of plasma membrane polarity in MDCK cells. To monitor clathrin function we used siRNA technology to chronically deplete clathrin heavy chains (CHC) and a reversible crosslinking approach to acutely "freeze" clathrin triskelia, based on the replacement of endogenous clathrin light chain (CLC) by CLC tagged with FK506-binding protein 12 (FKBP-CLCa) (Moskowitz et al., Mol Biol Cell, 2003). Both methods were efficient in disrupting clathrin function, as shown by an assay that monitors the maturation of the lysosomal hydrolase cathepsin D from the TGN. Clathrin suppression by both methods specifically disrupted the localization of basolateral proteins (e.g. Neural Cell Adhesion Molecule (N-CAM), Vesicular Stomatitis Virus G protein), without impairing the distribution of apical markers (e.g., p75 Neurotrophin Receptor). Live imaging assays to measure exit from the TGN (Kreitzer et al., Nature Cell Biology, 2000) in clathrin-suppressed cells demonstrated inhibition of the exit of GFP-NCAM but not VSVG-GFP or p75-GFP. The results suggest that clathrin regulates the sorting of basolateral membrane proteins in different intracellular compartments, according to the protein. Supported by NIH grant GM34107, the Dyson Foundation and Research to Prevent Blindness Foundation.

GENE STRUCTURE AND EXPRESSION

L71 Differential Role of Corepressor N-CoR and SMRT Complexes in Thyroid Hormone Receptor-mediated Transcriptional Repression

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The best characterized corepressors, N-CoR and SMRT, bind to unliganded nuclear receptors and repress transcription through recruitment of histone deacetylase 3 (HDAC3). A key question about the role of corepressor proteins in nuclear hormone receptor (NR)-mediated transcriptional repression is the differential role of N-CoR and SMRT complexes. Here we show that both N-CoR and SMRT are independently involved in transcriptional repression of multiple TR-target genes including BCL3 (B-Cell Lymphoma 3-encoded protein), Spot14 (thyroid hormone-inducible hepatic protein), FAS (fatty acid synthase) and ADRB2 (beta-adrenergic receptor). We demonstrate that either siRNA treatment against NCoR or SMRT is sufficient for the repression of multiple TR-target genes. By combination of sequencing mining and physical association as determined by chromatin immunoprecipitation (ChIP) assays, we mapped the putative TREs (androgen response elements) in the BCL3, Spot14, FASN, and ADRB2. Our data clearly suggests that the SMRT and N-CoR are independently recruited to various TR target genes through the siRNA and ChIP experiments. We also show that unliganded, corepressor-free TR is defective in repression and may interact with coactivator, P300. Furthermore, overexpression of N-CoR can restore repression of endogenous genes after knocking-down SMRT. Together, our data reveal the differential roles of corepressor complexes and provide the evidence for the possible interaction between unliganded, corepressor-free TR and coactivators. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A05-0125-A00718-05N1-00010A); Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2005-042-E00022); (R13-2003-054-0101-0, 2004) from the Basic Research Program of the Korea Science & Engineering.

L72 Formation of DNA Loop by the Interaction of 5’ Promoter Region Bound NF-kB and 3’UTR Bound Lef1/beta-catenin Facilitates COX-2 Expression

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Although transcription cycle is divided into multiple stages, physical and functional inter-connections between the steps allow efficient transcription. Several studies suggested the involvement of a link between transcription termination and initiation steps in efficient transcription reinitiation and acceleration of transcription rate. But little evidence is available in mammalian system until now. Here we show that the formation of a transcription loop between 5’promoter region and 3’UTR of COX-2 genomic locus by the interaction of NF-kB binding on the 5’promoter region and Lef1/beta-catenin binding on the 3’UTR, which facilitates efficient COX-2 transcription in mouse rib clonocytes. Lef1 plays a pivotal role in the formation of the transcription loop by facilitating the recruitment of NF-kB and RNA polymerase II. Lef1 siRNA treatment abolishes the in vivo loop formation and lowered COX-2 transcription. We confirmed loop formation by in vivo chromatin conformation capture assay and in vitro reconstitution of loop formation under atomic force microscopy. Our results suggest a regulatory role of 3’UTR- bound transcription factors in transcription loop formation through physical interaction with 5’promoter bound transcription factors, which improves the binding of RNA polymerase II and defines a transcription unit. The physical link between 5’ promoter region and 3’UTR in specific genomic locus may be a general phenomenon of the transcription in mammalian system and may provide the basis of connection between transcription initiation and termination steps and efficient reinitiation mechanism of transcription.

L73 p38γ Signaling Negatively Regulates MyoD Transcriptional Activity

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The myogenic transcription factor MyoD is responsible for the induction of skeletal muscle differentiation, a function it accomplishes in part via interactions with transcriptional co-regulators. Previous studies have identified multiple mitogen-activated protein kinase (MAPK) pathways regulating MyoD function during this process, including p38β/β. Another p38 family member, p38γ, is highly expressed in skeletal muscle relative to other tissues, yet little is known regarding its role in myogenesis. To further elucidate this role, we examined the mechanism through which p38γ signaling regulates MyoD and its ability to induce differentiation. Co-expression of p38γ with a constitutively active mutant of its upstream activator MKK6 repressed skeletal muscle differentiation in a MyoD-dependent manner. Using MALDI-TOF mass spectrometry, we identified two serine residues within the carboxy terminus of MyoD, which were directly phosphorylated by p38γ. Mutation of these residues to alanine reversed the inhibition of muscle differentiation by p38γ. Surprisingly, p38γ signaling resulted in enhanced association of MyoD with the promoter of myogenin, a direct MyoD target gene during the early stages of muscle differentiation, despite the fact this gene is repressed by p38γ activation. Moreover, increased methylation of histone H3 on lysine-9, a well-documented repressive histone modification, was also detected in response to p38γ signaling. Taken together, these data suggest that signaling through p38γ results in the formation of a repressive MyoD complex, which subsequently binds the promoter of the muscle-specific myogenin gene to repress its expression. Importantly, these results highlight a novel role for p38γ signaling in skeletal muscle differentiation, one that differs from p38α/β.

L74 Asymmetrical Distribution of the Transcriptionally Competent NORs in Mitosis

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Ribosomal genes are organized as tandem repeats termed Nucleolus Organizer Regions (NORs). In human cells NORs are accommodated on the short arms of the acrocentric chromosomes. Essential components of the RNA polymerase I transcription machinery, including Upstream Binding Factor (UBF), can be detected on some NORs during mitosis; these NORs, termed competent, are believed to be transcriptionally active during interphase. In cultured cycling cells, the number of competent NORs, and their distribution among the different chromosomes, does not vary significantly in the sequential cell cycles. In this present study we investigate whether this stable state
is achieved by equal distribution of active NORs during cell division. To answer this question we compared the state of NORs in daughter cells in telophase in transformed HeLa and diploid LEP cells. In both cell lines we found a small but significant difference between the daughter cells in the number of UBF-loaded NORs, and also in the extent of transcription activity of nucleoli as shown by bromouridine incorporation. This mitotic asymmetry may be due to the presence of relatively rare chromosomes with asymmetrical NORs in which only one of sister chromatids carries the signal of competence. Division of such chromosomes in mitosis should cause a decrease in number of the competent NORs in some cells. Our data thus indicate that a compensatory shift of some NOR-bearing chromosomes from non-competent to the competent state is required, and this shift apparently occurs during interphase. This work was supported by grants: 304/04/0692, AV0Z5010509, MSM0021620806, LC535, and WT 075834/04Z.

L75
AKAP12A/Gravin Up-regulates Transcription of Enzymes Involved in Cholesterol Biosynthesis through Activation of Sterol Regulatory Element Binding Protein-2
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The AKAP12/Gravin functions as a kinase scaffold and as a putative tumor suppressor. However, the biological role of AKAP12 is not well understood. To find the downstream signaling of AKAP12A, we performed microarray using Adenoviral-overexpression system. Interestingly, a cluster of genes involved in cholesterol biosynthesis, including HMG-CoA reductase (HMGR), HMG-CoA synthase (HMGS), and mevalonate pyrophosphate decarboxylase (MVD), were up-regulated by AKAP12A overexpression. In addition, AKAP12A also augmented the expression of LDL receptor (LDLR) and insulin-induced gene1 (Insig-1). These genes are known to be controlled by sterol regulatory element-binding protein-2 (SREBP-2). AKAP12A activated SREBP-2 in hepatocellular carcinomas (HCC), as demonstrated by its cleavage product and the up-regulation of the target genes, whereas the activation of SREBP-1 was not remarkably changed. Moreover, knock down of AKAP12A achieved by RNA interference (RNAi) inhibited the activation of SREBP-2. AKAP12A-induced SREBP-2 activation depended on SREBP cleavage-activating protein (SCAP), because inhibition of SCAP by RNAi blocked SCAP dependent activation of AKAP12A. Additionally, SREBP-2 activation was also suppressed by 25-hydroxycholesterol (25-HC) and/or cholesterol. In conclusion, our results suggest that AKAP12A regulates the transcription of enzymes involved in cholesterol biosynthesis pathway via the activation of SREBP-2.

L76
Transcription of Human Cc Chemokine CCL23 Gene Is Mediated by NFAT, NF-kB, and AP-1 in PMA-stimulated U937 Monocyte Cells
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CCL23 (also known as CKβ8, MPIF-1, or MIP-3) has been identified as a CC chemokine which exerts a number of biological activities via CCR1. The CCL23 gene is located on chromosome 17q11-q21, and is expressed in liver, lung, and monocye-derived dendritic cells. Expression of CCL23 is induced by external stimuli including PMA in monocyes, but its transcriptional regulation has not been studied to date. In the present study, we demonstrated that the region from -293 to +31 is important for induction by PMA. Mutational analysis of the promoter region revealed that cis-acting elements at the -269/-264 (NFAT site), -167/-159 (NF-kB site), and -51/-43 (AP-1 site) positions were critical sites for the CCL23 expression in U937 cells. The gel-mobility assay demonstrated the binding of the transcription factors to the consensus sites. Specific inhibitors for signal pathways reduced PMA-induced expression of CCL23, confirming involvement of these transcription factors. Our results demonstrate that NFAT, NF-kB, and AP-1 binding sites are essential for PMA-induced CCL23 expression in human monocyes.

L77
Site Directed Mutagenesis as a Method of Understanding the Mechanism of PqqC
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The genes pqxABCDE and F in Klebsiella pneumoniae are required for the synthesis of the bacterial vitamin pyrroloquinoline quinone (PQQ). PqqC is a redox cofactor in numerous bacterial dehydrogenases. The focus of this work is on the enzyme PqqC, which catalyzes the last step of PQQ formation. PqqC is unusual in that it catalyzes an eight electron oxidation and transfers electrons directly to molecular oxygen without the assistance of a redox active metal or cofactor. To be able to unravel the mechanism of PqqC, it is important to understand the catalytic role of certain amino acids in its active site. The histidines in the 24 and 154 sites of particular interest. These residues are proposed to act as base-catalysts in the PqqC reaction. To investigate the specific role of these histidines, site directed mutagenesis will be used to acquire single amino acid substitutions with alanine and asparagine. Primers have been designed to incorporate the mutations H24A, H24N, H154A, and H154N into PqqC, and mutagenesis is currently being performed.

RIBONUCLEOPROTEINS AND RNA LOCALIZATION

L78
Identification of a Hexanucleotide Repeat in the 3'UTR of Human Low Molecular Weight Neurofilament (NFL) That Affects mRNA Structure, Stability, and Interaction with 14-3-3 Proteins
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Changes in the stability of low molecular weight neurofilament (NFL) are found in amyotrophic lateral sclerosis (ALS) spinal cord tissue, and are associated with altered expression of trans-acting NFL 3'UTR binding proteins. We have identified two hexanucleotide motifs as the main cis elements in human NFL 3'UTR. These motifs appear to be unique to NFL, and are not comparable to known protein binding regions identified in mouse NFL mRNA suggesting possible differences in regulation of NFL mRNA between species. After incubation of hNFL mRNA with human spinal cord lysates, and LC/MS/MS of peptide digests, we identified 14-3-3 proteins as binding partners within the region of hNFL 3'UTR bearing the hexanucleotide motifs. Mutation of either the first or second motif resulted in decreased interaction, while mutation of both motifs resulted in significant loss of 14-3-3 interaction. Subsequent expression of wild-type hNFL 3'UTR, or mutant 3'UTR (each motif mutated singly or both together) in HEK293T cells showed that NFL mRNA stability is affected by these motifs. Wild-type 3'UTR appeared to destabilize NFL mRNA, while mutation of the first motif had little effect upon mRNA stability, and mutation of the second motif resulted in mRNA destabilization. Interestingly, changes in the second motif, but not the first, had marked effects on the predicted mRNA folding structure. These data strongly suggest that the first motif is involved in the destabilization of hNFL mRNA, while the second motif may be involved in maintaining mRNA folded structure. Taken together, these data identify a novel mRNA-binding function for 14-3-3 at specific motifs present in the 3'UTR of hNFL mRNA, with these same motifs appearing to affect mRNA stability. These data suggest in turn that 14-3-3 may participate in regulating NFL mRNA stability in normal tissue, and may play a role in the mis-regulation of NFL in ALS.

L79
TDP43 Is a Novel Human Low Molecular Weight Neurofilament (NFL) 3'UTR Binding Protein
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Alterations in the stability of low molecular weight neurofilament (NFL) mRNA have been linked to a selective suppression of the NFL mRNA steady state levels in spinal motor neurons in amyotrophic lateral sclerosis (ALS), and to the formation of neurofilament (NF) aggregates which are a neuropathological hallmark of ALS. We have identified the association of human NFL (hNFL) 3’UTR mRNA with human TAR DNA binding protein (hTDP43). TDP43 was originally described as a DNA binding protein, capable of affecting splicing regulation in the human cystic fibrosis transmembrane conductance regulator (CFTR) gene by interacting with (TG)\textsubscript{n}A\textsubscript{m} motifs in DNA. However, TDP43 also contains two RNA recognition motifs (RRM1 and RRM2), that interact with (UG)n (n = 6-12) motifs found in single stranded RNA. Using HEK293T cells (which express endogenous TDP43) in which we expressed human NF proteins, we observed that TDP43 interacts with NFL mRNA 3’UTR, but not with middle molecular weight NF (NFM) or high molecular weight NF (NFH) RNA. We next examined human spinal cord tissue from 6 ALS cases (1 familial SOD1\textsuperscript{A\textsubscript{V}} and 5 sporadic) for the expression of TDP43. In all cases, TDP43 is expressed in spinal motor neurons in lumbar and cervical spinal cord tissues. Immunohistochemistry shows that TDP43 is predominantly localized in the nucleus in cervical spinal cord tissues, while cytoplasmic distribution in lumbar spinal cord is evident. Additionally, lumbar cord tissues expressed lower levels of TDP43 compared to cervical cord tissues when examined by western blotting. The expression pattern of TDP43, its ability to bind NFL 3’UTR mRNA and not that of NFM or NFH, suggests that TDP43 plays a role in the modulation of NFL stability, and potentially contributes to the formation of NF aggregates through alterations in NF stoichiometry. Research supported by the Michael Hall’s ALS research endowment.

L80 Neuronal L1 Activates Oligodendroglial Fyn Leading to MBP Translation

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During CNS myelination, oligodendrocytes extend membrane processes that interact with axonal contact site which is followed by ensheathment resulting in a compacted multilaminellar myelin sheath. We have shown previously that the src-family non receptor tyrosine kinase fyn co-precipitates with the IgG superfamily membrane proteins F3/ contactin and N-CAM in distinct membrane subdomains referred to as “lipid rafts”. Antibody crosslinking of F3 results in activation of fyn kinase and fyn has also been shown to bind to microtubules and the microtubule associated protein tau. It has been shown that an interaction partner could bind to oligodendroglial F3 activating fyn and inducing the recruitment of the cytoskeleton towards the axon-glial contact site which leads to a polarisation of the cells and initiates the myelination process. The L1 cell adhesion molecule (L1-CAM) belongs to the immunoglobulin superfamily and is expressed by neurons. Here we show that fyn kinase is activated upon binding of a neuronltin L1-Fc fusion protein to cultured oligodendrocytes. Heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2) has been implicated in the transport of mRNAs containing a cis acting element in the 3’ UTR termed A2RE response element (A2RE) and A2RE containing myelin basic protein (MBP) mRNA is transported on microtubules to the distal tips of oligodendroglial processes in the hRNPA2 dependent manner in protein\textsuperscript{A}\textsubscript{ depict complex referred to as granules. We show that the presence of activated fyn leads to binding of hnRNPA2 and translation of an A2RE containing reporter in cultured oligodendrocytes. We suggest that binding of neuronal L1 to glial F3 leads to fyn activation resulting in a local activation of MBP mRNA translation at sites of glial-neuronal contact.

L81 Human Ribosomal Protein S3 (RpS3) Interacts with Uracil DNA Glycosylase (hUNG) and Stimulates Its Glycosylase Activity

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Base excision repair (BER) is one of the cellular defense mechanisms that repair damage bases in genomic DNA. Uracil residues are introduced into DNA by misincorporation of dUMP in place of dTMP during DNA replication or generated by deamination of cytosine residues. In BER pathway, uracil residues are recognized and removed by uracil-DNA glycosylase (UNG), which is a monofunctional enzyme that initiates the base excision repair pathway by hydrolyzing the N-glycosidic bond between uracil residues and sugar-phosphate backbone. Human ribosomal protein S3 (RpS3) has been reported to have an AP lyase activity and to increase the excision of 8-oxoG residues by the interacting with hOGG1 and APE1. However, the effect of RpS3 on the activity of other DNA glycosylases has not been reported. In this work, we investigated the physical interaction of RpS3 and hUNG and the effect of RpS3 on the glycosylase activity of hUNG. In GST-pull down and immunoprecipitation assay using transiently transfected HeK293 cells, RpS3 has been shown to interact with hUNG. To estimated the effect of RpS3 on the glycosylase activity of hUNG, hUNG and RpS3 proteins were purified from E. coli BL21 (DE3) cells transformed with hUNG and RpS3 expressing plasmids and were used in cleavage assay using [32P] labeled U/G mismatched duplex. DNA products cleaved by purified hUNG were increased by dose-dependent manner and were increased by the addition of purified RpS3. RpS3 did not show any glycosylase and AP lyase activities in the cleavage assay using U/G mismatched duplex. These suggest that RpS3 participates in hUNG mediated BER pathway and enhances the glycosylase activity of hUNG. RpS3 is thought to increase the hUNG activity by the increase of DNA binding activity or turn-over of hUNG through removing of hUNG bound to abasic site that generated remove uracil residues.

L82 Functional Domains of AUBPs Involved in Interleukin 2 mRNA Stability

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Gene expression is controlled at the transcriptional and posttranscriptional levels. The rate of mRNA decay is determined by cis-acting elements within the mRNA molecule, which are recognized by trans-acting factors. The most common cis element responsible for rapid mRNA decay in mammalian cells is the AU-rich elements (AREs), which are present in 3’UTRs of various mRNAs. Especially the expression of IL-2 is regulated precisely at transcriptional and posttranscriptional levels. Two ARE binding proteins (AUBPs), TTP and AURE, are shown to modulate turnover of ARE-containing mRNAs. The zinc finger protein TTP is capable of destabilizing mRNA but HUr, Drosophila ELAV-like protein, is shown to stabilize mRNA. We hypothesize that the terminal domain of TTP and the C terminal domain of HuR regulate IL-2 mRNA decay. In this study, we expressed MS2-fusion proteins and IL-2 probe which replaces ARE with MS2 coat protein-binding sites in the mRNA 3’ UTR to exclude direct binding of AUBPs with the ARE. According to this model, our data suggest that specific domains of TTP and HuR regulate IL-2 mRNA stability.

L83 Retrograde tRNA Movement: A Role for Xpo5/Mns5 and eEF1a/Tef1

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Transfer RNAs (tRNAs) are synthesized in the nucleus but function in the cytoplasm. The dogma that tRNA moves in a unidirectional manner from nucleus to cytoplasm was rewritten recently with the discovery of retrograde tRNA movement from cytoplasm into the nucleus (Shaheen and Hopper, 2005). Under stress conditions such as amino acid starvation, tRNAs are imported and sequestered inside the nucleus and the karyopherin Mtr10 plays a role in this process. Upon re-feeding tRNA are re-exported back to the cytoplasm (Whitney et al., in prep). However, this raised the question as to how tRNA sequestered inside the nucleus is sequestered in the nucleus upon amino acid starvation, an effect exaggerated in Msn5 deletion strains. Our results have also been substantiated with binding assays. Biochemical studies further support our hypothesis that Msn5 acts in conjunction with Tef1 in the re-export of tRNA from the nucleus to the cytoplasm.

L84 Retinoic Acid Regulates Dendritic Translation and Synapse Formation in the Hippocampal Neurons

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Dendritic message RNA (mRNA) translation constitutes an important mechanism for synaptic plasticity. There are 500 or so dendritic mRNAs, transported via microtubule-dependent mechanism in large repressive mRNP complex resembling granules (RNA granules), and activated near the synapse by appropriate stimuli. Because these mRNAs often encode receptors, scaffold proteins and signaling molecules essential for synaptic function, translation of these mRNA needs to be tightly regulated. Yet the regulatory mechanisms are largely unknown. We found that all-trans retinoic acid (at-RA) signals non-transcriptionally via RARs to activate dendritic translation and synapse formation in hippocampal neurons. Using Mass Spectrometry (MS), we identified components of RARα-associated complex isolated from the hippocampal neurons. We showed that RARα is tethered by Puro, an RNA binding protein known to regulate dendritic RNA transport, to the RNA granules. Furthermore, we identified AMPA receptor subunit 1 (GluR1) as one of RARα-target mRNAs by immunoprecipitation. Our results reveal a critical role of RARα in dendritic mRNA regulation and synaptic function.

**SIGNAL TRANSDUCTION IN DEVELOPMENT**

**L85 Non-membrane-associated Daily Binds to Lipoprotein Particles and Increases Hedgehog Signaling Efficiency**

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The secreted signaling proteins Hedgehog and Wingless play important roles in signaling during development, especially as controlling the formation and growth. The secreted signaling molecule is released from producing cells and spreads into adjacent non-expressing tissue where it activates target gene expression in a concentration-dependent manner. The Drosophila Lipoprotein, Lipophorin, bears lipid-linked morphogens on its surface and is required for long-range signaling activity of Wingless and Hedgehog. Lipophorin particles can also bind a wide variety of GPI-linked proteins. Whether any of these proteins affect morphogen signaling is unknown. The Drosophila glypicans Daily and Dally-like, which are GPI-linked heparan sulfate proteoglycans, function autonomously to regulate the spread of morphogens such as Wingless and Hedgehog throughout the tissue. Since it has been shown that GPI-anchored proteins are released from cell surfaces by proteolysis or in association with Lipoprotein particles, we investigated whether glypicans can also be released in vivo. Here, we show that the GPI-linked heparan sulfate proteoglycan Daily is released from cell membranes and binds to Lipoprotein particles both with and without its lipid anchor. Hedgehog signaling efficiency is reduced in Dally mutant discs, but can be rescued non-autonomously by expression of non-GPI-modified Daily. This Daily isoform colocalizes with Hedgehog, Patched and Lipophorin in endosomes and increases Hedgehog signaling efficiency without affecting Hedgehog distribution. These data show that Hedgehog signaling activity can be influenced by other Lipophorin-associated proteins, and suggest Lipoproteins provide a platform for regulation of morphogen signaling.

**L86 O-GlcNAc Modification Is Involved in Neurite Outgrowth of Dopaminergic Neuronal Cells**

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β-O-linked N-acetylglucosamine (O-GlcNAc) is a nucleocytoplasmic post-translational modification on serine and threonine residues that is dynamically regulated by O-GlcNAcase transferase and O-GlcNAcase. Many proteins are O-GlcNAcylated in response to various cellular process, include transcription, proliferation, apoptosis and signal transduction. In the case of neuronal cells, there are many O-GlcNAcylated proteins that are related to neurodegenerative diseases. Neuronal differentiation process is largely studied, but it is rarely known the relationship between O-GlcNAcylation and neuronal differentiation. To examine whether O-GlcNAc modification is involved in neuronal differentiation process, we utilized neurite outgrowth model system induced by all trans retinoic acid (tRA). In dopaminergic neuron cell line, O-GlcNAcylated inhibitors are co-treated with tRA to prevent the decrement of intracellular O-GlcNAcylation level, and the extent of neurite outgrowth was increase 17% compared to tRA-treated neurons. The total extent of neurites, the primary neurite length and the number of neurites per cell were increased slightly. The activation of c-Jun N-terminal kinase (JNK) in tRA-induced neurite outgrowth process is previously reported, and in this study JNK seems to be less activated when O-GlcNAcase inhibitor is co-treated with tRA. Thus, our data indicate that O-GlcNAc modification seems to be involved in neurite outgrowth in cultured dopaminergic neuronal cells.

**L87 Cellular Individuality in Directional Sensing**

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It is generally assumed that single cells in an isogenic population, when exposed to an identical environment, exhibit the same behavior. However, it is becoming increasingly clear that even in a genetically identical population, gene expression levels can vary significantly from cell-to-cell giving rise to non-genetic individuality. It is an open question whether a conceptually similar individuality can be observed in other cellular activities, such as signal transduction. For example, it is unknown how the fidelity of sensing an extracellular cue varies from cell-to-cell. Here we explore this individuality in the gradient sensing response of single Dictyostelium cells when exposed to repeated spatiotemporal pulses of the chemoattractant cAMP. We find the response of a single cell to be highly reproducible from pulse-to-pulse, in contrast, a large variability in the response direction and magnitude is observed from cell-to-cell, even when different cells are exposed to the same pulse. We propose that the effective signal a cell detects is the product of the extracellular cAMP signal and an intracellular signal that varies in direction and magnitude from cell-to-cell. Using this model we successfully predict the observed variability in directional sensing. This stochastic aspect of directional sensing might, on one hand, fundamentally limit the fidelity of signal detection and, on the other hand, might be beneficial by diversifying phenotypes in an isogenic population.

**L88 Type III Phosphatidylinositol 4-kinase α Is Required for Pectoral Fin Development in Zebrafish**

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Phosphatidylinositol (PtdIns) 4-kinases (P4Ks) catalyze the synthesis of PtdIns4P, an intermediate in the formation of PtdIns(4,5)P2, the precursor of the important second messengers, diacylglycerol and Ins(1,4,5)P3. There are four mammalian P4Ks that belong either to the type-I or -II class (α and β forms in each), the latter being sensitive to high concentrations of PI3K inhibitors. We found the ortholog of the mammalian P4KII β and studied its function in Zebrafish development. Whole-mount in situ hybridization showed that Zebrafish P4KII β (DrP4KII β) mRNA was expressed ubiquitously throughout the early embryonic development, and by 48 hours post fertilization (hpf), its expression was detected primarily in the brain, branchial arches, and the pectoral fin buds. Injection of antisense morpholinos directed against DrP4KII β caused multiple developmental defects, yielding brain, trunk and tail abnormalities at 24 hpf stage that varied in severity. One of the most prominent and reproducible effect of the injection of DrP4KII β morpholinos, was a defect in the formation of the pectoral fin by 72 hpf. Further analysis indicated that two markers acting in the FGF signaling pathway, MKP3 and sef, showed greatly reduced expression in the branchial arches and in the pectoral fin bud but not in the tail and trunk. Thus, the embryos injected with the DrP4KII β morpholinos. Current work focuses on the molecular mechanism explaining the selective sensitivity for P4KII β depletion of some but not other components of the FGF signaling pathway.

**L89 Unique Modes of Regulation of RasGRP2**

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The RasGRP family of guanine nucleotide exchange factors catalyse the activation of Ras and Rap GTPases, and thus regulate the wide variety of cell signaling pathways which are activated by these GTPases. RasGRP1, which activates Ras GT-Pase, is itself regulated by translocation to membranes. In lymphocytes, induction of RasGRP1 Monday
translocation by antigen receptors is dependent on binding of its C1 domain to the second messenger diacylglycerol. In contrast to RasGRP1, little is understood about RasGRP2 regulation. RasGRP2 activates Rap1, is expressed in lymphocytes and is implicated in integrin signaling and cell adhesion processes. Because RasGRP2 also has a C1 domain, it has been assumed that RasGRP2 will behave the same as RasGRP1 and translocate to membranes in response to DAG generating B cell receptor stimulation. Contrary to expectations, transduced RasGRP2 is not equivalent to RasGRP1 in that it does not translocate to the plasma membrane in response to DAG treatment or BCR stimulation of DT40 B cells. Instead, transduced RasGRP2 is found to be constitutively bound to the plasma and internal membranes of both DT40 B and Jurkat T cells. We are identifying the domains of RasGRP2 which regulate its unique pattern of membrane localization. In addition we are attempting to understand why RasGRP2 differs from RasGRP1 and does not respond to DAG treatment or B cell receptor stimulation.

L90 The Systematic Endopolyploidy in Different Tissues of Diploid and Tetraploid Phaeopsis aphrodite subsp. formosana

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Endopolyploidy, the duplication of DNA without cell division, is an observed fact in seed plants concomitant with its developmental processes and results in multiple copies of chromosomes. Endopolyploidization is manifested in different organs distinctively. So far, mature organ with higher degree of endopolyploidy is detected in Arabidopsis and other examples and apparently a common phenomenon among plants. In Orchidaceae, the largest flowering family among plants, diverse patterns of endopolyploidy are indicated according to different species. Information of patterns in diploid and autotetraploid plants can offer how the ploidy level affecting endopolyploidization. Phaeopsis aphrodite subsp. formosana 2X and 4X plantlets are used for analysis of systematic endopolyploidy. For the study, flow-cytometry is employed to determine nuclear DNA content. The protocorm including primordia of diploid P. aphrodite has large 2C signals, however, tissues excluded and distanced from primordia contain increasing 4C and 8C nuclei dramatically and 2C still. There are comparable ploidy patterns existed in protocorm between diploid and tetraploid plantlets. The ploidy patterns are similar between diploid and tetraploid P. aphrodite, but diverse among different tissues. The ploidy patterns progressively altered on different differentiation stages of matured leaves from 2C to 2C. The cells in root tip region with vigorous cell division ability showed pattern in 2C and 4C, from matured and elongation region of roots, absence of 2C and abundant in 8C and 16C, both in diploid and tetraploid P. aphrodite. Our results indicate that endopolyploidization takes place during root early developmental stages and suggest that polyploidization is associated with cell differentiation and aging.

STEM CELL BIOLOGY

L91 Investigating Human and Mouse Embryonic Stem Cell Fate with Chemical Tools

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Cell replacement therapy has gained increasing enthusiasm due to the potential for generating subtype-specific neurons from hematopoietic progenitor cells. A method for generating subtype-specific neurons from hematopoietic progenitor cells is desired to expand the pool of neuron precursors available for replacement therapy. We have discovered three compounds with the ability to differentiate mouse ES cells into LMX1α+ early DA neuron progenitors. We have also been successful in generating Sox1+ NSCs with chemically defined media and are expanding our NSC culture into a homogeneous population for further studies in subtype specification and region patterning studies. Our present small molecule hits are being examined for their extent of mature midbrain (En+) dopamine neuron induction. Compounds with the ability to induce midbrain DA neurons will be characterized for SAR (structure activity relationship), optimized for efficacy, potency and specificity. The most active compounds will be used to fish out novel proteins/pathways in the regulation of neurogenesis, midbrain patterning, and DA neuron specification.
The Immunomodulatory Potency of Human Mesenchymal Stem Cells Is Conditional on Culture Conditions and the Responsiveness to Immunostimulative Signal
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Mesenchymal stem cells (MSCs) have been reported for their suppressive roles on the induction of immune response including T cell proliferation. This aspect is notable for their clinical efficacy as an immunosuppressant, preventing allogenic transplant from being rejected. However, the efficacy of the useful function has yet to be confirmed due to the existence of contradictory results, necessitating a better grasp of details. To understand the contributing mechanisms, we investigated on the conditional immunomodulatory effect of human MSCs derived from cord blood (hCB-MSCs). The inflammatory signal was measured where peripheral blood mononuclear cells (PBMCs) were mitogen-stimulated in the presence or absence of hCB-MSCs. We observed that the induction of interferon-γ secretion by mitogen stimulation was down-regulated in the presence of hCB-MSCs. In this study, we found that the immunosuppressive effect of hCB-MSCs is reduced if the physical contact with PBMCs is not allowed and negligible on the PBMCs stimulated in low density culture. We also compared various hCB-MSCs for the responsiveness to the immunostimulatory signal and selected quality cells, by detecting the potency markers for hCB-MSCs as an immunosuppressant, for example, Indoleamine 2,3-dioxygenase, prostaglandin E2 synthase and tumor necrosis factor receptor 1. Furthermore, the profiling of cytokine secretion of the immunostimulated hCB-MSCs made it possible to screen novel soluble factors which may potentially account for the corresponding immunosuppressive role of hCB-MSCs. In this study, we showed that the immunomodulatory effect of hCB-MSCs is conditional for culture environment, especially for the state of target cells, and provided the criteria on which the optimal quality of hCB-MSCs for clinical application can be selected.

Intracellular Signalling Pathways That Control the Transition of Embryonic Stem Cells to Primitive Ectoderm-like Cells
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Development in the early mammalian embryo results in the production of several distinct populations of pluripotent cells including the inner cell mass (ICM), primitive ectoderm, and primordial germ cells. Primitive ectoderm represents an obligatory pluripotent intermediate from which the three germ layers are derived at gastrulation. However, the molecular mechanisms controlling the formation of primitive ectoderm from the ICM are poorly understood. Culture of mouse embryonic stem (ES) cells in the presence of MEDII, a medium containing a high concentration of SCF, results in the formation of a homogenous population of early primitive ectoderm-like (EPL) cells. EPL cells are the in vitro equivalent of primitive ectoderm as demonstrated by morphology, gene expression changes and differentiation potential. The amino acid L-proline has been previously identified as the bioactive component of MEDII responsible for EPL cell formation. In this work we investigate the intracellular signalling pathways and molecular mechanisms involved in the L-proline-induced formation of EPL cells. Experiments performed using a Kinome Profiler™ approach identified a number of signalling molecules that are activated in ES cells when L-proline is added. Biochemical and functional validation of a subset of these molecules has suggested a requirement for the PI3K/Akt and MAPK in the ES-to-EPL cell transition induced by L-Proline. Manipulation of these pathways, either by addition of L-proline or pathway antagonists, can be used to promote the maintenance of ES cells or induce the formation of EPL cells, providing methodologies for the directed and regulated differentiation of ES cells to primitive ectoderm. These methods, combined with technologies that direct the differentiation of EPL cells to the somatic lineages, will have application in the formation of therapeutically useful cells with clinical applications from human pluripotent cells.

Induction of Neural Precursor Cells Differentiation by Human Umbilical Cord Blood Mesenchymal Stem Cells
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There have been several reports that human cord blood derived mesenchymal stem cells have therapeutic effects in neuronal diseases, although therapeutic mechanism of mesenchymal stem cell is controversial. The present study was designed to elucidate the paracrine mechanism of human cord blood mesenchymal stem cells on neuronal diseases. For this purpose, we have co-cultured neuroblastoma × glioma hybrid NG108-15 cells with human cord blood derived mesenchymal stem cells. After co-culturing, NG108-15 cells changed into bi- or multi-polar neuron like morphology and increased the expression of neuron related proteins. Interestingly, proliferation rate of NG108-15 cells also increased after co-culture. Neural differentiation and proliferation capacity of NG108-15 were aroused on dose dependent manners. Moreover mouse cortical neural precursor cells also changed into neuron like morphology and increased the proliferation rate by co-culturing with human cord blood mesenchymal stem cells. Our results suggest that human cord blood mesenchymal stem cells can induce partial differentiation of neural lineage progenitor or stem cells and simultaneously increase the proliferation rate of these cells.

Leucine-rich Amelogenin Peptide Induces Bone Formation in Mouse Embryonic Stem Cell
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The developing enamel matrix is comprised by a heterogeneous group of proteins known to regulate inorganic composition of the teeth. The majority of the organic enamel matrix protein mix is amelogenin, a family of highly conserved proteins implicated to control enamel crystallographic morphology. Leucine-rich amelogenin peptide (LRAP) has been identified as one of the most abundant amelogenin splice products detected in the developing enamel matrix. LRAP has been shown to function in cell signaling, and bone markers induction in cementoblasts. The objective of this study is to determine the effect of LRAP on osteogenesis in embryonic stem (ES) cell. Mouse ES cells (RW4) were induced to differentiation according to a standard protocol. In brief, cells were exposed to different cell culture media to promote osteogenesis. ES cells cultured for 10 days or 20 days following embryoid body formation were subjected to osteogenic marker analysis by quantitative real-time PCR, mineral deposition analysis by alizarin red staining, and calcium concentration analysis by Quantichrom Calcium Assay Kit. The ES cells in response to the LRAP exposure for 10 days showed significant increase in the mRNA expression of osterix (Oxs), bone sialoprotein (BSP) and osteopentin (OPN), but not osteocalcin (OCN) and core binding factor alpha-1 (Cbfa1/Runx2), when compared to the untreated control ES cell. Continued exposure of the ES cell to LRAP for up to 20 days resulted in marked increase in Oxs and BSP expression. Analysis of the effect of LRAP on the terminal phenotype of the LRAP-treated ES cells indicated significant increase in calcium concentration and mineral deposition. In conclusion, these data suggest the unique function of LRAP as a signaling molecule implicated in bone and mineral tissue formation based on the ability of LRAP to alter ES cell differentiation.

Involvement of the ER Stress Responses in Neuronal Induction of Rat Bone Marrow Stromal Cells
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Induction of the endoplasmic reticulum (ER) stress has been observed during cell differentiation. We examined the ER stress gene expression during the neuronal induction of rat bone marrow stromal cells (rBMSCs). Based on the expression of neuronal markers, rBMSCs differentiated into neuronal cells within 24 h. Neuronal induction transiently increased the expression of BiP that reached to the maximum at 12 h, suggesting the induction of ER stress responses. In addition, the expression of CHOP/GADD153 was significantly up-regulated with the peak level at 9 and 12 h after the induction at mRNA
L100
Isolation of Neural Crest-like Multipotent Cells Derived from Embryonic Stem Cells
Y. Zhou, M. L. Sneed; CCMB, University of Southern California, Los Angeles, CA

The neural crest is a transient population of multipotent progenitors arising at the lateral edge of the neural plate in vertebrate embryos. After delamination and migration from the neuroepithelium, these cells contribute to a diverse array of tissues throughout the embryo. Fate determination and differentiation of mammalian neural crest have been hindered by the lack of an in vitro culture system. Embryonic stem (ES) cells, which are able to spontaneously differentiate in ESCs to direct their differentiation. This element (U1) is one of several human sequences that regulates the expression during dopaminergic neuron development of midbrain dopaminergic neurons, the cells destroyed in Parkinson’s disease. NR4A2 (Nurr1) is essential for the genesis and survival of midbrain dopaminergic neurons, but little is known about the mechanisms by which this gene is regulated. Our goal is to identify transcription factors that regulate NR4A2 expression and use them to direct the differentiation of ESCs to dopaminergic neurons. To this end, we first sought to identify cis-acting regulatory DNA sequences to which these factors bind. We report the identification of a 242 base pair element that regulates NR4A2 during dopaminergic neuron development. This element (U1) is one of several human sequences that were selected for analysis based on high sequence conservation among mammals. U1 is sufficient to drive LacZ reporter expression in the ventral midbrain of transgenic mouse embryos in a pattern consistent with Nr4a2 and other markers of midbrain dopaminergic neurons. Furthermore, we report that the mechanism for NR4A2 regulation is conserved across a wide range of vertebrate species, as U1 drove reporter gene expression in zebrafish in a pattern that closely recapitulates that of zebrafish nr4a2. We have begun to study the role of U1 in ESC differentiation to dopaminergic neurons and are in the process of generating stable lines in which U1 drives a reporter gene. Finally, we report our progress toward the identification of transcription factors that bind this sequence.

L102
Neurotrophins Induce Trk-dependent Cleavage of p75NTNR in PC12 Cells and in Primary Neurons
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The neurotrophins (NTs) are soluble proteins that mediate survival, differentiation and growth of neurons. NTs mediate their actions by binding specific Trk receptors, which are receptor tyrosine kinases (RTKs) and by binding p75NTNR, a member of the TNFR superfamily. p75NTNR lacks intrinsic enzymatic activity and mediates neurotrophin signaling by binding cytosolic adaptor proteins. Recent studies have shown that p75NTNR is substrate for NT-mediated cleavage and that these cleavage events are important for neuronal function. In this study, we have examined the role of NTs in mediating cleavage events. Using PC12 cells as a model system, we found that p75NTNR undergoes robust cleavage in response to NT treatment. We demonstrated that NGF-induced p75NTNR cleavage is dependent upon -secretase and that the secretase activity and, surprisingly, show that this effect required expression and activation of the TrkA RTK and required TrkA-induced MEK activation. Finally, we demonstrated that the p75NTNR ICD that is generated by NT binding to the transmembrane receptor is required for neurotrophin-induced modulation of small GTPases. Taken together, these data demonstrate that Trk receptors activation induces - and -secretase-dependent cleavage of p75NTNR and suggests that the role of p75NTNR in neurotrophin signaling. * These authors contributed equally to this work. This project was supported by grant MOP37850 from the Canadian Institute of Health Research (to PAB).
L103
The Effects of Huvec Cells on SH-SY5Y Neurite Outgrowth and Proliferation
M. Porta, R. Shurina, B. M. Fenner; Biology, Wheeling Jesuit University, Wheeling, WV

Many neurological conditions, including stroke and neurodegenerative disease, induce a hypoxic state in the brain. This leads to decreased cerebral blood flow and, consequently, decreases in oxygen and glucose to neurons. Even acute disruptions in glucose and oxygen availability can result in catastrophic neurological events. These include induction of oxidative stress, increased intracellular free-radicals, disrupted neurotransmission, and potentially, cell death. Neurodegenerative diseases, like Alzheimer’s and Parkinson’s, are believed to be complicated by hypoxic conditions. One potential compensatory mechanism against hypoxic insult may include the induction of angiogenesis. The formation of new blood vessels results in: 1) secretion of angiogenic factors like vascular endothelial growth factor (VEGF), 2) activation of endothelial cells to form tubules, and 3) the delivery of oxygen/glucose-rich blood to compromised regions of the brain. Recent evidence has shown that angiogenic factors have neurotrophic effects in the brain. Therefore, the induction of angiogenesis may rescue damaged neurons by increasing blood flow and directly improving neuronal survival and neurite outgrowth. The NO-cGMP pathway is proposed to be involved in angiogenesis and by directly inducing neuronal survival, proliferation, and neurite outgrowth. We hypothesize that endothelial activation will induce neuronal proliferation and neurite outgrowth. To investigate the effects of endothelial activation on neuronal proliferation and neurite outgrowth we co-cultured SH-SY5Y cells with activated HUVEC cells. Following HUVEC exposure, we analyzed neurite outgrowth by double- and triple-label immunofluorescence microscopy (IFL). IFL analysis showed that HUVEC cells induced extensive neurite outgrowth in SH-SY5Y cells, with maximal neurite outgrowth observed at 72hrs. The effect of HUVECs on neurite outgrowth was most pronounced in the cultures pre-treated with retinoic acid (RA). Neuronal cells exposed to HUVECs without RA showed increased cell proliferation in response to HUVEC treatment. Neurite outgrowth was evident at 72hrs, but not to the extent of RA pre-treated cells. Current studies are quantifying neuronal proliferation by the 96-well Aqueous CellTiter assay.

L104
NO-cGMP Pathway Modulates Actin Remodeling during Neuronal Differentiation
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Actin remodeling is a key mechanical and molecular process underlying migration, differentiation and synaptic plasticity of vascular and neuronal cells. Recent data on neurite outgrowth and cell motility suggest a role for the NO-cGMP pathway in neuronal differentiation and migration. NO activates the heme containing heterodimer soluble guanylyl cyclase (sGC), which in turn produces cGMP. We investigated whether the role of NO signaling in neuronal differentiation is dependent on its effect on actin architecture via cGMP modulation. Using a CNS catecholaminergic cell line (CAD), which differentiates in a serum-deprived medium, we report that NO-stimulated sGC expression was differentially modulated in non-differentiated and differentiated neuronal cells. Disruption of sGC expression by siRNA and of its activity by the inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinolin-1-one (ODQ), severely blocked neurite outgrowth in its early steps, thereby preventing cells from growing via neurotropic responses to angiogenic factors. We hypothesize that endothelial activation will induce neuronal proliferation and neurite outgrowth. To investigate the effects of endothelial activation on neuronal proliferation and neurite outgrowth we co-cultured SH-SY5Y cells with activated HUVEC cells. Following HUVEC exposure, we analyzed neurite outgrowth by double- and triple-label immunofluorescence microscopy (IFL). IFL analysis showed that HUVEC cells induced extensive neurite outgrowth in SH-SY5Y cells, with maximal neurite outgrowth observed at 72hrs. The effect of HUVECs on neurite outgrowth was most pronounced in the cultures pre-treated with retinoic acid (RA). Neuronal cells exposed to HUVECs without RA showed increased cell proliferation in response to HUVEC treatment. Neurite outgrowth was evident at 72hrs, but not to the extent of RA pre-treated cells. Current studies are quantifying neuronal proliferation by the 96-well Aqueous CellTiter assay.

L105
Optimal Treatment Period for Forskolin Induction of a Reactive-like Condition in F98 Gliona Cells
J. Neece, D. M. Garcia, J. R. Koke; Biology, Texas State University- San Marcos, San Marcos, TX

In response to injury, astrocytes in situ initiate proliferation and cellular hypertrophy; a phenomenon termed reactive astrogliosis. The intracellular signaling pathways are poorly characterized. It is known that phosphorylation of nuclear lamins occurs in mitotic prophase, and previously we have shown that mAB J1-31 may recognize a phosphorylated motif on GFAP, lamin B, and possibly other IF proteins (Garcia et al., Brain Res 2003, 976:9-21). Therefore, labeling of nuclear "dots" by J1-31 may be a useful indicator of the onset of "reactivity." We have further shown that activation of adenyly cyclase by forskolin in 2-day-old F98 cell cultures will increase the number and intensity of J1-31 nuclear "dots" that also label with anti-lamin B antibodies. Inhibitors suggest that PKA is not involved, but a cAMP-mediated Ca2+ channel may be. To further understand the intracellular pathways leading to phosphorylation of lamins in proliferating F98 cells, we conducted a time course experiment to determine the optimal duration forskolin treatment time that would elicit the strongest J1-31 labeling response. Gliona astrocyte F98 cells were cultured on slides and treated with 4µM forskolin in 10 minute increments for up to one hour. The cells were then fixed and labeled with the J1-31 monoclonal antibody. Confocal imaging shows J1-31 antigen labeling maximizes at 30 minutes, followed by a sharp attenuation. This work supported by the Texas Higher Education Coordinating Board #003658-04906-01, NSF-ESI #973122.

LEUKOCYTES

L106
Urokinase Suppresses Mononuclear and Eosinophilic Leukocyte Infiltration in a Rabbit Model
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Blood clotting is a prominent feature in inflammation. Fibrin and its degradation products are shown to have chemotactic activity to leucocytes. Urokinase and plasmin are also shown to modulate inflammatory responses in an experimental arthritis probably by their receptor-mediated signaling, however, their effects on inflammation are still controversial. To address this question, we studied effect of immobilized urokinase on leukocyte infiltration to implanted polyurethane tubes. Urokinase was immobilized on polyurethane tubes at an activity of 5.3±0.4 IU/cm² and small pieces of the tubes were embedded in rabbit back muscle for 3 months. As a control, non-treated polyurethane tubes were also embedded in the same animals. Animals were sacrificed at 1 week, 1 month and 3 month after the tube implantation and muscle tissues around the tubes were cut out for histological examination. Mononuclear, eosinophilic and neutrophilic leukocytes were counted and degree of fibrosis was scored in each tissue. Residual urokinase activity on the harvested tubes was measured by hydrolytic activity for a fluorescent substrate peptide. Mononuclear leukocyte accumulation around implanted polyurethane tubes peaked at 1 month, but was reduced significantly by urokinase immobilization. The treatment also reduced as well as delayed eosinophil accumulation, but did not affect fibrosis caused by implanted tubes. Neutrophilic leukocytes accumulation around the tubes was negligible, regardless of urokinase immobilization. Urokinase activity was detected significantly at least for a week but undetectable after 1 month of implantation. These results indicate that urokinase reduces mononuclear and eosinophilic leukocyte accumulation in polyurethane-elicited inflammatory responses, which may be due to receptor-mediated signaling by generated plasmin. Furthermore, persistently active urokinase on the material surface, suppressing inflammatory responses, may be beneficial for a long-term device implantation in the body.

L107
The Effects of Huvec Cells on SH-SY5Y Neurite Outgrowth and Proliferation
M. Porta, R. Shurina, B. M. Fenner; Biology, Wheeling Jesuit University, Wheeling, WV

Many neurological conditions, including stroke and neurodegenerative disease, induce a hypoxic state in the brain. This leads to decreased cerebral blood flow and, consequently, decreases in oxygen and glucose to neurons. Even acute disruptions in glucose and oxygen availability can result in catastrophic neurological events. These include induction of oxidative stress, increased intracellular free-radicals, disrupted neurotransmission, and potentially, cell death. Neurodegenerative diseases, like Alzheimer’s and Parkinson’s, are believed to be complicated by hypoxic conditions. One potential compensatory mechanism against hypoxic insult may include the induction of angiogenesis. The formation of new blood vessels results in: 1) secretion of angiogenic factors like vascular endothelial growth factor (VEGF), 2) activation of endothelial cells to form tubules, and 3) the delivery of oxygen/glucose-rich blood to compromised regions of the brain. Recent evidence has shown that angiogenic factors have neurotrophic effects in the brain. Therefore, the induction of angiogenesis may rescue damaged neurons by increasing blood flow and directly improving neuronal survival and neurite outgrowth. The NO-cGMP pathway is proposed to be involved in angiogenesis and by directly inducing neuronal survival, proliferation, and neurite outgrowth. We hypothesize that endothelial activation will induce neuronal proliferation and neurite outgrowth. To investigate the effects of endothelial activation on neuronal proliferation and neurite outgrowth we co-cultured SH-SY5Y cells with activated HUVEC cells. Following HUVEC exposure, we analyzed neurite outgrowth by double- and triple-label immunofluorescence microscopy (IFL). IFL analysis showed that HUVEC cells induced extensive neurite outgrowth in SH-SY5Y cells, with maximal neurite outgrowth observed at 72hrs. The effect of HUVECs on neurite outgrowth was most pronounced in the cultures pre-treated with retinoic acid (RA). Neuronal cells exposed to HUVECs without RA showed increased cell proliferation in response to HUVEC treatment. Neurite outgrowth was evident at 72hrs, but not to the extent of RA pre-treated cells. Current studies are quantifying neuronal proliferation by the 96-well Aqueous CellTiter assay.
A Spirochetal Outer Sheath Protein Selectively Inhibits Ral1 Activation in Murine Neutrophils

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The Ral GTPases play an important role in a wide range of biological processes, including essential mechanisms of innate immunity. Acting as “molecular switches”, these small GTPases regulate cytoskeleton reorganization, cell growth, gene transcription, motility, adhesion, and production of reactive oxygen species (ROS). *Treponema denticola* major outer sheath protein (Msp) is known to interact with host immune cells during periodontal disease, and it inhibits neutrophil chemotaxis and phagocytosis in vitro. The OBJECTIVE of the study was to analyze the effects of Msp on Ral activation in murine neutrophils. METHODS: For the p21-binding domain assay, murine neutrophils were isolated and incubated with 0.5, 1.0, 3.0 and 6.0 µg/ml Msp for 30 minutes following activation with fMLP for 60s and cell lysis. Activated RalGTPases were recovered using Glutathione-S-transferase (GST)-PBDbeads. Western analysis was performed using SDS-PAGE and immunoblotting with mouse monoclonal antibodies anti-Ral1 or anti-Ral2. Transfections were performed using Amaxa’s Nucleofector device. Murine primary neutrophils were isolated and transfected with PAKT-GFP and PAK-PBD-YFP, respectively. In incubation with 6.0 µg/ml Msp for 30 minutes. Cells were exposed to an fMLP gradient in a Zigmoid chamber and analyzed by fluorescence microscopy. Results were analyzed by ANOVA and Bonferroni test. RESULTS: Msp selectively inhibited fMLP-mediated Ral1 activation dose-dependently, reducing the Ral1 activation to basal levels at 3.0 µg/ml. Msp showed no effect on Rac 2 activation at the tested concentrations. Fluorescent images showed that Msp treatment inhibited the polarized accumulation of activated Rac and PEBK products at the leading edge during neutrophil chemotactic migration. CONCLUSIONS: Msp inhibition of chemotaxis may be due to suppression of the PEBK/Rac1 activation pathway in neutrophils. By investigating the underlying mechanisms, we may learn more about how bacterial proteins may modulate neutrophil functions and develop novel therapeutic strategies targeting Rac. Supported by CIHR MOP-5619 and CIHR MOP-53136.

Inhibitory Role of SK-126 in the Production of Inflammatory Cytokines Induced by LPS in Antigen-presenting Cells

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A variety of mediators released by immune cells trigger or enhance specific aspects of the inflammatory response. Dendritic cells play an essential role in the innate immune system in shaping adaptive immune responses and control production of cytokines in response to inflammatory stimuli. SK-126, a pyridine derivative based on lead structures of bacterial proteins, we investigated the involvement of MAPK, and transcription factor, NF-κB, after treatment with SK-126. SK-126 inhibited ERK1/2 activation and enhanced p38 activation after LPS stimulation, but it didn’t induce phosphorylation of SAP/JNK and NF-κB. Using specific inhibitors, we confirmed that SK-126 has dual effects in which it suppresses TNF-α production through the down-regulation of ERK1/2 and enhances IL-10 production through the up-regulation of p38. Therefore, our findings suggest that SK-126 may be a useful candidate to treat inflammatory diseases in which pro- or anti-inflammatory cytokines play a significant role in their pathogenesis.

CaMKII δ & γ Isoforms Regulate the Function of Human Dendritic Cells

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Dendritic cells (DC) are a critical bridge between innate & adaptive immune responses. However, the molecular mechanisms that regulate DC activation and functional responses are not fully understood. Utilizing gene-selective chemical inhibitors, our lab has demonstrated that Ca2+/Calmodulin-dependent protein kinase II (CaMKII) regulates the maturation and antigen presentation processes of human DC. Since CaMKII is comprised of 12 subunits derived from four genes (α, δ, β, γ), each of which encodes multiple splice variants, a more specific definition of CaMKII functions in DC via RNAi or targeted mutations requires characterization of isoform expression and its modulation during maturation. As determined by RT-PCR, human DC express mRNA for the δ and γ isoforms of CaMKII, but not the α or β isoforms. qRT-PCR analysis demonstrated that maturation of DC by both LPS and particle-bound tetanus toxoid (PB-TT) was accompanied by modulation of mRNA levels for the δ and γ isoforms. These data define the isoform expression of CaMKII in immature and mature human DC, and will enable assessment of isoform-specific functions and targeted modulation of their levels for therapeutic purposes.

Characterization of Macrocyclic Antimicrobial Peptides from Olive Baboon Leukocytes

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Defensins are antimicrobial peptides that are expressed in plants, insects, and vertebrates, and they have been implicated as effectors of innate immunity. In mammals, three structural subfamilies are expressed, the α, β, and γ-defensins. β-defensins are unique in that they are the only known macrocyclic peptides produced in animals. 0-defensins are expressed in leukocytes of Old World monkeys, but not in New World primates or prosimians. Humans and some apes express 0-defensin pseudogene mRNAs bearing a mutation that encodes a premature stop codon. We examined defensin expression in the olive baboon, a species commonly used as a model for the study of human diseases. RT-PCR analysis of olive baboon bone marrow RNA disclosed four prepro-0-defensin genes that differ from those characterized in other primates. 0-defensins are produced by binary, head-to-tail splicing of peptide segments obtained from paired precursors. Therefore, in theory, ten unique peptides could be generated from the pairing of four precursors. Five of the theoretical 0-defensins were identified in extracts of olive baboon leukocytes and purified to homogeneity. Immunohistochernical staining demonstrated that 0-defensins localize to the cytoplasmic granules of baboon neutrophils and monocytes. We synthesized the native (cyclic) form of the five isolated baboon 0-defensins and showed that they were biophysically indistinguishable from the leukocyte-derived molecules. The antimicrobial properties of the synthetic peptides were evaluated for their bactericidal and fungicidal properties in vitro. These experiments demonstrated that 1-4 µM of each peptide killed > 3 log of gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria, and fungi (*C. albicans*) in a 2 hour incubation. However, significant differences in target-specific potency were observed among the five baboon 0-defensins, suggesting that 0-defensin sequences diverged to neutralize a broad range of microbial species.

Murine Macrophage Activation in Response to Acute Restraint Stress

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Activated macrophages (mφs) are involved in multiple stages of an immune response and are capable of rapidly recognizing and destroying foreign pathogens and tumor cells. Following T cell activation activated mφs release reactive nitrogen intermediates, such as nitric oxide (NO) and produce a variety of cytokines including tumor necrosis factor-alpha (TNF-α). We examined the effects of acute restraint stress on events coupled to mφ activation. T-cell-derived cytokines such as interferon gamma (IFN-γ) along with bacterial lipopolysaccharide were used to activate mφs in vitro. Activation was...
was concluded therefore that cell spreading was dependent on Ca\textsuperscript{2+} just under the plasma membrane during Ca\textsuperscript{2+} influx causing activation required for the maximal rate of neutrophil spreading induced by IP\textsubscript{3}. Initial experiments have also suggested that calpain activity is signal, which can be shown to be the result of Ca\textsuperscript{2+} influx. Neutrophils in the flatter (spread) morphology of cells on the adherent surface. This

L112  
IP\textsubscript{3} -Induced Cell Spreading of Human Neutrophils Requires Ca\textsuperscript{2+} Influx

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Adherence of neutrophils to the endothelium is a necessary prelude to extravasation and requires a major morphological change of the neutrophil from the round quasi-spherical shape of circulating cells, to the flatter (spread) morphology of cells on the adherent surface. This event is triggered biochemically by the engagement of ICAM-1 on the endothelium with \( \beta\text{-integrin} \) on the neutrophil, which also induces a large rise in cytosolic free Ca\textsuperscript{2+}. As there is a similar Ca\textsuperscript{2+} signal during the slower spontaneous adhesion of neutrophils under experimental conditions, it has been suggested that the Ca\textsuperscript{2+} signal triggers an acceleration of the morphological change. In order to test this, neutrophils were loaded with caged IP\textsubscript{3} and the Ca\textsuperscript{2+} sensor, fluo4, from their respective acetoxy-methyl esters. Photolytic uncaging of IP\textsubscript{3} (360nm exposure) caused a transient cytosolic free Ca\textsuperscript{2+} elevation up to 600nM. This was composed of an initial localized Ca\textsuperscript{2+} puff (which presumably arose from release of stored Ca\textsuperscript{2+}) and a larger global Ca\textsuperscript{2+} signal, which can be shown to be the result of Ca\textsuperscript{2+} influx. Neutrophils that had settled on the glass coverslip but had not begun to spread, underwent a phase of rapid cell spreading at the time of IP\textsubscript{3}-induced glucose \( i^{\text{e}} \) elevation. Ni\textsuperscript{2+} blocked both the Ca\textsuperscript{2+} influx phase following IP\textsubscript{3} uncaging and the accompanying induced cell spreading. It was concluded therefore that cell spreading was dependent on Ca\textsuperscript{2+} influx. Initial experiments have also suggested that calpain activity is required for the maximal rate of neutrophil spreading induced by IP\textsubscript{3}. These data were consistent with a localized high concentration of Ca\textsuperscript{2+} just under the plasma membrane during Ca\textsuperscript{2+} influx causing activation of calpain, cleavage of peripheral cytoskeletal components and induction of spreading. Calpain activity may thus be a target for therapeutic intervention in modulating the rate of neutrophil extravasation at inflamed sites.

L113  
Multiparametric Flow Cytometric Analysis to Evaluate Responding Effector T Cell Populations

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Cytotoxic effector lymphocytes (CTL) are the primary cells of the acquired immune response for defense against viral infections and tumors. The accurate analysis of such an effector population is critical to the elucidation of correlates of protection for diseases involving cellular immunity. Once validated, these signatures can be routinely used as surrogate markers of efficacy in preventative and therapeutic strategies involving immune response. Multiparametric flow cytometry has enabled the accurate identification and evaluation of such finely targeted lymphocyte subpopulations. In the current study, the authors have evaluated the functional capacity of effector T cells using 5 color, 7 parameter flow cytometry by interrogating a combination of phenotypic and functional surface and intracellular markers. Peripheral blood mononuclear cells obtained from healthy donors were subjected to restricted polyclonal stimulation using Staphylococcal enterotoxin B or peptide specific stimulation for varying times ranging from 6-72 hrs. The T cell populations were analyzed using combinations of markers differentiating naive, effector, memory, activated and proliferating subpopulations along with functional evaluation utilizing intracellular cytokie, granzyme B, perforin and degranulation as assessed by activation-induced CD107 expression. Our findings indicate that there is a complex profile of effector T cells that varies with the donor and time post stimulation. The functional capacity of effector T cells is especially dependent on the "instinctive" state of the donor PBMC population with granzyme B upregulation being the most striking feature of the response. Such profiles when correlated with disease outcome could enable the targeted identification of effector subpopulations associated with therapeutic success thus leading to the development of "surrogate profiles" of efficacy.

L114  
Mechanism of Taxol-induced Apoptosis in Human Ovarian Carcinoma Cells

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Taxol is extensively used in clinical field for chemotherapy against ovarian, breast and lung cancer, and also it is well known that the compound induces apoptosis of these cancer cells. However, the exact mechanism of the apoptosis inducing effect is not fully understood. The c-Jun NH2-terminal kinase (JNK) of mitogen-activated protein (MAP) kinases is activated when cells are exposed to environmental stress and it can cause apoptosis by a mechanism that involved the mitochondrial pathways. But the physiological function of the JNK signaling pathway is unclear. One of the trials to elucidate the mechanism of the apoptosis induced by taxol, we studied on key mediators of apoptotic pathway, namely, JNK, Bcl-2, and AIF, by treatment to SKOV-3 ovarian carcinoma cell line with taxol. It was confirmed that taxol induced phosphorylation of JNK1, de polarization of mitochondria and apoptosis in the SKOV-3 cells at the concentration of 100 nM. And AIF was translocated to the nucleus from mitochondria. Our data also show that inhibition of phosphorylation of JNK was not caused depolarization of mitochondria or translocation of AIF, and even apoptosis. These data suggest that taxol induced a caspase-independent and AIF-dependent apoptosis and especially, JNK is one of key mediators in SKOV3 carcinoma cells.

L115  
Correlated Three-Dimensional Light and Electron Microscopy Reveals the Mechanisms of Cytochrome C Release during Apoptosis

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Mitochondria initiate and/or regulate the apoptosis (programmed cell death) through the release of cytochrome \( c \) and other proteins from the intermembrane and intracisrtal compartments. Two principal mechanisms have been proposed by which cytochrome \( c \) can be released from the outer membrane: (1) Opening of the mitochondrial permeability transition pore (mPTP) producing large amplitude swelling of the mitochondrial matrix rupturing the outer membrane, or (2) Formation of a large pore in the outer membrane through which proteins can exit. We have studied this process in HeLa cells in which apoptosis was initiated by various agents using correlated light and electron microscopy monitoring the release of cytochrome \( c \) in cells transfected with fluorescent cytochrome \( c \) fusion proteins and stained with TMRE to monitor mitochondrial membrane potential (\( \Delta \Psi_m \)). Calcein staining was used to monitor opening of the mPTP. Previously we observed that apoptosis initiated by etoposide, actinomycin, or staurosporine induced a dramatic transformation of the mitochondrial inner membrane during release of cytochrome \( c \) into a form in which the matrix was fragmented into individual vesicular compartments; swelling occurred only later after the loss of \( \Delta \Psi_m \). In this study, we used H\textsubscript{2}O\textsubscript{2} to induce apoptosis in HeLa cells as a model for ischemia/reperfusion injury. Initiation of apoptosis by H\textsubscript{2}O\textsubscript{2} results in immediate loss of \( \Delta \Psi_m \), subsequent activation of mPTP, and large amplitude swelling of the mitochondrial matrix that appears to be the cause of cytochrome \( c \) release as in mechanism (1). Cyclosporin A, an inhibitor of the mPTP, prevents the immediate loss of \( \Delta \Psi_m \), but does not prevent the release of cytochrome \( c \). Interestingly, Cyclosporin A inhibits mitochondrial swelling and induces formation of the vesicular structure suggesting release of cytochrome \( c \) via mechanism (2). Thus, our results indicate...
that both mechanisms for the release of cytochrome c exist in HeLa cells during apoptosis initiated by H2O2.

L116 Cytoplasmic HuR Expression Is a Prognostic Factor in Familial Breast Cancer
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Breast cancer is the most common cancer among women. Although the survival rate is getting better new clinically valuable prognostic parameters are needed. HuR is a ubiquitously expressed mRNA binding protein and its increased cytoplasmic expression has been associated with reduced survival in breast, gastric and ovarian cancers. Family history is one of the strongest risk factor for breast cancer development and two dominantly inherited genes, BRCA1 and BRCA2, have been identified and shown to predispose to breast and ovarian cancer. Here we have studied the prognostic relevance of cytoplasmic HuR expression in familial breast cancer. Our patient material consists of 641 familial breast cancer specimens with normal and mutated BRCA1/2 gene status. We found that in a patient group with normal BRCA1/2 gene (n = 525) the cytoplasmic HuR expression was seen in 39.5% of the cases and it associates with estrogen and progesterone receptor negativity, p53 positivity, tumor grade, anti-estrogen treatment and ductal type of the tumor, and it was an independent prognostic marker in this group. In patients with mutated BRCA1/2 gene (n = 98) the cytoplasmic HuR expression was seen in 63.3% of the cases and it could not predict survival. These results show that mutation in BRCA1/2 gene make HuR translocates from the cytoplasm even more efficient than what was seen in normal BRCA1/2 group. However, the reason that cytoplasmic HuR expression did not associate with survival anymore might be a consequence of the disturbed BRCA1/2 function and that followed severe changes to cell function. Results in the group with normal BRCA1/2 are similar to our earlier findings and supports the hypothesis that HuR can work as a prognostic marker and possibly contributes to the carcinogenic pathway of the hereditary BRCA1/2 negative breast cancer.

L117 The Expression and Localization of Melanoma Inhibitory Activity (MIA) and Its Functional Role in Breast Cancer
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Melanoma inhibitory activity (MIA), also called cartilage derived retinoic acid sensitive protein (CD-RAP), is a small, secreted protein that is normally expressed in chondrocytes and appears to be a pivotal player in their differentiation. Initially identified as a tumor-suppressor, MIA is now a major metastatic marker for melanoma. Melanoma patients expressing high levels of serum MIA have a poor prognosis and exhibit a reduced response to various chemotherapeutic agents. The conservation among us to look for the mutated MIA and metastatic potential in various breast cancer cell lines. The cell lines chosen ranged from relatively benign to highly metastatic. We examined the expression and localization of MIA by Western Blot Analysis, Reverse Transcriptase PCR (RT-PCR) and immunofluorescence. Western Blot and RT-PCR detected MIA in the MDA-MB-231, MDA-MB-468, Hs578T, BT-549, MDA-MB435, SKBR3 and T-47-D cell lines. Analysis of preliminary data suggest a positive correlation between MIA expression and tumor progression in these cell lines as in melanoma and some other cancer cells. Hence, MIA has the potential of being a predictive marker for patients' outcomes in breast cancer.

L118 Isolation and Characterization of Cells with Potential Stem/Progenitor-like Properties from an Ovarian Carcinoma Cell Line
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We cloned the ovarian carcinoma cell line SKOV3 at limiting dilution and isolated multiple clones with epithelial and fibroblastoid morphology, which by western blot resulted E-cadherin and N-cadherin positive, respectively. By immunofluorescence analysis, we found that E-cad positive clones maintained, as parental SKOV3 cells, heterogeneous E-cad expression, whereas N-cadherin positive clones were stably E-cadherin negative. Moreover, we found that the E-cad positive phenotype is associated to higher proliferative potential in vitro and in vivo, the E-cad positive clone E4 displayed ten fold higher tumorigenic potential than the N-cad positive clone E1, when injected s.c. in nude mice. Since in vitro E4 cells express at the mRNA levels the stem cell markers nestin, oct-4 and nanog, and at higher levels than E1 clone, we hypothesize that E4 cells are enriched for a subpopulation with stem/progenitor-like characteristics surviving into the stabilized cell line SKOV3. Starting from a solid E4 tumor fragment propagated in vitro in adherent conditions and in the presence of 10% FCS, spheroids released from the adherent mononucleate were dissociated and maintained in vitro in non-adherent conditions in serum-free medium with the addition of growth factors. At now, more than 20 passages of primary spheres have been performed. Cloning experiments demonstrated that single cells have self renewal capability giving rise to new sphere formation. Spheres express markers characteristic of multiple differentiation pathways and when grown in adherent conditions, clones with different phenotype were morphologically evident. When i.p injected, in nude mice, spheres maintained the capability to form tumors which, in vitro give rise to new sphere populations. Further characterization is being carried out in the perspective to identify potential cancer stem cells in samples derived from surgical specimens of ovarian cancer. Partially supported by AIRC and MRF2005.

L119 Identification and Characterization of Novel Protein (L3) Interacting with Human 8-oxoguanine DNA Glycosylase (hOGG1)
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Cellular DNA is damaged continuously by reactive oxygen species (ROS) that are generated by the by-products of respiration or other extrinsic factors. ROS-induced DNA lesions involve several base modifications in either free nucleotides or DNA including a relatively stable oxidized form of guanine, 8-oxo-7,8-dihydroguanine (8-oxoG). 8-oxoG is a major oxidative DNA lesion induced by ROS that has been strongly implicated in cancer and aging. Human 8-oxoguanine DNA glycosylase (hOGG1) initiates the repair of 8-oxoG by the removal of mutagenic 8-oxoG lesion situated opposite cytosine. Apyrine/apyrimidine (AP) site generated by the elimination of 8-oxoG in 8-oxoG/C mismatched DNA duplex is cleaved by human AP-endonuclease (APE1), which displaces hOGG1 bound to AP site and thus increases the turn-over of hOGG1. In this work, we identified a novel protein interacting with hOGG1 using a yeast two-hybrid system. The physical interaction of hOGG1 and L3 (we call this novel protein as L3) was confirmed by GST pull-down assay and co-immunoprecipitation assay using microbial and mammalian expression system. To estimate the effect of L3 on the glycosylase activity of hOGG1, hOGG1 and L3 proteins were purified from E. coli BL21(DE3) cells transformed with hOGG1 and L3 expressing plasmids and were used in cleavage assay using [32P] labeled 8-oxoG/C mismatched duplex. The physical interaction of hOGG1 and L3 has been decreased by 50% as compared to control by hOGG1 were increased by dose-dependent manner and were increased by the addition of purified L3. In trapping assay, the content of hOGG1 trapped with 8-oxoG/C mispaired DNA duplex was increased by the presence of L3. Interestingly, L3 interacted with other DNA glycosylases such as NEIL1, NTH1 and TDG in GST-pull down assay. These results suggest that L3 has a capacity to bind DNA glycosylases and stimulate enzymatic activities of DNA glycosylases.

L120 Phenotypic Changes of Malignant Breast Cancer Cell Line by Overexpression of NDRG2: Role of NDRG2 as a Tumor Suppressor Gene in Breast Cancer
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NDRG2 belongs to the NDRG family, a new family of differentiation-related genes, and it is highly expressed in the adult brain, salivary gland, and skeletal muscle. It has been implicated in cell growth, differentiation, and apoptosis, and is rapidly responsive to mineralocorticoid stimuli in the kidney. Most recently, it has been shown that its down-regulation is involved in the development of aggressive forms of brain cancer. At first, we examined NDRG2 expression in breast cancer tissue and a number of breast cancer cell lines. NDRG2
was generally expressed in normal breast tissues and breast cancer cell lines showing a weak malignancy, while highly metastatic cancer tissues and cell lines were found to be negative or weak positive for NDRG2 expression. To investigate the function of NDRG2 gene, the gene was overexpressed in NDRG2-negative MDA-MB-231 breast cancer cell line and stable cell lines were established. NDRG2-positive transfectants showed apparent morphological changes and decrease in proliferation rate compared to wild type cell line. We found increased level of E-cadherin expression in NDRG2-positive transfectants, and this may contribute to attenuate invasiveness of cancer cells. The transcriptional expression and activity of several matrix metalloproteinases were also modulated in NDRG2-positive transfectants. Moreover, we selected a few of interesting genes by DEG screening that makes it possible compare wild type with NDRG2-positive transfectants based on gene expression. Among these differential genes, we clearly observed the specific induction of BMP-4 expression in NDRG2-overexpressed cell line. Taken together, our data indicate that overexpression of NDRG2 as a tumor suppressor gene may contribute to the delayed tumor progression and further studies in relation to BMP-4 will provide more information for effective growth control of breast cancer.

METABOLIC DISEASES

L121

Lack of Erythropoietin Receptor in Non-hematopoietic Tissues Results in Insulin Resistance
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Erythropoietin (Epo), the main regulator of erythropoiesis, is required for the stimulation and differentiation of erythroid progenitor cells. This function is transduced by the specific cell surface Epo receptor (EpoR). However, Epo activity is not restricted to hematopoietic tissue. To study the broader function of Epo, EpoR null mice were rescued with a transgene expressing EpoR exclusively in hematopoietic tissue. These rescued mice exhibited normal appearance, complete blood counts and histological analysis of pancreas, liver, spleen, heart, kidney and brain. However, the body weights increased significantly faster than in control mice from the first week after birth, and continued beyond 16 months. Body composition at 8 months indicated the fat mass in rescued mice was 2 times that of controls in males and 3 times in females with no difference in lean mass. Rescued mice also had fasting hyperglycemia, higher serum insulin (2–3 times) and triglyceride (>30%) concentration, were glucose intolerant and did not show hyperphagia. In this study, we demonstrate that hyperphagia is not the reason of obesity. However, indirect calorimetry assay on these mice demonstrated a significantly decreased metabolic rate and activity compared with the control mice. Given the extensive expression of EpoR in endothelium, the central nervous system and other tissues, we conclude that Epo/EpoR signaling is necessary for basal metabolism and activity. Preliminary measurements of activity in younger mice suggest that reduction in physical activity may be the primary cause of obesity in these animals.

L122

Using Molecular Evolution to Dissect the Function of NPC2 (Niemann-Pick Type C2)
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Mutations in two genes (NPC1 and NPC2) cause defects in transport and processing of cholesterol and other lipids, resulting in the neurodegenerative disease Niemann-Pick Type C. Thus far, little is known about the physiologically relevant activities of these proteins or the precise mechanisms by which they cause disease. As a means to gain insight into these issues, we are studying the molecular evolution of the NPC1 and NPC2 proteins, and report here on our analysis of NPC2. We have identified the set of NPC2 proteins in vertebrate organisms, performed multiple sequence alignments and phylogenetic analysis, and used this information to calculate position-specific conservation scores. By mapping these scores onto the NPC2 crystal structure, we have identified evolutionary constrained surfaces of the human NPC2 protein. This analysis has allowed us to identify three regions that are likely involved in functionally significant protein-protein interactions. Using similar approaches to study the multi-gene insect NPC2 family, we have identified several regions that are conserved within subfamilies but divergent between them, suggesting that these regions are involved in functional differentiation between the insect NPC2 subfamilies. Finally, we have identified in NPC2 a hydrophobic "knob" that is well-conserved at the level of composition, but poorly conserved in primary sequence, suggesting that it may be involved in binding membranes. This analysis should provide the basis for directed experimental dissection of NPC2 function, which in the long term will hopefully lead to more effective NPC therapy. Supported by the Ara Parseghian Medical Research Foundation.

L123

Obesity Involves Antiapoptotic Mechanism to Avoid Apoptosis Due to ER Stress
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Obesity persists with chronic lipid biosynthesis and eventual overload in adipogenic cells. As with increased secretion of secretory proteins, the on-going lipid deposition eventually serves as a stress factor to the endoplasmic reticulum (ER). Under minor ER stress conditions, cells temporarily slow down the number of client proteins coming into the ER and increases the number of helper molecules. Sustained severe ER stress often results in apoptosis. In this report, we have discovered that the mitochondrial apoptotic pathway may be down regulated among ER stress-induced apoptosis by obesity. Under circumstances, an unusual link was observed between ER stress and the mitochondrial apoptotic pathway: obesity conditions inhibit the earliest apoptotic target gene products such as NOXA, PUMA, and BAX at the transcript level through p53. p53 is primarily nuclear and its level is elevated upon ER stress. The inhibition of NOXA and PUMA in ER stress-induced apoptosis was apparent. Overexpression of NOXA or PUMA, however, restored apoptosis as evidenced by the activation of DNA fragmentation and BAK expression. In activated T33 L1, ER stress-induced apoptosis is greatly suppressed corresponding with the level of p53. Reduced level of C/EBP homologous protein (CHOP) was also evident in the p53-independent apoptotic pathway. These results provide new evidence that adipogenic cells may utilize a novel antiapoptotic pathway via down-regulation of p53 to ensure survival, thus, accepting further chronic lipid deposition and resulting in eventual its overload. *This study was supported by BioGreen21 (2006) from RDA Korea.

L124

Development of a S1P3 Receptor Antagonist
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Sphingosine 1-phosphate (SIP) is a bioactive phospholipid released from activated platelets and is suggested to play roles in thrombosis and atherosclerosis. SIP has been shown to mediate diverse cellular responses including cell proliferation, migration, and blood vessel formation via a set of cell surface receptors (SIP1-5), which belong to the G protein-coupled receptor family. Among these, SIP3 is expressed at high levels in both vascular endothelial cells and vascular smooth muscle cells. However, the biological functions of SIP3 have not been fully elucidated because of the lack of selective agonists or antagonists for SIP3. Chemical library screening using calcium mobilization assay and rational drug design identified a SIP3-specific antagonist “Compound A”. Compound A specifically inhibited SIP3-mediated intracellular calcium mobilization and ERK activation. We developed CHO cells expressing SIP1, SIP2, and SIP3 receptors and confirmed the specific antagonism of this compound for SIP3. To investigate the role of SIP1 in modulating endothelial activation, we treated endothelial cells with Compound A and examined its effect on SIP-induced adhesion molecule expression. Compound A inhibited SIP-induced expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), whereas the SIP1-specific antagonist VPC23019 did not affect the expression of SIP induction molecules. Consistent with this observation, SIP-promoted adhesion of THP-1 monocytic cell to vascular endothelial cells was reduced in the
NEURONAL DISEASES

L125
Mechanisms of Axonal Transport of the Mammalian Prion Protein
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Prions have drawn much attention because of their involvement in a number of devastating neurological diseases including scrapie in sheep and Creutzfeldt-Jacob disease in humans. While both cellular (PrPc) and infectious (PrPSc) isoforms localize to axonal termini and PrPc has been shown to transport along axons by fast anterograde transport, the mechanisms underlying these processes have not been investigated. Moreover, understanding the spread of prion infectivity from extraneural sites of infection to the central nervous system (CNS) via axonal pathways is of critical importance for gaining insight into the pathology of prion diseases, yet this also remains to be determined. Our hypothesis is that at least partly, infectivity progression from gastrointestinal sites, to the CNS for example, occurs via axonal transport pathways. Through immunofluorescent and biochemical studies of sciatic nerve ligations in mice, we have shown that prion proteins transport along sciatic nerve axons, and accumulate at sites of injury consistent with anterograde movement. Furthermore, a visual assay using primary hippocampal neurons transfected with a YFP-PrPc construct shows that PrPc transports in antero- as well as retrograde directions in vesicle-like compartments. The dynamics of PrPc transport are affected in neurons that lack the function of kinesin light chain (KLC1), suggesting that kinesin 1 is involved in transporting the prion protein along axons. To identify other putative microtubule motors involved in transporting PrPc along axons and/or dendrites, we have built a lentiviral siRNA library of all kinesins and a number of dynein and dynactin components. We thank The Damon Runyon Cancer Research Foundation for funding of S.E.E.

L126
8-hydroxydeoxyguanosine Suppresses No Production and Cox-2 Activity via Rac1/Stats Signaling in LPS-induced Brain Microglia
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Free 8-hydroxydeoxyguanosine (oh8dG), a nucleoside of oh8Gua, present in cytosol is not incorporated into DNA. However, nothing is known about its biological function when it presents in cytosol as a free form. We demonstrate here for the first time that oh8dG inhibits lipopolysaccharide (LPS)-induced nitric oxide (NO) production and cyclooxygenase-2 (COX-2) activity, and both gene transcriptions in microglia. Furthermore, oh8dG reduced mRNA levels of pro-inflammatory cytokine, such as IL-1β, IL-6, and TNF-α, in activated BV2 cells. We also found that oh8dG suppressed reactive oxygen species (ROS) production through reduction of NADPH oxidase activities, and blocked Rac1/STATs signal cascade. Finally, oh8dG suppressed recruitment of STATs and p300 to the iNOS and COX-2 promoters, and inhibited H3 histone acetylation. Taken together, these results provide new aspects of oh8dG as an anti-inflammatory agent.

L127
Common Molecular Pathways Underlying Neuronal Ceredoid Lipofuscinosis?
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The neuronal ceroid lipofuscinoses (NCLs) comprise a group of recessively inherited children’s neurodegenerative disorders, characterized by accumulation of autofluorescent storage material and severe cortical atrophy. The incidence of NCLs is up to 1:12,500 and this disease group is considered the most common cause of childhood dementia. Three early onset forms, CLN1, CLN2 and CLN10 are caused by deficiencies of lysosomal enzymes, PPT1, TPP1 and Cathepsin D, respectively. CLN3, CLN5, CLN6 and CLN8, are caused by mutations in genes encoding proteins of the secretory pathway with unknown functions. The similar pathological and clinical profiles of different forms of NCL suggest a common disease mechanism. We have utilized Cln1 and Cln5 deficient mice to explore common NCL-associated molecular pathways. Comparative gene expression profiling of the cortex revealed several affected molecular pathways with prominent alterations in protein phosphorylation and cytoskeleton in both mouse models. Comparison of the microarray datasets from both Cln1 and Cln5 deficient mice revealed a common affected pathway, involved in cytoskeletal dynamics and neuronal growth cone stabilization. Furthermore, systematic GST-pulldown analyses demonstrated that mouse Cln5 interacts with several NCL proteins, including Cln1/Ppt1. These data suggest that the functions of different NCL proteins are linked to the same intracellular pathways.

OTHER DISEASES

L130
Characterization of the Phosphorylation and Activation States of IRAK-4: A Key Regulatory Kinase of Cell Signaling in the Innate Immune System
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In order to investigate the behavior of cellular prion protein (PrPc) in the neurites, real-time imaging with fluorescent cellular prion protein (GFP-PrPc) was employed in mouse neurons in the differentiated condition. Interestingly, the anterograde, kinesin-driven velocity was selectively but constantly reduced to a half (50 nm/sec) in the neurites, when compared with that in the cell body (100-140 nm/sec), whereas the retrograde, dynein-driven velocity remained at the same (1-1.2 µm/sec) as that in the cell body. The GFP-PrPc was transported in a microtubules-dependent but not actin-dependent manner. These data cannot be explained by the current perspective that such slow rate is due to rapid movements interrupted by prolonged pauses, or by the local obstacle with actin filaments impeding the vesicular motion. Instead, the step-like reduction in the anterograde velocity of GFP-PrPc may support the notion for the fast transport system that the velocity changes arise from changes in the number of active motor proteins attached to a vesicle even in the slow transport system. Whether the current mode of PrPc trafficking is a widespread phenomenon in the slow transport has yet to be further examined.
Interleukin-1 receptor-associated kinase 4 (IRAK-4) is a key regulatory kinase of the innate immune system. Mice and humans with deletions or mutations of IRAK-4 show severely impaired interleukin-1 and Toll-like receptor signaling and are sensitive to several types of pathogens. Here we describe the expression and characterization of the phosphorylation and activation states of the kinase domain (aa 154-460) of IRAK-4. The kinase domain was expressed in SF21 cells and purified to homogeneity. Different phosphorylated forms of the enzyme were separated by high-resolution anion exchange chromatography and then analyzed by LCMS. The predominant form of the enzyme purified from SF21 cells was phosphorylated at amino acids T345, S346 and T352, all of which reside in the activation loop of IRAK4. Incubation of de-phosphorylated IRAK4 in the presence of Mg2+ + ATP also produces enzyme phosphorylated at same activation loop residues. Catalytic studies with the de-phosphorylated enzyme revealed a brief burst of enzymatic activity at the onset of the lag phase in the initial rate when compared to studies with the phosphorylated enzyme. In addition, mutation of these three amino acids dramatically alters the activity of this kinase. This study demonstrates the importance of activation loop auto-phosphorylation in the regulation of IRAK-4.

L131
The Cellular Basis of Lung Fibrosis in an HPS Mouse Model, C57BL/6-HPS1p Ap3b1p-1
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Lung fibrosis is a cellular disease involving the replacement of epithelial tissue by the deposition of collagen from pulmonary myofibroblasts. We are studying the possible role of Alveolar Type II cells (ATII cells) and alveolar macrophages in the fibrotic process through cytokine signaling pathways using the double mutant mouse model C57BL/6 -HPS1p Ap3b1p-1 for Hermansky-Pudlak Syndrome (HPS), a genetic disorder in humans that is at risk for pulmonary fibrosis. Collagen, cytokine levels, and cell morphology were examined at different ages of these mice using hydroxyproline analysis, Masson Trichrome staining, ELISA, immunohistochemistry and BAL macropage primary culture. Wild type strain C57BL/6j and another mutant mouse strain with affected ATII cells, C57BL/6-Ly5-/- were used as controls. Lung section stains and hydroxyproline analyses both showed that only HPS animals developed fibrotic lungs, and only after one year of age. Lung cell abnormalities were seen at earlier stages in these double mutants. Alveolar macrophages were foamy, enlarged, and often multinucleated. The total TGF-beta-1 levels in the double mutants were higher than the controls from BAL at young ages, which were maintained during aging. Through flow cytometry, it was found that TGF-beta-1 concentrates in alveolar macrophages. Primary cultures of alveolar macrophages from the HPS animals exhibited greater longevity and cellular attachment than control cells. It appears that affected ATII cells are not a primary causative agent for fibrotic changes in HPS, while alveolar macrophages play a significant role in this process. Also, TGF-beta-1 appears to be a key cytokine in HPS lung fibrosis, as has been found in other forms of pulmonary fibrosis.

L132
Pigment Epithelium-derived Factor (PEDF) Induces THR-1 Macrophage Apoptosis and Necrosis by the Activation of the Peroxisome Proliferator-activated Receptor Gamma (PPARγ)
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PURPOSE: PEDF is recently found to contain anti-inflammation capability. PEDF suppresses inflammation cytokine production from macrophage. This study set out to investigate whether macrophage cell death can be a mechanism that PEDF achieves its anti-inflammation effect. METHODS: The human THR-1 monocytic leukemia cell-line was induced into differentiation by PMA. Apoptosis was detected by the annexin-V-propidium assay, TUNEL assay and by the cleaved poly-ADP ribose polymerase (PARP) expression. Necrosis was detected by PI staining. The expression of PPARγ was examined by immunoblot analysis and the reverse transcription-polymerase chain reaction. For siRNA transfection, 70% confluence THR-1 macrophages were transfected with double strand siRNAs by the use of oligofectamine. RESULTS: Cell apoptosis and necrosis was observed in PEDF-treated THR-1 macrophages. PEDF treatment leads to PPARγ expression. The apoptosis and necrosis was significantly abolished when cells were pretreated with either specific pharmacologic inhibitors of PPARγ or PPARγ small interfering RNAs. Increased caspase-9- and PARP-cleaved expressions were found in PEDF-treated THR-1 macrophages. Conclusion: We postulate that PPARγ activation and expression plays a novel role in PEDF-mediated THR-1 macrophage apoptosis and necrosis. The mechanisms as to how PEDF activates PPARγ and how PPARγ activation leads to apoptosis and necrosis will be presented in the meeting.

Monday

L133
Characterization of IdeS of M1 and M12 from Clinical Isolated Streptococcus pyogenes Strains
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Streptococcus pyogenes (GAS) is an important human pathogen capable of causing a wide spectrum of diseases ranging from mild pharyngitis to highly invasive and fatal complications such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). A vast reiteration of virulence factors of GAS evolved numerous mechanisms to evade host immune system to survive. Recently, a secreted cysteine protease named Ideg-degrading enzyme of S. pyogenes (IdeS) was identified and shown to enhance survival of the GAS. Here, we showed the genetic variation of IdeS. 137 GAS clinical strains which represented 19 M protein serotypes isolated from National Cheng Kung University Hospital in southern Taiwan were sequenced. Two distinct genetic complexes were identified by phylogenetic analysis. Two complexes including four subclasses were classified. One major variants of each complex (IdeS of M1 and M12) were chosen to clone the ideS gene and overexpress protein in E. coli. In contrast to the IgG-endopeptidase activity, it seems to be almost no difference between purified complex I IdeS and complex II IdeS. Although the activity of IdeS in each complex was similar, on the basis of the statistical analysis of GAS clinical strains, IdeS of M1 displayed correlation to invasive disease, whereas M12 displayed correlation to scarlet fever. Whether IdeS encoded by M1 or M12 serotype GAS strain seems to play a role in pathogenesis, which will be further investigated. Taken together, our results show that IdeS of M1 and M12 serotype GAS strains exhibit similar IgG-endopeptidase activity even though existence of genetic variation in protein-coding sequence between complex I and complex II, which suggests us maybe the genetic variation of IdeS encoded by M1 and M12 mediate pathogenesis through other way.

L134
Crucial Role of Peroxisidoxin II in the Differentiation of Osteoclast
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Receptor activator of NF-κB ligand (RANKL), a TNF-α superfamily, is an extracellular stimulus whose intracellular signaling relies on phosphorylation and redox systems. Like many other extracellular stimuli, TNF-α induces the generation of reactive oxygen species (ROS) (1). Recently, it has been reported that RANKL-induced ROS plays a crucial role on osteoclastogenesis. However, it is not known for the effects of highly sustained ROS in vivo. Here, we show the changes of signaling followed by RANKL in bone marrow-derived monocytes (BMMs) from peroxiredoxin II knock-out (PrxD2-) mice and decrease of bone density in vivo. In PrxII deleted BMMs, intracellular generation of ROS was highly sustained like as RANKL was stimulated. Moreover, Ca2+ oscillations were generated autonomously without RANKL and autonomously generated Ca2+ oscillations showed a similar characteristics with RANKL-induced Ca2+ oscillations. PLCγ1 and NFATc1 that trigger late-stage of osteoclastogenesis were presented constantly activated form in absence of RANKL. Finally, decrease of bone density was observed in PrxII- mice. These results suggest that PrxII attenuates the differentiation of osteoclast precursor cells and also activated osteoclasts and it may play as a modulator gene to adjust bone density. This study was supported by a grant of the Ministry of Health & Welfare, Republic of Korea (A050002)
Monday

L135

Differential Signaling in EPEC and EHEC Infection in Intestinal Cells

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Enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC) are pathogenic strains of E. coli that infect the intestinal mucosa. These bacteria have type III secretory systems (TTSS) that allow them to translocate virulence factors directly into host cells. We study the effect of infection with these bacteria on signaling networks of intestinal epithelial cells using a cue-signal-response approach to follow information flow in these cells. Different assays are used to measure dynamic changes in signals and responses that include components of the MAPK pathways (JNK, p38, ERK 1/2, Akt), Toll-Like-receptor/IL-1 pathway (IRAK-1, c-Jun), NFkB pathway (IkBα) and the TNFα/receptor mediated apoptosis (caspase 3, cleaved cytokeratin 18). The head based multiplexed assay Bio-Plex is used to quantitate phosphorylation of signaling proteins and cytokine production. Quantitative immunoblots allow for the detection of specific protein modifications in some of the intracellular effectors.

Cell apoptosis is measured by detection of the specific markers cleaved caspase 3 and cleaved cytokeratin-18 on fixed cells by flow cytomtery. We have done quantitative measurements of dynamic changes in phosphorylation of Akt, Erk1/2, IkBα and c-Jun, using Bio-Plex, on cells infected with EPEC 2348/69 and Alee strains for up to 3 hrs. Results suggest that these kinases present similar dynamic phosphorylation profiles for both infections. However, preliminary studies with cell apoptosis suggest that a pathogenic EPEC strain (2348/69) results in smaller percent of apoptotic cells, when compared to infection with EHEC (O157:H7) or the isogenic strain Alee, after 12 hrs. These studies suggest that signaling through the Kinases of interest at later time points (>3 hrs) may further affect cell survival decisions and that the effect of translocated virulence factors may be stronger in the apoptosis pathway.

L136

Stimulation of Lung Innate Immunity Protects Against a Broad Range of Infectious Microbes

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The lungs are exposed to infectious microbes with every breath, and innate immune mechanisms are important in defense of the lungs against infection. Stimulation of the innate immune system (IIS) can be helpful in protecting immunocompromised patients and the general public in cases of an aerosolized bioterror attack. We have demonstrated that these innate mechanisms can be stimulated dramatically in mice, even when there is severe neutrophil deficiency. Stimulation of the innate immune defense system with a purified aerosolized fraction of non-typeable S. pneumoniae lysate potentiated the immune response to infection. In addition to the classical signaling pathways (e.g. S. pneumoniae (Spn), B. anthracis, P. aeruginosa and A. fumigatus). We have termed this therapeutic ALIS (Aerosolized Lung Innate Immune Stimulant). Two hours after exposure to ALIS, mice challenged with an aerosol of live Spn showed a high level of resistance to lethality (83% survival in treated mice compared to 0% in untreated). Complete protection is observed when ALIS is given from 4 to 24 hours prior to infection, then slowly declines over 5 days. Treatment with ALIS for 24 hours prior to exposure to lethal doses of Anthrax and Aspergillus also provides complete protection against death. Protection does not depend on neutrophil recruitment, is localized to the pulmonary route of infection, and is associated with rapid bacterial killing and the presence of increased levels of antimicrobial polypeptides in the lung lining fluid. Therapeutic augmentation of local defenses of the lungs against microbial pathogens could be of value in settings of a bioterror attack, epidemic occurrence of a respiratory infection, or therapy for cancer or immunologic diseases associated with neutropenia or impaired adaptive immunity. Preclinical testing is being finalized as we initiate our next goal of analyzing ALIS in clinical trials.

L137

Profiling of Specific Antioxidants and NADPH Oxidase Subunits during Hypoxia in Mouse Renal Proximal Tubular Cells

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It is well known that the maintenance of normal renal blood perfusion and oxygenation is of importance in the homeostatic control of kidney. Although numerous studies have shown that reactive oxygen species (ROS) was generated during hypoxia, the precise mechanism mediating the adaptive response remains poorly understood. Presently, much has been reported about the role of hypoxia-inducible genes in controlling of redox regulation. We focused on the expression of NADPH oxidase subunits and specific antioxidants during hypoxia in mouse renal proximal tubular cells of normal and EC-SOD overexpression transgenic mice. We examined hypoxia using normal and EC-SOD transgenic mice under hypoxic condition (7% O2) for the indicated time periods. Our results show that Nos4 and p22phox transcripts were constitutively expressed in MCT cells during hypoxia. However, cytosolic subunits, such as p47phox, p40phox, p67phox, were not detected except Rac transcripts. Within the framework of antioxidants, the SOD3 levels were increased in mouse kidney after hypoxia exposure, but the expressions of SOD1 and SOD2 were not significantly changed in hypoxic condition. And we confirmed dynamic response of ROS production in normal and EC-SOD transgenic mouse kidney during hypoxia by using RT-PCR and Western blot. We assessed the expression level of peroxiredoxins (Prxs) to scavenge H2O2. Prx5 was increased in both of mouse kidney and MCT cells after low oxygen treatments. Prx5 was detected in the nucleus during hypoxia confirmed by immunohistochemistry, especially. Prx2 was increased in mouse kidney after hypoxic exposure whereas it’s not changed in MCT cells under hypoxic exposure. These results suggested that the level of specific antioxidant expression might be differently regulated during hypoxic and hypoxia-like exposure of renal cells and EC-SOD might scavenge ROS produced by NADPH oxidase subunits. This work was supported by KRF-2006-070-E00238. 1, 2) These authors are supported by the second stage of brain Korea 21.

L138

Vibrio vulnificus Induced Cellular Responses of HC11 Epithelial Cells Are Dependent on Ros- Generated via Non-phyagocytic NADPH Oxidase

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The marine bacterium Vibrio vulnificus is a human pathogen that causing a rapidly progressing fatal septicemia and wound infection. This pathogen infection is acquired via direct contact or the gastrointestinal route from contaminated seafood. Septicemia and wound infection are noted for the extremely high mortality rate of the microorganism. V. vulnificus infection in host tissues with extensive tissue damage to skin. Although various factors have been implicated as contributing to disease caused by V. vulnificus infection, the function related to reactive oxygen species is almost limited. In this study, we investigated the effect and cellular response of reactive oxygen species induced by V. vulnificus. Pathogen infection significantly induced ROS generation in a time dependent manner in HC11 cell and triggered host cells death. The pretreated cell with N-acetyl cystein (NAC), diphenyley iodonium (DPI) significantly inhibited the ROS generation and host cell death. Catalase, superoxide dismutase (SOD) 1, 2 and glutathione peroxidases (Gpx) levels were decreased in HC11 cells during the infection with pathogen but extracellular superoxide dismutase (SOD 3) was increased. Matrix metalloproteinases (MMPs) participated in degradation of extracellular matrix, which allows bacteria to come in contact and interact with cells. ROS has the potential to modulate the activity of critical signaling molecules leading to MMP expression. Therefore, we identified activation of MMPs using active gel assay. MMP-2, 9 were activated during infection and DPI pre-treated cell reduced activation of MMPs. Taken together, these results indicate that V. vulnificus significantly induces ROS generation via NADPH oxidase and this triggers the inactivation of TGF-β antiprotic oxidant responses. An MMPs activation mediated by ROS, contribute to mechanism of V. vulnificus invasion, quickly and efficiently though host tissue. This work was supported by the Brain Korea 21+ project.
L139

Establishment of a Convenient Hepatitis B Virus-producing Xenograft Model for Validation of Anti-HBV Drug Efficacy

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Human hepatitis B virus (HBV) has infected more than 350 million people worldwide. However, compared with anti-cancer drugs, the development of drugs for viral hepatitis still needs a breakthrough. In this study, we established a convenient hepatitis B virus animal model for validate the efficacy of anti-HBV drug. HepG2 2.2.15, which produce the infectious HBV particle, could cause the viremia in severe combined immunodeficiency (SCID) mice by subcutaneous transplantation. High levels of HBV DNA also caused an elevated Natural Killer (NK) cell activity compared with parental HepG2 cells. The increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also observed in this HepG2 2.2.15 xenograft model and which is relevant to the high levels of serum HBV DNA. 3TC, which is the first market drug for hepatitis B infection, was found effective in this HBV-producing xenograft animal model. The serum HBV DNA, HBsAg and NK cell activity were decreased by 3TC. Boehmeria nivea extract (BNE), which is widely used as folk medicine in south Taiwan, was assessed by using this Hepatitis B virus-producing xenograft model. BNE administered by intraperitoneal routes appeared to be more potent to inhibit serum HBV DNA levels compared with oral administration under the same dosage. In conclusion, establishment of this Hepatitis B virus-producing xenograft model can facilitate the development of anti-HBV drug.

L140

The Expression Profiling of Mouse Selenoproteins on Ros-induced Stress Conditions

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We presented the expression pattern of selenoprotein using mouse model system related to certain stressful conditions such as microbial infection and TPA/DMBA induced carcinogenesis. The 20 set of primers based on the sequences of each mouse selenoprotein in the NCBI GenBank database was designed to 3’ UTR which is located between UGA codon and selenocysteine insertion sequence (SECIS) element. The expression pattern of mouse embryonic fibroblast after Virus influenza A/Scotland/1/2005 was assessed. Northern blot analysis showed that thioredoxin reductase 3 (TR3) decreased markedly time-course dependent manner until one half hour. But that of 15-kDa selenoprotein (Sel15) was increased gradually from 30 to 60 min and then decreased. These results suggest that TR3 seems to be involved in initial stage of infection and Sel15 participated in intracellular death signaling on the basis of over-expression of its mRNA when the Ca2+ ion influx are begin. The study using mouse carcginogenesis model, four types of selenoprotein induced skin tumor using DMBA and TPA, respectively, are showed the altered expression pattern. The expression profile of thioredoxin reductase 1 (TR1) and thyroid hormone deiodinase 1 (Dio1) are sensitive to DMBA and that of cytosolic glutathione peroxidase (GPx1) and selenoprotein K (SelK) are susceptible to TPA. It is necessary to study the protein analysis of selenoprotein, which showed the altered expression pattern and the function of that are associated with redox status. The mechanism permits the recognition of translatinal stop and selenocysteine incorporation and the function of resultant truncated form must be elucidated. This work was supported by Korean Research Foundation Grant funded by Korean Government (MOEHRD, basic research promotion Fund) (KR-2006-070-C00086). 1, 2) These authors are supported by the second stage of brain korea 21.

L141

HDAC5 Nuclear Export Is Facilitated by Hypertrophic Agonists in Adult Rat Ventricular Myocytes

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Histone acetylation/deacetylation represents a central mechanism for the control of gene expression. In particular, class II histone deacetylases (HDACs) modify histones and transcription factors, causing transcriptional repression related to the maintenance of the normal adult cardiac myocyte phenotype. Export of class II HDACs from the nucleus is associated with derepression of the fetal gene program and induction of pathological hypertrophy in the neonatal rat cardiac myocyte. Because class II HDACs appear to be involved in pathological hypertrophy, we hypothesized that a GFP-HDAC5 ectopically expressed in adult rat ventricular myocytes (ARVMs) may serve as a platform for studying this maladaptive process. Adenoviral expression of GFP-HDAC5 results in its nuclear localization in ARVMs within 24hrs post infection, as determined by fluorescence microscopy and subcellular fractionation of nuclear and cytoplasmic protein compartments. By 48hrs post-infection, however, GFP-HDAC5 is mislocalized to the cytoplasm, suggesting that overexpression may disrupt proper localization. GFP-HDAC5 is exported from the nuclei of ARVMs treated with various hypertrophic agonists, including phorbol ester and the G protein-coupled receptor agonist endothelin-1 and prostaglandin F2α. Fluorescence quantitation reveals a ~30% export within 2hrs of agonist treatment that is statistically significant compared to control (p<0.001). These data suggest that GFP-HDAC5 is appropriately functional in ARVMs 24hrs post-infection, and that translocation events can be quantified for the study of hypertrophy or the identification of antihypertrophic mechanisms in adult cardiac myocytes.

MOLECULAR BIOLOGY

L142

Co-expression and Medium Optimization Increase Protein Yield, Solubility, Stability, and Functionality

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Cellular transcription and translation machineries may be viewed as protein synthesis assembly line. All cells have basic tools and materials for protein synthesis. However, a given cell may not have all tools and materials necessary and sufficient for a particular recombinant protein production. When a recombinant protein is expressed in a heterologous system, its yield may be low and the expressed protein may be insoluble, unstable and dysfunctional. We co-expressed different genes to introduce additional tools and supplied the medium with various trace metals, minerals and vitamins. These different genes serve as useful tools (chaperones) to increase protein solubility, stability and activity. Trace metals, minerals and vitamins may not be needed for helix formation, but they may serve as ligands, co-factors or prosthetic groups for recombinant proteins and therefore are critical for protein yield, solubility, stability and functionality. Retinoic acid receptor (RAR) is mostly insoluble when expressed in E. coli. Its residual soluble fraction cannot bind its ligand efficiently. Co-expression of RAR in the E.coli cell with its natural partner retinoid X receptor (RXR) resulted in a dramatic increase in production of soluble and stable heterodimer of both proteins. Hormone binding studies reveal they are completely functional and are indistinguishable with natural purified proteins. This observation is not limit to protein natural partners. Co-expression of transcription regulator LacI repressor will enhance the yield of a toxic protein induced by IPTG. Hsp90 can improve the yield and solubility of the protein which it interacts with. Rare codon-recognizing tRNA genes can increase the yields of proteins containing these rare codons. Supplying medium with various nutrients can almost always increase yield. Sometimes it can also improve solubility, stability and activity. These results suggest that co-expression and medium optimization may represent general strategies to produce soluble and functional proteins in a recombinant system.

L143

Free-flow-electrophoresis: A Highly Versatile Fractionation and Separation Method in Proteomics

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Progress in proteome analysis relies heavily on development of technologies to reduce the complexity of the sample prior to systematic application of such analytical techniques as gel electrophoresis, liquid chromatography and mass spectrometry. Free Flow Electrophoresis (FFE) offers a powerful approach to the separation of charged species including, peptides proteins, cellular organelles and whole cells. The FFE principle is based on the separation of a sample in a thin layer of gradient-forming buffers continuously flowing through a flat chamber in a laminar fashion, with the voltage applied perpendicular to the flow direction. As separation buffers and a sample are moving through the chamber, the electric field leads to a deflection of different sample components according to their charge. Due to its matrix-free fractionation principle and therefore the absence of any solid phase interactions, FFE offers considerable advantages over traditional chromatographic and gel-based techniques: separation from small molecules to particles; native separation conditions; exceptional sample recovery, fast fractionation times and high throughput. Application of the FFE separation methodology to a variety of sub-cellular proteomes are presented including cell organelles, membrane proteins and protein complexes.

L144  Enhancement of the Immunoglobulin Gene Conversion in Chicken DT40 by Inhibition of Histone Deacetylases (HDAC) and Its Application to Antibody Engineering

W. Lin,1,2 H. Seo,1,3 A. Nakamura,1,2 T. Yuyama,1 T. Shibata,4 K. Ohta1; 1Genetic System Regulation Laboratory, Riken Discovery Research Institute, Wako, Japan, 2REDs Group, Saitama Small Enterprise Promotion Corporation, Kawaguchi, Japan, 3Chiome Bioscience, Tokyo, Japan, 4Shibata Distinguished Senior Scientist Laboratory, Promotion Corporation, Kawaguchi, Japan, 3Chiome Bioscience,

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tried to identify novel carbohydrate binding proteins in human colon cancer cell, HCT-15, using this probe. We could detect some lactosamine specific carbohydrate binding proteins, and we used 2-dimensional electrophoresis and MALDI-TOF for identification of these proteins.


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Plant cells respond to cold stress by the expression of gene encoding specific stress proteins with possible protective functions. Simultaneous comparison of differentially expressed protein profiles of a freshwater alga, *Spirogyra varians*, grown under two different temperature conditions (4°C and 20°C) indicated the generation of cold stress-responsive proteins. We isolated a 20 KDa protein (pI 4.5) which was most strongly up-regulated in 4°C (about 500-fold higher than in 20°C). As the protein has never been reported before, we named it as SVCR1 (*Spirogyra varians* cold regulated) protein. The cDNA encoding SVCR1 was cloned using degenerated primers designed from the internal amino acid sequence of the protein and a λ Zap cDNA library of *Spirogyra varians*. The deduced amino acid had a high sequence similarity with early light-inducible proteins (ELIPs) of higher plant which are known as nuclear-encoded chloroplast proteins induced by light stress. The northern blot results showed that the accumulation of SVCR1 transcript could be induced by cold treatment (4°C) even under the dark condition.

L149 Cytotoxicity of Liposomal Taxol

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Taxol (paclitaxel) is one of the most used drugs used for the treatment of multiple cancers, including breast, ovarian, and non-small-cell lung cancer. However, development of resistance with prolonged use is common, requiring the use of taxol in combination with other treatments. Unlike many chemotherapeutics and/or their metabolite(s) which are known to interact with DNA, taxol does not directly target the genome. Instead, it binds tubulin subunits of microtubules to prevent depolymerization, leading to cell cycle arrest and apoptosis, or cell death. In addition to the development of resistance, taxol is difficult to administer due to its high lipophilicity, and it requires solubilization with Cremophor EL (polyethoxylated castor oil) and ethanol, which often lead to adverse side-effects, including life-threatening anaphylaxis. Incorporation of taxol in DPPC:DMPG liposomes will not only eliminate the adverse reactions associated with the Cremophor EL vehicle, but also decrease other toxicities that arise from the drug’s action. Moreover, liposomally-encapsulated taxol might have the potential to overcome resistance by facilitating the cellular delivery of taxol at the site of action. Liposomes have been shown to be very effective in the delivery of other chemotherapy agents, such as adriamycin. This project investigates the use of liposomes as a novel delivery method for Taxol. The liposomally-encapsulated taxol will be tested for efficacy on MCF-7 breast cancer cells and for its ability to overcome taxol-resistance. Initial studies on breast cancer cells have shown the lipid formulation to be non-toxic, and early preliminary results from MTT cytotoxicity assays, DNA laddering, and flow cytometry showed promising results in its use as a delivery method of the drug. The outcome of this study may demonstrate that liposomes might be used as a novel method for the administration of taxol, either alone or in combination with other agents for cancer treatment.

L150 Statistical Error Model for Real-Time PCR Based RNAi Validation

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RNA interference (RNAi) using synthetic siRNA or vector-based shRNA has growing popularity among cell biologists trying to understand the functional roles of their genes of interest. Central to any RNAi experiment is the validation of knock down efficiency. Although validation by Western analysis is indeed still necessary for successful gene function studies, real-time PCR provides a simpler method to validate the suppression of gene expression and therefore a more direct measure of actual RNAi success. However, due to limitations caused by variations in real-time PCR detection and siRNA delivery, some erroneous conclusions can easily be made when interpreting RNAi validation results. Here, we analyze all sources of variance in an RNAi validation process using both theoretical and experimental approaches to build a statistical error model for real-time PCR based RNAi validation. According to our model, greater than 80 percent transfection efficiency is essential for RNAi validation. We also demonstrate the absolute need for both biological and technical replicates. Based on our model, a practical guideline is provided for the minimal number of replicates required for a real-time PCR based RNAi validation process.

L151 Simultaneous Construction and Purification of Multiple Recombinant Adenoviral Vectors

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Recombinant adenovirus vectors continue to be widely used in gene therapy approaches and have demonstrated utility in the study of gene function. With the availability of gene and shRNA collections and the need for efficient delivery into a wide range of cell types, there is now a requirement for rapid and efficient scalability of adenoviral vector production. However, methods for the simultaneous production of multiple adenoviral vectors have been lacking due to cumbersome vector construction via bacterial recombination and purification strategies using cesium chloride. Here in this study, we report a method for higher-throughput construction and purification of adenoviral vectors. Adenovector cloning was performed utilizing *in-vitro* Cre-loxP recombination to directionally and precisely introduce a number of nucleic acid sequences into a first generation recombinant adenovirus genome. Using this approach, we achieved > 90% cloning success with little or no background as measured by colony PCR screening and endonuclease restriction analysis. Once the vectors were constructed, 24 recombinant adenoviral DNAs were then linearized and transfected into 293 cells. Rescue was deemed complete when CPE was observed 7 days following transfection. Each of the cell pellet-derived lysates from the primary amplifications were subsequently purified using a small-scale anion-exchange chromatography spin column. Under the appropriate buffer conditions, the purification columns provided high-yield of adenoviral particles with minimal non-specific viral and cellular culture formats, ranging from 96 well plates to 10 cm2 dishes with total infectious units ranging from 1e7 to 1e10, respectively. Purity of the small-scale samples was determined by SDS-PAGE to be comparable to larger scale anion-exchange purified preparations. Taken together, we have demonstrated a rapid, scalable, semi-high throughput approach to producing multiple purified adenoviral vector stocks that ultimately expand the utility of adenoviral vectors for gene therapy development and gene function studies.

L152 Studies on the Role of Ppql as a Putative Negative Regulator of the Mating Map Kinase Pathway in Budding Yeast

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The mating response of the budding yeast, *Saccharomyces cerevisiae*, upon stimulation by mating pheromone, relies on a MAP kinase (MAPK) signaling pathway. The mating MAPK signaling module is composed of three kinases and a scaffold protein Ste5. The Ste5 scaffold is essential for mating signaling and is thought to contribute to signaling specificity by formation of a protein complex through noncovalent interactions with component kinases. Precise control of magnitude and duration of signaling flux is critical for cell survival. To identify negative regulators of mating signaling a novel genetic screen was developed, in which all the 32 phosphatases in the yeast genome were tethered to scaffold signaling complex and changes in mating response is monitored. From the screen we have identified a phosphatase Ppql as a putative negative regulator of mating signal transduction. Our next goal was to know how Ppql acts as a negative regulator. Using FACS analysis and time course western blotting, we have observed cell-to-cell variations in the level of Ppql...
protein upon stimulation with mating pheromone. Real-time PCR analyses also showed that Ppq1 expression is stochastic. These finding along with our preliminary data based on immuno-precipitation analyses suggest that the stochastic, cell-to-cell fluctuation of Ppq1 expression results from variations in transcription and from changes in protein stability due to post translational modifications including phosphorylation, ubiquitination.

L153
Single-Cell Analyses of Mapk Signaling Pathways in Saccharomyces cerevisiae Using Cell Chips
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The mitogen-activated protein kinase (MAPK) signaling pathways is essential for cell growth, cell differentiation and survival in eukaryotes. The MAPK signaling pathways transmit signals from the cell surface to nucleus. In the yeast, there are at least four MAPK signaling pathways. The mating and high osmolarity responses in the budding yeast, Saccharomyces cerevisiae, depend on the MAPK signaling pathways. In this study, we analyzed the mating and high osmolarity responses in the budding yeast, S. cerevisiae, at single-cell level using cell chips. The cell chip analyses of the mating and high osmolarity responses were performed using fluorescent proteins fused to genes whose transcription is specifically upregulated by each signaling. Using the technique, we have determined the real-time gene expression patterns of the mating and high osmolarity responses at single-cell level. We observed that the mating and high osmolarity MAPK signaling showed a non-uniform, stochastic flux in the population yeast cells analyzed.

L154
Characterization of tlr0911 - a Phytochrome-like Protein in Thermosynechococcus elongatus BP-1
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Phytochromes are photoreceptors that sense changes in light conditions and adapt via photomorphogenesis. These biliproteins convert between inactive and active forms in response to different wavelengths. They covalently bind a linear tetrapyrrole chromophore derived from heme. The chromophore-binding GAF domain and the related PHY are the characteristics for phytochromes. Until recently, phytochromes were thought to be restricted to photosynthetic eukaryotes; however, genome sequencing and mutant analysis have uncovered genes similar to plant phytochromes in cyanobacteria and other prokaryotes. The term cyanobacteriochrome has recently been proposed for some cyanobacterial phytochrome-like proteins that show blue-green photoreversibility (Ishizuka et al. 2006). Five proteins were identified from Thermosynechococcus elongatus BP-1 gene sequence. T. elongatus BP-1 is a unicellular, rod-shaped cyanobacterium isolated from a hot spring in Beppu, Japan. Among the five, the protein for tlr0911 is the largest. The goal of this project is to co-express tlr0911 with plant and cyanobacterial chromophores and characterize the absorbance and chromophore binding properties. The full-length gene for tlr0911 was cloned into the pBAD-MycHisB vector. The vector construct was then transformed into a LMG194 E. coli strain containing a plasmid that coded for enzymes that synthesize the chromophores, phycocyanobilin (PCB), phytochromobilin (PØB), and biliverdin IX alpha (BV) from native E. coli heme. The preliminary result shows that Tlr0911 has some phytochrome-like spectral patterns. The olive green color of the expressed protein also supports this finding. Tlr0911 apparently does not fit the model of the cyanobacteriochromes. A better protein purification method is being investigated. Under this method, the Tlr0911 protein is fused to a self-cleavable Intein tag in which a chitin-binding domain allows affinity purification of the fused protein on a chitin column. Additional analysis such as Zinc-block and HPLC-mass spectrophotometry will be performed on the purified protein.
GAR-3b-mediated ERK1/2 activation is inhibited by cAMP via PKA.

and the inhibitory effect of forskolin was blocked by H89, an inhibitor

pathway might be responsible for GAR-3b-mediated ERK1/2

activation was specifically mediated by GAR-3b. We next examined

activation was inhibited by the biochemical pathway linking GAR-3b stimulation to ERK1/2

activation was inhibited by forskolin, an adenylyl cyclase activator, and the inhibitory effect of forskolin was blocked by H89, an inhibitor of cAMP-dependent protein kinase A (PKA). These results imply that GAR-3b-mediated ERK1/2 activation is inhibited by cAMP via PKA. Taken together, our data suggest that two signaling pathways, PLC/Ca\(^{2+}\)/PKC and cAMP/PKA, play important roles in GAR-3b-mediated ERK1/2 regulation in CHO cells.

L3

Isomform-specific Wiring of Phospholipase C-β and G-protein Coupled Receptor

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Phosphoinositide-specific phospholipase (PLC) plays a important role in signal transduction by catalyzing hydrolysis of a membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) to generate two intracellular second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP\(_3\)). Among PLC isozyme (β\(_1\), γ\(_1\), δ\(_1\), ε\(_1\) and ν), PLC-β plays a key role in G-protein coupled receptor (GPCR) mediated signaling. There are 4 PLC-β isoforms cloned so far (PLCβ1-4). Despite of almost similar structure and enzymatic activity, each PLC-β isoform shows unique tissue expression pattern and knock-out phenotype. Moreover recent report shows that some scaffold proteins physically link some GPCR to one of PLC-β. Thus it is reasonable to assume that each PLC-β isoform has unique role on GPCR signaling. To elucidate the roles of each PLC-β isotype on GPCR signaling, we developed siRNAs which induce isotype-specific knock-down of PLC-β. Using these siRNAs, we generated PLC-β1, PLC-β2, PLC-β3 knock down cells and measured PLC activity and intracellular Ca\(^{2+}\) mobilization upon BK treatment compared to control cells and PLC-β3 knock down cells, but upon LPA treatment, only PLC-β3 knock down cells showed the reduced response. Moreover LPA-induced cellular proliferation also reduced only in PLC-β3 knock down cells. To find out the molecular mechanism of these coupling, we investigated molecular complex including these GPCRs and PLC-β. As a result, we found that m-PAR-3 and NHERF2, PDZ proteins, are scaffold proteins, which physically links these GPCRs to its coupled PLC-β respectively. Taken together, we showed each PLC-β has a specific role on GPCR-mediated signaling even in a same type of cells. This study strongly suggests that multiple PLC-β isoforms in a cell are required for specific regulation of GPCR signaling.

L4

A Proteomic Approach of Immune Response in Drosophila melanogaster

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In response to microbial infections, Drosophila turns on a multifaceted immune response involving humoral reactions that leads to the destruction of invading organisms by lytic peptides. These defense mechanisms are activated via two distinct signaling pathways. One of these, the Imd pathway is responsible for defense against Gram-negative bacterial infections. This response occurs through a tightly orchestrated, complex signal transduction pathway, yet the components appertaining to it is not fully revealed. We sought to find proteins that are not previously reported to be involved in Imd pathway using proteomic techniques. We gave immune induction with Gram-negative bacteria to Drosophila SL2 cells, separated its total lysate with 2-DE, and compared with control group. We were able to identify manifold proteins using MALDI-TOF Mass Spectrometry. These proteins were assumed to be associated with many cellular events: translational elongation, protein folding, protein modification, CAM-P/mediated signaling, stress response, etc. Also, we found O-GlcNAc modification in these proteomes showed altered levels, by using specific anti-O-GlcNAc monoclonal antibody, CTD110.6. We are eagerly intending to identify the position of these proteins in Imd signal transduction pathway, and ultimately, find the functional role of these proteins involved in innate immune response.

L5

Light-dependent Subnuclear Remodeling and Genetic Reprogramming in Arabidopsis

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How the same genome can be reprogrammed to yield dramatically different outcomes is a central question in the post-genomic era. Among different layers of regulatory mechanisms, spatial and temporal
regulation of subnuclear organization has been suggested to play an important role in genome expression. However, the precise function of many subnuclear domains is still obscure. We use Arabidopsis light responses as a model system to investigate how nuclear bodies are involved in genetic reprogramming. The Arabidopsis genome possesses two different body plans in response to ambient light changes, a dark-grown non-photosynthetic lifestyle and a light-grown phototropotropic lifestyle. When a dark-grown seedling is exposed to light, it will switch from the dark program to the light program. The change in physiology is accompanied by dramatic changes in seedling morphology and changes in expression of thousands of genes. At the cellular level, one of the most striking effects of light is the relocation of phytochrome B (phyB), which is a red/far-red photoreceptor mediating the light responses. PhyB localizes in the cytoplasm in the dark; upon light activation, it accumulates in a few acute subnuclear foci, named phyB nuclear bodies. How phyB nuclear bodies are involved in light signaling is unknown. We initiated a novel confocal-microscopy-based genetic screen using a transgenic line expressing phyB:GFP to isolate mutants with altered patterns of phyB:GFP. Hemera, one of the mutants isolated from the screen, is defective in phyB:GFP nuclear body formation and blocked in the dark-to-light transition. The phenotypes of the hemera mutant suggest that HEMERA is a master positive regulator in the phyB-mediated genetic reprogramming process. HEMERA encodes a novel nuclear protein. The identification of HEMERA sets up the stage to further understand the function of phyB nuclear bodies and to unravel molecular mechanisms of light-dependent genetic reprogramming in plants.

L6 Mammary Epithelial-specific Deletion of the Focal Adhesion Kinase Gene Leads to Severe Lobulo-Alveolar Hypoplasia and Secretory Immaturity of the Murine Mammary Gland
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Here, we report mammary epithelial cell-specific deletion of the FAK gene utilizing the Cre LoxP method using MMTV (Mouse Mammary Tumor Virus) - Cre transgenic mice. Both sexes of the MMTV-Cre FAK floxed conditional knock-out (MFKCO) females (L6-1853) are育 and show no obvious abnormalities compared to MFKCO littermate control animals. In contrast, MFKCO females died at the age of 3 weeks. In vivo immunohistochemistry and western blotting revealed moderate to markedly reduced FAK expression throughout postnatal life within the mammary epithelium. Mammary gland development and can successfully nurse their progeny. In MFKCO females immunohistochemistry and western blotting revealed markedly reduced MAP Kinase phosphorylation and almost undetectable STAT5 phosphorylation. Based upon these results, we conclude that FAK deletion in the murine mammary gland causes inhibited lobulo-alveolar growth and, more importantly, no secretory maturation. The lobulo-alveolar hypoplasia is most likely due to the fact that FAK deletion causes disruption in the ERK1/2 signaling. Although it is known that integrity of the ERK1/2 signaling is indispensable for normal development, we found that the normal lobulo-alveolar development and parallel secretory maturation of the murine mammary gland are dependent on FAK. Studies are also underway to determine the exact role of FAK in prolactin-induced STAT5 phosphorylation.

L7 Profile of Protein Arginine Methylation Undergoes Time Dependent Changes in the Cytosol of Regenerating Rat Liver after Partial Hepatectomy
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Post-translational methylation of arginine residues of proteins is known to plays a crucial role in transcriptional regulation, signal transduction and protein-protein interactions. Previously, we showed that the 18-kDa protein is a major cytosolic protein undergoing in vitro methylation in regenerating hepatocytes and its methylation peaked at 1 day after partial hepatectomy, indicating that the activity of protein arginine methyltransferase was directly correlated with the degree of proliferation. In this study, our interest was to investigate the change of in vivo methylation profile in regenerating rat liver. Thus, we performed western blot using anti-asymmetric or symmetric dimethylarginine antibodies. In vivo asymmetric dimethylarginine formation in the proteins with molecular weights of about 18 or 32 kDa peaked at 1 day following partial hepatectomy, which gradually declined to a basal level within the next 7 days. Symmetric dimethylarginine formation detected by SYM10 was also prominent in 18-kDa protein and 32-kDa protein. The 18-kDa methylation peaked at 1 day and the 32-kDa methylation peaked at 3 day. On the other hand, SYM11 that bind to symmetric dimethylarginine in sequence different to that for SYM10, detected 15-50 kDa methylation which peaked at 3 day. It was not certain whether the asymmetric dimethylarginine containing 18-kDa and 32-kDa proteins were the same ones as symmetric-containing proteins with same molecular weights. The 18-kDa protein was shown again to be a major cytosolic protein undergoing in vitro methylation. Since the in vivo methylation profile of some proteins changes preceding proliferation of hepatocytes upon partial hepatectomy, it is tempting to speculate that sequential methylation of several proteins are involved in an early signal critical for liver regeneration and proliferation signal.

L8 Hsp82 Is Required for Ste11 Protein Stability and Mating Pathway Signaling in S. cerevisiae Yeast
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Ste11 is the MEKK in three MAPK signaling cascades that mediate mating, high osmolality glycerol (HOG), and filamentous growth responses in S. cerevisiae yeast. The yeast ATP-dependent chaperone Hsp90 (Hsp82) is known to co-purify with Ste11 and to be essential for the pheromone-induced transcriptional response. In this study, we further examine the roles of mating pathway signaling proteins, we made use of strains expressing either wild-type HSP82 or the temperature sensitive hsp82<sup>T70D</sup> allele. In vitro kinase assays revealed that Ste11 catalytic activity is higher when isolated in a complex with Hsp82 compared to that with Hsp42, which is a weak kinase activity is reduced when isolated in a complex with mutant Hsp82. Under in vitro kinase assay conditions, we demonstrate that Ste11 does not bind to Hsp82<sup>T70D</sup> as efficiently as that with wild-type HSP82, suggesting a role of Ste5 and Pbs2, respectively, and therefore strengthen the interpretation that Hsp82 binds directly to Ste11. We also show that Hsp82 co-purifies with other members of the mating pathway signaling cascade including Ste5 and Fus3, but not Ste7. We distinguish whether Hsp82 function is essential for Ste11 synthesis or for catalytic activity of Ste11, we show that Ste11 catalytic activity is lower when isolated in a complex with Hsp82<sup>T70D</sup> versus wild-type Hsp82; in contrast, Fus3 kinase activity is reduced when isolated in a complex with mutant Hsp82. In vitro kinase assay conditions, we demonstrate that Ste11 does not bind to Hsp82<sup>T70D</sup>. As a criterion, we confirm that Hsp82<sup>T70D</sup> is nonfunctional in vitro and therefore conclude that the catalytic activity of Ste11 and Fus3 is dependent on Hsp82 binding and function. Comparison of the steady state level and half-life of Ste11 in the wild type and mutant Hsp82 strains during pheromone induction showed that Hsp82 function is critical for newly synthesized Ste11 protein. Since Ste11 protein is rapidly degraded upon pheromone induction, the function of the Hsp82 chaperone is critical to maintain Ste11 protein levels for repeated cycles of MAPK activation.

L9 MiR-181a Reduces the T Cell Receptor Signaling Threshold and Enhances Sensitivity to Antigens
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T cell sensitivity to antigens is quantitatively regulated during T cell maturation to ensure the proper development of tolerance to self-antigens and immunity against foreign antigens. However, it remains elusive how T cell sensitivity to antigens can be tuned intrinsically and what molecules are the key players. Here we show that miR-181a, a member of abundant class of ~ 22 nucleotides endogenous small
regulatory RNAs, can quantitatively modulate T cell sensitivity to antigens by controlling multiple target gene expression at the posttranscriptional level. Increased miR-181a expression in mature T cells causes a marked increase in T cell activation and augments T cell sensitivity to peptide antigens. Moreover, T cell blasts with higher miR-181a expression become reactive to agonists—the inhibitory peptide antigens that are normally incapable of T cell activation alone but can block agonist ligand stimulation. The effects of miR-181a on antigen discrimination are in part achieved by dampening the expression of multiple negative regulators in the T cell receptor signaling pathway, including PTPN22 and the dual specificity phosphatases DUSP5 and DUSP6. This results in a reduction in the TCR signaling threshold, thus quantitatively and qualitatively enhancing T cell sensitivity to antigens. Interestingly, higher miR-181a expression correlates with greater T cell sensitivity to selecting ligands in thymocytes, suggesting that miR-181a may act as a ‘rheostat’ to tune T cell sensitivity at various stages of T cell development.

**CELL CYCLE CONTROLS**

L10  
**Promoting Mitotic Entry by Greatwall, a Phosphorylation-regulated Protein**  
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Greatwall is a serine/threonine kinase important for proper cell cycle progression. Mutations in the Drosophila greatwall gene cause prolonged transit from late G2 through nuclear envelope breakdown. In Xenopus egg extracts, Greatwall is required for both mitotic entry and maintenance. Depletion of Greatwall from mitotic extracts rapidly inactivates MPF due to the accumulation of inhibitory phosphorylations on Cdc2. Greatwall depletion also prevents cycling extracts from entering mitosis. Our recent studies show that addition of exogenous active Greatwall to cycling extracts causes premature mitotic entry; similarly, microinjection of active Greatwall induces Xenopus oocytes maturation in the absence of progesterone treatment. Interestingly, we found that Greatwall’s mitosis-promoting activity is independent of Plx1, which has been reported to be required for mitotic entry in Xenopus. On the other hand, addition of excess Plx1 can overcome the Greatwall depletion phenotype. These observations together suggest that Greatwall and Plx1 function in different but complementary pathways. Greatwall is cell-cycle regulated and only activated during mitosis by phosphorylation. The conserved kinase domain of Greatwall is split into two parts by a long non-conserved region. By generating point mutations, we have identified multiple potential regulatory phosphorylation sites in the kinase domain, some of which have been confirmed as physiological phosphorylation sites by mass spectrometry. Although the middle non-conserved region appears to bear most of the physiological phosphorylations, sectional deletion in the region failed to reveal any segments indispensable for Greatwall’s activity.

L11  
**The Kinase Activity of Polo Kinase Cdc5 Is Required for Regulating the Dynamic Localization of Bfa1 onto the Spindle Pole during Mitosis in Budding Yeast**  
K. Song; Dept. of Biochemistry, Yonsei University, Seoul, Republic of Korea  
Given that mitotic spindle misaligned, the spindle position checkpoint of budding yeast delays mitotic exit by inhibiting Tem1 GT-Pase via its GT-Pase activating protein (GAP) Bfa1/Bub2 that stay on both spindle pole bodies (SPBs). Since Bfa1/Bub2 complex is exclusively associated with on SPB toward bud at anaphase onset, its asymmetric pole bodies (SPBs). Since Bfa1/Bub2 complex is exclusively of budding yeast delays mitotic exit by inhibiting Tem1 GTPase via its...

L12  
**Inhibition of Kinase Activity of Aurora A by FAFl**  
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Aurora kinase A (Aur-A) is localized to the centroids in the G2/M phase, and its kinase activity regulates the G2 to M transition of the cell cycle. This study shows that FAFl binds to Aur-A and the interaction occurs predominantly at G2 and M phases. The amino acid 181-381 region where phosphorylation of FAFl occurs was mapped as the Aur-A interaction domain. This led us to examine whether the phosphorylation status of FAFl affects the Aur-A activity in Xenopus egg extracts, Greatwall is required for both mitotic entry and maintenance. Depletion of Greatwall from mitotic extracts rapidly inactivates MPF due to the accumulation of inhibitory phosphorylations on Cdc2. Greatwall depletion also prevents cycling extracts from entering mitosis. Our recent studies show that addition of exogenous active Greatwall to cycling extracts causes premature mitotic entry; similarly, microinjection of active Greatwall induces Xenopus oocytes maturation in the absence of progesterone treatment. Interestingly, we found that Greatwall’s mitosis-promoting activity is independent of Plx1, which has been reported to be required for mitotic entry in Xenopus. On the other hand, addition of excess Plx1 can overcome the Greatwall depletion phenotype. These observations together suggest that Greatwall and Plx1 function in different but complementary pathways. Greatwall is cell-cycle regulated and only activated during mitosis by phosphorylation. The conserved kinase domain of Greatwall is split into two parts by a long non-conserved region. By generating point mutations, we have identified multiple potential regulatory phosphorylation sites in the kinase domain, some of which have been confirmed as physiological phosphorylation sites by mass spectrometry. Although the middle non-conserved region appears to bear most of the physiological phosphorylations, sectional deletion in the region failed to reveal any segments indispensable for Greatwall’s activity.

L13  
**Autocrine Induction of TGFβ1 Induces Low Proliferation in Hypertrophic Scar Fibroblasts through the Focal Adhesion Protein, Hic-5**  
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Active TGFβ1 is a central mediator of fibrotic conditions, including lung, liver, and cutaneous fibrosis. Hypertrophic scars (HTSs) are characterized as both fibrocontractive and fibroproliferative cutaneous lesions. However, little is known about the mechanisms regulating the proliferation of fibroblasts derived from hypertrophic scars (HTSF). We report here that key functions of HTSF are constitutively activated by autocrine induction of TGFβ1 (Dabiri et al J. Investigative Dermatology, 2006). We now hypothesize that autocrine induction of TGFβ1 upregulates the focal adhesion protein Hic-5 to modulate the growth of HTSF. We show here that HTSF secrete and activate TGFβ1 to a greater extent than do normal human adult dermal fibroblasts (NHDf), that HTSF express high levels of Hic-5 constitutively, and that HTSF exhibit a markedly reduced doubling time in culture. We also find that Cyclin D1 and A levels are constitutively lower in HTSF compared to NHDf and the cyclin kinase inhibitor p21waf1 is upregulated in HTSF and decreased in the nucleus of autocrine TGFβ1 production in HTSF promotes replication, raising cyclin D1, cyclin A, and lowers p21waf1 levels. In addition, we also find that TGFβ1 regulates these cell cycle proteins in NHDf. Moreover, Hic-5 is located in the nucleus of HTSF, and knocking down Hic-5 with specific siRNA’s in these cells results in decreased p21waf1 levels, increased cyclin A levels, and a subsequent increased doubling time. Our results show that autocrine production of TGFβ1 upregulates the expression of Hic-5 and that Hic-5 is responsible for the decreased growth rate seen in HTSF.

L14  
**Spy1 Expression Prevents Normal Cellular Responses to DNA Damage: Inhibition of Apoptosis and Checkpoint Activation**  
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CA, Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA
Spy1 is the originally identified member of the Speedy/Ringo family of vertebrate cell cycle regulators, which can control cell proliferation and survival through the atypical activation of CDKs. Here we report a role for Spy1 in apoptosis and checkpoint activation in response to Ultraviolet (UV) irradiation. Using an inducible system allowing for regulated expression of Spy1, we show that Spy1 expression prevents activation of caspase-3 and suppresses apoptosis in response to UV irradiation. Spy1 expression also allows for UV irradiation resistant DNA synthesis (UVDS) and permits cells to progress into mitosis as demonstrated by phosphorylation on Histone H3, indicating that Spy1 expression can inhibit the S-phase/replisome and G2/M checkpoints. We demonstrate that Spy1 expression inhibits phosphorylation of Chk1, RPA, and histone H2A.X, which may directly contribute to the decrease in apoptosis and checkpoint bypass. Furthermore, mutation of the conserved Speedy/Ringo Box, known to mediate interaction with CDK2, abrogates the ability of Spy1 to inhibit apoptosis and the phosphorylation of Chk1 and RPA. The data presented indicate that Spy1 expression allows cells to evade checkpoints and apoptosis, and suggests that Spy1 regulation of CDK2 is important for the response to DNA damage.

L15
Spy1 Promotes Phosphorylation and Degradation of the Cell Cycle Inhibitor p27
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The cyclin dependent kinase inhibitor (CDK) p27Kip1 binds to the cyclin E/CDK2 complex and prevents premature S phase entry. Phosphorylation of p27 on T187 is essential for cell cycle progression through the G1/S transition. However, critical events that trigger phosphorylation of p27 at T187 on an inhibited cyclin E/CDK2 complex have remained unclear. We demonstrate that human Speedy (Spy1) directly activates CDK2 to phosphorylate CDK2 on T187 in vitro. Addition of Spy1 or a Spy1/CDK2 complex to an inhibited cyclin E/CDK2 complex promotes phosphorylation of p27 on T187. In addition, Spy1 can protect cyclin E/CDK2 from p27 inhibition. Inducible expression of Spy1 in U2OS cells results in increased T187 phosphorylation and subsequent degradation of endogenous p27, which is inhibited in the presence of the proteasome inhibitor MG132. Our studies establish Spy1 as a trigger for progression through the G1/S transition, through the degradation of p27 by enhancing T187 phosphorylation.

L16
Characterization of a Mutation in the Mouse Bub1 Gene
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Faithful segregation of chromosomes is a critical event in mitosis. Cells avoid missegregation and aneuploidy through the spindle checkpoint, which detects proper kinetochore-microtubule attachment and prevents the onset of anaphase with unattached kinetochores. Spindle checkpoint proteins of the Bub1 family bind to the kinetochore during prophase and required for the binding of other checkpoint proteins to the kinetochore. Core spindle checkpoint components are essential in nematodes, flies, and mice, with their loss resulting in developmental lethality through genomic instability and apoptosis. The spindle checkpoint is also thought to play a role in tumorigenesis, as genomic instability and aneuploidy are frequently seen in human cancers. We have developed a conditional mutation of the mouse spindle checkpoint gene Bub1 by flanking exons 2 and 3 with LoxP sites to examine Bub1’s potential role in aneuploidy and tumorigenesis. The majority of mice homozygous for the germline deletion of the mutant Bub1 allele (Bub1<sup>Δ/Δ</sup>) die shortly after birth with a failure to thrive phenotype, while heterozygous mice develop normally. Since deletions of other core spindle checkpoint genes result in early embryonic lethality, our data suggest that the mutant allele Bub1<sup>Δ/Δ</sup> is hypomorphic, although not nullomorphic, and Bub1<sup>Δ/Δ</sup> homozygous allele have a slower growth rate, likely due to increased senescence. Bub1<sup>Δ/Δ</sup> MEFs have a reduced but still active spindle checkpoint in response to both nocodazole and paclitaxel, and have very high levels of aneuploidy compared to wildtype cells. Also, preliminary analysis indicates that Mad2 often mislocalizes in heterozygous and homozygous Bub1<sup>Δ/Δ</sup> MEFs. The high rate of aneuploidy in the presence of a functional spindle checkpoint suggests that a weakened but still partially active spindle checkpoint may not be enough to protect the cell from aneuploidy, or that Bub1 has a yet to be defined role in microtubule/kinetochore attachment.

L17
Golgi Ceramidase/Sphingosine Mediates the PMA-induced Growth Arrest of HeLa Cells
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Sphingosine, a sphingolipid metabolite, has been implicated in growth arrest and apoptosis of mammalian cells in response to various stress stimuli. However, it is unclear about how the generation of sphingosine is regulated. In this study, we demonstrate that the human Golgi ceramidase that we cloned recently plays a critical role in regulating the generation of sphingosine. In growth arrest of HeLa cells in response to PMA, HPLC analysis demonstrated that sphingosine was rapidly and robustly generated in HeLa cells challenged with PMA. MTT assays showed that haCER2 overexpression in HeLa cells enhanced the PMA-induced generation of sphingosine and the PMA-induced growth arrest of these cells whereas RNAi-mediated knockdown of haCER2 exhibited the opposite effects. Confocal microscopy revealed that haCER2 expression caused Golgi fragmentation and reduced the number of both endosomes and lysosomes without affecting the structure of the endoplasmic reticulum. Western blot analysis showed that haCER2 express inhibited protein glycosylation that occurs in the Golgi complex and decreased the levels of the acid ceramidase and EE1A, a lysosomal and endosomal resident protein, respectively. These results suggest that sphingosine generated through the action of haCER2 disturbs the structure and function of the acid membrane compartments. These results are consistent with our previous works showing that membrane compartments play an active role during cell division, we postulate that their disturbance may be associated with cell growth arrest mediated by the PMA/haCER2/sphingosine pathway.

L18
Control of the Mid-Blastula Transition in Drosophila by Cyclin/Cdk Activity
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In the embryonic cycles of Drosophila melanogaster, the initial 13 divisions occur in a common cytoplasm and alternate between periods of rapid DNA synthesis and mitosis without intervening gap phases. Starting in cycle 10 the length of S-phase progressively increases until the Mid-Blastula transition (MBT) at cycle 14, when cell cycle division occurs. Maternal, maternal-driven embryonic cycles are associated with a cell cycle arrest and apoptosis in response to various stress stimuli. Sphingosine, a sphingolipid metabolite, has been implicated in growth arrest and apoptosis of mammalian cells in response to various stress stimuli. Our results suggest that sphingosine generated through the action of haCER2 disturbs the structure and function of the acid membrane compartments. These results are consistent with our previous works showing that membrane compartments play an active role during cell division, we postulate that their disturbance may be associated with cell growth arrest mediated by the PMA/haCER2/sphingosine pathway.
L19 Identification of Plk4 Interacting Partners
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Plk4 (Sak) is a member of the polo family of serine/threonine protein kinases (Plks). Plks control the cell cycle through the regulation of the anaphase-promoting complex (APC), DNA damage checkpoints, spindle formation and cytokinesis. Polo family members share a common domain architecture consisting of an N-terminal catalytic domain and one or two characteristic C-terminal motifs termed polo boxes (pb). Plk4 is the most structurally divergent family member as it has only one pb domain. Currently the substrates for Plk4 remain elusive. Given the similar localization and expression patterns of Plk1, Plk3 and Plk4 during the cell cycle, it seems likely that Plk4 may be targeting some of the same substrates as other Plks, thus placing these targets under tighter or opposing controls. For example, the biological functions of mammalian Plk1 and Plk3 appear to differ, although both proteins complement thecdc5-1 temperature-sensitive mutants of budding yeast. Additionally, the roles that Plk4 plays in DNA damage pathways and cell division have not been characterized. We examined potential interactions between Plk4 with several candidate proteins, previously identified as interacting partners of other Plk family members. We have established, using a communomeric approach, that Plk4 interacts with a number of candidates, including p53. We are currently testing whether these associated proteins are also targets for Plk4 kinase activity. Our hypothesis is that Plks in general associate with a number of common interacting partners and that this association likely results in different target residues being phosphorylated by the different Plk family members, thus resulting in tighter if not opposite control on stability/activity of their substrate(s).

L20 The Role of Dicer in Regulating Cell Senescence
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Dicer is a key enzyme that processes microRNA (miRNA) from longer double stranded RNA precursors. A Dicer conditional mouse model was generated in our lab to investigate the role of Dicer and miRNA molecules in the regulation of cell growth. Mouse embryonic fibroblasts (MEFs) ablated for Dicer grew much slower, displayed a flattened, enlarged morphology, and expressed senescence associated (SA) β-galactosidase activity. Dicer-null MEFs also displayed increased expression of plasminogen activator inhibitor 1 and the cyclin-dependent kinase inhibitors p16 and p19(ARF). However, deletion of the p19/p16 (Ink4a/Arf) locus or deletion of the p53 tumor suppressor gene fully rescued Dicer-null cells from premature senescence. These results indicate that Dicer and miRNAs regulate cell growth, and that the replicative senescence induced by loss of Dicer is mediated by Ink4a and p53 pathway. Analysis of the involvement of specific miRNAs in regulating Ink/Arf and p53-mediated control of cell growth is ongoing. This work was supported by grants from NIH-DK073324 and AHA-0625823T.

L21 Functional Genomic Screen Reveals the Role of a Novel Checkpoint Kinase, TA01 in Mitosis and Cell Cycle Progression
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A single defective chromosome-microtubule attachment is sufficient to trigger spindle checkpoint and delay cell cycle progression. How microtubule attachment is sensed and integrated into the steps of checkpoint signal amplification is poorly understood. In a functional genomic screen targeting human kinases and phosphatases, we identified a microtubule affinity regulating kinase, MARKK/TAO1 as an important regulator of mitotic progression, required for both microtubule attachment and checkpoint-induced delay. TA01-depleted cells fail to form correct attachments even when mitosis is prolonged by delaying anaphase. TA01 interacts with checkpoint kinase BubR1 and promotes enrichment of Mad2 at sites of defective attachments, a crucial step in checkpoint signaling. Identification of TA01 kinase reveals an additional regulatory step that precedes checkpoint signal amplification and safeguards genomic stability in human cells.

APOSTOPIS

L22 Lignans from the Medicinal Bark of Guaiacum Officinale L. (Zygophyllaceae) Induce Apoptosis and Indicates Cell Cycle Arrest in Human MD-MBA-231 Breast Cancer Cells
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The isolation and elucidation of chemical entities derived from natural products has been an invaluable tool for innovative therapeutic applications. For centuries, the heartwood of Guaiacum officinale L. (Zygophyllaceae) was utilized by natives in South America and the Caribbean Islands to treat a variety of ailments including tumour growth. These purported treatments led us to believe that Guaiacum may possess chemical properties worthy of investigation. Interests centered on elucidating the compounds responsible for the purported bioactivity against mammary tumors. Evaluation took place within a chemo-biological context, utilizing bioassay-guided fractionation. Three 2,5-diaryl-3,4-dimethylytetrahydrafuran lignan derivatives: Nectandrin B, Isonecandrin B and Machilin I were elucidated with multidimensional NMR. The compounds were screened for the induction of apoptosis using Annexin V/FTTC and PI staining and DNA content analysis with flow cytometry. Results showed that Nectandrin B and Isonecandrin B significantly inhibited human breast cancer MD-MBA-231 cell proliferation in a dose dependent manner from 1.2 to 30ppm (P<0.05) and surpassed the activity of Camptothecin, a well-known chemotherapeutic agent at the two highest concentrations. These two compounds at a dose of 30ppm resulted in a 30% higher ratio of apoptotic to total cells as compared to the control groups (P<0.05). Furthermore, a constant decreasing pattern of % cells in S-phase was observed at all doses, with Machilin I showing significance only at the 30ppm. Interestingly, while Nectandrin B and Isonecandrin B showed a significant decrease in percent cells in G2/M at 30ppm, Machilin I had the inverse relationship and showed a significant 3-fold increase. These results suggest that Guaiacum lignans possess the ability to suppress the proliferation of MD-MBA-231 and that stereochemistry appears to be an important factor in governing both the degree of apoptosis induced and the differences shown in cell cycle blockage. (The study was supported by NIH T37TW00076.)

L23 What Effects Do the Household Chemicals Permethrin and Propylene Glycol Have on U937 Cells and A549 Cells?
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People are continuously exposed to chemicals; many of these chemicals have the ability to cause acute and chronic inflammatory responses. An estimated 64,000+ people in the United States die prematurely yearly due to pollution and chemical exposures. Permethrin (PTN) and propylene glycol (PG) are synthetic chemicals that are found readily in household products such as cosmetics, beauty aids and insect repellent. Previous studies have determined that PG and PTN at low doses decrease immune cell recognition and responses to foreign antigens. At high doses, PTN is also known to cause inflammation and necrosis. The present study explores the mechanism of action of PTN and PG by measuring alterations in cellular growth, viability, and toxicity. U937 and A549 cells were cultured in 24-well plates at 0.5-1 x 105 cells/well for 2 hours. The cells were then exposed to 0 - 10 µg/ml of PG and/or 0 - 0.5 µg/ml of PTN. The cultures were incubated for 24, 48, and 72 hrs at 37°C and 5% CO2. At each time point, the cells were observed, counted, stained with calcine AM and ethidium homodimer-1 and photographed using fluorescence and light microscopy. Data suggest that U937 cells and A549 cells exposed to PTN, PG and PTN + PG exhibited a dose dependent decrease in cellular growth. G12RR017581P, DOE grant DE-FG01-03ER63580.
L24 Human Tribbles Ortholog TRB2 Is Induced by and Mediates Cytokine Withdrawal-induced Apoptosis in TF1 Cells
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Tribbles, an atypical protein kinase superfamily member, coordinates cell proliferation, migration and morphogenesis during the development of Drosophila and Xenopus embryos. Although mammalian Tribbles orthologs are highly conserved throughout evolution, except for the known involvement of the human TRB3 in metabolic fasting and ER stress responses, the physiological functions of the Tribbles family are largely unknown. Here we report the possible involvement of human TRB2 in apoptosis regulation in hematopoietic cells. TRB2 is selectively induced by removal of survival factors in factor-dependent cells TF-1 and CD4+ T cells. Ectopic expression of TRB2 induces apoptosis in many factor-dependent hematopoietic cells, but not in most adherent cells. The apoptotic features induced by TRB2 include Mcl-1 degradation, Bak/Bax activation, cytochrome C release and membrane potential dissipation. Overexpression of the survival genes, such as Bcl-2, Mcl-1, or constitutively active Akt prevent apoptosis induced by TRB2. Knockdown of endogenous TRB2 expression reduces GM-CSF deprivation-induced apoptosis and other related apoptotic events in TF-1 cells. Our data demonstrate that in lymphohematopoietic lineages TRB2 represents a novel apoptosis activator. The Tribbles family proteins therefore may play important roles in apoptosis regulation and development in hematopoietic system.

L25 3-Ethylpyridine Accelerates Cell Attachment Rate and Subsequently Induces Rapid Cell Death by Activating the Intrinsic and Extrinsic Apoptotic Pathways
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3-Ethylpyridine, a chemical found in cigarette smoke, significantly inhibits human umbilical vein endothelial cell (HUVEC) growth and survival on polystyrene dishes at attomolar doses during 48 hours of treatment (Toxic. Sci. 93: 82). The purpose of this study was to observe the possible involvement of human TRB2 in apoptosis regulation in hematopoietic cells. TRB2 is selectively induced by removal of survival factors in factor-dependent cells TF-1 and CD4+ T cells. Ectopic expression of TRB2 induces apoptosis in many factor-dependent hematopoietic cells, but not in most adherent cells. The apoptotic features induced by TRB2 include Mcl-1 degradation, Bak/Bax activation, cytochrome C release and membrane potential dissipation. Overexpression of the survival genes, such as Bcl-2, Mcl-1, or constitutively active Akt prevent apoptosis induced by TRB2. Knockdown of endogenous TRB2 expression reduces GM-CSF deprivation-induced apoptosis and other related apoptotic events in TF-1 cells. Our data demonstrate that in lymphohematopoietic lineages TRB2 represents a novel apoptosis activator. The Tribbles family proteins therefore may play important roles in apoptosis regulation and development in hematopoietic system.

L26 Role of E4BP4 in Regulation of Glucocorticoid-evoked Lymphoid Cell Apoptosis
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Glucocorticoid hormones (GCs) play an important role in T-cell selection and eliminate non-reactive and self-reactive thymic T-cells and peripheral lymphocytes via apoptosis. Synthetic GCs serve as therapeutic agents for some lymphoid leukemias. Using GC-sensitive CEM-C7-14 and GC-resistant CEM-C1-15 human T cell leukemic sister clones, we have shown that the bZIP transcriptional repressor, E4BP4, is preferentially up regulated in the GC-sensitive CEM-C7-14 cells. We now demonstrate that ectopic expression of mouse E4BP4 in the CEM-C1-15 cells re-establishes GC-evoked apoptosis. E4BP4 is an evolutionarily conserved homolog of the pro-apoptotic C. elegans death specification gene ces2, which is known to down regulate the downstream survival gene ces1, to allow the up regulation of the pro-apoptotic gene eg1, resulting in apoptosis in specific neuronal cells. Human orthologs of eg1, Bim and Puma (BH-3 only proteins of the Bcl-2 family), have previously been shown to be up regulated in CEM-C7-14 cells, but not in CEM-C1-15 cells, in conjunction with GC-evoked apoptosis. The role of human orthologs of ces1, Slug and Snail (survival genes), has not been explored in GC-evoked apoptosis. Our Western blot data affirm that GCs down regulate expression of the Slug protein in CEM-C7-14 cells, but not in CEM-C1-15 cells. These effects suggest the existence of an apoptotic pathway in CEM cells that is analogous to the C. elegans ces2>ces1>eg1 pathway. E4BP4 dependence of the regulation of Slug, and of other significant proteins mediating apoptosis, including cyclin D3, p27kip1, and c-Myc is currently being investigated. Supported by NIH MBR5-SCORE program, the CSUN office of Graduate studies, Research and International programs and the CSUN College of Science and Mathematics. LJN received the CSUN Retired Faculty Award to partially fund this project.

L27 Par-4 Interaction with GRP78 in the Endoplasmic Reticulum
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The glucose regulated protein 78 (GRP78) has historically been considered as a molecular chaperone in the folding, maturation and transport of proteins. GRP 78 alleviates endoplasmic reticulum (ER) stress, maintains ER function and protects against cell death. Recently, the ER stress pathways and GRPs have been linked to prostate cancer growth, proliferation and endoplasmic GRP78 expression confers resistance to prostate cancer. We now demonstrate that GRP78 interacts with the bZIP transcriptional repressor, E4BP4, in prostate cancer PC-3 cells. Par-4 is a leucine zipper domain protein that induces apoptosis in cancer cells but not in normal cells. Our in vitro studies show that GST-Par-4 and GST ΔZIP (which lacks the leucine zipper domain) but not the control GST protein, binds to GRP78 in GST pull down experiments with prostatic PC3 cell lysate. These interactions were further confirmed by co-immunoprecipitation assay with ectopic Par-4 and endogenous GRP78. The immunocytochemistry data reveals the colocalization of Par-4 and GRP78 in the ER compartment of prostatic cells. We suggest a pool of Par-4 is retained in the cytoplasmic compartment in association with GRP78. The precise role of Par-4 shutting and the core domains that interact with GRP78 is currently under investigation.

MITOSIS AND MEIOSIS

L28 Asymmetric 3D Gaussian Function Fitting Method Reveals the Detailed Spatial Orientation of Hec1 and Spc24 at Metaphase Kinetochores
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Using a 2D Gaussian function to approximate the objective Point Spread Function (PSF) to fit a fluorescent punctate signal is a common way to get its localization with sub-pixel accuracy. PSF represents the image of an infinite small light source. However, protein complexes like vertebrate kinetochores, despite their sub-resolution dimensions, still vary in sizes and shapes. As a result, their fluorescent intensity distribution is the convolution result of the shape with PSF and different from PSF itself. The asymmetric 3D Gaussian function, which has 3D orientation and different x and y deviations, is a better fitting function than the symmetric 2D Gaussian function. A Matlab program was developed to implement such algorithms with least-squares-fitting method. It yields 30% less fitting residual than 2D PSF fitting method by comparing the fitting results of Hec1 protein immunofluorescent
signal at kinetochores of human Hela cells. Hec1 and Spc24 are part of the ~50 nm long Ndc80 complex which is located in-part within the outer plate of the kinetochore where kinetochore microtubule plus ends are anchored. The Ndc80 complex is required for robust plus-end anchorage and a target for destabilization of attachment by the Aurora B kinase. Studying its orientation relative to the sister-sister kinetochore axis provides insights into Ndc80 function. Using 3D speckle high resolution co-localization (SHREC) methods, we found that the relative position between red fluorescent Hec1 and green fluorescent Spc24 along the sister-axis is on average 44nm (±5nm) in control metaphase Hela cells that have average sister kinetochore separation of ~1.5um. Taxol treated metaphase cells have an average projection distance of 47nm (±15nm), but an average separation of 0.8um. These results show that loss of centromere tension, but not attachment, by taxol treatment does not alter the orientation or length of the Ndc80 complex. Supported by NIH GM24364 and GM60648.

L29
Development of a High-Throughput Two-Color Mitotic Index Assay
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Mitosis is a highly regulated process that can be identified by chromosome condensation in the nucleus and the phosphorylation of histone proteins. Here we describe the development of a simple, two color high throughput assay that will enable users to quantify the number of cells in a population that are mitotic versus non-mitotic. The assay uses a marker antibody to detect the phosphorylation of histone H3 (serine 28) while propidium iodide (PI) is used to label all nuclei. Total cell number can be determined by counting nuclei in the PI channel while the degree of signal measured in the green channel (phospho H3) indicates which cells are mitotic. Experiments were performed to determine the optimal concentration of the primary antibody and to demonstrate how fixation and permeabilization conditions can affect the assay. We used paclitaxel (Taxol™), an agent that prevents mitotic spindle assembly, to show how the assay can be used to monitor the effect of a drug on mitosis. Hela cells were treated with salt (0.1M) for six hours and then fixed with formaldehyde (4%) followed by Triton-X 100, formaldehyde followed by methanol, formaldehyde followed by saponin (0.1%) or methanol only. Images were taken using a Nikon fluorescent microscope and plates were scanned on the IsoCyt for automated mitotic index analysis. Formaldehyde and Triton-X 100 resulted in a cytosolic rather than nuclear staining pattern, indicating that the nuclear membrane had not been permeabilized sufficiently to allow the antibody to access the target. Formaldehyde with saponin resulted in both nuclear and cytosolic staining, while methanol alone generated the most well-defined staining in the nucleus. By using this assay with the IsoCyt it was possible to correlate an increase in the mitotic index with increased exposure time to paclitaxel (2, 4, 8 and 24 hours).

L30
A Role of RASSF1A in Centrosome Separation
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The RAS association domain family 1A (RASSF1A) gene is located at chromosome 3p21.3 within a specific area of common heterozygous and homozygous deletions. RASSF1A most frequently suffers from chromosome 3p21.3 within a specific area of common heterozygous and homozygous deletions. RASSF1A overexpression is similar to what was seen in cells deficient in Aurora-A or Eg5. Interestingly, we found that RASSF1A was associated with Aurora-A but not Eg5. The catalytic domain of Aurora-A is required for this interaction. We hypothesize that RASSF1A regulates centrosome separation by modulating Aurora-A function. It is still under investigation how RASSF1A exerts its effect on Aurora-A.

L31
Long-term FLIP Experiments Reveal Two Compartments of Mad1 at Mitotic Kinetochore
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Mitotic cells actively prevent genomic damage caused by aneuploidy by way of the spindle assembly checkpoint (SAC). Previous work on SAC proteins has shown that Mad1 at kinetochores recruits Mad2, which then inhibits Cdc20’s ability to activate the anaphase promoting complex (APC/C). When activated, the APC/C initiates sister chromosome separation. Two proposed models attempt to explain how unattached kinetochores are able to successfully produce mature anaphase inhibitors. The “template model” of the SAC predicts that stable Mad1 binds and holds 50% of Mad2 at kinetochores while the other 50% rapidly binds and releases on this stable Mad2. This transient population can sequester Cdc20 in the cytoplasm and also act as a template to self-propagate more Mad2-Cdc20 inhibitory complexes. A competing “two-state Mad2” model states that all Mad2 binds and releases from stable Mad1 as dimers to bind Cdc20 in the cytoplasm, and that this complex cannot self-propagate. To test these models, we used fluorescence loss in photobleaching (FLIP) in nocodazole-treated PtK2 cells constitutively expressing YFP-Mad1 and Mad2 to evaluate what fractions of each protein were stable at kinetochores or exchanged with cytoplasmic pools. Our results did not yield data favoring either model, as approximately 30% of both Mad1 and Mad2 were found to be stable at kinetochores after the ten-minute FLIP period to bleach most cytoplasmic subunits. The continued observation of kinetochore fluorescence levels for fifty more minutes showed that exchange persists with cytoplasmic pools. The plot of YFP-Mad1 kinetochore fluorescence decay over sixty minutes was well fit to a double four-parameter exponential curve, suggesting biphasic characteristics. Nocodazole treatment thus potentially reveals two compartments of Mad1 in mitotic cells: a rapidly exchanging pool bound weakly to kinetochore fibers of kinetochores, and a more stable fraction bound with stronger affinity to the outer plate. Supported by grants from NIHGMS and HFSP.

KINETOCHORES

L32
Hierarchical Regulation of Human Kinetochore Function
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Chromosome-microtubule (MT) attachment is mediated by multiprotein complexes known as kinetochores, which assemble on centromeric DNA and act as a platform for microtubule attachment during mitosis. Kinetochore also monitor the state of chromosome-MT attachment and activate the spindle checkpoint in the presence of incorrectly attached chromosomes. Recent studies based on affinity-purifications of human kinetochore proteins revealed a novel kinetochore complex, termed the CENP-H/I complex, which contains up to 11 protein subunits (Foltz et al., 2006, Okada et al., 2006). An important question is whether all these proteins act as one functional unit, or whether there can be resolved as subcomplexes with specific kinetochore functions. We have investigated by RNA interference, time-lapse recording and various cell biological assays the function of three CENP-H/I complex subunits: CENP-H, Chl4R/CENP-N and Mem21R/CENP-O. Based on dependency experiments we find that the CENP-H/I complex consists of at least two structural layers: Mem21R depends on Chl4R or CENP-H for kinetochore recruitment, while CENP-H and Chl4R are dependent on each other, but not on Mem21R to bind kinetochores. This organization is also reflected at the...
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functional level. Depletion of Mcm21R abrogates the spindle checkpoint and impairs bipolar spindle formation, while Chl4R depletion leads to spindle checkpoint activation and severe congestion defects that are associated with normal spindle formation. This latter result is particular striking since these cells lack kinetochore bound Mcm21R - which, when depleted, is known to cause a failure in spindle formation. We propose that Chl4R depletion suppresses spindle assembly defect in Mcm21R depleted cells. Consistent with this idea, the double depletion of Chl4R and Mcm21R reinitates correct spindle formation and checkpoint activities. Together these results indicate that the CENP-H/I complex is composed of at least two structural and functional layers that regulate spindle checkpoint and chromosome attachment functions during mitosis.

ACTIN-ASSOCIATED PROTEINS

L33

Ezrin Function in the Retinal Pigment Epithelium (RPE) and Retina

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Ezrin, a member of the ezrin/moesin/radixin (ERM) family, localizes to microvilli of epithelia in vivo, where it functions as a bridge between actin filaments and plasma membrane proteins. In the posterior eye, ezrin has been localized to Müller cell microvilli and to apical microvilli and basal infoldings of RPE. Our hypothesis is that ezrin expression is required to establish and maintain RPE apical microvilli and basal infoldings and the Müller cell microvilli. To test this hypothesis, recently we followed the development of these structures in the eyes of ezrin knockout mice. Although these mice appear normal at birth, neonates fail to thrive postnatally and do not survive past weaning. In comparison to results obtained from WT L37 mice, we determined that loss of ezrin leads to substantial reductions in the apical microvilli and basal infoldings in RPE cells and in the Müller cell microvilli, as well as slow photoreceptor development. To further understand the role of ezrin in the eye, we have generated a RPE cell specific knockout mouse line. While RPE microvilli and basal infoldings are formed, they appear abnormal ultrastructurally and express low levels of RPE microvilli transporters. These mice will prove useful to understand the role of ezrin in RPE development and function. Supported in part by NIH grants EY06603, EY14240, EY15638, a Research Center Grant from The Foundation Fighting Blindness and funds from the Cleveland Clinic Foundation.

L34

Partial Co-localization of Profilin, VASP, and the Arp2/3 Complex Is Found at the Very Edge of Lamellipodia in Migrating Cells

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Dynamic reorganizations of the microfilament system drives cell migration, and are controlled by the pathways resulting from the signals caused by interactions of the cell surface with its surrounding milieu. A large number of proteins are involved in controlling actin polymerization. Profilin, VASP and the Arp2/3 complex are key components, which operate in this context. To locate profilin and/or the profilin:actin (PA) complex in B16F11 mouse melanoma cells, we have used affinity purified antibodies raised against profilin:actin (1). We compared the distribution of profilin/PA along with VASP and the Arp2/3 complex, respectively, in defined segments of the edge and quantified the fluorescence intensities generated by the different antibody labeling. In AIF3-stimulated cells, profilin and VASP were found to accumulate at the edge of the lamellipodium and showed extensive co-localization. This observation is in agreement with VASP functioning at the leading edge to recruit profilin:actin for the addition of new actin subunits to growing filament ends as recently was reported (2). Also the Arp2/3 complex co-localizes with profilin/PA, but to a lesser extent than VASP. References: 1. Grenklo S, Johansson T, Bertillson L, Karlsson R. Anti-actin antibodies generated against profilin:actin distinguish between non-filamentous and filamentous actin, and label cultured cells in a dotted pattern. Eur J Cell Biol. 2004; 83(8):413-423. 2. Grenklo S, Gecse M, Lindberg U, Wehland J, Karlsson R, Sechi AS. A crucial role for profilin-actin in the intracellular motility of Listeria monocytogenes. EMBO Rep. 2003; 4(5):523-529.

L35

Structural Studies on the Cytoskeletal Proteins Talin and Vinculin

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The dynamics of the interactions between integrin cytoplasmic domain and adapter proteins talin, as well as talin-vinculin interaction plays an important role in the assembly of cell-extracellular matrix, vinculin called focal adhesions (FA). Talin couples integrins to F-actin and contains ~11 vinculin binding sites (VBS). Studies on cells carrying a conditional talin1 allele confirm that it is essential for efficient cell spreading and FA assembly. In contrast, vinculin-null cells still assemble FA’s although they are smaller, more dynamic and the cells are more motile. This suggests that vinculin might be recruited to stabilize integrin-talin-actin complexes. Previously, we determined the crystal structure of the vinculin head (Vh) in complex with different synthetic VBS peptides. The interaction is mainly hydrophobic between the VBS’s amphipathic helix and the Vh helices 1-4, forming a new 5 helix bundle. The consensus sequence for a VBS is LxxAxxVxxVxLxxxA. NMR and crystal structures of various talin rod domains show that those key residues are buried within the core of the helical bundles that make up the talin rod. Indeed intact talin binds vinculin with low affinity (~9 μM) suggesting that most of the VBS’s present within the talin rod are cryptic. The release of VBS in talin may be a key trigger underlining integrin mediated signal transduction and assembly of the actin cytoskeleton. The mechanism of activation is still unclear, but our NMR, EPR and proteolysis studies show that vinculin binding induces a dramatic conformation change in VBS-containing talin rod domains. Moreover, mutations that stabilise the helical bundles within the talin rod dramatically reduce vinculin binding. Thus, one factor which appears to determines the availability of a VBS is the inherent stability of the helical bundle in which it is buried, and this varies from bundle to bundle.

L36

Monoclonal G3.5 Antibody and Cytoplasmic α-Actinin Antibody Label the Same Antigen in Rat Brain

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The G3.5 monoclonal antibody was raised in mice against multiple sclerosis plaques from human brain and spinal cord. This antibody recognises an antigen that has similar physical properties and a partial sequence identity with the microfilament associated protein, α-actinin. However, the antigen was originally found in glial and muscle tissue, and was isolated in association with type III intermediate filaments (desmin). In addition, the G3.5 antigen (and chicken gizzard α-actinin) has been shown capable of cross-linking desmin and f-actin in vitro, leading to the hypothesis that the G3.5 antigen is a novel isoform of α-actinin found only in cells expressing type III intermediate filaments. Localization of the G3.5 antibody was analyzed in rat brain using immunogold electron microscopy double labeling techniques. The images obtained showed co-localization of the G3.5 antibodies and cytoplasmic α-actinin antibodies around membrane structures in the brain tissue. The binding of the antibodies was within 30 nm of each other which corresponds to the length of α-actinin, strongly suggesting that the two antibodies were recognizing the same structure. These findings suggest that the monoclonal antibody G3.5 recognizes cytoplasmic α-actinin.

L37

Altered Interaction of FSGS-associated α-actinin-4 (K256E) with Endogenous Binding Partners in Podocytes

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Mutations in the ACTN4 gene, encoding the actin filament (F-actin) crosslinking protein α-actinin-4, are associated with a familial form of focal segmental glomerulosclerosis (FSGS). The disease-causing K256E mutation (α-actinin-4 K256E) dramatically increases its affinity for F-actin thereby altering the subcellular localization of α-actinin-4 while impairing cell migration, spreading and formation of foot process-like projections in cultured podocytes (Michaud et al, 2006). Additionally, α-actinin-4 likely functions beyond its actin bundling capacity since it interacts with a number of other proteins including the adherens junction protein β-catenin and the actin filament binding protein - synaptopodin, among others. We hypothesized that due to its mislocalization and enhanced association with F-actin in podocytes, the interaction of α-actinin-4 K256E with synaptopodin and β-catenin would be impaired. For this purpose we infected a conditionally-immortalized mouse podocyte cell line with adenovirus to over-express either wild-type (α-actinin-4 WT) or α-actinin-4 K256E. Immunoprecipitation and immunofluorescence approaches revealed that synaptopodin interacts and co-localizes with both α-actinin-4 WT and α-actinin-4 K256E. However, in cells expressing α-actinin-4 K256E there was a significant reduction in synaptopodin levels. In addition, immunofluorescence showed co-localization of β-catenin and α-actinin-4 WT, but not α-actinin-4 K256E, at the cell periphery including lamellipodia and cell-cell contacts. Similarly, there was significantly less β-catenin immunoprecipitated with α-actinin-4 K256E as compared to α-actinin-4 WT. These findings demonstrate that the K256E mutation sequesters α-actinin-4 away from specific subcellular domains such that it cannot interact with some of its endogenous binding partners. The abrogation of such interactions may underlie podocyte dysfunction in actinin-associated FSGS.

L38
Identification of Additional Calpain Cleavage Sites in Filamin A
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Filamin A (FLNa) is a protein that organizes actin filaments into networks. Previous studies indicated that a dynamic phosphorylation/depolymerization process may regulate the modulation of FLNa with other cytoskeletal elements. Depolymerization of FLNa by treatment with E. coli alkaline phosphatase resulted in the loss of its ability to crosslink F-actin into a low-speed sedimentable complex (platelet cytoskeleton) whereas phosphorylation of the native protein by cAMP-dependent protein kinase (PKA) strengthens this structural framework by protecting the molecule against proteolytic cleavage by calpain. In this case the phosphorylation site lies in the 100 kDa carboxy-terminal fragment produced by cleavage at residues 2152. In this regard, FLNa was described as a phosphoprotein whose phosphate content ranges from 18 to 40 moles of Pi/mole FLNa. Indeed, the analysis of the amino acid sequence of FLNa revealed multiple consensus sequences specific for diverse kinases, including three sites for PKA at residues 167, 2152 and 2336, whereas the calpain cleavage site has been localized to residues 1761-1762, 100 kDa away from the C-terminal end. There is an additional calpain site at 10 kDa from the C-terminal region but its cleavage is ineffective. Thus, calpain digestion results in polypeptides of 180 kDa, 100 kDa, 90 kDa and 10 kDa. Here we report that FLNa could be additionally hydrolyzed in alternative sites by calpain when the 180 kDa N-terminal and 100 kDa C-terminal regions of the molecule were expressed as recombinant peptides in an E. coli system. Additional cleavage sites could also be detected in FLNa from aged platelets that was previously found to contain a reduced level of phosphate. We propose that the high content of phosphate in FLNa masks susceptible sites that otherwise would be available for protease action.

ACTIN DYNAMICS AND ASSEMBLY

L39
Regulation of Cell Shape by Cdc42 Is Mediated by the Synergic Actin Bundling Activity of the Eps8:IRSp53 Complex
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Actin cross-linking proteins organize actin into highly dynamic and architecturally diverse subcellular scaffolds orchestrating a variety of mechanical processes, including lamellipodial and filopodial protrusions in motile cells. How signalling pathways control and coordinate the activity of these cross-linkers is poorly defined. IRSp53, a multi-domain protein, which can associate to the Rho-GTPases Rac and Cdc42, participates in these processes mainly through its N-terminal IMD (IRSp53 and MIM Domain). The isolated IMD displays actin bundling activity in vitro and is sufficient to induce filopodia in vivo. However, the regulation of this activity in the full-length protein remains largely unknown. Eps8 is involved in actin dynamics through its actin barbed ends capping activity and its ability to modulate Rac. Moreover, Eps8 binds to IRSp53. Here, we describe a novel actin cross-linking activity of Eps8. Additionally, Eps8 activates and synergizes with IRSp53 in mediating actin bundling in vitro, enhancing IRSp53-dependent membrane extensions in vivo. Cdc42 binds to and controls the cellular distribution of the IRSp53-Eps8 complex, supporting the existence of a Cdc42-IRSp53-Eps8 signaling pathway. Consistently, Cdc42-induced filopodia are inhibited following individual removal of either IRSp53 or Eps8. Collectively, these results support a model whereby the synergic bundling activity of the IRSp53-Eps8 complex, regulated by Cdc42, contributes to the generation of actin bundles, promoting filopodial protrusions.

Tuesday

L40
Polymerization of Formin Capped Actin: A Model for Accelerated and Decelerated Rates
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Formin family proteins act as processive cappers of actin filaments. The rate of actin polymerization upon processive capping varies within a broad range depending on the formin type and presence of profilin. While FH2 domains of various formins slow down polymerization by different extents, the FH1-FH2 domains in conjunction with profilin accelerate the reaction. We propose a model predicting that variation of a single physical parameter - the effective elastic energy of the formin-capped barbed end-results in the observed diversity of the polymerization rates. The model is based on the energy consideration of a continuous range of thermodynamic states adopted by the polymerizing barbed end upon processive capping by formin. In the heart of the model is the cycle accumulation and relaxation of the system's elastic energy. We show that the model accounts for the whole range of the experimental results on the kinetics of the formin-mediated filament growth, including the 4.5 fold acceleration by mDia1 formin in the presence of profilin. Fitting the theoretical predictions to the experimental curves provides the values of the effective elastic energies of different formin-barbed end complexes.

L41
Yeast Formins Bni1 and Bnr1 Utilize Different Modes of Cytoskeletal Interaction during the Assembly of Actin Cables
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The budding yeast formins Bni1 and Bnr1 control the assembly of actin cables. These formins exhibit distinct patterns of localization and polymerize two different populations of cables: Bni1 in the bud and Bnr1 in the mother cell. We generated a functional Bni1-3GFp that improved the visualization of Bni1 in vivo at endogenous levels. Bni1 exists as speckles in the cytoplasm, some of which co-localize on actin cables. These Bni1 speckles display linear, retrograde-directed movements. Loss of polymerized actin or specifically actin cables abolishes both retrograde movements and resulted in depletion of Bni1 speckles from the cytoplasm, with enhanced targeting of Bni1 to the bud tip. In contrast, Bnr1 did not detectably associate with actin cables, and was not observed as cytoplasmic speckles. Mutations that impair the actin assembly activity of Bni1 abolished the movement of Bni1 speckles, even when actin cables were present. Finally, fluorescence recovery after photobleaching demonstrated that Bni1 was very dynamic, exchanging between polarized sites and the cytoplasm, whereas Bnr1 was confined to the bud neck and did not exchange with a cytoplasmic pool. Based on these findings, we suggest that formins can have different modes of interaction with the cell cortex yet perform similar functions in actin cable assembly.

L42
Focal Adhesions and Actin Remodeling by the JNK MAP Kinase Pathway: A Role in Adenovirus E4orf4-dependent Cell Killing
at early timepoints, cells that overexpress the protein for a longer time
are remodeled into big complexes at the cell periphery, but also near the
juxtanuclear sites of actin remodeling. This was associated with
paullitin phosphorylation, recruitment of GT1, a paullitin-interacting
scaffold involved vesicular traffic, and accumulation of endosomes causing a
GPI-linked proteins to the forming juxtanuclear actin network.
Surprisingly, siRNA-directed knockdown of JNK1, but not JNK2,
dramatically inhibited E4orf4-dependent actin dynamics and apoptotic-
like nuclear condensation. In light of these results, we propose that the
early activation of JNK1 by E4orf4 is required to assemble a paullitin-
JNK1 complex that regulates FA dynamics and coordinates vesicular
traffic in the direction of actin remodeling. The results support a role for
the JNK pathway in the regulation of actin-dependent endocytic

L43
Functional and Physical Interactions between Gas7 and N-WASP
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Actin reorganization/polymerization is critical for many cell processes,
such as cell migration, cell differentiation, vesicle trafficking or
endocytosis. In order to maintain a normal cell process, regulation of
actin polymerization becomes important. Here, we found that Gas7
(growth arrest specific gene 7) can physically interact with an
important cytoskeleton regulator, N-WASP, and they are co-localized in
Neuro2A cell and mouse brain. We also demonstrated the interaction between
Gas7 and N-WASP is mediated through coiled-coil region.
Proline rich region, respectively. Moreover, Gas7 induced neurite-
like processes is also mediated through this interaction and is not
dependent on Cdc42. Thus, these data evince that Gas7 plays some roles in
the N-WASP mediated actin polymerization. How Gas7 acts to
N-WASP mediated pathway remains to be investigated.

DYNEIN
L44
A Role for the p24 Subunit of Dynactin in Apoptosis
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The function of dynactin’s smallest subunit, p24, remains largely
uncharacterized. p24 is part of the shoulder/sidearm sub-complex, along with p150 and dynamin. Previous studies have indicated that
overexpression of p24 causes microtubule disorganization and centroosomal fragmentation, as seen with other subunits of the
shoulder/sidearm. However, overexpression of p24 does not fragment
the Golgi complex, as seen when overexpressing dynamin or p150.
Cells overexpressing p24 are also less hardy than those overexpressing
other dynactin subunits and will generally die ~24 hours after
transfection. Previous results have indicated that these cells are undergoing apoptosis, as observed through poly-ADP ribose nuclear
localization in both synchronized and unsynchronized cell populations.
We have investigated this further, and show that while p24-
overexpressing cells exhibit high concentrations of cytoplasmic protein
at early timepoints, cells that overexpress the protein for a longer time
accumulate p24 in the nucleus. In these cells, some p24 aggregates co-
localize with aggregations of p53, suggesting that the p53-mediated
apoptotic pathway is used to kill those cells. We are currently examining other proteins in this pathway, both microscopically and
biochemically for interactions with p24. These results suggest that,
rather than an artifact of overexpression, p24-induced apoptosis may be
one role for this dynactin subunit.

L45
On the Presence of Cytoplasmic Dynein in Higher Plant Cells
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The microtubule-based cytoskeleton is a highly conserved system, and
components that comprise microtubules have been identified in all
major eukaryotic groups. Cytoplasmic dynein is essential in the transport of membranous organelles, formation of the Golgi apparatus,
assembly and function of the mitotic spindle, cilia and flagella. Higher
plant cells lack cilia and flagella, and full-length genes for cytoplasmic
dynein heavy chain (DHC) were not found in Arabidopsis genome.
However, the studies on Nicotiana pollen tubes revealed the presence
of DHC-related polypeptides, expressed during pollen germination
(Moscatelli et al., 1995). Thus, the presence of cytoplasmic dynein in
higher plant cells remains to be characterized. We used antibodies against the
motor and MT binding domains of DHC from Dictostelium
(antibodies were kindly provided by Dr. M. Koonce, Wadsworth
Center, USA) on the whole cell extracts and in cells of root meristem of
Trichum aestivum. We report that both antibodies react with a band of
relative molecular mass ~500 kDa in crude supernatant of meristem
cells and precipitate high molecular weight polypeptide from protein
extracts. Immunostaining showed that the antibodies associate with
vesicles, accumulated around the nucleus during interphase-early
prophase and around daughter nuclei in late telophase. From late
prophase to early telophase, vesicles localize at the polar region,
spindle periphery, and interzonal area during phragmoplast and cell
plate formation. Double immunostaining with antibodies to DHC and
to the Golgi apparatus shows that some vesicles labeled by the
antibodies to DHC belong to the Golgi apparatus. The distribution of DHC-positive vesicles is consistent with the data of
Vaisberg et al. (1996) on association of the mammalian DHC isoforms
with Golgi apparatus and other membranous organelles. We suggest that
DHC isoforms do exist in higher plants. This work was supported by
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L46
Ldb18 is a Component of the Dynactin Complex in Saccharomyces
cerevisiae
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In the budding yeast Saccharomyces cerevisiae, the mitotic spindle is
oriented in two steps. It is first positioned near the bud neck in a
process requiring Kar9 and is later pulled through the neck after the
onset of anaphase in a process requiring dynein/dynactin. Cells can
survive the loss of one of these pathways but loss of both is lethal. We
performed a genome-wide screen to identify gene deletions that are
synthetic lethal in combination with a temperature-sensitive allele of the
microtubule-associated protein Stu1. We identified ldb18Δ which
harbours a synthetic lethal with loss of other genes affecting spindle orientation, spindle elongation, and tubulin folding. LDB18 is a non-essential gene, although ldb18Δ cells grow slowly at
low temperature. In addition, in an unusually high percentage of large-
 budded ldb18Δ cells, spindle elongation occurs entirely within the
mother cell, indicating a defect in the dynein orientation pathway.
Consistent with this conclusion, ldb18Δ is synthetic lethal with loss of
proteins in the Kar9 orientation pathway but not loss of proteins in the
dynein pathway. Ldb18 shares modest sequence and structural
homology with the mammalian dynactin component p22/24, which has
been shown to bind p150Glued directly, but whose function in the
dynactin complex is unknown. Using the two-hybrid assay, we have
found that Ldb18 interacts strongly with the dynactin complex
components Jnm1 and Nap1,000, as well as itself, and to a lesser
degree with Jnm1 and Arp10. Ldb18 also co-immunoprecipitates Jnm1
and Arp1. Thus, based on genetic and biochemical evidence, we propose that
Ldb18 is the p22/24 equivalent in the dynactin complex of yeast.
Identification of the Genes of All Known *Chlamydomonas* Inner-Arm Dynein Heavy Chains

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The inner arm dynein of cilia and flagella comprises multiple species. In *Chlamydomonas*, seven species (called dynein a-g) have been identified by biochemical analyses, of which one species (dynein f, also called dynein I1) contains two different heavy chains (DHCs) and the other six species contain single heavy chains (Kagami and Kamiya, 1992). On the other hand, gene analyses have identified 11 DHC genes (*DHC1-DHC11*) assigned to inner-arm dyneins (Porter et al., 1996; Perrone et al., 2000). Only three DHC genes have previously been correlated with particular inner arm species: *DHC1* and *DHC10* with dynein f I1 and *DHC11* with dynein c. Here, we determined the DHC genes for the other inner-arm dyneins by peptide-mass fingerprinting. The DHCs of dynein a, b, d, e, and g were found to be encoded by *DHC6, DHC5, DHC2, DHC8*, and *DHC7*, respectively. In addition, we found that the DHCs encoded by *DHC4* and *DHC11* are present in the axoneme in small amounts. Thus all DHC genes except *DHC3* have been shown to have their products in the axoneme. Unlike other DHCs, most of *DHC4* and *DHC11* remain associated with the axoneme after high-salt extraction. *DHC11* is missing in the mutant that lacks p28, an inner-arm dynein light chain. A phylogenetic tree constructed using the sequence of all *Chlamydomonas* DHCs (including those predicted from the genome project data) shows that the DHCs can be largely classified into two groups: a multi-headed dynein group comprising outer-arm DHCs, two-headed inner-arm (species f) DHCs and cytoplasmic DHCs; and a single-headed group comprising the other nine DHCs. The latter type can be further classified into a subgroup comprising *DHC3* and *DHC7* (dynein g) and a subgroup comprising the rest of the DHCs. Dynein g and *DHC3* may perform some unique function in the flagellar motility mechanism.

**L47**

**G Protein Betagamma Subunit Interacts with Dynein Light Chain Tctex-1**

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The molecular determinants that allow heterotrimeric G protein βγ subunits to achieve effector specificity and regulate activity remain unclear. Here, we demonstrate a direct interaction between AGS2, a receptor-independent activator of G protein signaling, and Gβγ. We identified the key contact regions within each of the binding partners and showed that phosphorylation of Gβ serves to regulate negatively the Gβγ-AGS2 interaction. AGS2 is known also to be identical to Tctex-1, a light chain component of the cytoplasmic motor protein dynein. We demonstrate that Gβγ employs the same structural binding motif as the dynein intermediate chain (DIC) for Tctex-1 binding and competitions with DIC for Tctex-1 binding. Immunoprecipitation experiments show that Gβγ overlaps with the dynein-free pool of Tctex-1, and Gβγ and Tctex-1 show similar subcellular distribution in neurons, most prominently in growth cones. We propose that Gβγ modulates the dynein-independent role of Tctex-1 by regulating Tctex-1-dynein motor complex formation.

**MICROTUBULE-ASSOCIATED PROTEINS**

**L49**

**Key Interaction Modes of Dynamic +TIP Networks**

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Microtubules (MTs) are polarized structures organized by the centrosome. The minus-end is generally docked at the centrosome and the plus-end scans the cytoplasm to find targets such as the chromosomes. During mitosis, this network forms a bipolar spindle, attaches the kinetochores and allows equal segregation of the chromosomes. Ajuba is a LIM-family member first described in cell adhesion and migration. It has been recently involved in cell cycle progression as an activator of the Aurora A kinase, allowing mitotic commitment. We show that Ajuba localizes to the centrosomes and to the kinetochore microtubule tips (KMTs), and that Ajuba is a microtubule-associated protein (MAP). Owing to its contribution to the mitotic commitment and its MT-dependent localization, Ajuba may play a role during the metaphase/anaphase transition. We show that Ajuba interacts with two major players of the mitotic checkpoint. After inhibition of one of these proteins by siRNA, Ajuba is delocalized and the chromosomes are not properly aligned at the metaphase plate. Our sequence motifs. We recently have explored key binding modes of +TIPs by analyzing the interactions between selected CAP-Gly, EB-like, and carboxy terminal EEY/F-COO sequence motifs (1, 2). Using X-ray crystallography and biophysical binding studies we demonstrate that the β2-β3 loop of CAP-Gly domains determines EB-like motif binding specificity. We further show how CAP-Gly domains serve as recognition domains for EEY/F-COO motifs which represent characteristic and functionally important sequence elements in EB, CLIP-170, and α-tubulin. Our findings provide a molecular basis for understanding the modular interaction modes between α-tubulin, CLPs, EB proteins, and the dynactin-dynein motor complex and suggest that multiple binding sites in different combinations control dynamic +TIP networks at microtubule ends. We hypothesize that a local increase in concentration of EEY/F-COO sites together with secondary binding sites on EB and tubulin favors the transient accumulation of CAP-Gly containing +TIPs such as CLIP-170 and the dynein-dynactin motor complex at microtubule ends. Our findings further offer insights into the structural consequences of genetic CAP-Gly domain defects found in severe human disorders. This work was supported by the Swiss National Science Foundation through Grant 310000-109423 and within the framework of the NCCR Structural Biology program. 1. Honnappa et al., EMBO J. 24, 261 (2005) 2. Honnappa et al., Mol. Cell, 23, 663-671 (2006)

**L50**

**Driving and Coupling: A “Pac-man” Mechanism for Chromosome Poleward Translocation in Anaphase A**

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During mitosis, chromatin harnesses its kinetochore translocation at the depolymerizing microtubule ends for its poleward movement in anaphase A. The force generation mechanism for such movement has long been speculated. Analysis of the current experimental results shows that the bending energy release from the bound tubulin subunits alone could not provide sufficient driving force. Additional contribution from effective electrostatic attractions between the kinetochore and the microtubule is needed for kinetochore translocation. Interestingly, as the kinetochore passes to the inner side of the microtubule, the un-dissociated microtubule tip could bend out more due to the absence of kinetochore constriction. Consequently, the instantaneous distance between the kinetochore and the microtubule tip is much closer than the rest of the microtubule. This close contact thus yields much larger electrostatic attraction than those from the rest of the microtubule under physiological ionic conditions. As a result, the effective electrostatic interaction hinders the further kinetochore poleward translocation until the microtubule tip dissociates. Thus the kinetochore translocation is strongly coupled with the depolymerizing microtubule ends. This driving-coupling mechanism indicates that the kinetochore velocity is largely controlled by the microtubule dissociation rate, which explains the insensitivity of kinetochore velocities to its viscous drag, and the large redundancy in its stalling force.

**L51**

**Ajuba: A New Microtubule-associated Protein Involved in Mitotic Checkpoint**

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Microtubules (MTs) are polarized structures organized by the centrosome. The minus-end is generally docked at the centrosome and the plus-end scans the cytoplasm to find targets such as the chromosomes. During mitosis, this network forms a bipolar spindle, attaches the kinetochores and allows equal segregation of the chromosomes. Ajuba is a LIM-family member first described in cell adhesion and migration. It has been recently involved in cell cycle progression as an activator of the Aurora A kinase, allowing mitotic commitment. We show that Ajuba localizes to the centrosomes and to the kinetochore microtubule tips (KMTs), and that Ajuba is a microtubule-associated protein (MAP). Owing to its contribution to the mitotic commitment and its MT-dependent localization, Ajuba may play a role during the metaphase/anaphase transition. We show that Ajuba interacts with two major players of the mitotic checkpoint. After inhibition of one of these proteins by siRNA, Ajuba is delocalized and the chromosomes are not properly aligned at the metaphase plate. Our sequence motifs. We recently have explored key binding modes of +TIPs by analyzing the interactions between selected CAP-Gly, EB-like, and carboxy terminal EEY/F-COO sequence motifs (1, 2). Using X-ray crystallography and biophysical binding studies we demonstrate that the β2-β3 loop of CAP-Gly domains determines EB-like motif binding specificity. We further show how CAP-Gly domains serve as recognition domains for EEY/F-COO motifs which represent characteristic and functionally important sequence elements in EB, CLIP-170, and α-tubulin. Our findings provide a molecular basis for understanding the modular interaction modes between α-tubulin, CLPs, EB proteins, and the dynactin-dynein motor complex and suggest that multiple binding sites in different combinations control dynamic +TIP networks at microtubule ends. We hypothesize that a local increase in concentration of EEY/F-COO sites together with secondary binding sites on EB and tubulin favors the transient accumulation of CAP-Gly containing +TIPs such as CLIP-170 and the dynein-dynactin motor complex at microtubule ends. Our findings further offer insights into the structural consequences of genetic CAP-Gly domain defects found in severe human disorders. This work was supported by the Swiss National Science Foundation through Grant 310000-109423 and within the framework of the NCCR Structural Biology program. 1. Honnappa et al., EMBO J. 24, 261 (2005) 2. Honnappa et al., Mol. Cell, 23, 663-671 (2006)
data suggest that Ajuba is a MAP implicated in mitotic checkpoint in collaboration with mitotic checkpoint proteins.

CILIA AND FLAGELLA

L52
Hyptonic-activated Ca\(^{2+}\) Entry via TRPV4 Channels in Cholangiocytes: Implications for Osmoregulation

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Cholangiocytes, the epithelial cells in the liver lining the biliary tree, have primary cilia extending from their apical plasma membrane into the ductal lumen. We recently reported that cholangiocyte cilia are sensory organelles that respond to mechanical stimuli, (i.e., luminal fluid flow), by alterations in intracellular Ca\(^{2+}\) and cAMP signaling pathways. Proteins involved in the transduction of the mechanical stimulus include polycystin-1, a cell surface receptor, and polycystin-2, a Ca\(^{2+}\) channel, both expressed on cholangiocyte cilia. TRPV4 is a Ca\(^{2+}\)-permeable ion channel and has been implicated in Ca\(^{2+}\)-dependent signal transduction of osmotic stimuli. Since osmoregulation likely plays an important but obscure role in cholangiocyte function, our Objectives were to determine the expression and topography of TRPV4 in cholangiocytes, and functionally assess its possible role as an osmosensor. Results: Using freshly isolated mouse cholangiocytes and a normal mouse cholangiocyte cell line (NMC), we found that both TRPV4 message (by RT-PCR) and protein (by western blotting) are expressed in cholangiocytes. Transmission electron and immunofluorescent confocal microscopy studies on NMC-TRPV4 transfected cells and on isolated rat intrahepatic bile ducts showed that TRPV4 localized both on the apical membrane and on primary cilia of cholangiocytes. Functional studies demonstrated that NMC responded to hypotonicity by a two-fold increase in intracellular Ca\(^{2+}\) levels (p<0.01). When NMC and NMC overexpressing TRPV4 cells were transfected with shRNA in order to abolish TRPV4 expression, the Ca\(^{2+}\) rise induced by hypotonicity was totally inhibited (p<0.01). The osmotic response was also impaired when Ca\(^{2+}\)-free extracellular media was used. Conclusion: TRPV4 is expressed on the apical membrane and on the cilia of cholangiocytes and a hypotonic stimulus induces a rise in [Ca\(^{2+}\)]\(_i\) via a TRPV4 and extracellular calcium dependent process. These data suggest that TRPV4 could play an important role in ductal bile secretion via osmosensation by cholangiocytes.

L53
Calcium-dependent Activation of Chlamydomonas Flagellar Beating upon Mechanical Agitation

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Eukaryotic cilia/flagella are organelles that sense signals from surroundings as well as propagate bending waves that generate fluid flow. The flagellar beating of Chlamydomonas reinhardtii is regulated by Ca\(^{2+}\), phosphorylation and redox poise in response to alterations in the environmental conditions. Here we find a novel flagellar response of Chlamydomonas: elevation of beat frequency upon mechanical stimulation. After vigorous pipetting or stirring of cell cultures, the flagellar beat frequency of wild type cells increases from ~65 Hz to ~75 Hz, which lasts for ~3 minutes. Most of outer arm dynein-deficient mutants do not show this response whereas inner-arm dynein mutants and ada14, which has a reduced number of outer arms, show it. Lowered calcium concentration (10^{-4} M) and L-type calcium channel blockers (verapamil, diltiazem, and nimodipine) inhibit this response. Ni\(^{2+}\), a blocker of other types of Ca\(^{2+}\) channels, or Cd\(^{2+}\), a blocker of mechanosensitive channels, does not inhibit this response. These findings suggest that mechanical stimulation causes Ca\(^{2+}\) influx into the flagella through mechanosensitive L-type calcium channels, which then activates outer-arm dynein to raise the beat frequency. Ca\(^{2+}\) and outer arm dynein thus regulate flagellar movement in various ways: waveform conversion from cilia type to flagella type; dominance control between cis- and trans-flagella; and change in the beat frequency.

L54
Mechanical Compression Reduces Chondrocyte Primary Cilia Expression In Vitro

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Articular cartilage is found at the ends of long bones and functions to protect joints as well as providing a smooth surface for articulation. It is well known that mechanical loading plays a fundamental role in the physiological and pathological processes of articular cartilage, although the mechanisms are only partially understood. Chondrocytes, the cells of cartilage, each possess a primary cilium. Primary cilia are major organelles involved in the regulation of cell polarity, transport and secretion and have been implicated in cartilage regulation and maintenance. We hypothesize that chondrocyte primary cilium act as mechanosensors, initiating downstream signalling events, which in turn lead to altered synthesis and secretion of extracellular matrix. In order to test our hypothesis, bovine articular chondrocytes were seeded in agarose gel constructs and subjected to 0-15% cyclic compressive strain (unconfined, uniaxial; 1Hz) for up to 48 hours, using mechanical testing rigs. Constructs were analyzed in one of two ways. Some constructs were fixed and fluorescently labelled using primary antibodies against acetylated alpha tubulin or gamma tubulin to visualise primary cilia and centrioles, respectively. Others were assayed for DNA and proteoglycan synthesis. In unstrained cells, primary cilia expression increased with time in culture over 72 hours. In compressed constructs, the percentage of cells expressing primary cilium was significantly reduced by 30% (p<0.001) compared to unstrained controls. In addition, there was a concomitant compression-induced increase (120%) in DNA synthesis (an indicator of cell proliferation) and an increase (200%) in proteoglycan synthesis (an indicator of extracellular matrix synthesis). However, there was no significant change in the orientation of primary cilium or centrioles with respect to the direction of applied strain. We conclude that the reduction in primary cilia expression, as a result of cyclic compressive strain, is likely to have occurred via inactivation of intraflagellar transport.

L55
Analysis of Ciliary and Flagellar Genes in the Sea Urchin Genome


The sea urchin embryo has been a model of choice for studies of cilia and flagella for a half century. In most urchin species, the onset of ciliogenesis occurs on all blastomeres coordinately just prior to hatching and is regulated thereafter in a tissue-specific manner constituting "a subroutine in the program of development" (Stephens, 1995). Probing the recently completed genome of the purple sea urchin Strongylocentrotus purpuratus using the recently published flagellar proteome from the single-celled alga Chlamydomonas reinhardtii (Pazour et al., 2005), we have begun studying how embryos regulate the ciliogenetic developmental subroutine differently between germ layers. Genes representing all ciliary proteins were identified in the S. purpuratus genome. A sampling of the more than 500 novel flagellar-associated proteins identified in the Chlamydomonas flagellar proteome, including many shown to be transcriptionally up- or down-regulated upon deciliation, suggests that more than half of these previously uncharacterized proteins have direct counterparts in the S. purpuratus genome. Available S. purpuratus transcriptome data suggest that some ciliary proteins are transcribed preferentially at the onset of ciliogenesis while others are transcribed more broadly across embryonic development.

INTRACELLULAR MOVEMENT

L56
Serotonin Modulates Axonal Transport of Mitochondria in Cultured Hippocampal Neurons

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The sea urchin embryo has been a model of choice for studies of cilia and flagella for a half century. In most urchin species, the onset of ciliogenesis occurs on all blastomeres coordinately just prior to hatching and is regulated thereafter in a tissue-specific manner constituting "a subroutine in the program of development" (Stephens, 1995). Probing the recently completed genome of the purple sea urchin Strongylocentrotus purpuratus using the recently published flagellar proteome from the single-celled alga Chlamydomonas reinhardtii (Pazour et al., 2005), we have begun studying how embryos regulate the ciliogenetic developmental subroutine differently between germ layers. Genes representing all ciliary proteins were identified in the S. purpuratus genome. A sampling of the more than 500 novel flagellar-associated proteins identified in the Chlamydomonas flagellar proteome, including many shown to be transcriptionally up- or down-regulated upon deciliation, suggests that more than half of these previously uncharacterized proteins have direct counterparts in the S. purpuratus genome. Available S. purpuratus transcriptome data suggest that some ciliary proteins are transcribed preferentially at the onset of ciliogenesis while others are transcribed more broadly across embryonic development.
The trafficking of mitochondria likely plays a critical role in normal neuronal function. However, few extracellular signals have been identified as regulators of mitochondrial transport in neurons. In the present study, we have identified serotonin (5-HT) as an extracellular signal that modulates mitochondrial movement in hippocampal neurons in vitro. A recombinant lentivirus encoding enhanced yellow fluorescent protein that is targeted to the mitochondrion was used to stably label mitochondria in living cultures of hippocampal neurons. Oral treatment was recorded using time lapse microscopy. The rate and extent of mitochondrial movement increased in axons following administration of 5-HT. Addition of a 5-HT1A receptor-specific agonist, 8-OH-DPAT, resulted in similar effects, whereas pretreatment with the 5-HT1A receptor antagonist WAY100635 completely abolished the effects of both 5-HT and 8-OH-DPAT. These findings indicate that the effect of 5-HT on mitochondrial trafficking is mediated via the 5-HT1A receptor subtype. The kinesin KIF5 has been implicated in mediating mitochondrial transport on the microtubular network. Treatment of hippocampal neurons with 5-HT or 8-OH-DPAT resulted in an increase in the level of KIF5, while decreased levels of this kinesin were observed after administration of WAY100635. Decreased activity of glycogen synthase kinase 3β (GSK3β) and increased levels of acetylated tubulin were also observed after administration of 5-HT. These data are consistent with the hypothesis that 5-HT enhances mitochondrial locomotion by activating 5-HT1A receptors, which in turn modulate specific components of the microtubule-based transport system. Since 5-HT has been implicated in the regulation of a number of neural states and processes, including appetite, mood, sleep, learning, and memory, the finding that this neuromodulator also promotes mitochondrial movement provides a novel insight into a potential link between trafficking machinery and neuronal activity at the cellular level. Supported by the Neurosciences Research Foundation.

Peptide Zipcodes Sufficient for Axonal Transport: A Role for Amyloid Precursor Protein

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Membrane-bound organelles shuttle within cells from the surface to internal compartments. In neurons, this process is highly specialized to deliver gene products synthesized in the cell body out long processes to the presynaptic terminal. Herpes simplex virus (HSV), a neurotropic virus, co-opts cellular transport machinery to travel both ways in neuronal processes of 5-HT. These data are consistent with the hypothesis that 5-HT enhances mitochondrial locomotion by activating 5-HT1A receptors, which in turn modulate specific components of the microtubule-based transport system. Since 5-HT has been implicated in the regulation of a number of neural states and processes, including appetite, mood, sleep, learning, and memory, the finding that this neuromodulator also promotes mitochondrial movement provides a novel insight into a potential link between trafficking machinery and neuronal activity at the cellular level. Supported by the Neurosciences Research Foundation.

Concanavalin A Inhibits Melanosomal Aggregation in Isolated Retinal Pigment Epithelial Cells

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Retinal pigment epithelial (RPE) cells contain numerous melanosomes (pigment granules) that undergo bi-directional motility within apical projections. Movement of these granules is stimulated by light to control flux into the retina. Previous work has shown that melanosome aggregation within apical projections of dissociated RPE cells requires an intact actin cytoskeleton, and may be linked to retrograde actin flow (Canman and Bement, 1997, J Cell Sci 110:1907). Melanosomal aggregation in control and lectin-treated cells was quantified by the pigment index, which measures the ratio of the total area of apical projections occupied by melanosomes. Aggregation in lectin-treated cells was inhibited by 60% to 69% compared to control cells after ten and fifteen minutes of aggregation. Lectins have been shown to inhibit cortical actin flow by sterically clustering transmembrane proteins attached to the actin cytoskeleton, preventing motility. The inhibition of melanosome aggregation displayed in lectin-treated RPE cells suggests that aggregation may be reliant upon actin retrograde flow.

CYTOSKELETAL ORGANIZATION

Contributions of Whole-Cell Optimization via Cell Body Rolling to Polarization of T Cells

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Directed secretion of cytokotins or cytokines by T cells during immune response depends on migration of the centrosome in the T cell to the interface with the target cell. The objective of this work is to elucidate the mechanism of the centrosome translocation. Our computational analysis demonstrates that the centrosome will be positioned at the interface if the T cell attempts simultaneously a) to minimize its surface area...
area, b) to maximize the interface area, c) to maintain the cell volume, and d) to straighten the microtubules. Live three-dimensional microscopy and measurements show that the optimal position of the centrosome is achieved in large part (by about 40%) via rolling of the entire T cell body on the target surface, which movement appears to entrain the centrosome. These theoretical and experimental results draw attention to the previously unrecognized role of the whole-cell structure and whole-cell movements in the T cell polarization.

L61
Atypical Rho GTase Miro-1 and Mammalian Diaphanous-related Formins in Mitochondrial Dynamics
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Autoregulated mammalian Diaphanous-related formins (mDia1 and mDia2) localize to numerous membrane-bound organelles and the plasma membrane where they assemble non-branched actin filaments during cell migration and intracellular trafficking. Their activity is governed by direct binding to activated GTP-bound Rho proteins that disrupt intramolecular autoregulatory interactions. While mDia1 and mDia2 bind to numerous GTPases in vitro, it is unclear which interaction is physiologically relevant within cells. A recently characterized atypical Rho family GTase - Miro-1 (mitochondrial Rho) has been shown to have a role in trafficking of mitochondria. By indirect immunofluorescence, we have observed that endogenous mDia1 and mDia2 appear on mitochondria. We hypothesized that Miro-1 participates in the regulation of mDia1 and mDia2 to mitochondria through direct binding. Through a variety of approaches, including FRET, we show that Miro-1 interacts with mDia1 and mDia2 and that this event occurs on mitochondria. Ongoing studies using activated versions of mDia1 and mDia2 will test the hypothesis that they act as effectors for Miro-1 in the regulation of mitochondrial dynamics in cells.

NERVE CELL CYTOSKELETON
L62
A Rho Inhibitory Pathway That Acts in Hippocampal Synapses to Regulate Dendrite Branch Stability
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The RhoA (Rho) GTase is a master regulator of dendrite morphogenesis. Rho activation in developing neurons slows dendrite branch dynamics, leading to smaller, less branched dendrite arbors. Overexpression of constitutively-active Rho in mature neurons leads to spine loss and dendritic atrophy, but whether native Rho modules dendrite and synapse morphology in mature neurons is unclear. The Abl and Arg nonreceptor tyrosine kinases mediate changes in axon and dendrite morphology upon integrin-mediated adhesion. The apical dendrites of \( \text{arg}^{++} \) mouse hippocampal CA1 pyramidal neurons develop normally and are similar to wild type dendrites at postnatal day 21 (P21). However, \( \text{arg}^{-} \) hippocampal neurons lose apical dendrite branches as the mice age to adulthood. Similar reductions are not observed in \( \text{abl}^{-} \) or allo \( \text{arg}^{-} \) mice, demonstrating that Arg is selectively required for hippocampal dendrite maintenance and that loss of Abl function can suppress the \( \text{arg}^{-} \) phenotype. \( \text{arg}^{-} \) mice also exhibit a reduction in Schaffer collateral-CA1 (SC-CA1) synapses that parallels the reduction in apical dendrite branches. As expected, \( \text{arg}^{-} \) mice exhibit deficits in a hippocampus-dependent object recognition task, whereas wild type, \( \text{abl}^{-} \), and allo \( \text{arg}^{-} \) mice perform this task normally. The Rho inhibitor p190RhoGAP is a major substrate of Arg in the postnatal mouse brain. In response to integrin-mediated adhesion, Arg phosphorylates and activates the Rho inhibitor p190RhoGAP, leading to a reduction in active Rho. We present biochemical and genetic evidence that Arg acts through p190RhoGAP within the synapse to regulate synapse morphology and dendritic branch stability. These data suggest that adhesion-dependent signaling through Arg and p190RhoGAP promotes dendrite branch stability in mature neurons by keeping synaptic Rho activity in check.

ASCB 2006 Late Abstracts

L63
Hippocampal Organotypic Slice Cultures Display Regional Sensitivity to Stress Induced ADF/cofilin-actin Rod Formation
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We previously demonstrated that exposure of dissociated E-18 rat hippocampal neurons to ischemic conditions of anoxia, glutamate, peroxide, or nanomolar concentrations of soluble oligomeric \( \text{A}_{\beta} \) produces a rapid reorganization of the cytoskeletal proteins ADF/cofilin and actin into tapered cylindrical aggregates known as actin-cofilin rods. As these rods disrupt vesicle transport, they may be involved in synaptic loss and cognitive impairment. However, the inherent shortcoming of dissociated cells is ablation of the myriad of connections and support cells that help maintain neurons. Accordingly, in this study we utilized 8-14 day organotypic hippocampal slice cultures from 10 day postnatal rats. Rod formation can be observed within 10 minutes of anoxia or ATP depletion in live slices infected 3 days before observation with adenosivirus expressing cofilin-GFP. Rod formation also occurs in neurons within slices exposed to glutamate, peroxide, or oligomeric \( \text{A}_{\beta} \) at concentrations consistent with our work on the dissociated neuron rods. Rats in slices immunostained with an antibody to cofilin, but do not stain with fluorescent phalloidin, a characteristic of the cofilin-saturated rods that form in dissociated neurons. Furthermore, we demonstrate that the neuronal population in the hippocampus that forms rods in response to 48-hour exposure to 1 \( \mu \text{M} \) soluble \( \text{A}_{\beta} \) oligomers is localized to specific sub-regions. Taken together these findings suggest that rod formation is a rapid event that could explain the alterations in axonal transport, which are the earliest changes detected in many neurodegenerative diseases. Furthermore, the rapid formation of rods in response to anoxia suggests that they may be a significant contributor to the loss of neuronal function during stroke and severe brain injuries following prolonged anoxia. In addition, rods are also likely to mediate synaptic loss in vascular dementias and amyloidopathies such as Alzheimer’s disease. (Supported in part by NIH grants NS40371 and NS43115).

FOCAL ADHESIONS
L64
Measuring 3D Traction Force with Focal Adhesion Dynamics in Live Vascular Endothelial Cells
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Cells adhere to the extracellular matrix (ECM) through focal adhesions (FAs) that link the ECM to the actin cytoskeleton. FAs play an important role to transmit the dynamic force to the ECM during cell migration. Transfection of GFP-tagged focal adhesion kinase (FAK) enables the dynamic observation of FAs in live cells. The traction forces exerted by a cell during its motion can be determined from the cell-induced displacements of fluorescent markers embedded in an elastic substrate. Previous studies on cell traction force assayed only the tangential components of the cellular traction force i.e., forces in the plane of the substrate (X and Y directions), and it has been widely assumed that the normal component of the traction force (force in the Z-direction) is negligible in comparison with the tangential components. We have developed a novel method to measure the 3D force exerted by cultured vascular endothelial cells (ECs) and correlate it the dynamics of FAs. The 3D force on the surface of the substrate can be correlated with the dynamic features of the FAs, including their sizes and orientations, which are visualized via GFP-FAK and GFP-Paxillin in the migrating ECs. This 3D measurement of traction forces provides a novel way of assessing the full range of biomechanical dynamics of cells in conjunction with their biochemical activities. The results contribute to the understanding of the mechanical and biochemical basis of cell movements in health and disease.
L65 Visualizing the Spatial and Temporal Regulation of FAK Activity in Living Cells
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Focal adhesion kinase (FAK) is an important tyrosine kinase that regulates cell migration and survival. The intramolecular interaction between the FERM and kinase domains is believed to be a major mechanism for inhibition of FAK activity. This model is supported by the crystal structure of an autoinhibited form of FAK that contains the FERM and kinase domains. Based upon the structural studies, a Fluorescence Resonance Energy Transfer (FRET) based FAK biosensor has been developed to probe FAK conformational change. Introduction of well-characterized FAK mutations into the biosensor not only validates the crystal structure but also demonstrates that the biosensor is able to report FAK activity in vivo. Live cell imaging demonstrates that FAK in the active conformation is enriched in focal adhesions, whereas cytoplasmic FAK maintains the inactive conformation. Interestingly, a change of the FRET/CFP emission ratio is detected in living cells following serum or lysophosphatic acid (LPA) stimulation, demonstrating dynamic regulation of FAK conformation. The biosensor results provide direct evidence that FAK activity is regulated via conformational change in vivo. Moreover, the FAK biosensor establishes a reliable strategy to visualize the spatio-temporal dynamics of FAK activity.

CELL ATTACHMENT TO THE EXTRACELLULAR MATRIX
L66 Revealing Early Steps of αβ1 Integrin-Mediated Adhesion to Collagen Type I Using Single-Cell Force Spectroscopy
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We have characterized the early steps of αβ1 integrin-mediated cell adhesion to a structurally-defined collagen I matrix. In agreement with the role of αβ1 as a collagen I receptor, αβ1-expressing CHO-A2 cells spread rapidly on the matrix, while αβ2-negative CHO wild-type cells adhered poorly. Quantitating αβ1-mediated adhesion using single-cell force spectroscopy showed that matrix detachment forces were significantly higher for CHO-A2 than for wild-type cells. Probing CHO-A2 cell detachment forces over a contact time range of 600 sec demonstrated a non-linear adhesion response. During the first 60 sec cell adhesion increased slowly and forces associated with the smallest rupture events were consistent with the breakage of individual integrin-collagen bonds. Above 60 sec, a fraction of cells rapidly switched into an activated adhesion state marked by up to tenfold increased detachment forces. Elevated overall cell adhesion coincided with a rise of the smallest rupture force. Above the value required to break a single integrin-collagen bond, suggesting receptor clustering and cooperativity. Transition into the activated adhesion mode and establishment of receptor cooperativity required actomyosin contractility. We therefore propose a two-step mechanisms for the establishment of αβ1-mediated adhesion as weak initial, single-integrin-mediated binding events are superseded by strong adhesive interactions involving receptor cooperativity and myosin-driven contractility.

L67 Studies on the Mechanism of Lysophosphatidylcholine(LPC)-mediated Adhesion in Human Neutrophils
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Lysophosphatidylcholine(LPC), one of lysospholipids, has been known to be effective molecule in the treatment of sepsis in mice. Using MPO and Calcein assay, we revealed that LPC increased the adhesion of human neutrophils on plastic surfaces as well as on extracellular matrix-coated plastic surfaces in time and concentration-dependent manner and that the increased expression of integrins have no effect on the increase of adhesion through LPC administration to the human neutrophils. Scanning electron microscopy (SEM) analysis revealed that LPC involved in human neutrophils. Significant changes in morphology of human neutrophils by LPC have been observed by scanning electron microscopy. We assumed that these changes in morphology may contribute to increase adhesiveness of human neutrophils by LPC.

EXTRACELLULAR MATRIX AND CELL BEHAVIOR
L68 Growth Factor Modulation Under Three Dimensional Conditions of Fibroblast Proliferation, Differentiation, and Invasion
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TGF-beta is a potent regulator of extracellular matrix proteins such as TNC and collagen type I, and expression of alfa-SMA stress fibers. Only TGF-beta1 was able to induce expression of as well alfa-SMA, TNC and proteol-4-hydroxylase (P4H, an enzyme involved in collagen type I maturation). In contrast, combinations of TGF-beta+EGF or TGF-beta+bFGF induce only TNC and P4H but not alfa-SMA expression. TGF-beta increased collagen gel contraction, suggesting that alfa-SMA expression was functional. bFGF or EGF treatment alone had no effect on alfa-SMA, TNC and P4H expression. Basal invasion of fibroblasts from spheroids implanted inside collagen gels was stimulated by EGF or bFGF on growth, differentiation and invasion of hTERT immortalized human dermal fibroblast (hDFH) inside 3D collagen type I (2mg/ml) gels. Proliferation was stimulated by bFGF, and combinations of TGF-beta+EGF and TGF-beta+bFGF, but inhibited by TGF-beta and not affected by EGF as evidenced by MTT assay and analysis of the proliferation marker Ki67, and the cell cycle proteins cyclin D2, E and p21cip,waf-1. Furthermore, FACS analysis revealed that a TGF-beta induced GI-arrest and bFGF, TGF-beta+EGF and TGF-beta+bFGF increased the percentage of fibroblasts in S phase. Fibroblasts may differentiate into myofibroblasts producing excessive extracellular matrix proteins such as TNC and type I collagen, and expressing functional alfa-SMA stress fibers. Only TGF-beta1 was able to induce expression of as well alfa-SMA, TNC and proteol-4-hydroxylase (P4H, an enzyme involved in collagen type I maturation). In contrast, combinations of TGF-beta+EGF or TGF-beta+bFGF induce only TNC and P4H but not alfa-SMA expression. TGF-beta increased collagen gel contraction, suggesting that alfa-SMA expression was functional. bFGF or EGF treatment alone had no effect on alfa-SMA, TNC and P4H expression. Basal invasion of fibroblasts from spheroids implanted inside collagen gels was stimulated by EGF or bFGF on growth, differentiation and invasion of hTERT immortalized human dermal fibroblast (hDFH) inside 3D collagen gels. In contrast, when TGF-beta is combined with EGF or bFGF as well growth, invasion and ECM production, but not alfa-SMA dependent contractility, is stimulated.

L69 New Insights in the Link between Rab27b GTPase and Breast Cancer
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Rab27a has been described as a potential biomarker in breast cancer progression. We constructed a fusion protein of wild type Rab27b e-terminally fused with the reporter molecule eGFP. Transient overexpression of eGFP-Rab27b in MCF-
7/AZ induces cellular scattering and stimulates invasion in collagen type I and Matrigel. Based on these preliminary results we created MCF-7/7-AZ cells stably overexpressing eGFP-Rab27b. We selected single cell derived populations and confirmed overexpression of the eGFP-Rab27b fusion protein by Western blot analysis and immunocytochemistry. We studied the morphology and growth on 2D plastic substrate and in 3D collagen type I matrix, and invasion into collagen type I and Matrigel matrices. In agreement with our transient overexpression studies, MCF-7/7-AZ eGFP-Rab27b cells show a change in morphology on collagen type I matrix characterized by filopodia-like extensions and are invasive in both collagen type I and Matrigel. Rab27b overexpression stimulates growth of MCF-7/7-AZ cells in 3D collagen type I gels and on plastic substrate. In conclusion, transient and stable Rab27b overexpression induces a change in morphology and stimulates proliferation and invasion of human MCF-7/7-AZ breast cancer cells in vitro. Further research is ongoing to reveal the underlying mechanisms and physiological relevance.

L70 The Mechanism of Cell Interaction and Response on Decellularized Human Amniotic Membrane: Implications in Wound Healing

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ACELAGRAFT is a decellularized and dehydrated human amniotic membrane product, which is currently marketed by Celgene Cellular Therapeutics. ACELAGRAFT has been implicated as a potential wound healing product with several ongoing preclinical and clinical studies in the area of acute and chronic ulcers. Here we present a detailed structure-function analysis of ACELAGRAFT, its ability to interact with cells and its consequent cellular responses. Biochemical analysis of the composition of ACELAGRAFT indicates that it is essentially a collagenous biological material that retains several key bioactive molecules including fibronectin, laminin, GAGs and elastin, but is devoid of cytokines, growth factors or hormones. Our competitive cell binding & antibody blocking assays show that cells such as fibroblasts can recognize fibronectin in ACELAGRAFT and bind to it via integrin-fibronectin interactions similar to that observed in biological ECM-cell interactions. ACELAGRAFT promotes cytoskeletal spreading dynamics & morphologies similar to those achieved on substrates coated with purified, human fibronectin. Fibroblasts (and other cells) are able to actively assemble soluble fibronectin into a complex network of detergent-insoluble extracellular matrix fibrils on ACELAGRAFT. Further, ACELAGRAFT stimulates both cells to secrete key pro-inflammatory cytokines (IL-1 & IL-6) that are involved in recruiting cells to the wound site, promoting production of ECM and regulating and enhancing wound repair processes. Further, micro-array gene expression studies on cells bound to ACELAGRAFT show an increased expression of key wound healing cytokines in response to cell binding to ACELAGRAFT. Together, these studies allow us to identify and describe mechanisms by which ACELAGRAFT augments the wound healing process.

L71 Reducing Chondroitin Sulfate Synthesis Decreases the Inhibitory Activity of Astrocyte-derived Chondroitin Sulfate Proteoglycans

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Chondroitin sulfate proteoglycans (CSPGs) are up-regulated in the central nervous system (CNS) after injury and participate in the inhibition of axon regeneration. Removal of CSPG GAG chains in the injured mammalian CNS using the enzyme chondroitinase ABC (cABC) enhances axonal outgrowth and functional recovery after injury. We propose that decreasing the amount of CSPG GAG produced by cells by reducing the level of a key enzyme in the biosynthetic pathway, chondroitin polymerizing factor (ChPF), would have a similar action as cABC. Using a vector based short hairpin RNA we show a 70% reduction of ChPF mRNA in both primary rat astrocytes and the Neu7 astrocyte cell line two days post transfection. This reduction in mRNA levels leads to a reduction in ChPF protein levels and a decrease in the amount of CSPG GAG conditioned media (CM) of these cells as soon as three days post transfection as shown by western blotting and ELISA with CS-56 antibody. The total amount of NG2 core protein in the CM of Neu7 cells transfected with ChPF siRNA is not decreased, suggesting that knocking down key enzymes for GAG chain synthesis may not affect trafficking of core protein from these cells. CM from siRNA treated Neu7 cells is a less repulsive substrate for axons than CM from control cells. In addition, neurite outgrowth from cerebellar granule neurons is increased on or in CM from ChPF siRNA treated Neu7 cells. These data indicate that a small molecule inhibitor of ChPF given after injury may decrease CSPG GAG chains and thus, enhance axonal regeneration after injury.

L72 Placenta Derived Adherent Cell (PDAC) Interaction and Response on an Extracellular Matrix (ECM) Isolated from Human Placenta

M. Bhatia, C. Lugo, M. Pereira, H. Rana, S. Abramson, K. Labazzo, Q. Liu, W. Hofgartner, R. Harriri; Celgene Corporation, Summit, NJ

We have developed a scalable method to isolate extra-cellular matrix (ECM) from the human placenta, an organ well recognized to be rich in ECM proteins such as collagen, fibronectin, laminin and GAGs. The extraction process can be adjusted to generate variations of the ECM with altered biochemical compositions. The isolated ECM is easily molded into 2D and 3D structures that are stable in cell culture media. In this study, we have used this placental ECM to investigate ECM-cell interactions and consequent responses. We derived the cells used in this study from human placenta to have a phenotype described as CD200+, CD105+, CD10+ and CD34-. Cell binding studies showed that these placenta-derived adherent cells (PDACs) bind to and proliferate on the ECM. Further, the ECM stimulated the cells to secrete soluble fibronectin and actively assembled the fibronectin into a complex network of detergent-insoluble extracellular matrix fibrils. When attached to the placental ECM, PDACs secreted key cytokines at levels well above that on tissue treated tissue culture plates. These cytokines included MCP-1, IL-6 and IL-8, which are important participants in wound healing processes. Interestingly, our ECM-PDAC studies also indicated that the biochemical composition of the ECM influenced the morphology of PDACs, as well as, their physiology. For example, specific compositions of ECM enhanced the ability of PDACs to secrete and assemble fibronectin matrices, but also induced the cells to secrete cytokines. These results suggest that one could design a combination product of the placental ECM with PDACs to augment repair processes, for example in non-healing deep wounds and in diabetic ulcers.

L73 Involvement of Tenascin-C in Mediating Breast Cancer Metastasis to the Lung: Effects on Endothelial Cell Surface Stiffness

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The lung represents the most common site for breast cancer metastases, which are present in 60% of patients at autopsy. Although it is appreciated that metastatic breast cancer cells emobilize to the distal pulmonary microvasculature, recent reports also implicate active involvement of the proximal lung microvasculature in capturing these cells. The extracellular matrix (ECM) glycoprotein tenascin-C (TN-C) was recently identified as 1 of 54 genes that mediate breast cancer metastasis to the lung. The finding is of great interest because we have demonstrated preferential TN-C expression by injured pulmonary macrovascular endothelial cells (PAECs), compared to their microvascular (PMVEC) counterparts. Accordingly, we hypothesize that TN-C expressed by PAECs promotes the capture and subsequent dissemination of breast cancer metastases in the proximal lung microvasculature. Using rat PAEC or PMVEC monolayers, co-cultured with GFP-transduced human breast cancer cells of varied metastatic potentials (MCF-7, T47D, MDAMB-231), we demonstrated a significant 2-fold increase in cell spreading for all breast cancer cell types plated on PAECs versus PMVECs. Since tissue and cell stiffness can influence tumor cell spreading and migration, we measured the stiffness of PAEC and PMVEC monolayers via atomic force microscopy (AFM). This approach revealed that PAECs are 6-fold stiffer than PMVECs. Further breast cancer endothelial migration studies revealed that twice as many breast cancer cells invade through PMVEC (TN-C -ve) monolayers cultured on purified TN-C protein.
approximating migration through PAEC (TN-C +ve) monolayers. These co-culture findings were supported by in vivo studies in rat and human lungs, in which breast cancer metastases were found adjacent to the TN-C-expressing macrovascular endothelium. Thus, we suggest macrovascular TN-C involvement in mediating PAEC stiffness, culminating in increased breast cancer cell spreading, transmigration and metastasis.

EXTRACELLULAR MATRIX AND MORPHOGENESIS

L74

Involvement of Src-Tyrosine-Kinases in Mitotic Spindle Positioning

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Cell adhesion and cell division are intimately coupled processes. However, the signalling pathways between adhesion related cortical cues and mitotic spindle orientation still need to be investigated. We used adhesive micropatterns to control cell division and assess the role of the Src-tyrosine-kinase signalling pathway. We first wanted to confirm, in a well defined system for Src expression, the involvement of Src in spindle orientation on micro-patterns. We chose SYF cells: mouse embryonic fibroblasts, which lack the three ubiquitously expressed Src-tyrosine-kinases c-Src, Fyn and Yes. These cells showed a clear defect in spindle positioning compared to the WT fibroblasts. Furthermore, we could significantly rescue this defect by over-expression of avian c-Src in SYF cells. To further clarify the specific role of Src, we used the Src-tyrosine-kinase specific inhibitor SU6656 on HeLa cells that were spread on different micropattern shapes. When cells were spread on patterns with shape factors close to 1 but able to guide mitotic spindle orientation thanks to adhesive and non-adhesive sites, the inhibitor had a strong effect. Surprisingly, on elongated patterns (small shape factor), spindle positioning was almost not disturbed. In the latter case division axis was still predominantly oriented perpendicular to the longest cell axis. This cannot be simply explained by a different cell shape during mitosis, as cells become almost perfect spheres during mitosis whatever the underlying adhesive pattern. Thus the Src requirement for spindle orientation can only be observed in the absence of a strong geometrical bias. Altogether we conclude that Src-tyrosine-kinases are involved in mitotic spindle positioning in mammalian cells (mouse and human) by transducing, and maybe reinforcing, adhesion anisotropy.


CELL–CELL INTERACTIONS

L75

Extracellular HIV-1 Tat Enhances Monocyte Adhesion by Up-regulation of ICAM-1 and VCAM-1 Gene Expression via ROS-dependent NF-kappaB Activation in Astrocytes

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One of characteristic features of AIDS-related encephalitis and dementia is the infiltration of monocytes into the central nervous system (CNS). HIV-1 Tat was demonstrated to facilitate monocyte entry into the CNS. In this study, we examined the effect of HIV-1 Tat on the expression of adhesion molecules, generation of reactive oxygen species (ROS) and nuclear translocation of NF-kappaB in CRG-MG human astroglial cells. Treatment of CRG-MG cells with the Tat protein significantly increased protein and mRNA levels of ICAM-1 and VCAM-1, as measured by Western blot analysis and RT-PCR, indicating that Tat increases these protein levels at an mRNA level. In addition, Tat induced the translocation of NF-kappaB into the nucleus. Treatment of CRG-MG with NF-kappaB inhibitors led to decrease in Tat-induced protein and mRNA expression of ICAM-1 and VCAM-1. Furthermore, HIV-1 Tat protein increased ROS generation. Inhibition of Tat-induced ROS generation by antioxidants, NAC and Vitamin C suppressed Tat-induced NF-kappaB activation, ICAM-1 and VCAM-1 expression, and monocyte adhesion in CRG-MG. These data indicate that HIV-1 Tat can modulate monocyte adhesiveness by increasing expression of adhesion molecules such as ICAM-1 and VCAM-1 via ROS- and NF-kappaB-dependent mechanisms in astrocytes.

L76

ADAM15 Is a Mouse Sperm Protein with a Role for of Its Disintegrin Domain in Mouse Sperm-Egg Plasma Membrane Adhesion and Fusion

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A successful fertilization requires completion of several steps, beginning with the maturation and transport of gametes in the reproductive tract and ending with the interaction between the sperm and egg. This last step involves sperm-egg membranes adhesion and fusion. Members of the ADAM (A Disintegrin and Metalloproteinase) protein family, which includes molecules such as ADAMs 1, 2, and 3, have been found to be involved in sperm-egg binding and fusion. ADAM15 is other member of the ADAM family that might be involved in the sperm-egg interaction; however its presence in sperm has not been reported. In this work, the presence, localization, positioning and a role during fertilization for ADAM15 is demonstrated for the first time. ADAM15 was found to be processed post-translationaly by proteolytic removal of its N-terminal region, during sperm maturation. By indirect immunofluorescence, in vivo, ADAM15 was found in acrosome-reacted sperm. Furthermore, we assayed for a potential integrin binding site, using an analog peptide which included a TDD amino acid sequence from the disintegrin region of ADAM15. The peptide was able to bind to the egg plasma membrane and significantly inhibited sperm-egg fusion. These results show that the disintegrin domain of ADAM15 binds to the egg plasma membrane and that its binding is requiredgametes membrane fusion.

L77

Spinophilin Participates in Information Transfer at the Neuronal and Immunological Synapses

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Throughout the body antigen presenting cells (APCs) sample antigens and process them into peptides bound to MHC molecules. The "immunological synapse" (IS) refers to the adhesion site between APCs and T cells, where T cell receptors (TCRs) recognize their cognate peptide-MHC complexes. Adopting the term "synapse" to describe this site of adhesion and recognition is attractive, as both immunological and neuronal synapses form with temporal and spatial specificity. However, the extent to which these synapses are mechanistically related remains unclear. To investigate these varied synaptic settings utilize similar molecular mechanisms, we are studying spinophilin, a neuronal synaptic scaffolding protein, at the immunological synapse. In neurons, spinophilin is localized to dendritic spines where it organizes the cytoskeleton and regulates protein phagocytosis. In immune cells, we have discovered that spinophilin is recruited from the cytoplasm to antigen-specific contact sites between APCs and T lymphocytes during immune recognition, using RT-PCR, Western blotting, and immunofluorescence. Its localization at the immunological synapse is antigen-dependent, dynamic, transient, and precedes the redistribution of the T cell antigen receptor. Spinophilin is also required for optimal T cell stimulation since spinophilin +/- antigen presenting cells are defective in antigen presentation both in vitro and in vivo, as shown by co-culturing APCs and T cells and adoptive transfer experiments. Spinophilin thus plays an important role in immune recognition and suggests that APCs play an active role in the formation of the immunological synapse.

L78

Study of Notch1, Jagged2, Hes5, and Math1 Expression in Molar Tooth Germ of Mouse

Tuesday

As part of the scientific community, we are committed to advancing knowledge and understanding through the sharing of our research findings. This page contains abstracts from a scientific conference, detailing various studies that explore different aspects of cell biology, immunology, and neuroscience. The works presented span a range of topics, from the complexities of cell-cell interactions and matrix interactions to the intricacies of synaptic biology and immune responses. Each abstract highlights a specific research question and the methodologies employed to address it, offering insights into the multifaceted nature of biological systems.
Tuesday

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Odontogenesis or tooth development results from reciprocal and sequential interactions between the dental epithelium and mesenchyme. This process is regulated by morphodynamic mechanism, a combination of inductive and morphogenetic mechanisms. The Notch signaling pathway is an evolutionary conserved mechanism that plays an important role in cell-cell communication and consequently in determining cell fates in a wide range of tissues. One of the most established target genes for Notch are the HES genes. During embryonic stages Notch1 and HES5 weak expression was detected in the enamel organ but not in the basal cells that contact the dental mesenchyme. Jagged2 and Math1 were positive in the whole enamel organ. In the other hands, during postnatal stages Notch was strongly expressed in the stratum intermedium and briefly expressed in the ameloblasts at the cusp area and odontoblast layer except for the cusp area. Jagged2 was detected in the ameloblast and odontoblast layers and weakly expressed in dental papilla. Expression of Notch1 at in ameloblasts and odontoblasts during postnatal stages suggests an additional function of Notch1 in advanced stages of odontogenesis through inductive signaling mechanism. In our study, opposite expression of HE85 and Math1 indicates Math1 as an important downstream on the Notch signaling cascade directing the process of odontogenesis toward cell differentiation.

L79
Class IA PI3K Signaling Mediates Ephrin A1-induced Cell Retraction
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The evolutionarily conserved Eph receptor tyrosine kinase family, and their cognate ligands the ephrins, constitute the largest receptor-ligand family identified to date. The Eph receptor-ephrin signaling paradigm has been shown to be an important regulator of cell migration and tissue development. Specifically, stimulation with the ephrin A1 ligand has been shown to induce growth cone collapse in neuronal cell types, and lamellipodia/filopodia retraction in several other cell types in a Rh GTPase-, PLC2- and actin cytoskeleton-dependant manner. In the current study, we utilized mouse embryonic fibroblast (MEF) cell lines derived from pik3R2-/- (PI3K-p85β subunit knockout) and wildtype mice to determine the role of class Ia, PI3K signaling in ephrin A1-induced actin cytoskeleton and morphologic remodeling. Treatment of wildtype MEFs or NIH3T3 fibroblasts with ephrin A1 induced rapid actin cytoskeleton and morphologic remodeling. Treatment of the pik3R2-/- cells also exhibited disrupted actin rearrangement and nearly complete inhibition of ephrin A1-induced cell retraction and rounding. In contrast, pik3R2+ MEFs exhibited severely impaired Rh GTPase activation and PLC2 phosphorylation, and actin rearrangement, leading to marked cell retraction and rounding. In pik3R2+ cells also exhibited disrupted actin rearrangement and nearly complete inhibition of ephrin A1-induced cell retraction and rounding. These results demonstrate that loss of PI3K signaling through deletion of pik3R2 results in decreases in Rho activation and PLC2 phosphorylation, which in turn lead to impaired actin rearrangement and morphologic remodeling following ephrin A1 treatment. These data also suggest the selectivity of the pik3R2 subunit toward p85β in ephrin A1-induced PI3K signaling. In summary, these studies identify a pivotal role for PI3K, specifically PI3K-p85β, as a compelling element that regulates ephrin A1-induced activation of Rho and PLC2, leading to cell morphologic remodeling.

L80
Neuronal Expression of BT-IgSF (IgSF11) That Functions through a Homophilic Interaction
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Because of the similarity between the extracellular domains of IgSF11 and the T cell adhesion molecule LFA-1, and also function as signaling molecules. An important step in their biosynthesis is the reduction of 3-Ketosphinganine (3-KS) to dihydrospinganine. In yeast, this reaction is catalyzed by Tsc10p. In mammalian cells, this is believed to be catalyzed by FVT1, but it is not clear that this is the only enzyme capable of reducing the 3-KS intermediate. To address this question, and to compare the yeast and mammalian enzymes, the topology, organization and specificity of the two enzymes were investigated. These studies were facilitated by the aggregates through a homophilic interaction of BT-IgSF. Endogenous BT-IgSF expressed in primary cultured neurons also showed such interaction in formation of neuronal aggregation, adhesion to BT-IgSF coated substratum and neuronal sprouting in vitro. Immune electron microscopy revealed that BT-IgSF was observed in both cytoplasm and plasma membrane of neurons in Corpus Callosum and Hippocampus, while it was not always localized in an area where cellular interaction could take place between neurons and oligodendroglia. Either siRNA or BT-IgSF cDNA was also introduced to neuronal cells, and complementary results were obtained that changes in BT-IgSF level affected neuronal cell adhesion as well as neurite extension. These data, taken together, suggest that BT-IgSF may play a role in either formation or modification of neuronal network in the central nervous system.

CELL–CELL ADHERENCES JUNCTIONS

L81
Interaction of Protein 4.1R and Actin Stabilizes E-cadherin-dependent Intercellular Adhesion in Epithelial Adherens Junctuons
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Protein 4.1R (4.1R) consists of a diverse array of isoforms; their expression and localization in epithelial cells are cell density-dependent. 4.1R localizes to the nucleus and cytoplasm of non-confluent cells, but is confined almost exclusively to the lateral membrane of fully confluent cells. We characterized the cell density-dependent cellular distribution of 4.1R isoforms and examined the functional significance of their lateral membrane localization. 4.1R isoforms in MDCK cells are translated either from the AUG-1 (4.1R135 and 4.1R140) or from the AUG-2 (4.1R60 and 4.1R90). They lack exons 3, 14, 15, and 17a, and are comprised of different combinations of exons 16, 17b, 17c, and 18. Exon 17b encodes peptides that interact with actin and its expression becomes predominant as cells polarize. Exons 17c contributes to plaque membrane localization while the 3-KS reductases Involved in Sphingolipid Biosynthesis
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Sphingolipids are essential components of the eukaryotic membrane and also function as signaling molecules. An important step in their biosynthesis is the reduction of 3-Ketosphinganine (3-KS) to dihydrospinganine. In yeast, this reaction is catalyzed by Tsc10p. In mammalian cells, this is believed to be catalyzed by FVT1, but it is not clear that this is the only enzyme capable of reducing the 3-KS intermediate. To address this question, and to compare the yeast and mammalian enzymes, the topology, organization and specificity of the two enzymes were investigated. These studies were facilitated by the
ability of FVT1 to replace Tsc10 in the yeast biosynthetic pathway. Using a novel enzymatic assay and RNAi, we showed that FVT1 is indeed the principal 3-KS reductase activity in mammalian cells. We also show that both Tsc10p and FVT1 are integral endoplasmic reticulum (ER) membrane proteins. However, by inserting various topological probes along their lengths, these two orthologous proteins were found to have distinctly different topologies. Tsc10p has two membrane-spanning domains located in the C terminal half of the protein. In contrast, FVT1 appears to have a single 25-residue, normally located membrane-spanning domain between residues 1 and 25. When the N-terminal 25 residues of FVT1 were fused to a soluble heterologous protein (GFP), they correctly localized the chimera to the ER membrane. Coimmunoprecipitation experiments with epitope-tagged Tsc10p revealed that this protein is oligomeric. Analogous results were obtained for FVT1 indicating that, like other members of the short chain reductase family, these enzymes are most likely homodimers, or possibly tetramers. Coimmunoprecipitation experiments examining the interaction of Tsc10p with the individual components of serine palmitoyltransferase (SPT), failed to provide any evidence for association between the reductase and SPT. Taken together, these results suggest that 3-KS diffuses from its site of synthesis on SPT to the reductase.

L83

Functional Role of Domain III of the Bacillus thuringiensis Cry4Ba Toxin
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Similar to the other known structures of Bacillus thuringiensis Cry 8-endotoxins, the crystal structure of the 65-kDa activated Cry4Ba toxin comprises three domains which are, from the N- to C-terminus, a bundle of α-helices, a three-β-sheet domain, and a β-sandwich. To investigate the properties of the C-terminal domain III in isolation, a cloned Cry4Ba-domain III was over-expressed as a 21-kDa soluble protein in Escherichia coli and purified by FPLC. Using a surface plasmon resonance biosensor, the domain III fragment bound irreversibly to immobilised PE/PC/CH bilayer membranes via two-state binding mode with the affinity constant of ca. 160 nM. Similarly, the full-length Cry4Ba toxin bound to immobilised membrane with the affinity constant of ca. 760 nM as determined from two-state binding model. Moreover, treatment with proteinase K suggested that domain III region is involved in membrane binding and insertion. In vitro binding analysis via immuno-histochemical assay revealed that the Cry4Ba-domain III protein could bind to the apical microvilli of the susceptible Stegomyia aegypti larval midguts, albeit at lower-binding activity when compared with the full-length active toxin. These results demonstrate for the first time that the Cry4Ba mosquito-larvicidal protein conceivably participates in membrane binding and toxin-receptor recognition.

L84

Change in Permeant Size-Selectivity by Phosphorylation of Connexin 43 Gap-Junctional Hemichannels by PKC
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Gap-junctional channels, permeable to large hydrophilic solutes of up to M_r ~1000, are responsible for cell-to-cell communication. Phosphorylation of connexin 43 (Cx43) by PKC abolishes the permeability of gap-junctional channels and hemichannels to large hydrophilic solutes but not to small inorganic ions. Here, we reconstituted in liposomes purified hemichannels (average 0.8 hemichannels per liposome) with variable number of PKC-phosphorylated subunits (Cx43-P), determined their subunit composition by luminescence resonance energy transfer, and measured the % of sucrose-impermeable liposomes. Proteoliposomes reconstituted with the phosphorylated Cx43-P and non-phosphorylated Cx43 hemichannels from Cx43-dPC/Cx34-P mixtures at ratios of 5/1, 4/2 or 3/3 did not display significant sucrose retention. At Cx43-dPC/Cx43-P ratios ≤2/4, the percentage of sucrose retained was inversely proportional to the hemichannel content of Cx43-dP, but even for an average of 5 Cx43-P per hemichannel, sucrose retention was much less than that observed with proteoliposomes containing 6 Cx43-P hemichannels. Comparison of the data with the expected % of probe-impermeable liposomes, calculated from the binomial distribution, strongly suggests that all Cx43 subunits must be phosphorylated to abolish sucrose (M_r 342) permeability. We also show that Cx43-P hemichannels have a single N-terminal membrane-spanning domain thus suggesting that the small hydrophilic solute ethyleneglycol (M_r 62) passes through the pore formed by the Cx43-P hemichannels. These results indicate that phosphorylation of Cx43 by PKC alters the hemichannel size-selectivity and explain why PKC activity affects dye transfer between cells without consistent effects on electrical communication. This work was supported in part by NIH grants GM068586 and DC007150, and the American Heart Association, Texas Affiliate grant 045511S.

L85

Structures and Orientations within Phospholipid Membranes of the a7 Peptide from the Pore-forming Domain of the Bacillus thuringiensis Cry4Ba Toxin
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As can be inferred from the Cry4Ba crystal structure, both Tyr^296 and Phe^340 are oriented on the same side of α7. Together with their toxicity importance, it might imply a functional role for these two conserved aromatic residues in the membrane-binding step of the toxin as shown by numerous studies that the binding of membrane-inserting proteins to the lipid surface requires an aromatic cluster. In this study, ATR-FTIR results showed that 27-residue Cry4Ba α7 peptides in DMPG multilayers were a mixture of α-helices and β-forms. Moreover, changes in types of lipids used or the peptide-lipid ratio revealed variations in the peptide conformational ratio of α-helices to β-structure. Hydrogen/deuterium exchange experiments suggested that β-form was ~90% insertion in the membrane with an average tilt angle of 47°, whilst α-helical form was placed on the surface with ~49% insertion and both critical aromatic residues pointing towards the membrane. The results are consistent with 10-ns MD simulations of both conformations in DMPG bilayers, suggesting Tyr^296 and Phe^340 in α7 play a role in toxin-membrane interaction that is needed for membrane-pore formation of the Cry4Ba toxin.

L86

Proteomic Identification of a Novel O-GlcnAcylated Protein in Drosophila melanogaster
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β-O-linked N-acetylgalcosamine (O-GlcNAc) is dynamic post-translational modification in nucleocytoplasmic proteins such as transcription factors, cytoskeletal proteins, variable enzymes. These proteins are modified with O-GlcNAc at their Ser/Thr residues and this event changes their intracellular functions, including transcription, proliferation, apoptosis, cell signaling. Knock-out of the O-GlcNAc transferase (the nucleocytoplasmic enzymes for the addition of O-GlcNAc) result in stem cell and embryonic lethality so O-GlcNAc metabolism in Drosophila will likely provide important clues to the cellular functions of O-GlcNAc modification. In order to detect O-linked GlcNAc modifications in Drosophila and SL2 cell, immuno-blotting were performed with CTD 110.6 antibody. O-GlcNAcylated proteins in Drosophila and SL2 cell were analyzed using two-dimensional gel electrophoresis and MALDI-TOF-MS. As a result, ATP synthase beta subunit was identified as a novel O-GlcNAcylated protein in Drosophila and SL2 cell. Thus we will focus on revealing how ATP synthase beta modified with O-GlcNAc and the functional roles of O-GlcNAcylation on ATP synthase beta.

L87

Membrane Topology of OA1 Revealed by Partial Permeabilization of Plasma Membrane
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OA1 (GPR143) is a pigment cell specific glycoprotein consisting of 404 amino acid residues that is mutated in patients with Ocular
Albinism Type I, the most common form of ocular albinism. While its cellular localization is suggested to be endolysosomal and melanosomal, the physiological function of OA1 is currently unclear. Homology analysis indicates a weak similarity of OA1 to members of the G protein coupled receptor (GPCR) superfamily despite the fact that OA1 localizes only to intracellular organelles. Recent evidence obtained via purposeful mislocalization of OA1 to the plasma membrane suggests that OA1 functions as a GPCR. However, in view of its exclusively intracellular localization of OA1, the significance of these studies remains unclear. We analyzed the membrane topology of OA1 expressed in COS-1 cells, where it localizes to lysosomal organelles. To accomplish this topology analysis, we first established experimental conditions that selectively permeabilized the plasma membrane of COS-1 cells while leaving lysosomal membranes intact. We have undertaken the insertion of a hemagglutinin (HA) tag into the prototypic yeast cytoskeleton-associated region of OA1 molecule and assessed by immunofluorescence the accessibility of the HA tag inserted into OA1 to HA antibody. HA-tagged lysosome associated membrane protein 1 (LAMP1), a type I membrane protein, was employed as a control. The results show that OA1 spans intracellular membrane 7 times and that its C-terminus is oriented to the cytoplasm. Thus, OA1, properly localized intracellularly, is a 7 times spanning integral membrane protein consistent with its putative role as an intracellular GPCR.

L88 Subcellular Localization of Yeast Oxysterol Binding Protein Homologs
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While it is well established that sterols are distributed heterogeneously in the eukaryotic cell, the mechanisms by which this takes place are still somewhat unclear. Mutational studies in the model organism Saccharomyces cerevisiae implicated of a family of seven oxysterol-binding protein homologs (OSH) in sterol distribution and metabolism. Recent evidence suggests that one member of this family, Osh4p/Kes1p, acts as a cytosolic, non-vesicular transporter of sterol from the plasma membrane to ER. Here we confirm the ability of Osh5p, the OSH most similar to Osh4p, to bind free cholesterol and transfer it between small unilamellar vesicles. However, two other OSH proteins, Osh6p and Osh7p, show no ability to bind or transport cholesterol. Furthermore, since a putative sterol transporter might be expected to display an altered subcellular localization in response to changes in availability of extracellular cholesterol. We expressed GFP-tagged OSH proteins in yeast upc2-1 mutants (which are capable of aerobic sterol uptake), and found in minimal medium Osh4p co-localizes with the Golgi. However, in rich medium or minimal medium supplemented with cholesterol, Osh4p was cytosolically distributed with some plasma membrane association. In summary, these results lend weight to the hypothesis that Osh4p acts as a non-vesicular sterol transporter, and that Osh5p possibly shares this function. The function of Osh6p and Osh7p remains unclear, but their unresponsiveness to sterol points toward some other function in lipid homeostasis. Recent evidence has shown that Osh6p and Osh7p, which do not partition in cholesterol rich domains, are not a common feature of CNS myelin internodes which contain both PLP and P0. However, when we replaced PLP with similar amount of P0, incisures were present in optic nerve internodes. These incisures contained microtubules, membrane vesicles and other cytoplasmic organelles. These data support the hypothesis that P0 protein is essential for formation of SL incisures in PNS myelin. In addition, P0 can induce SL incisure formation in CNS myelin internodes when it replaces PLP as the major structural protein of compact CNS myelin.

L89 Chondroitin Sulfate Modification of CD74 is Required for Macrophage Migration Inhibitory Factor Signal Transduction
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Macrophage migration inhibitory factor (MIF) is an upstream activator of innate immunity, that antagonizes glucocorticoid activity, upregulates Toll-like receptor 4 expression and suppresses activation induced apoptosis. MIF has recently been cloned and found to interact with CD74 and CD44. CD44 interaction likely results in signal transduction, resulting in phosphorylation of ERK1 and ERK2 MAP kinases. Previous reports have also found a function interaction between CD74 and CD44 that requires CD44 modification by addition of chondroitin sulfate. In this work, we have examined whether chondroitin sulfate modification of CD74 is required for MIF-dependent ERK1/2 phosphorylation through CD44. We have expressed a mutated version of CD74 that lacks the serine acceptor for xylosylation, the first step in chondroitin sulfate addition, in cells expressing CD44. Our preliminary data indicates that MIF addition to these cells does not result in ERK1/2 phosphorylation, suggesting that CD74 interaction with CD44 via chondroitin sulfate is required for MIF-dependent activation.

MEMBRANE DOMAINS AND POLARITY
L90 P0 Protein Is Required for and Can Induce Schmidt-Lantermann Incisures Formation
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Myelin internodes are comprised of tightly compacted layers of glial cell membranes which surround axons in the peripheral (PNS) and central (CNS) nervous systems. All internodes in the PNS contain cytoplasmic channels called Schmidt-Lantermann (SL) incisures that connect outer and inner glial membrane domains. These channels are not a common feature of CNS myelin internodes. Molecularly, the myelin associated glycoprotein (MAG) is a major structural protein of Schmidt-Lantermann incisures, P0 protein is the major structural protein of compact PNS myelin, while proteolipid protein (PLP) is the major structural protein of compact CNS myelin. To investigate the potential role of MAG and P0 protein in SL incisure formation we examined PNS internodes from MAG-null and P0-null mice. SL incisures were present in MAG-null CNS internodes and absent from P0-null internodes. These data raised the possibility that P0 protein regulates SL incisure formation. To test this hypothesis, we introduced P0 protein into compact CNS myelin. As in wild type optic nerves, SL incisures were not present in optic nerves that contain both PLP and P0. However, when we replaced PLP with similar amount of P0, incisures were present in optic nerve internodes. These incisures contained microtubules, membrane vesicles and other cytoplasmic organelles. These data support the hypothesis that P0 protein is essential for formation of SL incisures in PNS myelin. In addition, P0 can induce SL incisure formation in CNS myelin internodes when it replaces PLP as the major structural protein of compact CNS myelin.

L91 Elastic Membrane Heterogeneity of Living Cells Revealed by Stiff Nanoscale Membrane Domains
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Multiple approaches have been developed to characterize membrane heterogeneity in living cells. Here, we have studied the elastic properties of plasma membrane domains using optical trapping, atomic force microscopy. This work demonstrates the existence of nanometric scale heterogeneous domains with specific biophysical properties. In particular, we focused on glycosylphosphatidylinositol (GPI)-anchored proteins, which play important roles in membrane trafficking, cell signaling and diseases and which were shown, using a wealth of methods, to preferentially partition in cholesterol rich microdomains. We found that GPI-anchored proteins resided in domains stiffer than the surrounding membrane, whereas membrane domains containing the transferring receptor, which do not partition in cholesterol rich domains showed no such features. The observed increase in stiffness with GPI-domains is consistent with previously documented specific lipid condensation and slow diffusion rate of proteins/lipids within these domains. Our data quantitatively document elastic membrane heterogeneity unveiling a putative link between membrane stiffness, molecular diffusion and signalling activation.

L92 aPKC-mediated Phosphorylation Regulates Asymmetric Membrane Localization of the Cell Fate Determinant Numb
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L95
Relationship between Lipid Composition, the CholesterolDependence of Perfringolysin O Membrane Interaction and Perfringolysin O Raft Affinity
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L93
Microvillar Size and Espin Expression Are Regulated by Testicular Factors in Principal Cells of the Adult Rat Epididymis
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In the epididymis, epithelial principal cells are studded with tall microvilli containing many components including aquaporins and solute carrier proteins that condition the luminal environment as an essential step in sperm maturation. Espins are a family of proteins that are expressed on microvilli of epithelial cells and are involved in microvillar development. The purpose of the present study was to define the regulation of microvillar growth and the expression of espin in the rat epididymis. Previous studies have shown that microvillar growth is dependant on testicular factors. Using a rat caput epididymal cell line we investigated the role of a soluble fraction of testicular homogenate on epididymal microvilli. Our results indicate that testicular factors stimulate the size of microvilli. To confirm that espin is expressed in the epididymis, RT-PCR and Western blot analyses were done on both RNA and protein isolated from the epididymis. Results showed that espin mRNA is present throughout the epididymis. In control rats, light microscope immunocytochemistry revealed that espin was expressed exclusively over the microvilli of principal cells of all epididymal regions. In orchidectomized rats, espin expression was significantly reduced, but restored to control levels in orchidectomized rats supplemented with high doses of testosterone. Efferent duct ligation also resulted in a dramatic decrease in espin expression but not to the extent of orchidectomy, suggesting that high luminal levels of testosterone were needed to maintain full expression of espin on microvilli. Together these data suggest that testicular androgens play an important role in the regulation of epididymal microvilli and that this role may be mediated via espin. Supported by CIHR, NSERC and NIH.
L94
Cholesterol, Src Family Kinases and Caveolin-dependent Transient Anchorage of Cross-linked Glycosyl-phosphatidylinositol-anchored Proteins
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How outer leaflet plasma membrane components, such as glycosylphosphatidylinositol-anchored proteins (GPIAps), transmit signals to the cell interior is an open question in membrane biology. By deliberately cross-linking several GPIAps under antibody conjugated 400-nm gold particles, transient anchorage of the gold-particle induced clusters of both Thy-1 and CD73, a 5′ exonuclease, occurred for periods ranging from 300 ms to 10 seconds in fibroblasts. Transient anchorage was abolished by cholesterol depletion, the addition of the Src family kinase (SFK) inhibitor, PP2, or in Src-Yes-Fyn knockout cells. It was also shown phosphorylated SFKs coincided with transient anchorage at high proportion. Caveolin-1 knockout cells exhibited reduced but not totally abolished transient anchorage time, suggesting caveolin-1 might serve as a trapping structure for transient anchorage complex components. By contrast, a transmembrane protein, the cystic fibrosis transmembrane conductance regulator (CFTR), exhibited transient anchorage that occurred without deliberately enhanced crosslinking: moreover, it was only slightly inhibited by cholesterol depletion or SFK inhibition and depended completely on interaction of its PDZ binding domain with the cytoskeletal adaptor, EB5. We propose that cross-linked GPIAps become transiently anchored via a cholesterol dependent, SFK-regulatable linkage between a transmembrane cluster sensor and the cytoskeleton.
L96
Rab5 Directly Binds to Caveolin-1 and Activates the Caveolaedependent Endocytosis
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Rab5 is involved in nutrition uptake, signal transduction, viral infection, drug delivery and other intracellular vesicle transportation during clathrin-dependent endocytosis. Recent studies have shown that Rab5 is also important in caveolaedependent endocytosis. Caveola is a 50-100 nm flask-shaped invaginations at the plasma membrane which is composed by a 22-24 kDa protein called caveolin-1. However, the molecular mechanism of caveolaedependent endocytosis is still remains unclear. Thus, we investigated the relation between caveolin-1 and Rab5 and their role during the endocytosis in the present study. First, caveolin-1 enriched fraction from rat spleen was isolated by using monoclonal beads coated with anti-caveolin-1 and the Rab5 co-immunoprecipitation was confirmed. Using recombinant rab5 as a probe far western blotting revealed that activated Rab5 bound to caveolin-1. Then their localization was examined through confocal
fluorescence microscopy. Both of them are co-localized on early endosome. Furthermore, the uptake of cholera toxin subunit B, a caveolae-dependent endocytosis marker, was enhanced by active Rab5. Further evidence comes from affinity column chromatography where caveolin-1 was detected activated Rab5 column in contrast with inactivated Rab5. Finally, we attempted to identify Rab5 binding domain on caveolin-1 by using various caveolin-1 deletion mutants. We found that Rab5 can bind through the caveolin-1 scaffolding and C-terminal cytoplasmic domain. Although, recent studies have shown that caveolin can undergo through an endocytic pathway, the role of Rab5 was not clear. Our results indicate direct interaction of Rab5 during the caveolae-dependent endocytosis machinery.

L97 The Internalization of Antennapedia-Green Fluorescence Fusion Protein Into Human Umbilical Vein Endothelial Cells P. A. Hartman, M. J. Porta, B. M. Fenner, R. D. Shurina; Biology, Wheeling Jesuit University, Wheeling, WV Antennapedia (AN) is a small, 16 amino acid polypeptide used in drug delivery systems. Previous studies have shown that AN chaperones proteins across cell membranes. Our study investigated the internalization of AN-Green Fluorescence Protein (GFP), a protein approximately 30 kDa. This fusion protein is significantly larger than any chaperone protein previously reported in HUVEC cells, a primary endothelial cells line. If AN can chaperone large proteins into HUVEC cells, AN may be able to deliver a much larger spectrum of drugs than previously accepted. We hypothesize that AN will be able to chaperone large proteins, such as GFP and AFAP-110, into HUVEC cells. The purpose of our study was to determine if the DNA for AN incorporated into a pGEX-6P-1 vector along with DNA encoding for Green Fluorescence Protein (GFP) could create a fusion protein that, when purified, was capable of crossing the membrane of HUVEC cells. Since this protein is nearly twice the molecular weight of previous proteins chaperoned by AN, our data will provide evidence for increased application of AN in HUVEC cells. Furthermore, we will investigate the mechanism of internalization by double- and triple-label immunofluorescence of vesicular compartment, including alpha-adaptin labeling of early endosomes. Western blot analysis of our protein showed the presence of GFP in our purified lysate. The AN-GFP fusion protein was taken up by the HUVEC cell as visualized by immunofluorescence microscopy. We are currently studying the localization of AN-GFP with early endosomes to determine if the AN-GFP fusion protein is internalized via receptor-mediated endocytosis.

PROTEIN FOLDING AND ASSEMBLY

L98 Role of Cyclophilin B in Endoplasmic Reticulum Stress J. Kim, T. Choi, K. Choi, Y. Ding, W. Cho; S. Kim; Dept. of Molecular Biology, Medical Research Center, Seoul, Republic of Korea Prolonged accumulation of misfolded proteins in the endoplasmic reticulum (ER) leads to ER stress-mediated apoptosis. Cyclophilins are protein chaperones that accelerate the rate of protein folding through their peptidyl-prolyl cis-trans isomerase (PPIase) activity. Here, we show that ER stress activates expression of the ER-localized cyclophilin B (CypB) gene through a novel ER stress-response element. Overexpression of wild-type CypB attenuates ER stress-induced cell death, whereas overexpression of an isomerase activity defective mutant CypB/R62A not only increases calcium leakage from the ER and ROS generation, but also decreases mitochondrial membrane potential leading to cell death after exposure to ER stress inducing agents. SiRNA-mediated inhibition of CypB expression renders cells more vulnerable to ER stress. Finally we also show that the CypB interacts with the ER stress-related chaperones, Bip/Gp78 and Grp94. Taken together, we conclude that CypB plays an essential role in protecting cells from ER stress via its PPIase activity.
Mechanisms of Polycystin Ciliary Localization in C. elegans

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Cilia are located at the distal ends of dendrites of C. elegans sensory neurons, where they perceive a variety of environmental cues and modulate worm behavior. Ciliary specialization involves targeting distinct sensory receptors and signaling molecules. How sensory receptors localize to cilia, and their targeting to the regulated secretory pathway in C12 cells. We propose that sorting of SgII into DCGs may recruit several discrete sorting determinant mediates the sorting of SgII into the regulated secretory pathway remains to be determined. In this study, we designed a series of full-length and truncated domains of human SgII fused to GFP, or to an engineered form of embryonic alkaline phosphatase (EAP) to identify sorting signals within the primary structure of SgII that mediate its targeting to DCGs. Using 3D deconvolution fluorescence microscopy and secretagogue-stimulated release assays, we have shown that SgII-GFP and SgII-EAP chimeras are correctly targeted to DCGs and other somatodendritic structures. In conclusion, our findings reveal new insights into the mechanisms that govern ciliogenesis. In Class II mutants, PKD-2::GFP is inappropriately retained in the cell surface upon insulin signaling. This positively-charged cluster, which is unique to PLCζ, was analysed by applying both a theoretical electrostatic model and measured empirically using a centrifugation assay. Incorporation of PtdInsP2 to phosphatidyliccholine/phosphatidylserine vesicles increases the molar partition coefficient (K) for PLCζ. In addition, bacterially-expressed recombiant fragments corresponding to the linker region of PLCζ, bind with higher affinity to polyvalent phosphoinositides on nitrocellulose filters. A peptide corresponding to the basic cluster of the linker region, PLCζ (374-385) binds to PC/PS vesicles with higher affinity than PLCζ. However, this effect is reversed upon incorporation of PtdInsP2. These results suggest that the cluster of basic residues may play a role in targeting of PLCζ to acidic membrane lipids.

Potential Membrane Lipid Interaction Role for Charged Residues in the X-Y Linker Region of PLCζ


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Insulin induces a redistribution of the GLUT4 glucose transporter from intracellular compartments to the surface of adipocytes by decreasing GLUT4 endocytosis and increasing its exocytosis rate. The GLUT4 FQQI motif in its amino-terminal domain (amino acids 25-41) is necessary for its ability to interact with acidic lipids. We designed a series of full-length and truncated domains of human SgII and GLUT4 to study the role of positively-charged clusters in their targeting to DCGs. We propose that sorting of SgII into DCGs may recruit several discrete determinants that mediate its targeting to the regulated secretory pathway in C12 cells. We designed a series of full-length and truncated domains of human SgII fused to GFP, or to an engineered form of embryonic alkaline phosphatase (EAP) to identify sorting signals within the primary structure of SgII that mediate its targeting to DCGs. Using 3D deconvolution fluorescence microscopy and secretagogue-stimulated release assays, we have shown that SgII-GFP and SgII-EAP chimeras are correctly targeted to DCGs and other somatodendritic structures. In conclusion, our findings reveal new insights into the mechanisms that govern ciliogenesis. In Class II mutants, PKD-2::GFP is inappropriately retained in the cell surface upon insulin signaling. This positively-charged cluster, which is unique to PLCζ, was analysed by applying both a theoretical electrostatic model and measured empirically using a centrifugation assay. Incorporation of PtdInsP2 to phosphatidyliccholine/phosphatidylserine vesicles increases the molar partition coefficient (K) for PLCζ. In addition, bacterially-expressed recombiant fragments corresponding to the linker region of PLCζ, bind with higher affinity to polyvalent phosphoinositides on nitrocellulose filters. A peptide corresponding to the basic cluster of the linker region, PLCζ (374-385) binds to PC/PS vesicles with higher affinity than PLCζ. However, this effect is reversed upon incorporation of PtdInsP2. These results suggest that the cluster of basic residues may play a role in targeting of PLCζ to acidic membrane lipids.

Investigation of Membrane Trafficking of Dpl1-Dihydrosphingosine Phosphate Lyase in the Cell

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Sphingosine and sphingosine 1-phosphate (SIP) have emerged as important regulators of several biological processes in mammalian cells. Sphingosine causes growth arrest while sphingosine 1-phosphate is a key mediator of proliferative, angiogenic and acute inflammation responses. SIP has also been implicated in pathology of diabetes and infection. Likewise, in Saccharomyces cerevisiae sphinganine (DHS),...
**L106**

**Mucolipin-2 Localizes to the Arf6-associated Pathway and Regulates Trafficking of GPI-proteins**

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In mammals, the mucolipin family includes three members (MCOLN1-3). While mutations in MCOLN1 and MCOLN3 have been associated with mucolipidoses type IV and the variant-waddler mouse phenotype, respectively, little is known about the function and cellular distribution of MCOLN2. Here we show that MCOLN2 traffics via the Arf6-dependent pathway and co-localizes with major histocompatibility class I (MHC1) in both vesicles and long tubular structures. Expression of a constitutive active Arf6 mutant or activation of endogenous Arf6 by transfection with EFA6 or treatment with aluminum fluoride caused accumulation of MCOLN2 in enlarged vacuoles that also contain MHC1 and CD59. Moreover, over-expression of MCOLN2 promoted efficient activation of Arf6. Finally, depletion of endogenous MCOLN2 by expression of a specific shRNA caused a strong reduction in the levels of several endogenous glycosylphosphatidylinositol-anchored (GPI) proteins such as CD59, CD55, and alkaline phosphatase, and resulted in missorting of GPI-anchored proteins.

**L107**

**PKC-ε Membrane Localization during IgG-mediated Phagocytosis Is Dependent on εPS and εC1B**

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Protein kinase C ε (PKC-ε) is necessary for efficient antibody (IgG)-mediated phagocytosis in macrophages. Activation of the enzyme requires membrane localization mediated by the N-terminal regulatory domain (RD). The current study addresses the mechanism of PKC-ε localization at the phagosome membrane. We previously demonstrated that PKC-ε accumulation requires diacetylglcerol binding to the εC1B (Cheeseman et al., 2006). Studies utilizing chimeras of PKC-δ (non-localizing) and PKC-ε (localizing) conjugated to green fluorescent protein (GFP) were used to determine if εC1B is sufficient for localization. Chimeric PKC-ε containing εC1B (εεC1B) localizes to phagosomes. In contrast, PKC-δ expressing εC1B (δεC1B) did not accumulate suggesting that εC1B is necessary, but not sufficient, for localization. Truncation and deletion mutants of PKC-ε were used to identify the region(s) of PKC-ε involved in phagosome localization. These studies revealed that the pseudosubstrate (εPS) also acts as a localization domain. Loss of εPS, either by truncation and deletion, abrogated PKC-ε accumulation at targets. Additionally, substitution of the PS and C1B region of PKC-δ with the corresponding regions from PKC-ε produced a chimeric PKC (δεPS εC1B) that transiently accumulated at phagosomes and was indistinguishable from wt PKC-ε. Interestingly, the PKC-δ chimera containing only εPS (δεPS) failed to localize to phagosomes, suggesting that both εPS and εC1B are necessary for phagosomal targeting. These results suggest that εPS and εC1B are necessary and sufficient for localization of PKC to forming phagosomes. This is the first report of a PKC PS domain contributing to isoform-specific localization. Lipid overlay and GST-pull down assays are underway to ascertain the εPS lipid anchor or protein binding partner. This work is supported by NIH GM50821 and T32HL07194.

**TISSUE-SPECIFIC GENE EXPRESSION**

**L108**

**Protocol and Initial Analysis of ADH Isozymes of Chrysoma pauciflosculos**

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Previous studies have shown that plant species, or populations of the same species differing in geographical locations, often contain slight variations in the amino acid sequences of the same protein. This occurrence results in the production of isozymes, variant forms of the same enzymes. *Chrysoma pauciflosculos*, a small evergreen shrub that grows on sandy ridges in the southeast, is considered a rare plant species in North Carolina. Two sites of *Chrysoma*, located approximately one mile apart in Robeson County, NC, have been located. No published information on isozyme studies or population genetics exists for this species. In this pilot study, using a protocol that has been devised for homogenized plant tissue, lysates are electrophoresized on 12 % starch gel and/or 5.5 % acrylamide gel, then stained for alcohol dehydrogenase (ADH) activity. Our initial results indicate several variables that influence the detection of ADH isozymes from *Chrysoma*. Seasonal differences, storage conditions, and extraction methods have a great effect on the viability of ADH isozymes. This work will supply information regarding population genetics for this species.

**L109**

**Characterizing the Transcriptional Profile of Invasive Tumor Cells in Breast Cancer**

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The tumor microenvironment is known to play an important role in the progression to metastasis. A number of studies have correlated the presence of macrophages in tumors with poor prognosis, until now there was no direct observation of how macrophages are involved in haematogenous metastasis. We have used multiphoton microscopy and immunohistochemistry to show that tumor cell motility is linked to the presence of neighboring macrophages and that perivascular macrophage clusters in mammary tumors are sites of tumor cell intravasation. These results have demonstrated that the paracrine interaction between macrophages and tumor cells lying in close proximity defines a microenvironment that is directly involved in the intravasation of cancer cells in mammary tumors. We have also performed co-culture of the carcinoma cells and macrophages to identify a paracrine loop between the two cells. Macrophages express epidermal growth factor (EGF), which promotes the formation of elongated protrusions and cell invasion by carcinoma cells. Colony stimulating factor 1 (CSF-1) produced by carcinoma cells promotes the expression of EGF by macrophages. In addition, EGF promotes the expression of CSF-1 by carcinoma cells thereby generating a positive feedback loop. Recently we have developed a multiplex fluorescent in situ hybridization technique to identify a number of transcription sites within cells in culture. This technology has also been used on fixed tissues for multiple transcription site analysis. We report here the presence of CSF-1 and EGF expressing cells in the proximity of the blood vessels in a mouse PyMT transgene mammary tumor model, identified using multicolor transcription site analysis. Work is in progress to extend this technology to identify the gene expression pattern of multiple genes in the primary breast tumor.
L110

NO2-dependent NF-κB Activation Is Involved in NTHi-induced DEFB4 Up-regulation of the Middle Ear Epithelial Cells via a TLR2-independent Signaling Pathway

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Nontypeable Haemophilus influenzae (NTHi) is a common pathogen causing middle ear infection in children and exacerbating chronic lung disease in the elderly. Recently, we reported that the toll-like receptor 2 (TLR2)-dependent signaling pathway plays a critical role in NTHi-induced DEFB4 up-regulation in the middle ear epithelial cells. However, inhibition of TLR2 did not completely block NTHi stimulation, hinting to an alternative pathway. We investigated NO2, a cytoplasmic receptor and its downstream signaling molecules since NO2 is required for MDP-induced DEFB4 up-regulation in primary keratocytes. Immunohistochemistry showed induction of DEFB4 in the rat middle ear modulate when infected with NTHi. Immunoprecipitation showed that human middle ear cells induced DEFB4 upon exposure to the NTHi lysate. NTHi-induced DEFB4 up-regulation was inhibited by transfection of the NO2-specific siRNA, but not by TLR4-specific siRNA. Moreover, NO2 expression was up-regulated by stimulation with the NTHi lysate, in a time-dependent manner. Treatment of hemolysin increased membrane permeability and resulted in a synergistic effect on DEFB4 up-regulation upon exposure to suboptimal dose of the NTHi lysate. This synergistic effect was inhibited by NO2-specific siRNA. Transfection of the RICK-specific siRNA inhibited NTHi-induced DEFB4 up-regulation, whereas inhibition of Card12 resulted in a 2 to 3 fold enhancement of DEFB4 expression. The inhibitors of NF-κB and IKK showed blockage of NTHi-induced NF-κB translocation to the nucleus. Treatment with these inhibitors resulted in an inhibition of NTHi-induced DEFB4 up-regulation. The presence of NTHi resulted in phosphorylation of IkBa. Furthermore, NTHi-induced DEFB4 up-regulation was inhibited by MG-132 and the dominant negative mutant construct of IkBa. In conclusion, in the middle ear epithelial cells, NTHi induces DEFB4 expression via a NO2-dependent NF-κB activation, mediated by the RICK-IKK-IκBα signaling pathway. Further studies are underway for characterizing the enhancer region of DEFB4 responsive to NTHi molecules and identifying a NO2-specific ligand for NTHi molecules.

L111

Synergistic Effect of IL-1α on Nontypeable Haemophilus influenzae-induced Up-regulation of Human β-Defensin 2 in Middle Ear Epithelial Cells

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Background: We recently showed that beta-defensins have antimicrobial activity against nontypeable Haemophilus influenzae (NTHi) and that interleukin 1 alpha (IL-1α) up-regulates the transcription of beta-defensin 2 (DEFB2) according to new nomenclature of the Human Genome Organization (Homo sapiens). Human cytokine macroarray analysis was performed to detect the released cytokines in response to NTHi exposure. Real time quantitative PCR was done to compare the induction of IL-1 alpha or beta-defensin 2 mRNAs and to identify the signaling pathways involved. Direct activation of the beta-defensin 2 promoter was monitored using a beta-defensin 2 promoter-Luciferase construct. An IL-1 alpha blocking antibody was used to determine the role of cytokine in beta-defensin 2 expression. Results: Middle ear epithelial cells released IL-1 alpha when stimulated by NTHi components and this cytokine acted in an autocrine/paracrine synergistic manner with NTHi to up-regulate beta-defensin 2. This synergistic effect of IL-1 alpha on NTHi-induced beta-defensin 2 up-regulation appeared to be mediated by the p38 MAP kinase pathway. Conclusion: We demonstrate that IL-1 alpha is secreted by middle ear epithelial cells upon exposure to NTHi components and that it can synergistically act with certain of these molecules to up-regulate beta-defensin 2 via the p38 MAP kinase pathway.

L112

Multiple Signalling Pathways Differentially Regulate Epithelial Sertoli Cell Genes Encoding Steroidogenic Acute Regulatory (StAR)-related Lipid Transfer Proteins

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StARD4 and StARD5 are subfamily members of the steroidogenic acute regulatory protein (StAR/StARD1)-related lipid transfer (START-domain-containing) protein family, and are implicated in lipid transport, metabolism and signalling. Our recent studies demonstrated cytokine-mediated coordinate regulation of StARD1 and -D5, but not StARD4, expression in the highly specialized testicular epithelial Sertoli cell. To further address how differential gene expression with lipogenic and non-lipogenic START proteins is regulated, we compared the expression of StAR, StARD4 and D5 as compared to StAR, primary rat Sertoli cells were treated with the sterol lanosterol (LAN) or the fatty acid (FA) arachidonic acid (AA). Flow cytometry, Quantitative-Real-time RT-PCR and Western analyses were used to determine the kinetics of these treatments on intracellular signalling and gene expression. AA resulted in time-dependent increases in StARD4 mRNA and protein levels, but no changes in StAR or StARD5 protein levels. In contrast, biphasic changes in StARD4 and D5 mRNAs for all three SAR genes were observed following LAN treatment; in a temporal manner, changes in StARD4 and StARD5 proteins were seen. Neither LAN nor AA affected steady-state levels of StAR protein. AA resulted in a rapid, but transient, increase in phosphorylation of the eJUN-N-terminal kinase (pJNK), the serine-threonine kinase mitogen-activated protein kinase (MAPK, specifically, p42/p44-ERK-1, -2), and the eAMP-response element binding protein (CREB) as well as transient effects on reactive oxygen species (ROS) and nitric oxide (NO). Interestingly, LAN resulted in similar activation of JNK and ERK but a temporally distinct activation pattern for CREB, StARD4 and StARD5, but not StARD1. Taken together, our findings are suggestive of both transcriptional and posttranscriptional mechanisms involved in the differential regulation of the Sertoli cell START proteins. Further studies are required to determine individual components of the StAR/StARD regulation in the Sertoli cell to elucidate their functional relevance to this epithelial cell's critical supporting role in spermatogenesis. Studies supported by NIH R01 HD-39024 (PLM).
sialylated targets), adjacent to Siglec-6 expression on invasive placental trophoblast cells. Ligands were also found in uterine endometrium, and on cell lines of trophoblastic or endometrial origin. Siglec-6 levels are generally low in placenta from elective surgical deliveries without known labor, and highest following completion of labor. Thus, Siglec-6 expression was upregulated in the placenta during human evolution, presumably to interact with sialylated ligands for specific signaling functions and/or to regulate leptin availability. The negative signaling potential of Siglec-6 may have been recruited to human-specific placental expression, to slow the tempo of the human birth process. The leptin-binding ability of Siglec-6 is also consistent with this hypothesis. The mechanism may involve human-specific transcription factor recognition site changes in the Siglec-6 promoter region.

L114 Molecular Analysis of the RbBP6 in Human Malignancies Z. Mbiita,1 Z. Dlamini,1 D. Pugh,2 J. Rees;1 University of the Witwatersrand, Johannesburg, South Africa, 1University of the Western Cape, Cape Town, South Africa

Retinoblastoma binding protein 6 (RbBP6) is a gene that encode RbBP6 protein product that has been previously documented to bind retinoblastoma protein (pRB) and localises on chromosome 16p12.2. RbBP6 can also interact with p53. This has been documented by several groups, whereby they showed that P2P-R, truncated RbBP6 form lacking the N-terminal Domain With No Name (DWN) domain, can bind the two tumour suppressors, p53 and pRB. This strongly suggests the involvement of this gene in cell cycle regulation. The aim of this study was to determine the expression pattern of the RbBP6 in human cancers. The objective was to localize RbBP6 in these cancers and determine its role in cancers, apoptosis and cell cycle. 

In situ hybridization (ISH) and immunocytochemistry (ICC) were used for localizing the RbBP6 in human cancers at mRNA and protein levels respectively. Western blotting was performed to further determine the expression of the RbBP6 protein products in cultured cancer cells. In both cultured cancer cells and tissues, RbBP6 localized in the cytoplasm contrary to nuclear localization observed in normal cells and tissues except in the kidney tissues whereby localization was exclusively cytoplasmic. apoptotic and mitotic cells showed increased expression levels of the RbBP6 with some evident nuclear localization. Western blotting on different human cancer cell lines showed laddering when using an RbBP6 antibody raised against the 13kDa DWN of the RbBP6. These results suggest that RbBP6 is either translocated to the cytoplasm or in cancer cells it loses its nuclear localization signal for nuclear localization after protein synthesis or it targets cytoplasmic proteins. The latter may be suggested by the fact that the DWN domain is targeted by other proteins like ubiquitin since it shares 16% similarity with ubiquitin as reported previously. Its increased expression in apoptotic and mitotic cells further suggests its role in apoptosis and cell cycle control.

STRUCTURE OF NUCLEAR ENVELOPE

L115 Fusion between Nuclear Membrane Vesicles and Protein-free Liposomes Results in Nuclear Assembly E. Rafikova,1 K. Melkov,1 C. Ramos,2 L. Chernomordik1;1 National Institute of Health, Bethesda, MD, 2University of California, San Diego, La Jolla, CA

To study nuclear assembly we used an in vitro Xenopus laevis egg nuclear reconstitution system. When membrane vesicles and cytosol are mixed in the presence of sperm chromatin, fully functional nuclei with expanded nuclear envelopes, nuclear pores, and the ability to actively transport substrates are formed. A 5-fold decrease in the concentration of membrane vesicles resulted in the formation of nuclei that were 2-3 times smaller in diameter than nuclei formed at an optimal concentration of membrane vesicles. These small nuclei had nuclear pores, but they failed to actively import substrates. Recently we reported that while protein-free dioleoylphosphatidylcholine liposomes with associated cytosolic proteins fuse at the surface of the chromatin, they do not form functional nuclei. Thus, neither lowered concentrations of membrane vesicles nor liposomes support the later stages of nuclear assembly. However, addition of liposomes to diluted membrane vesicles compensated for the membrane shortage and yielded nuclei indistinguishable in size from those prepared at the optimal membrane concentrations. Active nuclear transport was also restored. Formation of these functional nuclei involved fusion between liposomes and membrane vesicles. We propose that while soluble proteins can drive membrane fusion, active nuclear transport and chromatin swelling mediated by integral proteins of specialized membrane vesicles are indispensable for formation of full-size functional nuclei.

L116 Meiotic Chromosome Dynamics at the Nuclear Envelope in C. elegans A. B. Allison,1 A. F. Dernburg,2,3 University of California, Berkeley, Berkeley, CA, 1Lawrence Berkeley National Laboratory, Berkeley, CA

Meiosis is a specialized cell division that halves the number of chromosomes to enable sexual reproduction. Our lab has observed interactions between meiotic pairing center proteins such as HIM-8 and the ZIM proteins and components of the nuclear envelope (NE), including lamin and SUN-1. In C. elegans, the C34D4.14 gene encodes a protein with a predicted SUN domain, suggesting it may localize to the NE. Mutations in C34D4.14 cause several meiotic phenotypes, including a high incidence of males (Him) and high percentage of dead eggs. Meiotic pairing center and NE proteins likely play a role in tethering chromosomes to the nuclear periphery during meiotic prophase, but the functional relevance of this phenomenon remains unclear. In other organisms such as maize, telomeres gather at the NE during meiosis in a structure referred to as the "meiotic bouquet" which may contribute to chromosome pairing and synopsis. Therefore, we investigated telomere localization during meiosis in C. elegans using immunofluorescence and fluorescence in situ hybridization techniques. Preliminary studies in wild type and C34D4.14 mutant animals suggest that telomeres do not localize to the NE during meiosis in C. elegans. Further studies focus on the role of C34D4.14 in chromosome-NE dynamics during meiosis.

ORGANOGENESIS

L117 Angiogenesis and Endothelial Signaling in the Developing Zebrafish Interrenal Tissue Y. H. Huang, Y. W. Liu; Department of Life Science, Tunghai University, Taichung, Taiwan

Increasing evidence points to a direct role for paracrine signaling between blood vessels and surrounding organ cells, during embryonic development and cell differentiation. The zebrafish is a useful system for examining the effects of vascular dysfunction on development, because they receive enough oxygen from the environment and continue to develop in normal fashion for several days even in the absence of blood circulation. The adrenal gland is the key endocrine tissue involved in mediating the stress response in mammals. The adrenal gland has a complex vasculature, the interaction between vasculature and adrenal gland during early organogenesis remains however unclear. The structure of adrenal gland is composed of the outer adrenocortical and the inner medullary tissues. The adrenocortical equivalent in zebrafish is called the interrenal, because it is embedded in the cephalic region of the kidney. In zebrafish embryo, primordial interrenal cells first appear as bilateral intermediate mesodermal cells expressing 

ff1b in a region ventral to the third somite, which then begins to express the steroidogenic enzyme gene, 3F-hsd. In order to observe the angiogenesis in the developing zebrafish interrenal tissue, we used transgenic line where EGFP is controlled by the zebrafish fkl1 promoter to mark the endothelial cells, and 3F-hsd histochemical staining for identifying the location of interrenal tissue. In addition, we used the cloche mutation to analyze how interrenal tissue respond to endothelial signaling. According to my preliminary results, the differentiation of the interrenal primordial in clo was normal, but the tissue morphology was altered since 30 hpf. In addition, the invagination of endothelial cells into the interrenal tissue, which occurred by 4 dpf, appeared not to be involved in the earlier central migration of interrenal primordia.

L118 Role and Regulation of Barx1 in the Zebrafish Pharyngeal Arches S. M. Sperber, I. B. Dawid; Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD
The Barx1 transcription factor has previously been shown to modulate the expression of cellular adhesion molecules and participate in specification of tooth-type, but little is understood of its role in patterning the pharyngeal arches. To determine the role of barx1 in zebrafish craniofacial formation, we examined its expression during embryogenesis and performed a functional analysis by microinjecting morpholino oligonucleotides to attenuate its translation. Barx1 is expressed in the cranial neural crest, the pharyngeal arches, the anterior aspect of the pectoral fin buds and the gut wall. Additionally, transient expression is observed in the posterior lateral line ganglia and developing tail. During neural crest migration and early pharyngeal arch patterning, embryos microinjected with barx1 morpholinos exhibit reductions in cranial neural crest markers that include crestin and dib2a. By 2.5 days post fertilization barx1 morpholino injected embryos exhibit poor facial outgrowth and micrognathia. Histological analysis reveals reductions in chondrocyte differentiation and condensation within the arches. Affected larvae stained with Alcian blue exhibit small and dysmorphic cartilage elements, and expression of chondrogenic markers such as col2a1 is perturbed. To determine how barx1 is regulated within the developing arches, we implanted beads imbued with growth factors such as FGF8 and BMP2. The bead experiments demonstrate the positive and negative influence of the growth factors on barx1 expression, and its role in modulating chondrocyte condensation within the pharyngeal arches. Together these results suggest a necessary role for barx1 at early stages of cranial neural crest patterning and chondrogenesis within the developing zebrafish viscerocranium. Investigating the zebrafish barx1 provides new insights into the human BARX1 ortholog that may be implicated in agnathia, micrognathia or other craniofacial syndromes.

INVERTEBRATE DEVELOPMENT

L119
Regulation of Midline Axon Guidance by a Novel Translation Inhibitor Krasavietz in the Drosophila Embryo
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Axon extension and guidance require a coordinated assembly of F-actin and microtubules as well as regulated translation. The molecular basis of how the translation of mRNAs encoding guidance proteins could be closely tied to the pace of cytoskeletal assembly is poorly understood. Previous studies have shown that F-actin/microtubule crosslinker Short Stop (Shot) is required for motor and sensory axon extension in the Drosophila embryo. Here, we provide biochemical and genetic evidence that Shot functions with a novel translation inhibitor, Krasavietz (Kra), to steer longitudinally directed CNS axons away from the midline. Kra can bind directly to the C-terminal domain of Shot. shot and kra mutations lead to weak robo-like phenotypes and synthetically affect midline avoidance. We also show that shot and kra dominantly enhance the frequency of midline crossovers in embryos heterozygous for slit, and that in kra mutant embryos, Robo-positive axons cross the midline that normally expresses the repellent Slit. Finally, Kra interacts with the translation machinery and inhibits translation in vivo. Together, these data suggest that Shot-Kra interactions control the translation of mRNAs encoding proteins necessary for the Robo-mediated response of the growth cone. We propose that Shot functions as a direct physical link between translational regulation and cytoskeleton reorganization.

L120
A Neural Crest-like Cell Lineage in the Invertebrate Chordate Ciona intestinalis
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The “new head hypothesis” by Northcutt and Gans states that neural crest cells (NCCs) are unique to vertebrates and were important for their evolution from invertebrate ancestors. NCCs arise during neurulation and later differentiate into a multitude of tissues that account for much of the structural complexity that distinguishes craniates from invertebrate chordates. NCC origins remain unclear. Here we show for the first time that the pigment cell lineage of a urochordate ascidian, Ciona intestinalis, meets the four part definition of NCCs - origin at the neural plate border, migration, NCC regulatory gene expression, and multipotency. This careful analysis of the pigment cell lineage has been carried out with single-cell resolution within a single ascidian species. Because vertebrate embryos have large numbers of cells, it is technically difficult to profile the development and gene regulation of individual NCCs. Our results support a new hypothesis that the last common ancestor of vertebrates and urochordates had an embryonic cell lineage that employed an extensive gene regulatory network dedicated to generating neural crest-like cells that are remarkably similar to extant NCCs. Additionally, key differences in the utilization of Fox D and Sox E transcription factors early in the regulatory network of vertebrate NCCs and not the developing pigment cell lineage of Ciona intestinalis may represent co-option that has occurred in the evolution of vertebrate NCCs.

ENDOPLASMIC RETICULUM

L121
A Role for the MAPK Slt2p in the Inheritance of Yeast Cortical ER
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Studies in the yeast Saccharomyces cerevisiae have shown that the inheritance of ER, mitochondria, and vacuoles involves the capture of a tubular structure at the bud tip. Ptc1p, a serine/threonine phosphatase, has previously been shown to regulate mitochondrial inheritance by an unknown mechanism. Ptc1p regulates the high osmolarity glycerol (HOG) MAPK pathway and has also been implicated in the cell wall integrity (CWD) MAPK pathway. Here we show that the loss of Ptc1p or the Ptc1p binding protein, Nbp2p, causes a prominent delay in the delivery of ER tubules to the periphery of daughter cells and results in a dramatic increase in the level of phosphorylated Slt2p, the MAPK in the CWD pathway. Either loss of Slt2p or inhibition of the CWD pathway by addition of sorbitol, suppresses the ER inheritance defect in the ptc1Δ and nbp2Δ mutants. Our findings indicate that Ptc1p and Nbp2p regulate ER inheritance through the CWD MAPK pathway by modulating the MAPK, Slt2p.

L122
Regulation of Prostaglandin D2 Production by Phosphorylation and Ubiquitination of Hematopoietic Prostaglandin D Synthase in Human Megakaryocytes
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Hematopoietic prostaglandin D synthase (H-PGDS) is responsible for the biosynthesis of PGD2 in megakaryocytes. Here, we investigated the regulation mechanism of PGD2 production in human megakaryocytic MEG-01 cells after stimulation with A23187. H-PGDS was rapidly translocated by stimulation with A23187 to the membrane of endoplasmic reticulum (ER), where its upstream enzyme, cyclooxygenase (COX), was also located. H-PGDS was bound to caveolin-1 and phosphorylated by protein kinase C (PKC). Production of PGD2 and phosphorylation of H-PGDS reached a maximum level at 2 min after treatment with A23187, gradually decreased, and terminated after 4 min. The phosphorylation site in human H-PGDS was identified as Thr176 in the carboxyl terminus. Substitution of Thr176 to Ala residue of H-PGDS abolished the abilities for phosphorylation and translocation to ER. Thus, phosphorylation at Thr176 residue of H-PGDS may be required for efficient PGD2 production. In addition, COX-1 and H-PGDS were rapidly degraded through the ubiquitin-proteasome system. A23187-triggered ubiquitination of H-PGDS was cleared by the co-treatment with bisindolylmaleimide I, PKC inhibitor. These results indicate that PGD2 production mediated by H-PGDS is regulated through several sequential steps, i) translocation of H-PGDS to the membrane of ER by increasing intracellular Ca2+ level, ii) binding of H-PGDS with caveolin-1, iii) phosphorylation of H-PGDS by PKC, iv) functional coupling of COX-1 and H-PGDS to achieve efficient production of PGD2, and v) rapid degradation of COX-1 and H-PGDS through the ubiquitin-proteasome system.

L123
Identification of a Novel Lymphoid-specific Component of the BIP:GRP94 ER Chaperone Complex
A subset of mammalian ER chaperones is organized into a large complex, of which BiP and GRP94 are the major components. This complex is found in all cells we examined and binds to unfolded substrates as a preformed complex instead of assembling on them. When the chaperone complex was examined in plasma cells, we identified a novel ~23 kD protein, termed Erp23, that is only expressed in lymphocytes. Erp23 possesses a single CXXC motif, which is a hallmark of thioreductases. PDI, a well-characterized thioreductase that enhances oxidative folding of antibodies, was previously shown to be a component of the BiP/GRP94 complex in plasmacytomas. However, given the vast quantities of antibodies produced and the large number of disulfide bonds required in their synthesis, it is possible that plasma cells have unusually high demands for thioreductases. In support of this, the expression of Erp23 is dramatically up-regulated during B to plasma cell differentiation. Interestingly, its enzymatic activity, molecular form, and interaction with the BiP-Ig HC complex are all uniquely redox-regulated. Reduction of the ER leads to multimerization of Erp23 and greatly increases its presence in the chaperone complex. Erp23 dimers are stable after reoxidation and are involved in mixed disulfides with aggregated Ig heavy chains, which resolve into properly assembled heavy chains over time. Recombinant Erp23 protein enhances oxidative folding of Fab molecules in a CXXC-dependent, redox-regulated manner. Together these data demonstrate that Erp23 is a uniquely activated thioreductase with isomerase activity. Since the interaction of Erp23 with the BiP complex increased as production of IgM increases during plasma cell differentiation, Erp23 is likely to play an important role in oxidative folding and quality control of immunoglobulins.

L124 Herp and Derlin 1 Are Involved in the ERAD Pathway for Non-glycosylated Substrates
Y. Okuda-Shimizu, L. M. Hendershot; Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN

The biosynthesis of proteins in the endoplasmic reticulum (ER) is tightly monitored by a mechanism termed ER quality control to assure that only properly folded and assembled proteins reach their final destination. Those proteins that fail to mature properly are identified and retrotranslocated for degradation by the 26S proteasome via a process known as ER associated degradation (ERAD). Recent studies have revealed novel aspects about the mechanism and cellular compartment that play a role in disposing of unfolded glycoproteins that use the calnexin/calreticulin pathway. However, much less is understood about the ERAD pathway for identifying and targeting non-glycosylated proteins, which are the substrates of BiP, for degradation. To investigate this pathway, we used the non-secreted NS1 xLC, which is a non-glycosylated BiP substrate that was previously shown to be degraded by 26S proteasome. The NS1 xLC is composed of two Ig domains and exists in both a partially (ox1) and completely oxidized (ox2) form, although neither is secreted. We found that the ox1, but not the ox2, form is ubiquitinated and is associated with both Herp and Derlin1 in these cells. Herp is turned in a complex with ubiquitinated proteins and with the 26S proteasome, suggesting that it plays a role in linking substrates with the proteasome. Furthermore, our data suggest that the ox2 form is targeted to the ER in order to be degraded. Moreover, we found that over-expressed Herp protein interacted with two other BiP substrates (i.e., λLC and unassembled Ig HC), but not with a non-secreted α1-antitrypsin variant that is a substrate of the calnexin/calreticulin system and has been shown to interact with Derlin 2 and 3. This raises the possibility that there is some distinction in the pathways used to dispose of these two different types of ERAD substrates.

L125 What Keeps Reticulons in the Peripheral ER?
C. Voss, W. Prinz; NIH, NIDDK, Bethesda, MD

The endoplasmic reticulum (ER) in yeast has two domains, one perinuclear and the other in the cell cortex. Each has distinct structures. Cortical ER is largely a dynamic network of tubules. Recently, it has been shown that a highly conserved family of proteins, the reticulons, is required for maintaining tubules in the ER of both yeast and higher eukaryotes. We are interested in understanding how these proteins shape tubules and function in the cell. Reticulons are integral membrane proteins and are located primarily in the cortical ER, despite the fact that the ER is composed of a single continuous membrane. To better understand how and why reticulons are enriched in the peripheral ER, we screened for mutations in Rtn1p that cause it to be equally distributed between the two ER domains. Using an Rtn1-GFP fusion, we used fluorescence microscopy to identify two mutants; one has five mutations and the other has three. We have begun to use site-directed mutagenesis to find out which of these mutations causes the altered distribution of the Rtn1-GFP fusions. Interestingly, both of the mutant fusions are still functional, that is, they complement an Rtn1 deletion strain. We have also ruled out that the mutants are mislocalized because they are degraded. Currently, we are working to determine if these mutations affect the ability of Rtn1p to interact with itself or other proteins.

L126 Secretory Alkaline Phosphatase Induces Endoplasmic Reticulum Stress
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Endoplasmic reticulum (ER) stress is a typical cellular response when cells undergo severe physiological conditions, especially of unfolded protein accumulation in the lumen of ER. In this study, alkaline phosphatase (AP), a cytosolic protein, was overexpressed via the ER-dependent secretory pathway with signal peptide attached to the amino terminal. Under this expression conditions, AP induces ER stress in 293T cell. Various ER stress markers increased concomitant with AP expression in ER. Levels of Bip and ER 60 increase at least three times. When subjected to chemical ER stress induced by DTT or tunicamycin, cells expressing secretory AP exhibit significant ER stress syndromes as evidenced by the enhanced expression of Bip and ER60. Secretion of genetically modified protein during ER stress may constitute a negative signal to and metabolism and induce a systemic response. However, no change was apparent on the level of translocon machinary. Functionality of AP was assay on the three fractions of secretion, microsomes and cytosol. Enzymatic activity was very low in the microsomic fraction. Secreted fraction did not show any different from that of cytosolic portion. In summary, these result strongly imply that overexpression of genetically engineered protein may involve a significant stress to ER and to cell physiology in general. To ensure a continuous success of genetic engineering, further in depth study is necessary on ER stress induced by genetically modified proteins. *This study was supported by the 2006 BioGreen21 Grant from RDA Korea

PARASITOLOGY

L127 Role of Host Cytoskeleton and Ionic Homeostasis in Toxoplasma gondii Egress
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Some of the devastating effects of an infection by the obligate intracellular parasite Toxoplasma gondii are directly and indirectly caused by the parasites exiting their host cells. This process, known as egress, can be initiated by inducing ionic fluxes in both the host cell and the parasite. For instance, it is known that loss of potassium as a consequence of artificial permeabilization of the host cell membrane induces the parasite to exit. This is corroborated by our results showing that the potassium ionophore nigericin induces parasite exit, indicating that potassium efflux is sufficient to cause egress. Nevertheless, the parasite genes involved in detecting and responding to the ionic changes are not known. Accordingly, we have isolated several mutants with altered sensitivity to potassium fluxes. We have characterized one mutant in particular that can activate its motility machinery in concentrations of potassium that normally paralyze the parasite. This mutant has an insertion in a membrane protein, which function we are currently studying. To examine whether potassium efflux is not only sufficient but also necessary for egress we studied the effect of various inhibitors and ionic compositions on natural egress. Our results showed that potassium efflux from the cell is not necessary for egress. Nevertheless, our experiments suggest that the host cytoskeleton plays a role in egress. Parasites can invade and replicate within parasites treated with the irreversible actin inhibitor mycologide B or the
irreversible tubulin inhibitor vinblastine but cannot exit those cells. We are currently investigating the role of the host cytoskeleton in Toxoplasma egress as well as identifying other components of the host cell cytoskeleton necessary for this process. In conclusion potassium efflux from the host cell is sufficient but not necessary for the intracellular parasite Toxoplasma to exit. Moreover, an intact host cell cytoskeleton is required for normal egress.

L.128
Proteomic Analysis of Phagosomes from the Parasite Entamoeba histolytica Identified Tmk96, a Novel Membrane Protein with a Role in Erythropagocytosis

D. R. Boettner,1 C. D. Huston,2 A. S. Linford,1 W. A. Petri3; 1Microbiology, Immunology, & Infectious Diseases, University of Virginia, Charlottesville, VA, 2Microbiology and Molecular Genetics, University of Vermont, Burlington, VT, 3Internal Medicine, Division of Infectious Diseases, University of Virginia, Charlottesville, VA Entamoeba histolytica is the causative agent of amoebiasis and is annually responsible for approximately 100,000 deaths, worldwide. Our previous work demonstrated that phagocytosis occurs following contact-dependent host cell killing and is dependent on the exposed phosphatidylinerine (PS) in a manner similar to metazoan apoptotic cell ingestion. Our goals for this study were to identify possible participants in phagocytosis and elucidate their function(s) in this process. A proteome of phagocytosed was assembled by incubating carboxylated, magnetic beads with amoeba for different time intervals. Following ingestion of the beads, amoebae were disrupted and intact phagosomes were isolated by a magnet. These phagosome preparations were depleted and sequenced by liquid chromatography-mass spectrometry. The resultant data was sorted for membrane proteins which appeared early in the process. Tmk96, a putative transmembrane kinase was found in early (5 minutes), but disappeared later at 15 and 30 minutes. We utilized anti-TM96 serum to illustrate that Tmk96 was both associated with the membrane of the amoeba and co-localized to fluorescent erythrocytes on the surface of trophozoites. Pre-incubation of amoeba with this antibody inhibited amoebic ingestion of carboxylated beads (36% ± 8% vs. pre-immune serum, p<0.01). In addition, both the umbilical and the expression of a carboxy-truncation of Tmk96 (TMK96(1621)) in amoeba also significantly significantly prevented the engulfment of PS-exposing human erythrocytes. This work implicates Tmk96 in erythropagocytosis by this parasite, thus opening the door to our future work elucidating the mechanism of Tmk96 activation.

L.129
Evidence for a Role of the Plasmodium berghei Aquaglyceroporin (PbAQP) in the Intraerythrocytic Development of the Malaria Parasite

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The malaria parasite can utilize host plasma glycerol for lipid biosynthesis and membrane biogenesis during the asexual intraerythrocytic development. The molecular basis for glycerol uptake into the parasite is undefined. We hypothesize that the Plasmodium aquaglyceroporin provides the pathway for glycerol uptake into the malaria parasite. To test this hypothesis, we identified the orthologue of aquaglyceroporins in Plasmodium berghei (PbAQP), and examined the role of PbAQP by performing targeted deletion of the PbAQP gene. PbAQP and PAQP are 62% identical. In contrast to the canonical NPA (Asn-Pro-Ala) motif in most aquaporins, the PbAQP has NLA (Asn-Leu-Ala) and NPS (Asn-Leu-Ser) in those positions. PbAQP expressed in Xenopus oocytes was permeable to water, glycerol and urea suggesting that PbAQP is an aquaglyceroporin. In P. berghei, PbAQP was localized to the parasite plasma membrane. To further investigate the biological role of PbAQP we generated PbAQP-null parasites by deleting the PbAQP gene using a targeted disruption approach. The PbAQP-null parasites were viable, however, they were highly deficient in glycerol transport. In addition, they proliferated more slowly compared to the wild-type parasites and mice infected with PbAQP-null parasites survived longer (10 days vs. 16 days). Taken together, these findings suggest that PbAQP, which is present at the plasma membrane of P. berghei, provides the pathway for the entry of glycerol into the organism and contributes to the growth of the parasite during the asexual intraerythrocytic stages of infection. In conclusion, we demonstrate here that the P. berghei aquaglyceroporin plays an important role in the blood stage development of the rodent malaria parasite during infection in mice, and could be added to the list of targets for the design of anti-malarial drugs.

L.130
A Dynamin-related Mechanosenzyme in the Primitive Eukaryote Giardia lamblia Is Essential for Endocytic and Exocytic Transport

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Dynamins are a family of conserved large GTPases which function as mechanosenzymes in scission processes and membrane tubulation. The primitive unicellular parasite Giardia lamblia has a single dynamin-related protein (GiDRP) with an unusual domain structure. Giardia lacks a conventional Golgi apparatus but generates Golgi-like delay compartments dubbed encystation-specific vesicles (ESVs) transiently during differentiation of the proliferating trophozoite to an environmentally resistant infectious cyst form. The stage-regulated ESVs selectively accumulate and mature newly synthesized extracellular matrix proteins before regulated secretion to the cell surface. Because giardial DRP re-localizes from the endosomal system to emerging and matured ESVs during encystation, we hypothesized that GiDRP functions in both endocytosis and regulated secretion. Here we show that conditional expression of a dominant-negative GiDRP variant indeed produces a severe encystation phenotype. Transgenic cells are still able to form ESVs but encystation is completely blocked at a late stage prior to regulated exocytosis of the cyst wall material. Subsequently, instead of ~15 ESVs in normal Giardia lamblia, or two very large compartments are detected in transgenic cells. In trophozoites, endogenous GiDRP localizes with clathrin at endocytic organelles in the cell periphery. Functional analysis shows that GiDRP is essential for receptor-mediated endocytosis, but not for bulk uptake of fluid phase markers into to endosomal-lysosomal organelles. Electron microscopy and live cell imaging also demonstrate gross morphological changes and functional impairment of the endocytic system in cells expressing the dominant-negative GiDRP. This demonstrates that giardial DRP plays a key role in membrane scission and tubulation function in endocytic and exocytic protein transport. The observation that interference with GiDRP function leads to grossly enlarged membrane compartments in Giardia suggests an additional essential function in organelle homeostasis.

L.131
Bioynthesis and Maturation of Golgi-like Vesicles in the Primitive Protozoan Giardia lamblia

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Giardia lamblia (syn. G. intestinalis, G. duodenalis) has no conventional Golgi apparatus, but generates encystation specific vesicles (ESVs) de novo, export cyst wall proteins (CWP). Here we show evidence supporting the notion that ESVs are minimal compartments of a regulated secretory pathway with none of the morphological characteristics of Golgi cisternae, but they nevertheless show certain characteristics of Golgi organelles, e.g. sensitivity to brefeldin A and association with peripheral Golgi-markers. Since ESVs contain only pre-sorted material destined for the cyst wall they are presumably dedicated delay compartments for post-translational modification and ER export of cyst wall proteins. The CWP are specialized compartments of a regulated secretory pathway with none of the morphological characteristics of Golgi cisternae, but they nevertheless show certain characteristics of Golgi organelles, e.g. sensitivity to brefeldin A and association with peripheral Golgi-markers. Since ESVs contain only pre-sorted material destined for the cyst wall they are presumably dedicated delay compartments for post-translational modification and ER export of cyst wall proteins. The CWP are specialized compartments of a regulated secretory pathway with none of the morphological characteristics of Golgi cisternae, but they nevertheless show certain characteristics of Golgi organelles, e.g. sensitivity to brefeldin A and association with peripheral Golgi-markers. Since ESVs contain only pre-sorted material destined for the cyst wall they are presumably dedicated delay compartments for post-translational modification and ER export of cyst wall proteins. The CWP are specialized compartments of a regulated secretory pathway with none of the morphological characteristics of Golgi cisternae, but they nevertheless show certain characteristics of Golgi organelles, e.g. sensitivity to brefeldin A and association with peripheral Golgi-markers. Since ESVs contain only pre-sorted material destined for the cyst wall they are presumably dedicated delay compartments for post-translational modification and ER export of cyst wall proteins.
protein of Giardia are all essential for discrete steps in this process. The current data can be interpreted as a comprehensible sequence of events that indicates the presence of both conserved mechanisms for Golgi assembly and distinct Giardia-specific features consistent with a rudimentary form of this organelle.

L132

Bt Crystal Toxin Cry5B Mutants Hypertoxic to C. elegans

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Crystal (Cry) proteins made by Bacillus thuringiensis (Bt) are the most widely used biological pesticides in the world. Cry5B is an important Cry toxin that has been recently shown to intoxicate the nematode Caenorhabditis elegans (C. elegans) and the human hookworm Ancylostoma ceylanicum. What has been lacking is a systematically large-scale study of Cry toxins to dissect how various amino acids can result to the function. The ultimate goal of this study is to uncover functions of a Bt Cry5B toxin at the amino acid level using C. elegans as a host. We have carried out random mutagenesis in Cry5B and performed a C. elegans bioassay to screen for hypertoxic Cry5B variants. Hypertoxic variants that adversely affect worm health more than wild type Cry5B were identified to further study. The variants were validated by a secondary plate assay protocol. The protein expression was analyzed by SDS-PAGE to exclude over-expressed variants. One of the candidates, H1P03H05, has been mutation-corrected, and the result has confirmed its enhanced toxicity. Then we have sub-cloned H1P03H05 variant into a Bt expression vector to express the hypertoxic variant at high levels for purification. We have conducted a pilot study by LC50 assay with purified wild type Cry5B and H1P03H05 Cry5B at 20°C and 25°C. We have found significant toxicity differences between the wild type Cry5B and H1P03H05 Cry5B variant. Characterization of the Cry5B variants and the amino acids that enhance their toxicity will have implications for the elucidation of mechanisms of Cry toxin action and also improve the therapeutic potential of Cry5B against human parasitic nematodes.

CANCER

L133

Regulation of Breast Cancer Invadopodia Formation and Function: The Role of the Extracellular Matrix

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Invadopodia are actin-rich protrusions that serve as focal points for extracellular matrix (ECM) degradation. The formation and function of invadopodia are dependent on the signaling of src kinase, Arp2/3-dependent actin polymerization, and the proper localization and function of matrix degrading enzymes. How cell adhesion to the ECM contributes to invadopodia dynamics is not well understood; moreover, it is not known how the physical properties of the 3D matrix, specifically the rigidity of the ECM, regulate invadopodia function. We have tested the role of the ECM in breast cancer invadopodia formation by varying 3 different properties of the standard ECM substrate degradation assay: 1) the concentration of the surface coated ECM molecules (fibronectin, Laminin 5 and Matrigel on top of crosslinked 3-D gelatin); 2) the density of the 3-D gelatin matrix; 3) and the pore size of the 3-D gelatin matrix, through alterations in chemical crosslinking. We find that increasing the surface concentration of fibronectin (FN) significantly decreases the invadopodia mediated degradation of the 3D gelatin, while Matrigel and Laminin 5 have less of an effect on degradation. Conversely, increasing the density (concentration) of the 3-D gelatin matrix increased the degradation area of cells in experiments both with and without surface FN. Decreasing the pore size of the 3-D gelatin matrix by varying the percentage of crosslinking decreases the invadopodia mediated degradation. These data support a model in which the chemical and physical properties of the ECM play important roles in modulating invadopodia function.

L134

IQGAP1 Modulates Activation of B-Raf

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IQGAP1 modulates several cellular functions, including cell-cell adhesion, transcription, cytoskeletal architecture, and selected signaling pathways (Trends Cell Biol 2006; 16:242). We previously documented that IQGAP1 binds directly to both extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) kinase (MEK), and regulates their activation by epidermal growth factor (EGF) (Mol Cell Biol 2005; 25:7940). Therefore, we examined B-Raf, the molecule immediately upstream of MEK in the Ras/MAPK signaling cascade. In this study we characterize the interaction between IQGAP1 and B-Raf, and explore the role of IQGAP1 in B-Raf activation. Analysis was performed in vitro with pure proteins, by co-immunoprecipitation from cell lysates, and with an in vitro kinase assay. Our data reveal that B-Raf binds directly to IQGAP1 in vitro and co-immunoprecipitates with IQGAP1 from cell lysates. Incubation of cells with EGF reduces B-Raf binding to IQGAP1 by 17%. Similarly, both calmodulin and calcium ionophores inhibit the interaction between B-Raf and IQGAP1. Importantly, IQGAP1 modulates B-Raf function in cells. EGF is unable to stimulate B-Raf activity in IQGAP1-null mouse embryo fibroblasts, while binding to IQGAP1 significantly enhances B-Raf activity. Overexpression of mutant IQGAP1 constructs that lack binding to B-Raf inhibit activation of B-Raf by EGF. Collectively our data identify a novel interaction between IQGAP1 and B-Raf and suggest that IQGAP1 functions as a regulator of B-Raf.

L135

Physiological Function of LKB1 in Drosophila

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Tumor suppressor LKB1 is a serine/threonine kinase mutated in the Peutz-Jeghers syndrome, which predisposes those affected to a wide spectrum of benign and malignant cancer. Mammalian LKB1 has been implicated as a regulator of multiple biological processes including the control of cell cycle, growth arrest, apoptosis, differentiation, and polarity. To further dissect the molecular function of LKB1, we utilized a Drosophila model system, and successfully generated the gain-of-function and null mutants of LKB1. Interestingly, the overexpression of LKB1 did not result in any alteration in cell growth and cell cycle, but induced prominent apoptosis and tissue destruction. Conversely, LKB1-null animal displayed various defects in apoptosis and epithelial morphogenesis, and these defects were completely rescued by the transcriptional LKB1 expression. Therefore, we concluded that the involvement of LKB1 in cell death- and cell polarity-controlling pathways. To reveal the downstream mechanism underlying these phenotypes, we performed a genetic screening, and found the JNK pathway to mediate the LKB1-dependent cell death. We are currently trying to identify the molecular link between LKB1 and JNK, and searching other downstream targets that mediate the polarity function of LKB1.

L136

RSK Inhibits ERK-MAP Kinase Signaling during Drosophila Development

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Although p90 ribosomal S6 kinase (RSK) is known as a critical downstream mediator of the Ras/extracellular signal-regulated kinase (ERK) pathway, its endogenous role and precise molecular function remain to be elucidated. Through the generation of both gain-of-function and null mutants of RSK, we successfully characterized its physiological role in Drosophila. Surprisingly, RSK-null flies survived into the adult stage with developmental abnormalities related to an enhanced ERK-dependent cellular differentiation such as the ectopic formation of photoreceptor- and vein-cell. Conversely, overexpression of RSK dramatically suppressed the ERK-dependent differentiation, which was further augmented by a mutation altering the Ras/ERK pathway. Consistent with these physiological phenotypes, our genetic and histological analyses clearly revealed that RSK negatively regulates ERK-mediated developmental processes and gene expressions by blocking the nuclear localization of ERK in a kinase activity-independent manner. In addition, we further demonstrated that the RSK-dependent suppression of ERK nuclear migration is mediated by the direct physical association between ERK and RSK. Collectively, our study reveals a novel regulatory mechanism of the Ras/ERK pathway by RSK, which negatively regulates ERK activity by acting as a cytoplasmic anchor in Drosophila.

L137

Syndecan-1 in Endometrial Cancers

J. Kim, D. Choi, J. Kim, C. Min; Ajou University, Suwon, Republic of Korea

Syndecan-1 is a transmembrane proteoglycan, which is highly expressed in the endometrium. It is known to have a role in cell adhesion, morphogenesis, and angiogenesis. Recently, it has been reported that Syndecan-1 is overexpressed in human endometrial cancer tissues and in breast cancer cell lines. In this study, we examined the expression of Syndecan-1 in human endometrial cancer tissues using immunohistochemical analysis. We found that Syndecan-1 expression was positively correlated with the grade of endometrial cancer. Additionally, we examined the clinical significance of Syndecan-1 expression in endometrial cancer patients. The results showed that high Syndecan-1 expression was associated with a poor prognosis, suggesting that Syndecan-1 may be a potential prognostic marker for endometrial cancer. Furthermore, we investigated the mechanism by which Syndecan-1 affects endometrial cancer cell behavior. We found that Syndecan-1 expression increased cell proliferation and invasion in endometrial cancer cells. These findings suggest that Syndecan-1 may play a crucial role in the development and progression of endometrial cancer.
Syndecan-1 is one of the four-membered transmembrane heparin sulfate proteoglycans that acts as a receptor for a wide pool of ligands, thus promotes the growth of various cancers. In this study, we investigated expression level of syndecan-1 in human endometrial cancer tissues by immunohistochemistry. Our results showed that syndecan-1 was significantly upregulated in cancer tissues compared to hyperplasia (P<0.0001). Alteration of syndecan-1 expression in human endometrial cancer 1A (HEC-1A) cells by RNA interference or overexpression with transfection revealed that overexpression of syndecan-1 promoted the cancer growth by enhancing activation of both Erk and Akt. Therefore, it is suggested that syndecan-1 is closely associated with endometrial carcinogenesis by, at least, promoting the growth of endometrial cancer cells. [supported by RTI04-03-05 from MOCIE]

L138
NF-κB Promotes Epithelial-Mesenchymal Transition and Migration in Pancreatic Carcinoma Cells
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The transcription factor NF-κB (nuclear factor κB) plays a pivotal role in a wide spectrum of biological functions and has recently been associated with cancer progression. The objective of this study was to explore the role of NF-κB in the epithelial-mesenchymal transition (EMT) and the migration of pancreatic cancer cells, which are considered important features of the malignancy of these cells. The NF-κB activity in pancreatic ductal adenocarcinoma cells was modulated by expressing a constitutively active mutant of IKK2 (CA-IKK2) or a trans-dominant mutant of IκBα (TD-IκBα), leading to a constitutive activation or repression of NF-κB, respectively. Cells expressing CA-IKK2 showed an impressive change in morphology characterized by the loss of cell-cell contacts and the development of filopodia and lamellipodia. This was accompanied by a loss of the epithelial marker protein E-cadherin and a gain of the mesenchymal marker protein vimentin, thus recapitulating two of the defining hallmarks of EMT. Cells expressing TD-IκBα showed a slightly more polarized phenotype than control cells and also showed downregulation of vimentin. The NF-κB-induced EMT phenotype was dependent on the RAS/RAF/MEK/ERK pathway, as it could be blocked by the MEK-inhibitor PD98059, but occurred independently of the p38 MAP kinase and the PI3K/AKT pathway. The zine finger transcription factor δEF-1, which has previously been implicated in EMT, was upregulated in cells expressing CA-IKK2. To assess whether the observed phenotype also had functional consequences, we analyzed the migratory properties of our cells and found that migration of the CA-IKK2-expressing cells was enhanced strongly. Our findings implicate interesting questions about the role of NF-κB in the biology of pancreatic cancer and suggest that it might be important for tumor progression due to its capacity to promote epithelial-mesenchymal transition and cell migration.

L139
CIB1 Modulates Cell Survival through Binding and Regulating PDK1
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CIB1 is a calcium-binding EF-hand protein that shares significant sequence homology with calmodulin and calcineurin B. CIB1 is widely expressed and binds to a variety of proteins including several serine/threonine kinases, such as PAK1, and the polo-like protein kinases FNK and SNK. To further understand the function of CIB1, we analyzed the CIB1 sequence (www.scsite.mit.edu) and identified a potential consensus binding motif for PDK1 (Fxx/FD/E/S[V/Y/F]) between residues 110 and 115. PDK1 phosphorylates and activates members of the AGC protein kinase family and plays an important role in regulating cell growth and survival, proliferation, and cell cycle regulation. However, co-expression of a non-myristoylated CIB1 G2A mutant with PDK1 resulted in a loss of CIB1 localization to cellular membranes and the redistribution of PDK1 to the cytosol, suggesting CIB1 regulates PDK1 localization in cells. In addition, treatment of cells with a variety of apoptotic stimuli revealed that CIB1 overexpression promoted cell proliferation while CIB1 depletion inhibited cell proliferation. Furthermore, CIB1 overexpression blocked apoptosis induced by apoptotic stimuli in control cells but not in PDK1-depleted cells. In contrast, CIB1 dependence of enhanced apoptosis in response to apoptotic stimuli, and this response was partially overcome by PDK1 overexpression. In conclusion, these results demonstrate that CIB1 is a critical regulator of cell survival, in part via a PDK1 signaling pathway.

L140
A Mutation in the Renin Receptor Gene Associated with X-Linked Mental Retardation-Epilepsy (XMRE) Leads to a Reduction in Nerve Growth Factor (NGF)-induced ERK1/2 Activation
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The renin-angiotensin system is essential for blood pressure control and water-electrolyte balance. The discovery of a mutation in the renin receptor (ATP6AP2 gene) in a family with X-linked mental retardation and epilepsy (XMRE) points to a novel role for the renin receptor in the brain. We have previously shown that the mutation in the renin receptor (ReR) resulted in an in-frame deletion of exon 4 (ReRΔ4), due to an alternate exon splice enhancer site. In order to further characterize ReR function and its potential role in neuronal cells, we cloned and expressed wild type or mutant ReR cDNA in rat PC-12, pheochromacytoma cells. No morphological differences between PC-12 cells expressing ReRwt or ReRΔ4 were evident. Nerve growth factor (NGF)-stimulated PC-12 cells expressing ReRwt exhibited about a 30% increase in ERK1/2 phosphorylation compared to mock-transfected control cells, whereas their counterparts expressing ReRΔ4 demonstrated approximately 30% lower phosphorylation levels compared to control cells. Furthermore, in NGF-induced PC-12 cells, ReRwt shifted in localization to the tips of neurites. Under the same conditions, ReRΔ4 did not appear to accumulate in any particular pattern, but was distributed throughout the entire cell body and neurites. In unstimulated PC-12 cells expressing ReRwt and ReRΔ4, it was determined by co-immunoprecipitation that ReRΔ4 could dimerize with itself or with ReRwt. When ReRwt and ReRΔ4 were co-expressed in PC-12 cells, the NGF-induced redistribution of ReRwt was no longer evident, suggesting that ReRΔ4 may have influenced the intracellular targeting of ReRwt, perhaps via dimerization with ReRwt. Together, our data suggest that the ReRΔ4 mutation associated with XMRE results in a loss of renin receptor that may impair the ability of neurons to respond to NGF via the ERK1/2 pathway. These findings support the important role of the renin receptor in cognitive function.

L141
Neuroprotective Effects of Silibinin on the Lipopolysaccharide-induced Neuro-toxicity in Dopaminergic Neuronal Cells
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Silibinin and related flavonolignan from a major part of the silybum marianum extract silymarin, which have been used to treat liver diseases. Hepatoprotective and inflammation effects have been reported by a lot of papers in recent years. We studied on the neuroprotective effect of lipopolysaccharide-induced neuro-toxicity using MSAs assay, revers transe transcription polymerase chain reaction (RT-PCR), western blot, and nitric oxide detection on mouse BV2 microglia cells and SH-SY5Y human dopaminergic neuron cells. Silibinin, dose-
dependent 

mRNA expression levels in lipopolysaccharide-induced BV2 microglia cells co-cultured with SH-SY5Y human dopaminergic neuronal cells and also sibillin significantly reduced lipopolysaccharide-induced COX-2 activation in RT-PCR and western blot. This with effects of neuroprotection related to anti-inflammation, we suggests that sibillin might be a useful candidate for the development of a drug on the related neurodegenerative diseases.

L142

Neurogenesis in Alzheimer’s Disease Mouse Model-p25 Transgenic Mice

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Cyclin-dependent kinase 5 (Cdk5) belongs to the family of cdk serine/threonine kinases. Cdk5 functions particularly in terminally differentiated neurons and is required for different cellular processes: neuronal migration, apoptosis, synaptic plasticity, etc. Cdk5 kinase activity is tightly regulated by two regulatory subunits: p35 and p39. Increased Cdk5 activity has been reported in Alzheimer’s disease with the accumulation of a truncated form of p35, p25. Our lab has generated an inducible transgenic mouse line expressing p25 in the postnatal forebrain. These animals showed severe neuronal loss in the cortex and hippocampus (Neuron, 40:471). However, if the neurogenesis is affected by p25 overexpression is not clear. Here, we test the number of adult neural stem cells in these animals using in vitro and in vivo assay. To compare the proliferation of endogenous neural progenitors in the wild-type (WT) and p25 transgenic (TG) mice, BrdU was used to label endogenous neural stem cells. When comparing the BrdU labeling index in several regions between the WT and p25 TG mice, a significant increase was observed in the SVZ, hippocampus and cortex of the p25 mice. These BrdU positive cells were negative for GFAP and gamma-H2AX. We also used an in vitro neurosphere assay to confirm some of the above in vivo findings. In the dentate gyrus (DG) and the cortex of p25 mice, there was an increase of neurosphere numbers compared to the WT mice. No significant difference was observed in the subventricular zone (SVZ) and the non-DG region. In the two-stranded labeling experiment, much fewer BrdU positive cells survived in the p25 TG mice. Most BrdU positive cells were positive for active caspase-3 displaying the condensed appearance. Collectively, our data supported that in the p25 mice, there was a hyperproliferation of neural stem cells, especially in non-neurogenic regions, but with survival problem.

L143

Neuroprotective Effect of Cirsium japonicum Lipopolysaccharide-induced Inflammation in BV2 Microglia Cells

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Cirsium japonicum may be a useful candidate for the development of a drug on the related neurodegenerative diseases.

L144

Evidence for Two Isomers of the Cellular Prion Protein with Distinct Pro- and Anti-Apoptotic Functions

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The physiological function of the cellular prion protein (PrPC) remains enigmatic as both pro- and anti-apoptotic functions have been proposed. We resolve this paradox by demonstrating that PrPC is expressed in two topological isomers with unique functional manifestations. Expression of two topological isomers was identified in mice expressing wild type PrPC, a fully secreted isoform, designated FcPrP and a small fraction as a single spanning transmembrane isoform designated cmPrP. Our data further indicate that both isoforms are equally recognized as physiologic isoforms. By introducing mutations that favor expression in either FcPrP or cmPrP the functional role of each form was dissected. Transgenic mice that favor cmPrP expression develop spontaneous neurodegeneration with evidence of apoptotic cell death in the cerebellum. Further investigation in cultured mammalian cells identified a caspase 3 dependent pathway by which cmPrP triggers apoptosis in a dose dependent manner. In contrast, FcPrP reveals a protective role against apoptosis as seen in the hippocampus of transgenic mice challenged with kainic acid. We observe that cmPrP favoring mice are most vulnerable to kainic acid mediated neurodegeneration whereas FcPrP favoring mice are the least vulnerable to the same treatment, establishing a correlation between the propensity to form FcPrP and protection from cell death. Further analysis in cultured mammalian cells confirmed a protective role of FcPrP against oxidative stress. In conclusion our study provides evidence that wild type PrPC has the intrinsic capacity to be expressed as either FcPrP or cmPrP and that each isoform can partake in independent pathways. FcPrP participates in a neuroprotective pathway against oxidative stress whereas cmPrP activates a caspase 3 dependent apoptotic pathway.

OTHER DISEASES

L145

Transcriptome Analysis of Hypoxia-induced Responses in the Human Choriocarcinoma Cell Line JEG3

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Early placental development occurs under hypoxia. Hypoxia plays a major role in trophoblast cell proliferation and uterine artery invasion to establish blood flow to the developing placenta. We hypothesize that trophoblast cell proliferation and differentiation are mediated through the transcriptional regulation of genes responsive to hypoxia. To determine differential gene expression that is consistent with this hypothesis, we used an in vitro suppression subtractive hybridization method to identify hypoxia-responsive cDNAs from cultured human placental choriocarcinoma (JEG3) cells under normoxia and hypoxia. Of the 192 cDNA clones being sequenced, 64 were unique sequences with 99 to 100% similar to sequences in GenBank. Both semi-quantitative RT-PCR and real-time PCR were used to confirm 7 hypoxia induced genes: one novel protein (FAMD83), three previously known to be hypoxia-inducible (hexokinase 2; HK2, hypoxia-inducible protein 2; HIG2, DNA-damage-inducible transcript 4; DDIT4), and three genes not previously identified as hypoxia-inducible (Adipose differentiation-related protein, leucine rich repeat and PYD containing 7; NALP7, Pim-2 oncogene) and 8 hypoxia suppressed genes: X-ray repair complementing defective repair; XRCC6 or Ku70 was known to be down-regulated during hypoxia and the rest of seven genes not reported as hypoxia down-regulated genes (a protein disulfide isomerase; PDIAG, two heat shock proteins; HSPA8 and HSPA9, two proteasomes; PSMAM and PSMA5, lactate dehydrogenase B; LDLH, adipopectin receptor 1; ADIPOR1). These results provide evidence that genes controlling metabolism, signal transduction, transport, translation and transcription are regulated under hypoxia. In additional, novel differentially expressed genes were identified to generate new insights into the involvement of hypoxia in gene regulation during early normal and abnormal placental development.
The Effect of Antifungal Drug Caspofungin on Disseminated Candidiasis: An FTIR Study
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The continuous increase in the incidence of human candidiasis is associated with a high mortality rate. In the current management of fungal infections, the azoles, e.g. conazole and iraconazole and amphotericin B/liposomal amphotericin have been used generally. Recently, caspofungin, an echinocandin anti-fungal agent, has been approved as an effective alternative treatment option to these drugs. In the present study, the effect of caspofungin on disseminated candidiasis was investigated. Drug therapy was evaluated on two therapy groups as group 1: caspofungin at 0.5 mg/kg/day (low dose) and group 2: caspofungin at 1.25 mg/kg/day (high dose). Liver samples from control, infected and therapy groups were evaluated by Fourier Transform Infrared (FTIR) Spectroscopic method which is widely used to precise determination of macromolecular content in biological systems at molecular level (Toyran et al., 2006). The results revealed that infection causes compositional changes in the tissues by decreasing the lipid content and ratio of the saturated lipids to unsaturated lipids. An increase in the lipid/protein ratio was also observed. In the low dose therapy of systemic Candida infections, there was no significant change in the lipid content. In addition the lipid/protein ratio showed lower value than infected samples. In the aspect of lipid concentration, the high dose therapy was found more effective in restoring the disease-induced changes. Moreover, in infectious disease samples lipid peroxidation was also observed. However, the both drug therapy were found to be not effective for recovering the peroxidation. These results indicate that FTIR spectroscopy is a promising technique for the evaluation and diagnosis of disseminated candidiasis. Toyran N, Lasch P, Naumann D, Turan B, Severcan F. “Early rainbow trout liver, revealed by FT-IR spectroscopy: a comparative estradiol induced compositional, structural and functional changes in starvated ileal tissue. Cakmak G, Togan I, Severcan F.”

Identification of Human Cell Factors Involved in the Vacular Biology of Listeria monocytogenes Infection
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Listeria monocytogenes is an intracellular bacterial pathogen that replicates within the cytosol of infected host cells. Rapid escape from the phagocytic vacuole to access the cytosol is essential for efficient intracellular replication. Previously, we conducted a genome-wide RNA interference (RNAi) screen to identify host factors required for intracellular infection using L. monocytogenes and Drosophila host cells. One striking result of the RNAi screen was the identification of vesicular trafficking components, including several Rab and exocyst proteins, which appeared to affect escape from the phagosome/vacuole. Since infection of Drosophila cells is similar to infection in mammalian host cells, it is likely that many homologous host factors will be conserved in their requirement for intracellular infection in humans. To confirm this premise, and to potentially identify human-specific host factors required for infection, we have developed a high-throughput screening protocol for RNAs using HEK-293 human epithelial cells and small (siRNA) designed to target lipids to the aneurysm of the brain. An increase in the lipid/protein ratio was also observed. In the low dose therapy of systemic Candida infections, there was no significant change in the lipid content. In addition the lipid/protein ratio showed lower value than infected samples. In the aspect of lipid concentration, the high dose therapy was found more effective in restoring the disease-induced changes. Moreover, in infectious disease samples lipid peroxidation was also observed. However, the both drug therapy were found to be not effective for recovering the peroxidation. These results indicate that FTIR spectroscopy is a promising technique for the evaluation and diagnosis of disseminated candidiasis. Toyran N, Lasch P, Naumann D, Turan B, Severcan F. “Early rainbow trout liver, revealed by FT-IR spectroscopy: a comparative estradiol induced compositional, structural and functional changes in starvated ileal tissue. Cakmak G, Togan I, Severcan F.”

In Situ Identification of the Staufen1/pr55Gag/Genomic RNA Complex in HIV-1-Expressing Cells
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The host protein Staufen1 interacts directly with the major HIV-1 structural protein, pr55Gag, the key regulator in genomic RNA selection for encapsidation into newly-formed virus particles. Staufen1 also found in complexes containing the genomic RNA in both cells and purified virus. Few details are known about host protein involvement in genomic RNA encapsidation but these results indicate that Staufen1 regulates a host protein system that affects the virion assembly and RNA encapsidation. In the present study, we find that Staufen1 is a critical component of the virion assembly and that it is necessary for the efficient formation of the structural protein, pr55Gag, the key regulator in genomic RNA selection for encapsidation. The results of the present study demonstrate that Staufen1 is a critical component of the virion assembly and that it is necessary for the efficient formation of the structural protein, pr55Gag, the key regulator in genomic RNA selection for encapsidation.
**Tuesday**

L150

**Particles Induced - SF20/IL25 Production by Alveolar Macrophages Is Regulated through Nuclear Factor-κB Signaling**

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Bone marrow stroma-derived growth factor (SF20/IL25) regulates lymphoid cell lineage proliferation. We hypothesized that SF20/IL25 amyloidogenic particles induced airway inflammation and that alveolar macrophages play an important role in particles-induced lung inflammation via direct induction of SF20/IL25 production. Also we evaluated signaling pathways involved in SF20/IL25 up-regulation in lung inflammation after particles treatment in the lung tissues and alveolar macrophages. We used RT-PCR and immunohistochemistry to examine the expression of SF20/IL25. Treatment of lung tissue with TiO2 resulted in a significant increase in SF20/IL25 protein as measured by immunohistochemical stain. RT-PCR also demonstrated the increase of SF20/IL25 mRNA and SF20/IL25 protein in lung tissue after TiO2 treatment, which were decreased by co-treatment with an antioxidiant, N-acetyl-L-cysteine (NAC). In vitro study, the enhanced expression of SF20/IL25 in purified alveolar macrophages 24 hours after stimulation with TiO2 particles was completely (or partially) abolished by co-treatment of NAC and inhibitors of nuclear factor-kappa B. In conclusion, the above findings indicate that particles stimulate alveolar macrophages to produce SF20/IL25 via nuclear factor-B-dependent - generation of reactive oxygen species.

L151

**Interferon-gamma Inhibits Intestinal Restitution by Preventing Gap Junction Communication between Enterocytes**

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Background and Aims: Necrotizing enterocolitis (NEC) is characterized by interferon gamma release and inadequate mucosal healing due to impaired enterocyte migration. Because adjacent enterocytes migrate together, inter-enterocyte communication may be required for mucosal healing. Many cells communicate via connexin43-containing gap junctions. We hypothesize that enterocyte migration requires inter-enterocyte communication and inhibits migration in vitro and in vivo by factor-B in dose-dependent fashion. These findings indicate that particles stimulate alveolar macrophages to produce SF20/IL25 via nuclear factor-kappa B - dependent - generation of reactive oxygen species.

L152

**Lipid A-associated Proteins from Porphyromonas gingivalis Stimulate Release of Nitric Oxide by Inducing Expression of Inducible Nitric Oxide Synthase**

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The purpose of this study was to examine the effects of lipid A-associated proteins (LAP) from Porphyromonas gingivalis, a major cause of inflammatory periodontal disease, on the production of nitric oxide (NO) and expression of inducible nitric oxide synthase (iNOS) in the murine macrophage cell line RAW264.7. We also attempted to throw light on the signaling mechanisms involved in P. gingivalis LAP-induced NO production. The LAP from P. gingivalis 381 were prepared by standard hot phenol-water extraction of endotoxin isolated by the butanol method. We found that P. gingivalis LAP can induce iNOS expression and stimulate the release of NO without additional stimuli. Exposure of P. gingivalis LAP to heat or trypsin resulted in significant, but not total loss of activity. Interestingly, polymyxin B was also capable of inhibiting P. gingivalis LAP-induced NO production from RAW264.7 cells. LPS extracted from this bacterium was significantly more potent than the LAP in stimulating release of NO. We demonstrated that multiple signaling pathways such as NF-kB, mitoerulin polymerization, protein tyrosine kinase, protein kinase C, and MAPK cascades are involved in P. gingivalis LAP-stimulated NO production. The production of NO required L-arginine. Analysis by SDS-PAGE has shown that P. gingivalis LAP comprise four very prominent bands at approximate molecular weights of 52, 47, 32, and 29 kDa together with other faint bands. It would be necessary to determine which of the components in this population of proteins is responsible for the stimulation of NO induction. The ability of P. gingivalis LAP to promote the production of NO may be important in the pathogenesis of inflammatory periodontal disease. "This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea. (03-PJ1-PG1-CH08-0001)"

L153

**Lysosome Repair Enables Host Cell Survival and Bacterial Persistence following Chlamydia trachomatis Infection**

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Chlamydiae are obligate intracellular bacteria that replicate within the confines of a membrane-bound vacuole termed the inclusion. The final event in the infectious process is the disruption of the inclusion membrane and release of a multitude of infectious elementary bodies, each capable of eliciting a new infectious process. A subset of strains of Chlamydia trachomatis are released from the host cell without causing host cell death. Chlamydial infection is associated with inflammatory events that impair host defense, and require inter-enterocyte communication, that Interferon gamma is a requisite to lysosome/plasma membrane fusion and host cell survival. A consequence of this lysosome-mediated repair process, was the retention of residual bacteria within the surviving host cell, providing a unique niche for intracellular persistence of C. trachomatis.

L154

**Temperature and Glucose Level Are Important for Hypha Development and Sap5 Expression in Candida albicans and the Sap5 Is An Important Virulent Factor in Co-culture of C. albicans and THP-1 Human Mononuclear Cells**

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The most common species that causes candidiasis is C. albicans. Secreted asparlty proteinases (Saps) from C. albicans are encoded by a multi-gene family and are considereed to be the putative virulent factors for candidiasis. In this study, we demonstrate that the serum and BSA are not indispensible for inducing SAP genes expression and hypha formation in C. albicans. By Western blotting, we identified that the Sap5 and Sap6 were highly expressed and secreted during true-hypha development when C. albicans was culturing at 37°C for 1 to 2 hr in glucose-limited media. Hence, we deduce that in vivo condition the temperature and glucose level, but not the serum, are essential
determinants to trigger hyphal development and to increase the expression of SAP genes in C. albicans. The RT-PCR analysis revealed that the expression levels of SAP1, SAP4, SAP5, SAP6, SAP9 and SAP10 were appeared noticeable increasing within 30 min in the C. albicans and human THP-1 monocytes co-cultured condition, but not in the simply serum-contained hypha induced state. Hence, the expression pattern of SAP genes defined in this co-cultured condition may be more imitated the status of disseminated infection of C. albicans in humans. Furthermore, in culture assay higher phagocytosis ratio was examined in the sap6 mutant (2.9±0.4 candida/cell) comparing with the strain SC5314 (1.7±0.2 candida/cell) and sap5 mutant (2.0±0.3 candida/cell). We also identified that the Sap2 was dominantly expressed and secreted in YPD medium at 25°C without BSA for induction. The Sap2 protein was barely detected and the Sap2 mRNA level was severely decreased in sap6 mutant culturing in YPD medium at 25°C. Therefore, the Sap6 may play functional role in Candida cells, and lacking Sap6 may impact the survival of C. albicans in phagocytosis by human THP-1 monocytes.

L155 Transcriptional Profile of Right Ventricular Tissue During Acute Pulmonary Embolism and Pulmonary Hypertension in Rats
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Acute pulmonary embolism (PE) is the third most prevalent cardiovascular disease in the US. Moderately severe PE is often associated with pulmonary hypertension (PH) in excess of 50 mm Hg with resultant right ventricular (RV) dysfunction and right heart damage. Clinical studies show that mortality after PE increases when RV dysfunction is present. We have developed a model of experimental PE in rats using intra-jugular vein injection of 25 micron polystyrene microspheres, which rapidly and irreversibly deposit in the lung vasculature, obstructing pulmonary blood flow. At sufficiently high doses, microsphere obstruction results in PH with peak right ventricular systolic pressure that can exceed 55 mm Hg. Microsphere PE/PH is associated with a profound neutrophil and monocyte-mediated inflammatory response which can be assayed by anti-inflammatory antibodies in the circulation. In this current study we have used high density DNA microarrays to assess the alterations in gene expression in hypertensive right ventricle tissue during PE/PH. PH caused 1505 genes to be up-regulated 18 hours after microsphere infusion while 1694 genes were decreased in expression (both defined as ≥2-fold change versus control samples, p<0.05, n=5 heart/group, 3 time points, 3 treatments, 9 groups total). A total of 61 genes were induced by at least 10-fold while 344 genes were induced by at least 5-fold. The magnitude of down-regulated genes did not reach 10-fold for any gene. Significantly, seven monocyte-activating CC chemokines were up-regulated as well as the neutrophil chemotaxins CINC-1 and CINC-2. Numerous genes involved in tissue remodeling and repair, including matrix contributors and tissue inhibitor of metalloproteinase-1, were also increased. Increased gelatinase activity was detected by gelatin zymography of right ventricle protein homogenates. This data identify possible important mediators of both cardiac inflammation during PH and of mediators of subsequent tissue repair.

L156 Silencing of Constitutive NF-κB and JNK Signaling by PKN1 via Phosphorylation of TRAF1
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Importance of NF-κB and JNK signaling in numerous physiological and pathological processes has been confirmed with the studies using genetically engineered mouse systems. Moreover, chronically elevated activity of these signaling has been shown to associate with cancer and chronic inflammatory disease. However, the molecular basis of how these signalings are coordinated and properly regulated has not been fully understood. Here, by using RNA interference (RNAi) technique, we demonstrate that a serine/threonine protein kinase, PKN1, is required for prevention of constitutive activity of IKK and JNK. We also identified the TRAF1 as a substrate for PKN1, whose gene disruption has revealed previously that it is required for down-regulation of both IKK/NF-κB and JNK signaling. Accordingly, phosphorylation of TRAF1 by PKN1 was required for the silencing. Furthermore, this silencing was dependent on TNFR2. Mutagenesis of phospho-acceptor residue in TRAF1 decreased the ratio of TRAF1 against TRAF2 associated with TNFR2. Moreover, this decrease was enhanced when recently identified novel (carboxyl-terminal) TRAF2-binding site, T2bs-C, was removed from TNFR2. Collectively, our results demonstrate that PKN1 silences constitutive activity of IKK/NF-κB and JNK through phosphorylation of TRAF1, which in assay stimulates the cooperation with T2bs-C, a negative modulator of TNFR2 signaling. TRAF1 phosphorylation by the abundantly expressed kinase PKN1 thereby plays a critical role in the negative regulation of tonic activity of the two central inflammatory pathways.

PRE-COLLEGE AND COLLEGE SCIENCE EDUCATION

L157 All-or-Nothing Grading Structure Enhances Student Performance on Laboratory Reports
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The communication of research findings is an integral step in the process of science; therefore, the development of scientific writing skills is an essential component of biological education. Various approaches have been recommended for teaching undergraduate students how to write laboratory reports in the format of a scientific paper. However, after nearly ten years of traditional writing instruction involving multiple assignments and extensive teacher feedback, we remained frustrated by the limited progress most of our students made in the development of their scientific writing skills. Consequently, we implemented a novel approach for assessing student writing in introductory biology. Our “all-or-nothing” grading strategy gives each student several opportunities to write a high-quality laboratory report in the format of a scientific paper. If the student succeeds on the first attempt, then the scientific writing component of the course has been completed. (However, the student must still answer “content” questions on the laboratory investigation.) If the student doesn’t write a high-quality report, then it is returned with a prioritized evaluation. The student should then use this feedback, which focuses foremost on content and organization, when preparing the next laboratory report. We have collected several years of data using this approach for writing assessment and compared it to student success rates from years prior to its implementation, as well as success rates in other introductory biology courses taught at the same time. Using this novel approach for writing assessment, we found that a significantly greater percentage of our students successfully produced a high-quality laboratory report. Furthermore, the results of a self-reporting survey on student writing attitudes indicated that the all-or-nothing strategy significantly increased student motivation. Altogether, we are enthusiastic about the potential of the all-or-nothing assessment approach combined with prioritized feedback to efficiently improve the development of scientific writing skills.

L158 Workshop Design: Computational Biology for Biologists and Mathematicians
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A week long workshop in computational biology was held at Howard University as part of the RISE program in computational biology. This workshop focused on modeling cellular dynamics and the development of collaborations between graduate students and faculty in mathematics and biology. Computational biology inherently involves aspects of mathematics, computer science and biology. The aim was to provide attendees with sufficient training to initiate their own modeling projects and pursue effective collaborations with resource providers and colleagues across disciplines. The workshop was designed after the BioQUEST professional development model that has succeeded in bringing together faculty from these distinct disciplines to develop new curricular materials. The workshop structure consisted of morning introductory lectures and discussions followed by hands-on exercises and work sessions with currently available modeling tools: Stella (R. Holmes), Excel and JDesigner (H. Sauro) and Virtual Cell (A. Cowan)
and I. Moraru). Experimental and computational models of cell cycle (J. Sible) and signaling cascades provided the topical context for working with numerical models. Materials and schedules can be found online at http://eot.bu.edu/ceb/ACCB/. Participants in work groups of two and three developed initial modeling projects that drew on their experimental and mathematical backgrounds. In a Likert-scale survey, participants agreed or strongly agreed that the workshop met their expectations and goals to 1) learn more about biology 2) learn the use of modeling tools and 3) seed collaborations. Students reported verbally and through the survey concern that insufficient time was spent addressing biological research from a mathematical and computer science perspective. Suggested workshop modifications included more group time, presentation of mathematical research and expansion into a full length course. Work supported by NSF Grant No. 0439204 and NIH HRD-0401697.
ONCOGENES AND TUMOR SUPPRESSORS

WEDNESDAY, DECEMBER 13

L1 The Role of ETS 1 and Rb Proteins in the Hibernating Thirteen-lined Ground Squirrels

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Thirteen-lined ground squirrels (Spermophilus tridecemlineatus) hibernate for 6–7 months over the winter. During hibernation growth and differentiation are minimized. ETS transcription factors control specific genes that perform roles including growth and differentiation, anti/pro apoptotic function, angiogenesis etc. The Retinoblastoma (Rb) is one of the genes controlled by ETS. Tumor suppressor gene RB and the corresponding protein pRb have an important role in cancer research. Protein pRb is usually found in a complex with E2F. Phosphorylation of Rb-E2F complex releases E2F and the epidermal growth factor (EGF) then phosphorylates pRb. Protein pRb is important for growth suppression. Hence, we hypothesized that ETS1 and pRb may play an important role as growth suppressors in thirteen-lined ground squirrels during hibernation. To assess this, we measured the levels of ETS1 and Rb protein levels using Western blotting in six different organs of euthermic versus hibernating squirrels. We also measured the amount of Epidermal growth factor receptor and phosphorylated Rb (pRb) at three potential serine phosphorylation sites: S780, S795 and S807 in euthermic and hibernating animals. During hibernation ETS1 protein levels were significantly increased in heart, kidney, liver and muscle (P< 0.05) as compared with euthermic values. Protein pRb levels were significantly high in brain and liver but largely unchanged in kidney and muscle. EGFR levels were significantly increased in Brain and Kidney and the phospho forms of pRb also increased in Brain and Kidney during hibernation. This type of preliminary research will lead to the discovery of anti-cancer drugs in the future.

L2 PTEN Downregulation by Free Fatty Acids in the Liver Induces Steatosis and Is a Potential Risk Factor for Hepatocellular Carcinoma

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The tumor suppressor PTEN is a phosphoinositide phosphatase controlling cell survival and proliferation but also insulin sensitivity in peripheral tissues. In the liver, PTEN knock out was shown to improve insulin sensitivity but was also associated with steatosis and hepatocellular carcinoma (HCC), two hepatic disorders frequently observed in diabetes and obesity. Whether insulin resistance and related disorders in vivo correlate with dysregulations of PTEN expression in the liver is unknown. Herein, we examine PTEN expression in the liver of various rat models displaying metabolic syndromes and investigate in vitro the molecular mechanisms linking dysregulation of PTEN expression in the liver and the development of steatosis and potentially of HCC. Among various rat models displaying metabolic syndromes, dysregulation of PTEN expression in the liver was present only in rats with hepatic steatosis. In vitro studies with HepG2 cells, indicated that free fatty acids (FFA) induced Pten downregulation through a mTOR/NF-kB-dependent mechanism, with the concomitant development of steatosis, Overexpression of wild type PTEN, but not of lipid phosphatase-deficient PTEN, in FFA-treated HepG2 cells could prevent the development of steatosis. Inversely, PTEN downregulation by siRNA strongly affects lipid metabolism, promotes steatosis and cellular proliferation. Together our data demonstrate that hepatic steatosis, but not diabetes nor insulin resistance, strongly correlates with PTEN downregulation in the liver of rodents, and that PTEN plays a critical role in lipid metabolism and cellular proliferation in cultured hepatocytes.

L3 Identification of the Molecular Determinants Regulating HPV E6 Recognition of its PDZ Domain-containing Targets

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The E6 proteins of high-risk mucosotropic HPV types (e.g. HPV-16 and HPV-18) interact with a large number of cellular proteins. Some targets are shared with E6 proteins from low-risk virus types, but PDZ domain-containing proteins are targeted exclusively by high-risk viruses. These include the Dlg and hScrib tumour suppressors, MAGI-1, -2 and -3, MUPP1 and PATJ. High-risk HPV E6 proteins have PDZ-binding motifs at their extreme C termini (ETQL in HPV-16 E6; ETQV in HPV-18), and we have previously used end-swap mutations to show that the identity of the last amino acid has a profound effect upon the ability of the protein to bind different targets. Most strikingly, the higher affinity of 16E6 for hScrib and 18E6 for Dlg can be completely reversed by swapping only the last amino acid residue of E6. In addition, several groups have shown that although one of the PDZ-containing targets have several PDZ domains only one of them is bound with high specificity by E6. In an attempt to elucidate the mechanism behind these observations, we have generated a series of mutants within the C-terminal region of HPV E6, and analysed their effects upon the binding and degradation of a range of PDZ targets. At the same time we have solved the crystal structures of an HPV-18 E6 peptide bound to PDZ domains from two 18E6 target proteins. Our results show that residues upstream of the core PDZ-binding motif are involved in binding, and that mutation of these residues can affect the ability of E6 to bind to or induce the degradation of various PDZ-containing targets. This implies that considerable discriminatory effects are mediated by regions of the protein outside the canonical binding motif and suggests the possibility of specific therapeutic interference with certain interactions, both in HPV disease and in other malignancies.

L4 SEI-1 Suppresses Doxorubicin-induced Senescence in MCF 7, Human Breast Cancer Cell

S. Lee, J. Jung, D. Jin, S. Yim, M. Lee; Division of Biological Sciences, Research Center for Women's Diseases, Seoul, Republic of Korea

In this study, we demonstrate novel functions of SEI-1 protein, which is an oncoprotein and cell cycle regulator. First, we found that the level of SEI-1 was decreased during doxorubicin-induced senescence, implying that SEI-1 is a regulatarily involved in senescence process. Secondly, senescent phenotype, which is morphological changes, increased senescence marker, and irreversible cell cycle arrest, induced by doxorubicin was not observed in SEI-1 expressing cells. It was previously reported that senescence is associated with ROS accumulation. This increase in ROS was unchanged by SEI-1 in our system. These data indicate that SEI-1 is involved in down-stream of ROS induced by doxorubicin. Furthermore, p38, Akt, and NFKB was found to be activated during doxorubicin -induced senescence, but not cells expressing SEI-1. Consistently, a pharmaceutical inhibitor of JNK, SP600125 was found to inhibit doxorubicin -induced senescence, but not inhibitor of p38, Akt, and NFKB in SEI-1 expressing cells, implying that p38 kinase is a down-stream target of SEI-1. Therefore, SEI-1 inhibit doxorubicin -induced senescence by preventing JNK kinase activity in MCF 7, human breast cancer cell lines, but we do not know whether this suppresses the activity of JNK kinase directly or indirectly. These results suggest that oncogenic potential of SEI-1 is due to its ability to inhibit senescence.

L5 Oncogenic Potential, SEI-1, Potentiates Resistance for Cell Death Induced by Anti-Cancer Drugs

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It was previously reported that SEI-1 was identified as a CDK4-binding protein, a transcriptional coactivator, and an oncprotein. Apoptosis inhibitory proteins, like Bel-2 family, almost have oncopgenic effect and not only facilitate cell survival and contribute to tumor development, but also render tumor cells resistant to therapy. In this study, we
Spatial Regulation of Dbl Mediated Cellular Transformation

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PTEN deficiency predisposes to a subset of human cancers but the mechanism that underlies such selectivity is unknown. Using a mouse model that conditionally deletes Pten in a tissue-restricted manner with a Cre-loxP system, we previously discovered that the mice develop carcinomas at high frequency in the prostate but at relatively low frequency in the bladder, despite early and complete penetrance of hyperplasia in both organs. Cell proliferation was initially high in the bladder of newborn Pten-deficient mice but within five days is inhibited by p21 induction. In contrast, proliferation remains elevated in Pten-deficient prostate, where p21 is never induced, suggesting that p21 induction is a compensatory bladder-specific mechanism to inhibit proliferation caused by Pten deletion. Breeding of p21-deficient mice with the Pten-deficient mice confirmed that proliferation was restored in the bladder epithelium of double deficient mice after 5 days of age, showing that p21 was at least partially responsible for inhibiting cell proliferation in the bladder. Furthermore, the AKT/mTOR growth pathway, which is highly activated in Pten-deficient prostate, was not activated in bladder epithelium. Another difference we observed between prostate and bladder tissues in response to Pten deletion was that Focal Adhesion Kinase (FAK) was induced in the prostate but not the bladder of Pten-deficient animals. Our results reveal alternative downstream signaling pathways activated by Pten deficiency that lead to tissue-specific susceptibilities to tumorigenesis.

Distinct Signaling Pathways Are Activated in Response to Pten Deficiency in Urogenital Organs

L.6

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PTEN deficiency predisposes to a subset of human cancers but the mechanism that underlies such selectivity is unknown. Using a mouse model that conditionally deletes Pten in a tissue-restricted manner with a Cre-loxP system, we previously discovered that the mice develop carcinomas at high frequency in the prostate but at relatively low frequency in the bladder, despite early and complete penetrance of hyperplasia in both organs. Cell proliferation was initially high in the bladder of newborn Pten-deficient mice but within five days is inhibited by p21 induction. In contrast, proliferation remains elevated in Pten-deficient prostate, where p21 is never induced, suggesting that p21 induction is a compensatory bladder-specific mechanism to inhibit proliferation caused by Pten deletion. Breeding of p21-deficient mice with the Pten-deficient mice confirmed that proliferation was restored in the bladder epithelium of double deficient mice after 5 days of age, showing that p21 was at least partially responsible for inhibiting cell proliferation in the bladder. Furthermore, the AKT/mTOR growth pathway, which is highly activated in Pten-deficient prostate, was not activated in bladder epithelium. Another difference we observed between prostate and bladder tissues in response to Pten deletion was that Focal Adhesion Kinase (FAK) was induced in the prostate but not the bladder of Pten-deficient animals. Our results reveal alternative downstream signaling pathways activated by Pten deficiency that lead to tissue-specific susceptibilities to tumorigenesis.

Acacetin-induced Apoptosis in MCF-7 Cells Is Mediated by the Activation of Caspase Cascade, the Decrease of Mitochondrial Membrane Potential and JNK/c-jun Activation Signaling

H. Shim, J. Park, Y. Han; Department of Advanced Technology Fusion, Konkuk University, Seoul, Republic of Korea

Acacetin, 5,7-dihydroxy-4'-methoxy flavone compound, possesses anti-proliferative, anti-cancer, anti-oxidative and anti-inflammatory activities. Although a broad range of biological and pharmacological activity of p21 53 stability was reduced in Ras-mediated senescence cell under stress conditions. Interestingly, endogenous B23/Nucleophosmin expression level was not changed, and its location was remained at nucleolus in Ras-mediated senescence cells under UV damage. In addition, the binding affinity of epithelial cells to epithelial cells of p53 protein was slightly decreased in senescence cells, whereas the interaction between MDM2 and p53 was stable. In conclusion, B23/nucleophosmin protein may not play a role as a sensor against the damages in senesced cells.

DNA Damage Induced Localization of ING1 in Promyelocytic Leukemia (PML) Nuclear Bodies

L9

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The Inhibitor of Growth (ING) family of tumor suppressors that our lab discovered in 1996 has been implicated in such cellular processes like chromatin remodeling, cell cycle regulation, apoptosis, replicative senescence and DNA repair. ING1, the founding member of the ING family, interacts with Proliferating Cell Nuclear Antigen (PCNA) and gets translocated to the nucleolus after ultraviolet (UV) light induced DNA damage. It also cooperates with p53 in inducing apoptosis after DNA damage. Here we report that ING1 localizes to Promyelocytic Leukemia nuclear bodies (PML NBs) after UV induced DNA damage. PML NBs are nuclear domains that are present in almost all mammalian cells. Their lack leads to impaired immune function, genomic instability and increased susceptibility to carcinogenesis. Recent studies have implicated that PML NBs could act as dynamic sensors of DNA damage. Many DNA damage signaling proteins have been shown to localize to PML bodies. Posttranslational modification by sumoylation has been shown to be necessary for this localization in some cases. We find that a fraction of the nuclear pool of ING1 gets localized in PML bodies ~ 3hrs after UV irradiation. A 70-80% increase in the level of ING1 proteins in colocalization with Proliferating Cell Nuclear Antigen (PCNA) and gets localized to the nucleolus after ultraviolet (UV) light induced DNA damage. We find that ING1 interacts with PML under stress conditions. Interestingly, endogenous B23/nucleophosmin expression level was not changed, and its location was remained at nucleolus in Ras-mediated senescence cells under UV damage. In addition, the binding affinity of epithelial cells to epithelial cells of p53 protein was slightly decreased in senescence cells, whereas the interaction between MDM2 and p53 was stable. In conclusion, B23/nucleophosmin protein may not play a role as a sensor against the damages in senesced cells.

APOPTOSIS

L10

Acacetin-induced Apoptosis in MCF-7 Cells Is Mediated by the Activation of Caspase Cascade, the Decrease of Mitochondrial Membrane Potential and JNK/c-jun Activation Signaling

H. Shim, J. Park, Y. Han; Department of Advanced Technology Fusion, Konkuk University, Seoul, Republic of Korea

Acacetin, 5,7-dihydroxy-4'-methoxy flavone compound, possesses anti-proliferative, anti-cancer, anti-oxidative and anti-inflammatory activities. Although a broad range of biological and pharmacological
activities of acacetin has been reported, the mechanism by which acacetin induces apoptosis is largely unclear. In this work, we investigated the molecular mechanisms of acacetin-induced apoptosis in human breast cancer MCF-7 cells. Acacetin inhibited cell proliferation and induced apoptosis in a dose-dependent manner. Acacetin-induced apoptosis was characterized with changes in DNA fragmentation and PARP (poly-ADP-ribose polymerase) cleavages. Acacetin treatment caused the activation of pro-caspase 8/9/7 and increased Bax/Bcl-2 ratio by the decrease of Bcl-2. In acacetin-treated cells, the generation of reactive oxygen species (ROS) was elevated and inhibited by the pre-treatment of N-acetylcysteine (NAC). Acacetin treatment decreased the mitochondrial membrane potential (ΔΨm). The loss of ΔΨm was correlated with the release of cytochrome c and apoptosis inducing factor (AIF) from mitochondria into cytoplasm. In addition, the phosphorylation of mitogen activated protein kinase (MAPK) c-Jun N-terminal kinase (JNK) and c-Jun, was increased in acacetin-treated cells, but not extracellular signal regulating kinase 1/2 (Erk1/2) and p38 MAPK with exception of a slight increase at early time of acacetin treatment. Activations of JNK and c-Jun by acacetin were reduced by the pre-treatment of Sp600125, a specific JNK activation inhibitor. JNK/c-Jun signaling was thought to be a major MAPK pathway contributing to the apoptotic mechanism induced by acacetin-treated MCF-7 cells. These findings suggest that apoptosis-induced apoptosis in MCF-7 cells is mediated by the activation of caspase 8/9/7, the decrease of mitochondrial membrane potential, which is induced by ROS generation and caspase 8 activation, and JNK/c-Jun activation signaling.

L10
Tip60 Inhibits p73β-induced Apoptosis by Interacting with MDM2
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The p53-related p73 protein is an important mediator of apoptosis, development and tumorigenesis. In the case of sympathetic neurons, p73β plays a critical role as an apoptotic activator by interacting with MDM2, a negative regulator of p73β. Expression of p73β is increased during the neuronal differentiation and developmental period through the expression of DeltaNp73. Here, we have asked whether Tip60 (Tat-Interacting Protein, 60kDa), which has intrinsic histone acetyl transferase (HAT) activities, regulates transcriptional activity of p73β as a co-activator or a co-repressor to modulate transcription. In this study, we demonstrate that Tip60 represses naphthoquinone analog (NA) induced apoptosis mediated by p73β. We also show that Tip60 represses p73β transcriptional activity through its association with MDM2, negative regulator of p73β. This repression is due to the enhancement of binding affinity between p73β and MDM2 by forming ternary complex with Tip60 in nucleus. Finally, we show that Tip60/MDM2/p73β complex was increased in differentiated neurons which are induced by all-trans-retinoic acid (RA) treatment in SH-SY5Y human neuroblastoma cells. From the above results, we can conclude that Tip60 represses p73β transcriptional activity expression level, followed by inhibition of p73β transcriptional activity as forming ternary complex, and also modulate p73β induced apoptosis in neuronal differentiation.

L11
Epothilone Inhibits Neointimal Hyperplasia by Inducing Arterial Smooth Muscle Cell Apoptosis via p53-dependent Caspase-3 Activation
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Epothilone (EPO), a novel synthesized MTSA, on platelet derived growth factor (PDGF)-BB-induced VSMC proliferation and on balloon-injury-induced neointimal hyperplasia in a rat model, and then subsequently investigated the induction of apoptosis and role of p53 in this process. EPO-B and EPO-D (1-100 nM) effectively inhibited PDGF-BB-induced VSMC proliferation. Treatment with these EPOs (20 µg/rat) also potently reduced balloon-injury-induced neointimal hyperplasia in the rat carotid artery, and potently induced the apoptosis of VSMCs in vitro and in vivo. EPOs also potently induced p53 nuclear accumulation, and the anti-proliferative effects of EPOs were reduced by the p53 inhibitor and by knock down with p53 siRNA. Further study, showed that the p53-regulatory apoptotic proteins Bax and caspase-3 were concomitantly activated by EPO treatment in cultured VSMCs, and treatment with p53 inhibitor attenuated the EPO-induced activation of caspase-3. Moreover, p53 and caspase-3 expressing cells number were concomitantly and significantly elevated in the neointimal hyperplastic areas of EPO-treated rat carotid arteries. Conclusions: These results suggest that the molecular mechanisms underlying the anti-proliferative and anti-neointimal hyperplasia effects of EPOs are due to the apoptotic cell deaths of hyper-proliferated VSMCs via the activation of the p53-dependent caspase-3 pathway.

L13
Receptor-mediated Apoptotic Volume Decrease Is Associated with Activation of ATM and Decondensation of Chromatin in NIH 3T3 Fibroblasts
K. Schou, L. Schneider, S. T. Christensen, E. K. Hoffmann; Institute of Molecular Biology, Copenhagen University, Copenhagen, Denmark

Apoptotic volume decrease (AVD) is an early hallmark of cells entering apoptosis. Volume decrease is driven by the release of potassium and chloride ions, which is followed by loss of cellular water, and evidence suggests that loss of ions and subsequent changes in the intracellular ionic milieu play an active role in apoptotic progression. Apoptosis is also accompanied by degradation of DNA into internucleosomal DNA fragments, but it remains a paradox how cells degrade their tightly packed chromatin as nuclease degradation in vitro occurs only at regions of decondensed chromatin. In this report we investigated how apoptotic ion loss converge upon chromatin packing/decondensation prior to apoptotic DNA degradation as well as upon activation of the DNA/chromatin surveillance protein ATM dependent DNA damage response. We also demonstrated that an intact ATM pathway is required for TRAIL-mediated apoptosis in NIH3T3 fibroblasts. Using turbidimetric assays we show that chromatin from NIH3T3 cells decondenses at ionic losses known to occur during apoptosis, and that treatment of cells with 50% hypotonic media (168 mOsm) activates ATM, indicating that ATM is sensitive to ionic and/or chromatin changes. Further, immunofluorescence microscopy analysis demonstrated that TRAIL-induced apoptosis in NIH3T3 fibroblasts is associated with decondensed chromatin as judged by phosphorlated ATM. Activation of ATM appeared not to be the result of DNA damage or nuclease degradation as judged by an alkaline comet assay. Finally immunofluorescence microscopy analysis of granular heterochromatin histone H4 modification, trimethyl histone H4 lys20, showed that hypotonicity as well as TNF-α/CHX treatment reduced aggregation of constitutive heterochromatin, indicating that chromatin is decondensed early in receptor-mediated apoptosis. In conclusion heterochromatin is decondensed and ATM is activated during TNF-α/CHX induced apoptosis, suggesting that change in chromatin could be triggered by potassium and chloride ion loss during AVD.

L14
Silibinin Sensitizes Human Glioma Cells to TRAIL-mediated Apoptosis via CHOP-dependent DR5 Upregulation and Downregulation of Survivin
Y. Son, E. Kim, S. Kim, K. Choi; Ajou University, Suwon, Republic of Korea

Silibinin, a flavonolignan from milk thistle, is a potential chemopreventive agent. Here we show that treatment with subtoxic doses of silibinin in combination with the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces rapid apoptosis in TRAIL-resistant glioma cells, but not in human astrocytes. Therefore, this combined treatment may offer an attractive strategy for safely treating gliomas. While the proteolytic processing of procaspase-3 by TRAIL was partially blocked in glioma cells, treatment with silibinin efficiently recovered TRAIL-induced activation of caspases. We found that silibinin treatment, compared with silibinin plus TRAIL, upregulated CHOP and DR5, a death receptor of TRAIL. The silibinin-mediated sensitization to TRAIL was efficiently reduced by
Regulation of Degradation of Apoptosis Signal-Regulating Kinase 1 (ASK1) by Its Novel Interaction Partner, Gα13

M. A. Kutuzov, A. V. Andreeva, T. A. Voyno-Yasenetskaya; Pharmacology, University of Illinois at Chicago, Chicago, IL

ASK1 expression levels were not required. Co-expression of ASK1 with mutationally activated G13Q226L increased the levels of ASK1 (endogenous or overexpressed in COS-7 cells) and reduced the rate of ASK1 degradation. Ubiquitinated high molecular mass forms of ASK1 were less pronounced in the presence of G13Q226L. Association of ASK1 with CHIP (carbonyx terminus of Hsp70-binding protein), a ubiquitin ligase previously shown to mediate ASK1 degradation, was reduced in the presence of G13Q226L. Prolonged treatment of human umbilical vein endothelial cells with thrombin downregulated both endogenous G13 and ASK1, but not Gβ or Hsp90. Coexpression of ASK1 and 5-HTr receptor (known to be coupled to G13) in COS-7 cells enhanced ASK1 expression. Our findings indicate that ASK1 expression levels can be regulated by G13 via control of ubiquitination and proteasome-dependent ASK1 degradation.

MITOSIS AND MEIOSIS

Nucleoporins Are Targets of the APC and Regulate Cell Cycle Progression

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Nucleoporins (Nups) mediate nucleocytoplasmic trafficking in interphase. In mitosis, upon nuclear envelope breakdown, the role and regulation of Nups remain to be elucidated. An important subcomplex of nucleoporins is the Nup107-160 complex, which, in mitosis, is involved in spindle assembly and nuclear pore reassembly. Here we show that the levels of key constituents of the Nup107-160 complex are cell cycle-regulated. We find that one of the mechanisms regulating nucleoporin levels is proteolysis via the anaphase-promoting complex (APC) during mitosis. Key constituents of the Nup107-160 complex interact with the APC, and their proteolysis is regulated by cdc20 and cdh1. Like the Nup107-160 complex, the APC is localized at kinetochores, spindle pole (centrosomes), and proximal spindles. Disruption of the levels of key constituents of the Nup107-160 complex leads to an acceleration of prophase to prometaphase transition and, most importantly, results in a delay of G1 progression. Thus, regulation of these critical nucleoporin levels in mitosis sets the stage for proper G1 progression and nucleocytoplasmic trafficking in G1.

A Transient Protein Interaction Required for Bipolar Spindle Formation

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During normal cell division a microtubule based, bipolar mitotic spindle is indispensable for precise chromosome segregation. Human Rael is a WD-repeat beta propeller protein that localizes to the nuclear pore complex in interphase cells through its interaction with the nucleoporin nup98. In mitosis Rael associates with microtubules, and plays a role in mitotic spindle polarity; cells lacking Rael arrest in mitosis with multipolar spindles. To investigate the relationship between Rael and multipolar spindles, we searched for binding partners of Rael. We immunoprecipitated Rael in a complex with the spindle component Nuclear Mitotic Apparatus protein (NuMA). Using RNAi-mediated knockdowns in cultured mammalian cells we found that NuMA down regulation can rescue a Rael multi-polar spindle phenotype and that conversely this same phenotype, when induced by NuMA overexpression is rescued by concomitant Rael overexpression. These results are consistent with a novel role for Rael in regulating NuMA availability during mitosis, and suggest a critical role for Rael in bipolar spindle formation.

Biophysical Characterization of Mitotic Aster

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Accurate chromosome segregation during cell division is essential for cell viability, and is driven by the specialized microtubule-based apparatus called the spindle. Microtubules in the spindle are arranged in a highly ordered bipolar fashion with their minus ends focused at spindle poles. Microtubule minus end focusing at poles occurs through a centrosome-independent self-organization process that relies on microtubule motors to cross-link and sort microtubules. Our lab has used a cell-free system derived from synchronized cultured cells to show that multiple motor and non-motor proteins cooperatively act to control steady-state focus of microtubule minus ends at poles. Here, we describe the biophysical properties of spindle poles formed in these extracts using optical trapping techniques. We supplement the cell-free extract with biotin-conjugated tubulin and use the optical trap to anchor 0.6 micrometer diameter neutravidin-conjugated silica beads to microtubules at the periphery of the aster array. With the position of the trap fixed, trapped beads attached to microtubule asters exhibited directed movements of 20 nm or more, experiencing loads over 1 pN. When a sustained 1- to 2-pN load was applied to the bead, larger movements were observed. In some cases, the speed of the movements was consistent with that of molecular motors present in the extract (50 to 100 nm/s). Surprisingly, these displacements were observed in both the poleward and anti-poleward direction. Thus, at steady-state, microtubules at mitotic spindle poles execute both poleward and anti-poleward force. Currently, we are combining our ability to manipulate the cell-free extract with optical trapping to determine how specific motor and non-motor proteins contribute to these steady-state behaviors.

Chrysotile Small Fibers Interfere with Cell Mitosis: A 3-D Reconstruction Study

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The serpentine chrysotile is a type of asbestos fibers assumed to be safe for human health, since it is not associated with lung fibrosis. The chrysotile clearance in human lung results in small fibers, less than 5mm, that can be harmful to cell. A previous study of our laboratory showed that the interaction of these kinds of fibers with mononuclear cells may interfere with cellular process, such as mitosis division and cytokinesis. So in the present study it was analyzed the 3-D interaction of lung cancer cells with chrysotile fibers and the effects of long term exposure on the cell proliferation, cell death and DNA ploidy. The analysis of cell/fibers interaction was performed by 3-D reconstruction of the confocal optical sections using the Imaris software (Bitplane). Cell preparations were subjected to immunolabelling with anti-gama tubulina and anti-laminin, and stained with phalloloid-FTC and prodidium iodide.After treatment, the apoptosis frequency increased
from 0.26% in controls to 4.64%. Focusing only on mitotic cells, a high frequency of cells in metaphase/anaphase with centrosome amplification was observed and most of them contain chrysotile fibers inside the cell. Fibers containing inter cellular bridges were also frequent in chrysotile treated cells. The analysis of cell/fibers interaction was performed by 3-D reconstruction of the confocal optical sections using the Imaris software (Bitplane). An interesting observation was the presence of amplified centrosomes in treated cells that can be associated to increased frequency of cell with DNA content higher than 5µm. Our results suggest that chrysotile fibers intervene in mitotic process, causing centrosome amplification and consequently resulting in changes in DNA ploidy of daughter cells throughout a multinucleation process.

**G1-S PHASE AND G2-M**

L20

**Human TopBP1 Participates in Both Cyclin E/CDK2 Activation and Preinitiation Complex Assembly during G1/S Transition**

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Although human TopBP1 with eight BRCT domains has been mainly reported to be involved in DNA damage response pathways, the requirement of TopBP1 homologues for DNA replication and S phase progression in other organisms suggests additional functions of TopBP1. Using the RNA-interference (RNAi) system, here we show that human TopBP1 is required for G1 to S progression in a normal cell cycle. TopBP1 deficiency inhibited cells from entering S phase by up-regulating two CDK inhibitors, p21 and p27, resulting in inhibition of cyclin E/CDK2. The decreased cyclin E/CDK2 activity also prevented activation of Cdc7/Dbf4 suggesting that cyclinE/CDK2 acts prior to and is required for Cdc7/Dbf4 activation. The p21 induction in response to TopBP1 depletion was transcriptionally regulated, while p27 protein was stabilized via phosphorylation on Ser10. Co-depletion of p21 and p27 with TopBP1 restored both cyclin E/CDK2 and Cdc7/Dbf4 activities, however, cells remained arrested at the G1/S boundary due to defective chromatin-loading of replication components such as DNA pol-a and PCNA. Therefore our results suggest a dual role of TopBP1 necessary for the G1/S transition: one for activating cyclin E/CDK2 kinase and the other for loading replication components onto chromatin to initiate DNA synthesis.

L21

**Genetic Interaction between Cdc24p and the Checkpoint Kinase Cds1p**

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cdc24+ is a novel gene in the fission yeast Schizosaccharomyces pombe. Cdc24p is implicated to have an important role in DNA replication and is essential for genome stability. Cds1p is a checkpoint protein required for arresting the cell cycle in the presence of DNA replication fork stalling or DNA damage during S phase. In addition, Cds1p is also responsible for recovery of S-phase arrested cells. Our preliminary result shows that cdc24Δ cds1Δ double mutants exhibit a synthetic growth defect. Specifically, cdc2Δ single mutants are viable at 32°C, but the cdc24Δ cds1Δ double mutant cannot form colonies at this temperature. This is indicative of a genetic interaction. Additionally, published results show that cdc24 truncation mutants have a novel chromosome breakage phenotype by pulsed-field gel electrophoresis analyses. The chromosome breakage phenotype is suggestive of DNA damage; therefore we hypothesize that Cds1p is activated in the cdc24 mutant background. Here we expressed Cds1p in wild type and mutant cdc24 strains, and we performed Western blot analyses to test our hypothesis. Detection of the phosphorylated form of Cds1p is observed by a mobility shift on Western blot analyses. Our preliminary results indicate that Cds1p is activated in the cdc24 mutants. The chromosome breakage phenotype is observed in cdc24 mutants as well as in other mutants in conserved replication proteins dna2 helicase and DNA ligase. Our analysis of the generation of the chromosome breakage and the cellular response to this damage will help us understand the role of these replication proteins in genome stability.

L22

**Knockdown of MCM10 Exhibits Delayed and Incomplete Chromosome Replication Followed by G2 Phase Arrest**

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MCM10 is required to form pre-initiation complex for initiation of chromosome replication in *Schizosaccharomyces pombe* and *Xenopus laevis*; however, the function of mammalian MCM10 has not been addressed. To investigate the role of MCM10 in human chromosome replication, we used small interfering RNA (siRNA) in MCM10-knockdown experiments and found that knockdown accumulated S and G2 phase cells. Chromosome replication was slowed during early and mid S phase and was aberrant during late S phase, which appeared to be caused by less efficient or defective initiations on replication origins, especially the case for late S replication.

Defective and incomplete chromosome replication, which was followed by DNA breaks and cell death, activated a checkpoint pathway composed of Chk1 and Cdc25 to inhibit Cdk1. The function of Cdk1 is necessary for the transition from G2 to mitotic phase, with Cdk1 inhibition arresting MCM10-depleted cells in G2 phase. Our results indicate that MCM10 is essential for human chromosome replication and cell cycle progression.

L23

**Long-Term Live-Cell Imaging: Green Light and G1 Progression**

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Hinchcliffe, et al. reported that centrosome removal did not prevent BSC-1 karyoplasts - cells without a centrosome - from progressing to mitosis, but subsequently to G1 arrest in most cells (2001, Science, 291:1547-1550). Recent work from our lab has shown that the centrosome per se is not needed for untransformed cells to progress through G1. However, centrosome loss is functionally a stress for the cell that can work additively with other stresses to cause a G1 arrest (Uetake, et al. 2006, in revision). Here we investigate the extent to which green (546nm) light used for long-term phase contrast imaging of karyoplasts acts as a stress that promotes a G1 arrest. Using imaging conditions identical to those used in the earlier study, 10/12 karyoplasts arrest in G1 for over 60 hours while same-preparation control cells progress through interphase with normal kinetics. When we reduce the green light intensity used to follow the cells by 45 fold, all karyoplasts proceed through mitosis and 18/21 enter a subsequent S-phase. These results indicate that green light can act as a stress that contributes to the G1 arrest previously observed for BSC1 karyoplasts. We are currently using karyoplasts to investigate whether red light (610nm) is a more benign wavelength to use for imaging cells for prolonged periods of time. Our observations point to the importance of limiting green light illumination intensity and/or duration in conducting long-term live-cell observations of live mammalian cells.

L24

**Pin1 Stabilises Emi1 during G2 Phase by Preventing Its Association with SCF beta-trep**

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The Anaphase-Promoting Complex (APC) inhibitor Emi1 is required to induce S and M phase entries by stimulating accumulation of cyclin A and cyclin B through APCDb1/cdc20 inhibition. In this report we show that Emi1 proteolysis can be induced by cyclin A/cdk. Paradoxically, Emi1 is stable during G2 phase, when the players of its degradation, cyclin A/cdk, Plx1 and SCFbtrcp, are active. Here we show that Emi1 proteolysis can be induced by cyclin A/cdk. However, Emi1 is stable during G2 phase and that this association protects Emi1 from being degraded during this phase of the cell cycle. We propose that S and M phase entries are mediated by the accumulation of cyclin A and cyclin B through a Pin1-dependent stabilisation of Emi1 during G2.
Role of Ssn2p in Ca²⁺-mediated Regulation of Cell Cycle and Polarized Growth in Saccharomyces cerevisiae
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The Ca²⁺-activated pathways of Saccharomyces cerevisiae cause a growth defect, which is accompanied by a delay in onset of mitosis and hyper-polarized bud growth through the activation of Cdc2p, a G1 cyclin, as well as Swe1p, a negative regulatory kinase that inhibits the Cdc28p-Clb complex. Calcinurin and Mpk1 coordinately activate Swel at the transcriptional and post-transcriptional level, respectively, and both pathways are essential for the cell-cycle delay. ZDS1 (also known as Oss1 and Hst1), is important in repressing the transcription of SWEL in G2 phase. In the presence of extend calcium, ZDS1 deletion strain shows a G2-phase delay and form a highly elongated bud. To clarify the mechanism for the Ca²⁺ signal-mediated regulation of G2 cell-cycle progression, we isolated and characterized the mutants (designated secz) defective in the Ca²⁺-signaling pathways. In our genetic screen for the gene that causes a defect in this pathway, an allele of Ssn2 gene was identified as a mutation that suppressed the Ca²⁺-related phenotypes of ZDS1 deletion strain. The Ssn2 gene encodes a subunit of the RNA polymerase II mediator complex. Snn2p is known to play a role as the mediator of some transcriptional factors recruited to RNA polymerase II. However, the relationship between Ssn2p and Ca²⁺ signaling is not clear. We found that the elevation of the Cln2p and Swe1p levels by exogenous CaCl₂ in Ssn2 deletion strain, compared to those of wild-type strain, was reduced. The result of this study indicated that Ssn2p is involved in the activation of Cln2p and Swe1p. We are currently investigating the role of Ssn2p in the Ca²⁺-dependent regulation of cell-cycle and morphogenesis.

Suppression of Ca²⁺-dependent Cell-Cycle Regulation by Methyl Quadrangularate O in Budding Yeast
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ZDS1 gene of Saccharomyces cerevisiae is important in repressing in G2 phase the transcription of SWEL gene, encoding a negative regulatory kinase that inhibits the Cdc28p-Clb complex. In the presence of exogenous CaCl₂, cellular Ca²⁺ level is elevated and causes the hyperactivation of cellular Ca²⁺ signaling. In the presence of external CaCl₂, ZDS1 deletion strain exhibits characteristic Ca²⁺-dependent phenotypes, such as Ca²⁺-induced growth inhibition, G2 cell-cycle arrest and formation of a highly elongated bud (1). Because Ca²⁺-activated Ca²⁺ signaling in yeast causes a defect of cell growth, the inhibitors of this pathway can be detected by the ability to suppress the deleterious effects caused by Ca²⁺, allowing cell growth in the presence of external CaCl₂. Based on this assumption, an efficient drug screening for the inhibitors of the Ca²⁺-dependent cell-cycle regulation of ZDS1 deletion strain was developed, in which active substances can be identified by the positive effect on cell growth. (2) By screening plant extracts prepared from various traditional medicinal plants, Methyl quadrangularate O (MqO) was isolated from Combretum quadrangularare (Vietnamese herb medicine) and identified as the active substance. By the analyses of the effect of MqO on various mutant strains with a defect in the Ca²⁺-dependent cell-cycle regulatory pathway, it was suggested that MqO exerts effect by inhibiting the activity of Swe1p. The toxic effect of overexpression of Swe1p by Gal1 promoter-driven SWEL gene was suppressed by MqO. By Western blot analysis of Swe1p, it was suggested that MqO downregulated the Swe1p level. The analysis of detailed mechanisms for the regulation of Swe1p by MqO is in progress. (1) Mizunuma, M. et al., Nature, 303-306 (1998) (2) Shitamukai, A. et al., Biosci. Biotechnol. Biochem., 1942-1946 (2000)

High Temperature Arrests the Cell Cycle in G2 Phase in Cultured Insect Cells
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Insect cell lines are usually cultured at 26-28°C, and the cells cannot proliferate at 37°C. The effects of high temperature on the growth of the silkworm BmN cell line were investigated. Cell proliferation was accelerated as the temperature increased from 10 to 30°C, but the growth rate decreased at 34°C, and proliferation stopped at 38°C. The cells treated at 38°C had abnormal morphology, such as bilateral cytoplasmic protrusions, and they gradually aggregated and floated in the medium. Nevertheless, the trypan blue cell viability test revealed that most of these abnormal cells were viable. These results suggest that at high temperatures the cell cycle of BmN cells stops at a particular point. The point of arrest was determined by cell cycle analysis using laser-scanning cytometry. At the optimal growth temperature of 26°C, the highest percentage of the cell population observed was in G1 phase. By contrast, after incubation at 38°C for 24 h, cells accumulated in G1 phase, and the G1 population increased as incubation was prolonged. The cell cycle of cells arrested in G1 phase recovered after incubation at 26°C. Analysis of a culture synchronized with hydroxyurea showed that BmN cells incubated at 38°C proceeded slowly from G1 to G2 and stopped before M phase. Interphase cells or cells arrested in G1 at 38°C had diffuse chromatin stained with propidium iodide in the nucleus, while cells in mitosis had highly condensed chromosomes. Treatment of G1-arrested cells with okadaic acid, which is a protein phosphatase 1 and 2A inhibitor, induced normal chromosome condensation that was observed at mitosis. These results suggest that high temperature influences the G2/M cell cycle transition involving Cdc2 kinase activity and stops cell proliferation at G2 phase in insect cells.

IQGAP1 Stimulates Actin Assembly through the N-WASP-Arp2/3 Pathway
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IQGAP1 is a conserved modular protein over-expressed in cancer and involved in organizing actin and microtubules in motile processes such as adhesion, migration and cytokinesis. A variety of proteins have been shown to interact with IQGAP1, including the small G proteins Rac1 and Cdc42, actin, calmodulin, beta-catenin, the microtubule plus-end binding proteins CLIP170 (cytoplasmic linker protein) and APC (adenomatous polyposis coli). However the molecular mechanism by which IQGAP1 controls actin dynamics in cell motility is not understood. Quantitative co-localization analysis and down regulation of IQGAP1 revealed that IQGAP1 controls the colocalization of N-WASP with the Arp2/3 complex in lamellipodia. Coimmunoprecipitation suggests an in vivo link between IQGAP1 and N-WASP. Pull down experiments and kinetic assays of branched actin polymerization with N-WASP and Arp2/3 complex demonstrated that the C-terminal half of IQGAP1 activates N-WASP by interacting with its Br/Crb2 domain in a Cdc42-like manner, while the N-terminal half of IQGAP1 antagonizes this activation by associating with IQGAP1 C-terminal region. We propose that signal-induced relief of the autoinhibited fold of IQGAP1 allows activation of N-WASP to stimulate Arp2/3 dependent actin assembly. C.L.C, D.S. and A.F. contributed equally to this work.

Actin Dynamics-dependent Fast Actin Uncapping in Dendritic Nucleation Network
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Actin forms the dendritic nucleation network and undergoes rapid polymerization-depolymerization cycles in lamellipodia. In order to elucidate the mechanism of actin disassembly, we measured molecular kinetics of major filament end-binding proteins, Arp2/3 complex (Arp2/3) and capping protein (CP). Single-molecule speckle microscopy (Science 295, 1083, 2002) yielded 0.048 s⁻¹ and 0.58 s⁻¹ for the dissociation rates of Arp2/3 and CP, respectively, in lamellipodia of XTC fibroblasts. This CP dissociation rate is three orders of magnitude faster than in vitro. CP dissociates slower from actin stress fibers than from the lamellipodial actin network, suggesting that CP dissociation...
correlates with actin filament dynamics. We found jasplakinolide, an actin depolymerization inhibitor, rapidly blocked the fast CP dissociation in cells. Consistently, coexpression of LIM-kinase prolonged CP speckle lifetime in lamellipodia. These results suggest that cofillin-mediated actin disassembly triggers CP dissociation from actin filaments. We predict that filament severing and end-to-end annealing might take place fairly frequently in the dendritic nucleation actin arrays.

**Rapid Activation of mDia1 to Restore Cellular Actin Polymers Regulated by G-actin**

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mDia1 belongs to the formin family proteins (formins) that share proline-rich formin homology 1 (FH1) and formin homology 2 (FH2) domains. Many actin-based cellular structures such as yeast actin cables, cytokinetic cleavage furrows and actin bundles in mammalian cells are dependent on formins. Recent studies characterized that FH2 or FH1-FH2 domains nucleate actin filaments and they processively remain associated to the growing barbed-end of filaments (Higashida et al., Science 303, 2007-2010, 2004). However, the precise mechanisms that regulate formin-mediated actin filament formation, both temporally and spatially within the cell, are still unknown. Here we show that cells possess an acute actin polymer restoration mechanism involving mDia1. By using single-molecule live-cell imaging, we found that several treatments which increase the concentration of G-actin induced processive movement of mDia1. Induction of processive movement occurred within 10 seconds. These data suggest that the increase in the G-actin concentration works as a cue to activate mDia1. Cells may possess an acute mDia1-mediated mechanism that restores actin polymers regulated by G-actin.

**Quantitative Analysis of S. cerevisiae Actin Patch Motility Reveals Distinct Roles for Arp2/3 Regulators in Actin Assembly and Endocytosis**

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The Arp2/3 complex is a key component required for the formation of dendritic actin networks, generating branches and seeding the formation of new filaments. The proper regulation of Arp2/3 activity is likely to be critical for efficient generation of force and movement by actin networks, evidenced in part by the presence of several Arp2/3 regulators in many cells. An outstanding question in our understanding of the dendritic nucleation model is how the regulation of Arp2/3 activity, by a variety of regulatory proteins, specifically influences the function of actin networks and how the activities of these regulators are coordinated or overlap in vivo. To further our understanding of how specific Arp2/3 regulators influence network formation and the generation of movement, we have utilized the yeast actin patch as a model system. Actin patches, which correspond to sites of endocytosis, contain a branched actin network and undergo a series of reproducible changes in protein composition and motility. Mutations in the Arp2/3 regulators present in the yeast actin patch; Las17/WASP, the type I myosins, Pan1, Abp1, and Coronin (Crm1), have been generated. In these mutants the motility of GFP-labeled patch proteins that undergo distinct behaviors and mark the main phases of patch motility, Abp1 and Sl2a, have been used to assay the effects of improper Arp2/3 regulation on actin network assembly and patch movement. The movement of these GFP-labeled proteins has been followed using high-speed confocal microscopy coupled with computer assisted particle tracking software and then analyzed quantitatively utilizing plots of mean-squared displacement (MSD) as a primary indicator of both the character and amount of motion. This sensitive technique has allowed us to determine the specific requirements of Arp2/3 regulators for the distinct phases of actin patch motility and has revealed several previously unappreciated phenotypes.

**CONVENTIONAL MYOSIN**

**L32**

Sm2 Interaction with Actin Isoforms in Contracting Rat Aorta Smooth Muscle Cells and Tissue

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We have examined the differences in association of the myosin II tail isoforms (SM1 and SM2) with the actin isoforms, α- and β-actin, in A7r5 cells and in rat aorta tissue using fluorescence resonance energy transfer (FRET). In the A7r5 cells, no significant changes in the association of the SM2 myosin isoform with each actin isoform were noted when comparing control cells to cells contracted with 10^-4M phorbol-12,13-dibutyrate (PDBu). Similarly, in tissue the association of SM2 with α-actin did not change significantly (control, 21 ± 2%, contracted, 12 ± 4%, p<0.1). However, the association of SM2 with β-actin was decreased when comparing control tissue (22 ± 4%) with contracted tissue (5 ± 5%, p<0.007). This suggests that these two actin isoforms behave differently during the contraction of aortic smooth muscle. Further studies are in progress to examine the association of SM1 with each of these actin isoforms in rat aorta tissue.

**MUSCLE: BIOCHEMISTRY AND CELL BIOLOGY**

**L33**

Phospholipase D1 and 2 Regulate Myoblast Fusion at Different Phases

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Membrane fusion plays a critical role in both the development and repair of skeletal muscle. During myogenesis, myoblasts need to form multinuclear myotubes. When muscle tissue is damaged, this fusion process reoccurs to repair and regenerate muscle fibers. Myoblast fusion takes place in two phases controlled by separate molecular pathways - the initial myoblast-myoblast fusion that forms nascent myotubes, and the subsequent myoblast fusion with pre-existing myotubes to generate myofibers with increased cell size. Using shRNA to specifically knock down endogenous genes, we demonstrate that Phospholipase D (PLD), which catalyzes the hydrolysis of phosphatidylcholine (PC) to generate the signaling lipid phosphatidic acid (PA), is essential for both phases of myoblast fusion. Knockdown of PLD1 delayed first phase fusion whereas knockdown of PLD2 blocked it, at least in part by affecting the signaling pathways that induce myogenin expression. Moreover, the PLD1-RNAi myotubes remained small in size and contained a decreased myonuclear number even after days of differentiation, indicating that PLD1 is required for second phase myoblast-myotube fusion. Cell mixing experiments revealed that nascent PLD1-RNAi myotubes were able to recruit wild-type mononucleated cells; in contrast, mononucleated PLD1-RNAi cells had a defect in their ability to fuse with wild-type nascent myotubes. Currently, we are dissecting the PLD-dependent signaling pathway that leads to myogenin expression and are investigating the mechanisms through which PLD1 regulates the second phase of myoblast fusion. The data forthcoming from these experiments will yield insights into the general mechanisms operating in myoblast fusion, which are important in designing treatments for the impaired muscle growth associated with aging, atrophy and disease. In addition, the ability to promote cell fusion could improve muscular gene therapy based on introduction of exogenous myoblasts or stem cells.

**L34**

A C-terminal Phosphorylation Site in Human Cardiac Troponin I That Controls Phosphorylation of Additional Clusters by cAMP-dependent Protein Kinase A

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Cardiac troponin I (cTnI) is a fundamental regulatory protein in cardiac muscle contraction and relaxation. CnTl, together with cardiac troponin T (cTnT) and cardiac troponin C (cTnC), form the troponin complex. The protein binds preferentially to cTnC or actin in response to the changing levels of calcium during muscle contraction. Phosphorylation of cTnI may be especially important in modulating cardiac function.
Within the cardiac isofrom there is an amino-terminal extension that includes two serine residues at positions 23 and 24 which serve as substrate for protein kinase A (PKA) when its kinase activity is stimulated in response to β-adrenergic agonists. PKA phosphorylation of cTnI results in a reduction in myofilament calcium sensitivity and an increase in cross-bridge cycling. Cardiac TnI is also a substrate for protein kinase C (PKC) at Ser43/Ser45 and Thr144. In this case, phosphorylation of cTnI results in an inhibition of the actin-myosin interaction. Mg\(^{2+}\)-ATPase activity and thin filament sliding speed. Here we report that replacement of Thr144 with aspartic acid, mimicking a constant state of phosphorylation at this residue, completely prevents phosphorylation of Ser23/Ser24 by PKA. On the other hand, the replacement Thr144Ala induced an enhanced state of phosphorylation at these serine residues by PKA. We postulate a hierarchical model of phosphorylation in which Thr144 modulates the state of phosphorylation of the amino-terminal extension of cTnI in response to its own phosphorylation state.

**TUBULIN**

**L35 Insight into the Gsalpha-Tubulin Interaction**

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Gsalpha, the stimulatory G proteins, interact with tubulin with high affinity (KD~115). Tubulin modifies Gsalpha signaling and Gsalpha regulates microtubule assembly. The purpose of this study is to identify the functional regions on Gsalpha that interact with tubulin. Tubulin was bound to a peptide array membrane containing putative interacting regions of Gsalpha as well as corresponding domains on Gsalpha (which does not bind to tubulin). Based on this, we found 14 peptides from Gsalpha that have enhanced binding to tubulin compared to the corresponding Gsalpha peptide. We have synthesized peptides from the switch II and switch III region. We found that these Gsalpha-derived peptides bind to tubulin and tubulin concentration dependent manner. Interestingly, replacing amino acid residues from Gsalpha to Gsalpha peptide lead to decrease in the binding of this peptide to tubulin suggested that these amino acid residues are important for the binding of Gsalpha to tubulin. These results provide insight into the Gsalpha-tubulin interaction. They also provide potential targets for future drug development.

**L36 Modeling the Conformational Flexibility of Tubulin’s Carboxy Terminal Tail and Its Role in Protein Interactions**

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Tubulin is a structural protein involved in the formation of cytoplasmic protofilaments known as microtubules (MT). Several isoforms of tubulin have been characterized, with sequence variation between tubulin isoforms occurring throughout the entire protein. However, the extreme C-termini display the greatest sequence divergence and physical flexibility, preventing their structural determination by crystallography. While their precise function is unknown, C-termini are not essential for MT assembly in vitro but are required for correct MT function in vivo. Using molecular modeling techniques, we have attempted to examine the structural properties of the C-termini and their specific role in binding the motor protein kinesin. Due to tubulin’s large size, it is impossible to obtain adequate sampling of the C-terminal region while performing Molecular Dynamics (MD) simulations over the entire protein. We have therefore performed Replica Exchange Molecular Dynamics (REMD) simulations on the C-termini of nine human β-tubulin isoforms and docked them onto a tubulin-kinesin structure, which did not contain tubulin’s C-terminal region. During REMD, 43 replicas, covering a temperature range from 270 K to 380 K, were simulated for 10 ns, providing an aggregate time of 430 ns per isotype. From these simulations, we produced models of the secondary and tertiary structure of the C-terminal tails and quantified other physical properties such as their length and flexibility. In particular, we have shown that βIII and βVI have the greatest flexibility and more secondary structure than previously thought, perhaps contributing to their role in MT destabilization. Furthermore, we have assessed the atomic scale interactions between tubulin and kinesin. These results offer an initial demonstration of how the structural and physical properties of tubulin’s C-terminal tails may mediate interactions with Microtubule-Associated Proteins (MAPs) such as kinesin.

**MICROTUBULE-ASSOCIATED PROTEINS**

**L37 Towards In Vitro Reconstitution of Regulated S. pombe Microtubule Dynamics: The Synergy of Mal3 and Tea2**

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Plus-end-tracking proteins (+Tips) form a complex at the plus end of microtubules (MT) and regulate microtubule dynamics. Since it is difficult to determine the precise molecular function of each protein from cellular studies we are trying to establish an in vitro reconstituted system utilising only proteins from *S. pombe*, which will permit us to compare directly in vitro dynamics with those in live *S. pombe* cells. Wild type *S. pombe* cells have one beta and two alpha tubulin isoforms. We have created an alpha 2 tubulin deletion strain and recovered highly purified single isoform tubulin from it in amounts of ~10 mg per prep. The purified tubulin contained no significant post-translational modifications. The tubulin assembles to form microtubules that have normal structure by negative stained electron microscopy. *S. pombe* tubulin MT dynamics were measured using video enhanced DIC microscopy. The value for the critical concentration of elongation was significantly lower than reported for mammalian brain tubulin (Walker et al. 1988) In *S. pombe* cells the +Tip EB1 homologue Mal3 interacts with Tea2, a kinesin-7 family member (Busch et al., 2004), and in the absence of either protein short MTs are formed (Beinhauser et al., 1997, Browning et al., 2000). Using a light scattering assay we found promotion of MT assembly at either a 1:1 molar ratio of Mal3:tubulin heterodimers or a 1:10 molar ratio of Tea2:tubulin heterodimers. When mixed, Mal3 and Tea2 act synergistically to promote MT polymerization. At concentrations of 0.5 μM Mal3 or 0.1 μM Tea2, each protein binds to MTs but produces little or no enhancement of MT assembly when added to 3 μM of tubulin heterodimers. However adding both 0.5 μM Mal3 and 0.1 μM Tea2 to 3 μM tubulin caused a substantial enhancement of MT assembly.

**L38 Dynein-activity in Anaphase B Requires a Clip170-homologue in Ustilago maydis**

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In the fungus *Ustilago maydis* mitosis occurs in the daughter cell and is accompanied the migration of the spindle pole bodies into the daughter cell, where they form the mitotic spindle. This pre-mitotic migration is mediated by dynein, which is thought to exert force on the long astral microtubules, thereby pulling the spindle pole bodies into the bud and supporting spindle elongation in anaphase B. We describe here that migration in prophase and spindle elongation in anaphase B require the presence of Clip1, a Clip170-homologue in *U. maydis*. In the absence of Clip1 pre-mitotic migration is impaired, whereas dynein localization at MT plus-ends is not affected. In anaphase, reduced amounts of dynein are off-loaded to the cortex, and spindle elongation rates are reduced to half, suggesting that dynein activity is impaired in the absence of Clip1. Indeed, we found that Clip1 is required for anchorage of the putative dynein activator Liss1, which confirms previous results in interphase cells of *A. nidulans* (Efimov et al 2006; Mol Biol Cell 17:2021). Consequently, overexpression of Liss1 in a clip1 null mutant partially rescues the pre-mitotic migration defect. Thus, in contrast to the yeast *S. cerevisiae*, Clip170-like protein has important roles in dynein activation, but not in dynein targeting or anchorage.
 Regulation of Spindle Positioning in Budding Yeast by Modification of Kar9

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During asymmetric cell division, correct orientation of the division axis relative to the polarity axis of the cell requires alignment of the spindle. Proper division in budding yeast cells, which constitutively divide asymmetrically, depends upon positioning of the mitotic spindle to the bud neck and its alignment with the mother-bud axis. Migration of the metaphase spindle towards the bud is achieved by the myosin V motor Myo2. Myo2 pulls the spindle on astral microtubules emanating from one spindle pole body (SPB) to the bud. The pulling on only one SPB prevents relocation of the entire spindle to the daughter cell. The asymmetry of Myo2 activity on one side of the spindle depends on Kar9, a protein functionally related to APC. Kar9 localizes asymmetrically to the old SPB and the emanating astral microtubules. Kar9 associates to microtubules indirectly through its interaction with the microtubule +end protein Bim1. Kar9 asymmetry is controlled through its phosphorylation by Cdc28, the main Cdk in yeast, associated to its cyclin Ccb4. How Cdk activity controls Kar9 asymmetry is not well understood. Here, we show evidence that Kar9 is modified by protein conjugation and that this modification interferes with Kar9 asymmetry. Mutants disrupting protein conjugation pathways perturb the asymmetry of Kar9 localization and change Kar9 modification profile judged by SDS-PAGE. Further, cells lacking the isopeptidase Ubp14 accumulate modified forms of Kar9. Since asymmetric localization of Kar9 to one SPB is more pronounced in ubp14 deletion mutants than in wild type cells, we propose that Kar9 localization and function is regulated by modification through protein conjugation.

CYTOSKELETAL ORGANIZATION

Regulation of Phospholipase D by Dynamin in the Integrin Signaling

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Phospholipase D (PLD) is a ubiquitous enzyme which hydrolyzes phosphatidylcholine to generate choline and phosphatidic acid. Several reports suggest that PLD induces cell movement, adhesion, and stress fiber formation which events are regulated by Rho family GTPases through the cytoskeletal rearrangement but the role of PLD in cytoskeletal rearrangement remains unknown, and relationship between PLD and Rho GTPase is also unclear. Here we verified that PLD interacts with RhoA. In addition to that, we found that PLD is involved in LPA-induced RhoA signaling. It was also confirmed that PLD is required for stress fiber formation. Together, our study reveals a new role for PLD in LPA-induced RhoA signaling pathway.

Regulation of Phospholipase D by Dynamin in the Integrin Signaling

C. Lee, J. Kim, P. Suh, S. Ryu; POSTECH, Pohang, Republic of Korea

Dynamin has been considered as a mechanochemical enzyme, which is involved in endocytosis. However, recently, there were reports which highlight dynamin's role as a regulator such as controlling of cell spreading. There are also evidences that PLD activity is important in the cell spreading. All these previous findings imply the relationship of PLD and dynamin within the integrin signaling pathway. Here, we confirmed that the dominant-negative K44A dynamin inhibited cell spreading. It was also verified the significance of PLD activity in cell spreading. Altogether, we found that dynamin is involved in the integrin induced PLD activity. This is the first to report the role of dynamin in the integrin mediated PLD signaling.

ORGANIZATION AND REGULATION OF THE EXTRACELLULAR MATRIX

Gender-specific Regulation of Vascular Gene Expression with Aging in the Monkey

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Increased vascular stiffness with aging is more prominent in males than females, but no study has investigated the genes responsible for such gender differences. We tested the hypothesis that the transcriptional adaptation to aging differs in males and females, using a monkey model (M. fascicularis) in which the transcription of the aorta was compared by microarrays between young (6.6 ± 0.5 yr old) and old (20.0 ± 0.5 yr old) males (M) or females (F) (n=6/group). The index of vascular stiffness (β), calculated from aortic pressure and dimensions, increased in old M (46±7) vs. young M (21±4) more than in old F (29±6) vs. young F (17±3; P<0.05 old M vs. young F). About 600 genes were expressed differentially between old and young animals, but less than 5% of these genes were shared between M and F. Aging M showed an upregulation of genes responsible for switching smooth muscle cells from the “contractile” to “secretory” phenotype, and of genes regulating the extracellular matrix, which relates to stiffness. In particular, aging M but not F showed a 60% decrease in collagen type 3, an antagonist of vascular stiffness, and a 3 fold increase in collagen type 8, which promotes the neo-intimal migration of smooth muscle cells, which was confirmed by qPCR and western blotting. Several genes encoded by sex-linked chromosomes showed a very significant difference between genders already in young animals. Therefore, major differences of gene regulation exist between M and F in vascular aging, including qualitative modifications of collagen isoform expression and a difference in collagen/elastin ratio, which may underlie the physiological gender differences in aging arteries. Differences in genes regulating vascular structure were found already in young animals, implying that the gender differences in vascular stiffness that develop with aging may be programmed at an early age.

Regulation of Cell Morphology and Matrix Reorganization in 3-D Culture by PDGF and Rho-kinase

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The purpose of this study was to assess the role of PDGF and Rho-kinase in modulating 3-D cell morphology and global and local cell-induced collagen matrix reorganization. Human corneal fibroblasts were plated at low (7.0 X 10^4 cells/ml) and high (3.5 X 10^5 cells/ml) densities inside 400μm thick fibrillar collagen matrices and cultured for 1 day in serum-free media (S-), and S- plus PDGF, Y-27632, or both PDGF and Y-27632. Cells were then fixed and stained with phalloidin. Fluorescent (for F-actin) and reflected light (for collagen fibrils) 3-D optical section images were acquired using laser confocal microscopy. Changes in cell morphology including cell length and height (extension of processes along z-axis) and local collagen matrix remodeling were assessed using MetaMorph. The decrease in matrix height was used to quantify global matrix remodeling (contraction). Cells cultured in S-
Wednesday

generally had a bipolar morphology and rarely formed stress fibers. Following PDGF treatment, cells were more elongated and had branching cell processes. Following low density culture with Y-27632 or both PDGF and Y-27632, cells developed a more dendritic morphology and the cell height was significantly increased as compared to cells in S-alone (p < 0.001). At high density, cells cultured in both PDGF and Y-27632 developed a bipolar morphology and cell height was decreased as compared to low density culture. Interestingly, both local and global matrix contraction in PDGF was significantly less than in low density culture (p < 0.05) but greater than S-in high density culture (p < 0.05). Overall, the data shows cell-density dependent changes in cell morphology and matrix reorganization in response to PDGF and Rho-kinase.

L45
Expression Profiling of Extracellular Matrix Genes Across the Human Growth Plate
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The growth plates of long bones are located near each end of the bone and are responsible for the majority of linear growth. The growth plate can be divided into four functional regions or zones based on the characteristics of the chondrocytes that comprise them. The zones are (beginning from the articular end and progressing in the direction of differentiation): reserve, proliferative, pre-hypertrophic, and hypertrophic. A fifth zone, the perichondrium is located in apposition to the growth plate at its circumferential surface. The progression of growth plate chondrocytes through these different regions, and hence growth, is regulated by an exquisite interplay of signaling pathways, many of which are modulated by components of the extracellular matrix. Well characterized defects in numerous extracellular matrix components lead to abnormalities in bone growth or development known collectively as osteochondrodysplasias. Currently, there are more than 200 known osteochondrodysplasias, over half of which remain to be explained at the molecular level.

Through a combination of laser microdissection, linear mRNA amplification, and Affymetrix U133 plus 2.0 Genechip analysis, our laboratory has examined the expression profile of the five zones of the long bone growth plate from two normal patients. Out of a possible 54,000 probe sets, the average number called positive by MAS 5.0 for all five arrays is 12,193 for patient 1 and 18,454 for patient 2. The homologous inter-array correlations for the log2 expression levels are ≥ 0.91±0.01. The data indicate expression of over 100 extracellular matrix genes many of which have not previously been implicated in growth plate function. Furthermore, numerous extracellular matrix genes are differentially regulated across growth plate zones indicative of zone specific function. Here we present the long bone growth plate expression profiles for the extracellular matrix components.

L46
Beta-Actin Protein Concentration Fluctuates during Contraction of Loaded and Unloaded Fibroblast Populated Collagen Gels
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Fibroblasts use a cytoplasmic actin-myosin force generating machinery to contract type-I collagen gels. Beta-actin is the major actin expressed in fibroblasts and exists as monomers or microfilaments capable of force generation. In the present study we ask if total beta-actin protein concentration changes in fibroblasts during contraction of unloaded or loaded fibroblast populated collagen gels (FPCGs). FPCGs were formed from adult rat tail tendon fibroblasts and type-I collagen in 48-well culture plates. Two glass beads weighing 19 mg were placed on top of each loaded FPCG. Harvested FPCGs were subject to SDS-PAGE and quantitative beta-actin Western blotting. Cell number was determined by DNA assay and FPCGs were photographed to track gel contraction. FPCGs contracted 80% in the first 24 hours of culture and loaded FPCGs contracted faster than unloaded FPCGs. The beta-actin concentration in fibroblasts rose and fell twice during the 96 hour culture period. The first rise in beta-actin concentration occurred during the first 24 hours of contraction where it went from 100 million molecules/cell to 240 million molecules/cell. Beta-actin protein levels rose and peaked 6 hours earlier in loaded than in unloaded FPCGs. From 24 to 72 hours beta-actin levels returned to 100 million molecules per cell. This was followed by a second rise in beta-actin levels to 300 million molecules/cell at 74 hours and fall to 125 million molecules/cell at 96 hours. In conclusion, we have shown that beta-actin content of fibroblasts in contracting FPCGs rises and falls twice during a 96 hour culture period spanning a range of 100 million to 300 million beta-actin molecules/cell. The first rise in beta-actin concentration coincides with early reduction of FPCG volume and is accelerated in loaded FPCGs. Supported by NIU and the NIU Foundation.

EXTRACELLULAR MATRIX AND CELL SIGNALING

L47
NG2 and Integrin α4 Regulate FAK Phosphorylation by Distinct Mechanisms in Apoptosis Induced by an Altered Fibronectin Matrix
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Altered fibronectin (FN) matrices induce apoptosis of primary human fibroblasts via a novel signaling pathway that is characterized by transcriptional downregulation of the tumor suppressor p53. However, the receptors that mediate this process are not known. Two classes of receptors, integrins and proteoglycans, can potentially interact with FN to regulate this apoptotic response. Focal adhesion kinase (FAK) is a major nonreceptor tyrosine kinase activated after integrin-mediated adhesion to FN. However, regulation of FAK phosphorylation through proteoglycans in apoptosis has not been extensively studied. We hypothesized that both integrins and the NG2 proteoglycan regulate FAK phosphorylation in apoptosis induced by an altered FN matrix. Also, since NG2 signals through PKCα and PKCα is known to regulate FAK phosphorylation; PKCα may be a common signaling molecule between integrins and proteoglycans. Fibroblasts were transfected with NG2, integrin α4, and PKCα cDNAs to examine the regulation of FAK phosphorylation mediated by NG2 and integrin α4. We used western blotting and flow cytometry to determine protein expression and a flow cytometry based apoptotic assay to assess the percentage of apoptotic cells. Altered FN matrices induced apoptosis in fibroblasts through upregulation of NG2 and downregulation of integrin α4. NG2 overexpression potentiated the apoptosis mediated by an altered FN matrix by decreasing the phosphorylation level of FAK Tyr (397). NG2 overexpression or treatment with an altered FN matrix also suppressed PKCα expression. However, overexpression of integrin α4 enhanced FAK phosphorylation independent of PKCα. These data suggest that NG2 and integrin alpha 4 are involved in an altered FN matrix-mediated apoptosis in fibroblasts. In addition, NG2 and integrin α4 regulate FAK phosphorylation by PKCα-dependent and PKCα-independent mechanisms (Study supported by NIH grant R01 DE013725 to YLK).

L48
RIP and FAK Regulate Altered Fibronectin Matrix-induced CD95/Fas Signaling in Squamous Cell Carcinoma
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Cell adhesion to extracellular matrix (ECM) proteins such as fibronectin generates signals important for many cellular processes, including migration, proliferation, differentiation, and survival. Previously, we showed that an altered fibronectin matrix, consisting of a mutated, nonfunctional high-affinity heparin-binding domain and the V region of fibronectin (V'H), induced anoikis in human SCC cells by modulating integrin alpha v-mediated phosphorylation of FAK and ERK. Here we report that an altered fibronectin matrix (V'H) activates a CD95/Fas signaling pathway involving FAK, RIP and caspase-8. In V'H treated cells, dissociation of RIP and FAK interaction leads to increased association of RIP with Fas, which triggers RIP cleavage and results in caspase-8-dependent anoikis/apoptosis. Overexpression of FAK in FAK null cells (FAK−/−) restores the association of RIP with FAK, inhibits the RIP cleavage, and rescues cells from V'H-induced anoikis/apoptosis. Furthermore, RIP deficient mouse embryonic fibroblasts (RIP−/−) were resistant to altered fibronectin matrix-induced anoikis. These findings provide evidence that an altered fibronectin matrix (V'H) induces CD95/Fas signaling-mediated anoikis in human SCC cells regulated by FAK, RIP and Fas interactions (Supported by NIH R01# DE014429 to YLK).
INTEGRINS

L50
Beta 1 Integrin Down-regulation Increases Radiosensitivity through Activation of JNK
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The β1 integrins are positive modulators of cell proliferation and cell survival. We tested the ability of β1 integrins to promote tumor growth and radiosensitivity to radiation. The lack of β1 expression inhibited tumor growth whether the tumor cells were embedded in Matrigel or not, suggesting that the failure to form tumors is not the result of incomplete extracellular matrix synthesis. The effect on tumor growth was not due to reduced cell adhesion or cell survival. In 3-D cultures, we find that β1 shRNA expressing prostate cancer cells formed normal glands as compared to the irregular morphology of the glands shown by mock-transfected cells. We also provide evidence that failure to express β1 increased radiosensitivity of cancer cells; apoptosis induced by radiation at doses of 5 or 10 Gy was significantly increased as evaluated by the extent of PARP cleavage, caspase activation, and DNA fragmentation. The results obtained in 2D were also reproduced in 3D cultures, where we found induction of apoptosis measured by PARP cleavage and TUNEL staining. The mechanism by which down-regulation of β1 increases radiosensitivity involves JNK, but not ERK activation. Expression of a JNK inhibitor JBD-JP1 or treatment with the JNK inhibitor SP600125 prevented apoptosis induced by irradiation in the absence of β1. JNK activation appears to be the cause rather than the result of undergoing cell death, since JNK was activated even in the presence of ZVAD, a caspase cleavage inhibitor. In conclusion, these results show that downregulation of β1 integrins promotes reversion of cancer cells to a normal phenotype and increases radiosensitivity through JNK activation (Support: from Army, PCRP DAMD PC040221; NIH, RO1 CA-89720 and RO1 CA-109874).

L51
Role of SHP-2 in Concanavalin A-Induced Invadopodium Formation
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Invadopodium is a membrane protrusion with a matrix degradation activity formed in invasive cells. We explored a novel role of SH2 domain containing protein tyrosine phosphatase (SHP)-2 in invadopodium formation induced by Concanavalin A (Con A). SHP-2 is widely expressed cytoplasmic protein tyrosine phosphatase with two SH2 domains which is implicated in a wide variety of signaling elucidated by growth factor, cytokine, hormone, extracellular matrix etc. and activates Erk, Akt and p38 signaling pathways. In cells expressing mutant SHP-2 in which 65 amino acids in the SH2-N domain were deleted, we found that Con A-induced formation of invadopodium was severely impaired in contrast to wild-type cells. To explore the molecular mechanism, we examined Con A-induced expression of membrane type-1 matrix metalloproteinase (MT1-MMP), a transmembrane protein which is responsible for the invadopodium formation. We found, Con A-induced MT1-MMP expression was severely impaired in SHP-2 mutant cells, whereas MT1-MMP expression in wild-type cells was activated by Con A in a time- and dose-dependent manner. We also observed that Con A-dependent transcription of MT1-MMP was impaired in SHP-2 mutant cells. In wild-type cells, Con A activated SHP-2, Erk and p38. In contrast, Con A-dependent activation of these signaling were absent in SHP-2 mutant cells. In addition, wild-type cells were pretreated with SB203580, a specific inhibitor for p38, significantly inhibited Con A-dependent expression of MT1-MMP but U0126, a specific inhibitor for MEK-1, could not. Finally, reintroduction of wild-type SHP-2 in SHP-2 mutant cells could rescue the activation of p38 and the expression of MT1-MMP induced by Con A. Taken together, our results strongly suggest that SHP-2 plays a critical role in MT1-MMP expression and in invadopodium formation induced by Con A.

L52
Antitumor Efficacy of DX-2400, a Potent and Selective Human Antibody MMP-14 Inhibitor Discovered Using Phage Display Technology
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Selective inhibition of the activity of matrix metalloproteinases (MMPs) provides an attractive non-cytotoxic approach for improving the therapy of aggressive metastatic cancer. Current MMP inhibitors have been approved for oncology probably for reasons of insufficient specificity and/or dose-limiting side effects. The challenge in the MMP field is to design highly potent and selective inhibitors with optimal pharmacokinetics and minimal toxicity. This provides a unique opportunity for protein-based inhibitors that can have levels of selectivity that are not possible to attain with small molecule drugs. Utilizing our human Fab-displaying phage library and using a recombinant active catalytic domain of human MMP-14 as a target, we have discovered DX-2400. DX-2400 binds and specifically inhibits MMP-14 activity (Ki=0.9±0.3nM) and does not inhibit activity of a panel of other MMPs tested. DX-2400 blocks pro-MMP2 activation on MMP-14/MMP-2 expressing cancer cells and therefore has the potential to inhibit extracellular matrix proteolysis. In addition, DX-2400 blocks in vitro invasion of select cancer cell lines through collagen type IV. We evaluated the anti-cancer activity of DX-2400 in the orthotopic MDA-MB-231 breast cancer model. DX-2400 reduced tumor progression by 70% compared to an isotype control, with activity comparable to doxorubicin. Importantly, DX-2400 resulted in no body weight loss through the treatment. This fully human antibody represents an innovative approach for the selective inhibition of MMP activity.

L53
Enhanced Membrane-Type 1 Matrix Metalloproteinase Expression by Hyaluronan Oligosaccharides in Breast Cancer Cells Facilitates CD44 Cleavage and Tumor Cell Migration

Metalloproteinases

L49
Microscopy Based Measurement of Myosin Light Chain Phosphorylation in Adherent Cells
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In anchorage dependent cells, myosin generated contractile forces on the ECM effect both events proximal to adhesion such as the formation of stress fibers, focal adhesions, and temporally distal events such as entry in to S-phase. Hence tools to measure myosin activity are of great value. Changes in myosin light chain (MLC) phosphorylation underlie changes in its activity. Western Blotting (WB) has been useful in measuring MLC phosphorylation. However, in the process of sample preparation for WB, cells are lysed and hence population distribution, location, and morphology data of individual cells is lost. There are few published microscopy-based protocols for measuring phosphorylation signaling in adherent cells. We screened several specimen fixation and permeabilization methods for microscopy based quantitative measurement of MLC phosphorylation in adherent cells. Specimen fixation with ethanol, methanol or acetone leads to cell rounding and lysis. Parafomaldehyde fixation and Triton X-100 permeabilization preserved cell morphology and MLC phosphorylation the best. We utilized the automated microscopy methods for cell morphology previously developed in our lab to automate images acquisition and MLC signal integration over individual cells. To illustrate the utility of the method, we examined MLC phosphorylation in a population of A10 rat aortic smooth muscle cells and found a reproducible non-Gaussian distribution of signal per cell. Inhibition curves for Y27632, an inhibitor of Rho kinase, inhibited MLC phosphorylation to half the maximum at about 1µM in single cells, which agrees well with the published IC50 for the inhibition of contractility. There was a linear relation between cell area and MLC phosphorylation, also in line with what has been reported in literature using other methods.

Enhanced Membrane-Type 1 Matrix Metalloproteinase Expression by Hyaluronan Oligosaccharides in Breast Cancer Cells Facilitates CD44 Cleavage and Tumor Cell Migration
C. Liu, J. Chen, C. Su, H. Wang, Anatomy, Yang-Ming University, Taipei, Taiwan, Surgery, Veterans General Hospital, Taipei, Taiwan, Hyaluronan (HA), a component of the extracellular matrix, plays an important role in cell-cell adhesion and cell migration. Membrane type 1-matrix metalloproteinase (MT1-MMP) is often expressed in invasive cancer cells and in endothelial cells during angiogenesis. CD44, a transmembrane receptor for HA, is implicated in various adhesion-dependent cellular processes including cell migration, tumor cell metastasis and invasion. Recent studies have shown that CD44 is highly expressed in cancer cells and can be proteolytically cleaved at the ectodomain by MT1-MMP; this process of inducing CD44 cleavage plays a critical role in cancer cell migration. We hypothesized that HA modulates MT1-MMP expression to facilitate breast cancer cell migration. By cDNA microarray analysis, the MT1-MMP gene expression was up-regulated after HA stimulation in MDA-MB-435s breast cancer cells. Using Western blot and immunofluorescence staining, we found that after HA stimulation, MT1-MMP expression in the membrane of breast cancer cells was increased. In response to HA stimulation, significant up-regulation of MT1-MMP mRNA was observed. Our data also provide evidence that HA enhances MT1-MMP; elevated expression of MT1-MMP enhances CD44 cleavage and cell migration. In conclusion, we have identified a new function of HA in the induction of MT1-MMP expression in MDA-MB-435s breast cancer cells and CD44 cleavage to increase cell migration during the invasion process. Based on these results we propose that the HA-induced increase in MT1-MMP expression, which, at least for breast cancer cells, may be a critical step in the formation of metastatic colonies.

L54 Soluble Expression of Tissue Inhibitor for Metalloproteinase 2 by In Vitro Mutagenesis and Gene Shuffling
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Tissue inhibitor of metalloproteinase 2 (TIMP-2) is a 21kDa protein that inhibits matrix metalloproteinase 2 (MMP-2). Expression of mammalian proteins in E. coli often results in production of insoluble proteins in the form of inclusion body. TIMP-2 folding requirement for the formation of 6 disulfide bonds is likely to be incompatible with the reducing environment of E. coli. This incompatibility, however, could be overcome by introducing mutagenesis. With this regard, we attempted to express soluble TIMP-2 in E. coli by applying the staggered extension process (StEP), one of the in vitro PCR-based recombinant mutagenesis. To facilitate the high throughput screening, the CAT gene is fused to the resulting recombinant TIMP-2 gene at the C-terminus. Selection based on chloramphenicol resistance enabled us to obtain 128 bacterial cells that showed a drastic increase in expression level of soluble, presumably correctly folded, TIMP-2. Therefore, StEP technique, combined with CAT-based screening, might be a simple and effective method for the expression of mammalian proteins in E. coli.

L55 Collagenase-1 and -3 Differentially Upregulate Markers of Osteoblastic Differentiation
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Objective: Collagen deposition and potential degradation via protease activity are considered to be important in osteoblastic differentiation. In this study we examined the role of collagenase activity and specifically defined the contribution of the two key collagenolytic proteinases of the matrix metalloproteinase (MMP) family, collagenase-1 (MMP-1) and -3 (MMP-13) in the regulation of osteoblastic differentiation of human periodontal ligament (PDL) cells. Methods: PDL cells plated at of 30,000 cells/cm² were exposed to increasing concentrations of exogenous collagenase-1 in the absence and presence of dexamethasone. Additionally, cells were transfected with MMP-1 cDNA to collagenase-1 or with siRNA to MMP-1 and/or MMP-13. After5 days of culture, the culture-conditioned medium was assayed by Western blotting and a collagen degradative assay. Cell extracts were used for measuring alkaline phosphatase (AP) activity, and RT-PCR to mRNA expression for Osx, osteocalcin (ON), osteopontin (OP), bone sialoprotein (BSP), osteocalcin (OC), MMP-1 and MMP-13 mRNA levels. Results: Increasing concentrations of exogenous collagenase or endogenous collagenase-1 caused a dose-dependent decrease in AP activity. Conversely, a dose-dependent increase in AP activity was observed with increasing concentrations of MMP-1 or MMP-13 siRNA. The knockdown of MMP-1 also resulted in significant increases in Colla1, OP, BSP, OC and ON. MMP-13 knockdown a significant decrease in ON expression and an increase in OP levels. Osx expression was significantly increased when MMP-13 was suppressed but decreased with knockdown of MMP-1. Simultaneous suppression of both MMPs resulted in a significant increase in the levels of all osteoblastic markers assayed. Collagenase activity showed negative correlations to AP, Colla1, OP, BSP and OC. Conclusion: These data show that collagenase activity regulates osteoblastic differentiation of PDL cells, and that the knockdown of MMP-1 or -13-gene activity results in a differential regulation of osteoblastic markers. (Studies supported by NIH R01 DE16671).

CELL–CELL INTERACTIONS

L56 Rap1 GTPase Activation in Endothelial Cells
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Rap1 is a small molecular weight GTPase which has been well-characterized with respect to its role in integrin mediated adhesion events, particularly involving cell-substrate attachment. However, within the last two years it has become appreciated that Rap1 is also involved in non-integrin mediated adhesive events such as cadherin-mediated cell-cell adhesion. We and others have shown that Rap1 regulates the formation of cadherin-mediated cell junctions in both epithelia and endothelia (Wittchen et al., JBC, 2005). We show that Rap1 activation occurs during endothelial cell junction assembly, and that this activity is required for mature junction formation. Furthermore, treatment of cells with a Rap1-activating compound strengthened junctions, as measured by an increase in transendothelial resistance, decrease in permeability, and inhibition of leukocyte transmigration across the endothelial cell barrier. Another known modulator of endothelial cell morphology and barrier function in vivo is fluid shear stress. We hypothesized that the well-documented barrier strengthening effects of shear stress may be related to the activity of Rap1 GTPase. Application of shear stress to endothelial cell cultures resulted in varying degrees of Rap1 activation, which correlated with enhanced monolayer integrity as observed by immunofluorescence. We can use this model to further explore the function of Rap1 for many different aspects of endothelial cell biology, such as leukocyte adhesion and transmigration.

L57 Visualization of Spatiotemporal Dynamics of Endogenous Foxp3+ Regulatory T Cells in Solid Tumors
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The inhibitory function of CD25+Foxp3+CD4+ regulatory T cells (Treg), while being important for preventing autoimmunity, is detrimental for anti-cancer immune responses. However, their interactions with tumor cells remain unclear. Here, we used mice expressing GFP under Foxp3 control elements to visualize the spatiotemporal dynamics of endogenous Tregs within solid tumors. We asked whether Treg cells form stable conjugates with tumor cells to obstruct access of other lymphocytes. GFP-Treg reporter mice were generated by inserting GFP into a bacterial artificial chromosome (BAC) encompassing the Foxp3 locus. As a tumor model, we used the MCA-205 fibrosarcoma, which, depending on the route of injection, forms lung or subcutaneous tumors in C57Bl6 mice. The GFP-Treg mice were inoculated with MCA-205 cells expressing red fluorescent protein and resulting tumor growths were imaged by 3D time-lapse multiphoton microscopy. The BAC Foxp3-GFP mice expressed very bright fluorescence that correlated with endogenous Foxp3 and was sufficient for multiphoton microscopy. GFP-Treg reporter mice were then used to study tumor-mediated infiltration of Treg cells. We found that these Treg cells preferentially accumulated within tumor nodules, sparing the surrounding tissue. The density of GFP+ cells approached 35×10³/m².
cells per mm³ of lung tumor, which represented over 10-fold increase over tumor-free lung. The spatial distribution of GFP-Tregs depended on the site of tumor growth: lung tumors had pronounced infiltration of Tregs across the tumor mass, whereas subcutaneous tumors had a lesser intra-tumor infiltration but were surrounded by a 100 um zone of highly recruited Tregs. Infiltrating GFP-Tregs were highly motile, squeezing in between tumor cells with remarkable plasticity. However, Tregs did not stop to form stable interactions with tumor cells and did not appear to physically obstruct access to tumor cells indicating that other factors are responsible for their suppressor function in situ.

L58

Regulation of Mouse Gicerin/CD146 Gene by Forskolin

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Gicerin/CD146 is a cell adhesion molecule belonging to the immunoglobulin superfamily. Gicerin consists of five immunoglobulin-like loop structures in an extracellular domain, a single transmembrane region, and a short cytoplasmic tail. Gicerin/CD146 participates in neurite extension and cell migration resulting in structural organization of nervous system. Expression of Gicerin/CD146 in the nervous system is highly observed during development and dramatically decreased after birth. To elucidate the mechanism of regulation of gicerin/CD146 expression, we cloned a 14kb mouse gicerin/CD146 gene. It contained 8kb gicerin/CD146 gene composed of 16 exons and 6kb upstream region. The gene has a TATA less promoter with several Sp1 and a Cre-binding site. Since the upstream region of gicerin gene has a cre-binding site, we tried to examine the effect of forskolin on gicerin mRNA expression. To characterize this, we examined the promoter activity by using luciferase as a reporter gene. Chimeric luciferase reporter gene with 1.5 kb upstream region of gicerin/CD146 construct was transiently transfected in PC12 cells. This 1.5kb revealed the three-fold increase of promoter activity responsive to the stimulation by forskolin compared with non-stimulated after thirty minutes. The peak response was observed within the first 30 min after stimulation and the increase was observed at least continued for six hours. This quick response was distinct from the stimulation with NGF.

L59

From Microfluidics to Yeast Mating: The Role of Bar1 Protease in Pheromone Homeostasis and Robust Mating Partner Detection

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We have asked how cells of the budding yeast, Saccharomyces cerevisiae, select and mate with each other. Chemotactic cells such as bakers yeast and nematophiles detect signals over long ranges and move quickly towards them. These cells respond over a wide range of stimulus strengths, and maximize their sensitivity by adjusting the affinity of their receptors. Conversely, interaction of cells within the same species, such as neuronal growth cones looking for target cells or fungal cells searching for mating partners, often involves detecting signals over short ranges. These cells face two problems: first the strength and equality of the local signal depends on the number and density of target cells, second the responding cells must be able to pick one of several equally attractive targets. Budding yeast has two mating types, a and α. The Bar1 protease, which degrades α factor and is produced by a cells, is essential for efficient mating but there has been little work on how the production and distribution of Bar1 allows a cells to mate under a wide range of conditions, from isolated couples to dense mating mixtures containing millions of cells. We used a combination of microfluidics, microscopy, and theory to ask how yeast cells detect pheromone gradients, polarize, and mate. We found that, in the absence of Bar1, a cells responded to α factor with a sharp transition between budding and shmooing, and only polarized precisely to a very narrow range of gradients. In mating mixtures, a cells regulate their production of Bar1 to keep the pheromone concentration at the surface within this narrow range, and use surface-bound Bar1 to distinguish between equally attractive partners. We argue, using simulation, that the use of proteases to control ligand concentrations is a general mechanism that allows cells to robustly find their targets.

L60

A Sperm-Egg-like Adhesion Required for Neuron-Glia Interaction and Dendrite Development

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Glia, which outnumber neurons ten-to-one in the human brain, make intimate physical contacts with synapses, actively respond to synaptic signaling, and express factors that regulate synapse formation and dendrite shape. To identify genes controlling neuron-glia interactions we have developed a set of tools for studying glial function using a C. elegans sense organ consisting of only 12 neurons and two glial cells and in which the pattern of neuron-glia contacts is developmentally fixed. Using a genetic screen for abnormal neuron-glia morphology we identified a pair of single-pass transmembrane proteins, DVF-7 and DEX-1, required for the interaction of sensory dendrites with a glial cell at the tip of the nose. In mutants lacking these proteins, neurons detach from the glial cell early in development and thus fail to form full-length dendrites. DVF-7 is expressed in neurons, localizes to the tips of growing dendrites, and bears a zona pellucida (ZP) domain like those of the proteins that compose the vertebrate egg coat. DEX-1 is expressed in glia and bears a zonadhesin domain like that of the sperm protein that binds the ZP, as well as two nidogen domains, involved in neurite outgrowth in other systems. Weak alleles of dvf-7 and dex-1 strongly interact genetically, suggesting the genes act cooperatively, likely mediating neuron-glia binding directly through a ZP-zonadhesin pairing. The DEX-1 nidogen domains may additionally participate in glia-to-neuron signaling to stimulate dendrite growth. DEX-1 and DVF-7 are closely related to α- and β-tectorin, proteins in the vertebrate inner ear associated with familial deafness, suggesting their function is conserved in humans.

L61

The Endocytic Pathway Mediates Cell Entry of dsRNA to Induce RNAi Silencing

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Many metazoan cells can take up exogenous dsRNA and use it to initiate an RNA silencing response. The mechanism for dsRNA uptake is ill-defined. Here, we identify the pathway for dsRNA uptake in Drosophila melanogaster S2 cells. Biochemical and cell biological analyses, as well as a genome-wide screen for components of the dsRNA uptake machinery, indicated that dsRNA is taken-up by an active process involving receptor-mediated endocytosis. Furthermore, pharmacological inhibition of endocytic pathways disrupted exogenous dsRNA entry and the induction of gene silencing. Importantly, this dsRNA uptake mechanism appears evolutionarily conserved, since knockdown of orthologs in Caenorhabditis elegans inactivated the RNAi response in worms. Thus, this entry pathway is required for systemic RNAi, in both single-cell and multicellular organisms. In larvae, pharmacological evidence suggests that dsRNA entry is mediated by pattern-recognition receptors. The possible role of these receptors in dsRNA entry may link RNAi silencing to other innate immune responses.

TIGHT JUNCTIONS

L62

Mupp1 Expression Is Induced by Hypertonic Stress and Involved in the Osmoresponse in Renal Cells

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Proteomic analysis of Inner Medullary Collecting Duct (IMCD3) cells adapted to increasing levels of tonicity (300, 600, and 900 mOsm/kg H₂O) revealed several proteins as yet unknown to be upregulated in response to hypertonic stress by antibody array technology. Of these proteins, the most robustly upregulated was the expression of MUPP1, a multi-PDZ domain containing adaptor protein with numerous cellular interactions and a principal role in tight junctions scaffolding and activity. Western blot analysis confirmed increased expression of MUPP1 with increased tonicity in both acute and chronic settings in IMCD3 cells. Likewise, the protein is also
regulated in vivo, as in mouse and human kidney tissues, MUPP1 expression was many fold greater in the papilla as compared to the cortex and increased further in the mouse papilla following 36 hr of thirsting. Data from quantitative PCR also revealed increased MUPP1 message in IMCD3 cells adapted to increasing hypertonicity. The expression of message increased substantially following 8 - 10 hrs of exposure to acute sublethal hypertonic stress (550 mOsm/kgH2O). Protein ½-life in IMCD3 cells was 19.9 hrs. Increasing medium tonyicity with sodium chloride, urea, sucrose, mannitol, sodium acetate and choline chloride stimulated MUPP1 expression. The c-Jun N-Terminal Kinase (JNK) specific inhibitor SP600125 eliminates MUPP1 translation in IMCD3 cells. In summary, we describe the upregulation of a tight junction-related protein with multiple cellular functions that may play an important role in the osmoreponse in renal cells.

L63 Tightening Mechanism of Claudin-5
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A key question of the tight junction research is the tightening of the paracellular cleft. The transmembrane protein claudin-5 is known to close the blood-brain barrier for small molecule transport. To test the hypothesis that the tight junction-related protein with multiple cellular functions that may play an important role in renal cells.

L64 Differential Expression Patterns of Claudin-7, -8, -12, -13 and -15 in the Mouse Intestine
K. Sugimoto,1 H. Chiba,1 H. Fujita,1 N. Sakai,1 T. Kojima,1 H. Yokozaki,2 N. Sawada1;1 Department of Pathology, Sapporo Medical University School of Medicine, Sapporo, Japan, 2Division of Surgical Pathology, Kobe University Graduate School of Medicine, Kobe, Japan
Among tight-junction proteins claudins, which play a key role in paracellular cleft. The transmembrane protein claudin-5 is known to close the blood-brain barrier for small molecule transport. To test the hypothesis that the tight junction-related protein with multiple cellular functions that may play an important role in renal cells.

L65 Regulation of Claudin-1 Promoter in the Rat Epididymis
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Claudin-1 (Cldn-1) is a tight junctional protein present in the epididymis. Limited information exists regarding the regulation of Cldn-1 transcription. In the epididymis, the regulation of the 5' flanking region of genes coding for tight junctional proteins is unknown. The present objectives were to investigate the transcriptional regulation of the Cldn-1 gene in the rat epididymis. A 1.8kb sequence of the 5' flanking region of the rat Cldn-1 gene was cloned. The transcriptional start site is an adenine located at the -198 position relative to the first codon and 26 bp downstream of the putative TATA box. It is the only start site in the rat epididymis. The Cldn-1 promoter was inserted into a luciferase gene expression vector and transfected into a rat caput epididymal cell line (RCE-1). Sequential deletion analysis revealed that minimal promoter activity was achieved with the construct containing -61 to +164 bp of the promoter. This sequence contained a TATA box and two consensus Sp binding sites. Electrophoretic mobility shift and supershift assays confirmed that Sp1 and Sp3 were present in RCE-1 cell and epididymal nuclear extracts, and that these bind to the 5' Sp1 binding motif of the promoter. Site directed mutagenesis of the 5' Sp1 binding site resulted in a 4-fold decrease in transactivation of the minimal promoter sequence. These findings indicate that Sp1/Sp3 bind to the Cldn-1 promoter region and that this interaction influences the expression of Cldn-1 in the rat epididymis. Supported by NSERC and CIHR.

L67 Effects from the Exposure of Nanoparticles on Human Intestinal Cells
B. A. Koeneham,1 Y. Zhang,1 K. Hristovski,1 P. Westerhoff,2 Y. Chen,2 J. C. Crittenden,2 D. G. Capco; 1Molecular and Cellular Biology, Arizona State University, Tempe, AZ, 2Civil and Environmental Engineering, Arizona State University, Tempe, AZ
Synthetic nanomaterials have been commercialized for delivery and medical diagnostics. However, the safety and risk of commercialized products has not been examined in detail. If the current trend continues, it will result in the discharge of synthetic nanomaterials into the environment, potentially threatening human and environmental health. The size effect is considerably more important to nanoparticle toxicity than the actual composition of the material. Consequently, understanding whether nanoparticles enter the body and the medium by which they do this is important.

L66 Regulation of Claudin-1 Expression by AMP-activated Protein Kinase
B. Zheng, L. Cantley; Harvard Medical School, Boston, MA
AMP-activated protein kinase (AMPK) is a heterotrimeric serine/threonine kinase that plays important roles in maintaining cellular energy balance. The activity of AMPK is modulated both by the cellular AMP to ATP ratio and by upstream kinases. Upon activation, AMPK increases energy supply by stimulating ATP-producing pathways and inhibiting ATP-consuming pathways. Recently, one of the AMPK upstream kinases was identified as LKB1, a homolog of C. elegans and Drosophila Par-4 proteins that are essential to the establishment of cell polarity during embryogenesis. Here we have used MDCK epithelial cells as a model system to investigate whether AMPK, as a substrate of LKB1, plays a role in the regulation of epithelial polarization. We found that AICAR, an activator of AMPK, promoted the assembly of tight junctions as indicated by measurement of trans-epithelial resistance (TER) and immunofluorescence analysis of ZO-1 translocation after calcium switch. Expression of a kinase-dead mutant of AMPK (D157A) inhibited the development of TER and ZO-1 translocation after calcium switch. Furthermore, the levels of AMPK phosphorylation (T172) increased during calcium-induced cell polarization, and this increase was dependent but the mutation abolished the increase of phospho-AMPK level. These results together support a role of AMPK in the regulation of epithelial tight junction assembly and provide an intriguing link between cellular energy status and tight junction assembly in epithelial cells.

L68 Regulation of Claudin-1 Promoter by AMPK
B. Zheng, L. Cantley; Harvard Medical School, Boston, MA
AMP-activated protein kinase (AMPK) is a heterotrimeric serine/threonine kinase that plays important roles in maintaining cellular energy balance. The activity of AMPK is modulated both by the cellular AMP to ATP ratio and by upstream kinases. Upon activation, AMPK increases energy supply by stimulating ATP-producing pathways and inhibiting ATP-consuming pathways. Recently, one of the AMPK upstream kinases was identified as LKB1, a homolog of C. elegans and Drosophila Par-4 proteins that are essential to the establishment of cell polarity during embryogenesis. Here we have used MDCK epithelial cells as a model system to investigate whether AMPK, as a substrate of LKB1, plays a role in the regulation of epithelial polarization. We found that AICAR, an activator of AMPK, promoted the assembly of tight junctions as indicated by measurement of trans-epithelial resistance (TER) and immunofluorescence analysis of ZO-1 translocation after calcium switch. Expression of a kinase-dead mutant of AMPK (D157A) inhibited the development of TER and ZO-1 translocation after calcium switch. Furthermore, the levels of AMPK phosphorylation (T172) increased during calcium-induced cell polarization, and this increase was dependent but the mutation abolished the increase of phospho-AMPK level. These results together support a role of AMPK in the regulation of epithelial tight junction assembly and provide an intriguing link between cellular energy status and tight junction assembly in epithelial cells.

L69 Regulation of Claudin-1 Promoter by AMPK
B. Zheng, L. Cantley; Harvard Medical School, Boston, MA
AMP-activated protein kinase (AMPK) is a heterotrimeric serine/threonine kinase that plays important roles in maintaining cellular energy balance. The activity of AMPK is modulated both by the cellular AMP to ATP ratio and by upstream kinases. Upon activation, AMPK increases energy supply by stimulating ATP-producing pathways and inhibiting ATP-consuming pathways. Recently, one of the AMPK upstream kinases was identified as LKB1, a homolog of C. elegans and Drosophila Par-4 proteins that are essential to the establishment of cell polarity during embryogenesis. Here we have used MDCK epithelial cells as a model system to investigate whether AMPK, as a substrate of LKB1, plays a role in the regulation of epithelial polarization. We found that AICAR, an activator of AMPK, promoted the assembly of tight junctions as indicated by measurement of trans-epithelial resistance (TER) and immunofluorescence analysis of ZO-1 translocation after calcium switch. Expression of a kinase-dead mutant of AMPK (D157A) inhibited the development of TER and ZO-1 translocation after calcium switch. Furthermore, the levels of AMPK phosphorylation (T172) increased during calcium-induced cell polarization, and this increase was dependent but the mutation abolished the increase of phospho-AMPK level. These results together support a role of AMPK in the regulation of epithelial tight junction assembly and provide an intriguing link between cellular energy status and tight junction assembly in epithelial cells.
Unfortunately, this issue remains poorly understood. This investigation provides fundamental information about the potential risks of nanomaterials in drinking water. This study focuses on metal nanoparticles, particularly titanium dioxide and aluminum oxide that are used in sunscreens and cosmetics. In this investigation we report the development of an assay method to examine the potential nanotoxicity of metal nanoparticles using a system that mimics the components of the digestive tract. The assays developed work at various levels to monitor both major changes in cell function such as cell death and minor changes in functions such as an alteration in the cell-cell junctions. In order to measure the integrity of the epithelial junctions in the tissue layer, transepithelial electrical resistance (TEER) was utilized. Immunocytochemistry was also performed with a confocal microscope to determine the effects of the nanoparticles on the junctional complexes (catenins and occludins). Scanning electron microscopy (SEM) was utilized to determine the effects on the microvilli. Results indicate that long-term exposure to nanomaterials allows the particles to pass through the monolayer of cells, reduce the TEER, and cause a shortening of microvilli.

L69 Computational Protocol for the Prediction of the Atomic Structures of Helical Membrane Proteins Based on Low-Resolution EM Maps

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Integral membrane proteins pose a major challenge for protein-structure prediction. The importance of this problem stems from the fact that high-resolution structures are known for only a handful of these proteins. This, in turns, makes it difficult to develop rules or empirical potentials to predict the packing of transmembrane helices. However, when a low-resolution electron-microscopy (EM) map is available, it can be used to provide restraints which, in combination with a suitable computational protocol, make the structure prediction feasible. Here we present such a protocol, which proceeds in three stages: (1) generation of an ensemble of helices by flexible fitting canonical helices into each of the density rods, and for a range of rotation angles around the main helical axes and shifts along the axes; (2) determination of admissible pairings of conformations for each helix pair (keeping only those that yield low packing scores), fast optimization of side chains, and scoring of the resulting conformations; and (3) energy minimization of the lowest-scoring conformations using an energy function that includes, besides the usual terms, a penalty term acting on the backbone atoms through a “tethering map” derived (indirectly) from the given low-resolution EM map. This protocol was successfully validated on three test cases: a dimer (Glycophorin-A, GpA), a tetramer (KcsA), and a pentamer (MscL). By this method, we obtained a full-atom prediction of the structure of the transmembrane (TM) domains of the gap-junction channel Cx43, a homohexamer with four TM helices per subunit. Even though symmetry was not imposed during these calculations, the final structure had an almost perfect 6-fold symmetry, the deviation from perfect symmetry being 0.035 Å.

L70 Modulation of the Hyperpolarization-activated Currents by Caffeine in the Rat Sensory Neurons

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Hyperpolarization-activated current (Ih) is important in the control of resting membrane potential and excitability in heart cells and neurons. Recent studies have demonstrated that Ih channels are also permeable to Ca2+ as well as K+ ions and modulated by Ca2+- and calmodulin. Ca2+ controls many physiological events, such as neurotransmitter release. Using whole-cell patch clamp technique, we studied the effects of store released Ca2+ on Ih channel. External application of caffeine (30 mM) strongly reduced the amplitude of Ih, regardless of the presence or absence of extracellular Ca2+. Caffeine-dependent inhibition of Ih was not altered by buffering intracellular Ca2+ with 1,2-bis-(2-aminophenoxy) ethane-N,N,N',N'-tetracetic acid (BAPTA). Ca2+ store depletion caused by thapsigargin did not prevent the inhibitory effect of caffeine on Ih. Inhibition of Ca2+ release from ryanodine-sensitive store by dantrolene and blockade of protein kinase C failed to abolish the inhibition of Ih by caffeine. Elevation of cytosolic Ca2+ by ATP or ionomycin did not cause inhibition of Ih. These results suggest that Ca2+ mobilization from intracellular storage is not responsible for the inhibitory effect of caffeine on Ih.

L71 Multiple Determinants of the β2-subunit Facilitation of the Ca1.2 Calcium Channel Voltage Gating: Characterization of the C-terminal β2 Determinants

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Ca1.2β2β3δ subunits are cytoplasmic proteins encoded by the family of 4 genes (β2-β3). β2-subunits facilitate voltage gating of the L-type calcium channels by competing with the N-terminal of the pore-forming α1 subunit that blocks the channel. Previously we described fully functional abdominal subunit α2dδ as well as a non-sporadic subunit that lacks a large central (β3δ) as well as N-terminal (β2δα) parts (Harry et al., 2004). Here, by deletion analysis of β2δα, we have identified a 42-amino acid C-terminal distal essential determinant of the human cardiac β2 subunit (β2CED) that facilitates voltage gating of the recombinant Ca1.2 channel. When expressed in COS1 cells, the channel composed of only the α1C and auxiliary αδβ3δ subunits was silent unless membrane depolarization to as high as +100 mV apPLIED to the α1C subunits a holding potential of -90 mV. Co-expression of β2CED (or its N-terminally tagged variant, ECFP-β2CED) facilitated voltage gating of the channel that generated calcium currents peaked at +40 mV. The calcium current through the αδβ3δ-β2CED channel activated much more slowly than that with the β2δα subunit, and did not show calcium dependent inactivation. Since ECFP-β2CED was membrane-targeted by the αδ subunit and co-immunoprecipitated with αδ, these data are consistent with association of β2CED into a functional channel complex. The β2δα subunit retained its activity after ablation of the β2CED determinant suggesting for the first time that multiple structures of β2 are responsible for the gating facilitation and membrane targeting of the Ca1.2 channel. The β2CED determinant shares no homology with beta subunits encoded by other genes, and represents a functional element of the β2-subunit that facilitates human cardiac Ca1.2 channel.

MEMBRANE CHANNELS
Ca,1.2 regulation that may be progressively changed by additional motifs.

**MEMBRANE FUSION**

L72

Visualizing Nucleotide Dependant Movement of N-ethylmaleimide Sensitive Factor by Cryoelectron Microscopy

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The AAA (ATPases Associated with a variety of cellular Activities) family member N-ethylmaleimide Sensitive Factor (NSF) is a protein helicase that regulates SNARE-mediated vesicular fusion in all cells. At present, the mechanism by which NSF utilizes ATP hydrolysis to generate the mechanical force necessary to disassemble SNARE complexes remains unclear. We used cryo-electron microscopy to generate intermediate-resolution 3D maps of the NSF hexamer in two nucleotide states (ATP and ADP-bound) that designate the beginning and end points of its catalytic cycle. Six large protrusions, corresponding to the N-terminal domains of hexameric NSF emerged from the sides of the D1 ring in both maps. Cryo-EM also revealed dynamic movement in the N-terminal region that contains the α-SNAP-binding domain of NSF. Manual docking of the trimeric crystal structure into the AMP-PNP map reveals that three of the N-terminal domains can form a "cap" enclosing the orifice above the D1 domain which is open only in the ADP state. The crystal structure of the N-terminal domain, fit well into this "cap" density, suggesting that during SNAP-SNARE binding that each alternate arm binds one SNAP protein.

L73

Mutations in the Saccharomyces cerevisiae V-ATPase Suppress sec9-4

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The terminal step of exocytosis is membrane fusion of secretory vesicles with the plasma membrane. Although the formation of SNARE complexes between SNARE proteins on opposed membranes is integral for fusion, other proteins are likely to have an accessory or regulatory role in SNARE complex formation. We took a genetic approach to identify regulators of SNARE function by utilizing Saccharomyces cerevisiae and isolating genetic suppressors of sec9 mutations. sec9 encodes the yeast homologue of SNAP-25, a t-SNARE involved in fusion of secretory vesicles with the plasma membrane. Mutants that grow at the sec9-4 restrictive temperature were isolated and subsequently screened for secondary phenotypes. Three different mutations were isolated that suppress the sec9-4 phenotype and also display a cold-sensitive phenotype on their own. Complementation testing of two of these cold-sensitive suppressors resulted in the isolation of rescued plasmids that contain the entire coding region of two different V-ATPase subunits. The V-ATPase is a multi-subunit complex that acidifies membrane compartments via the hydrolysis of ATP. However, recent studies in different systems have implicated the V-ATPase as having a role in membrane fusion. Our results demonstrate a genetic interaction between the V-ATPase and SEC9, providing a further link between the V-ATPase and membrane fusion.

**ER TO GOLGI TRANSPORT**

L74

The Role of Bap31 in MHC I Traffic and Quality Control

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Cytotoxic T lymphocytes (CD8+ T cells) detect viral infection by continuously scanning somatic cell surfaces for major histocompatibility complex class I (MHC I) molecules presenting non-self peptides. MHC I is assembled with peptide in the endoplasmic reticulum (ER). However, the mechanism by which MHC I molecules are targeted to ER exit sites, exported through the secretory pathway, and ultimately to the cell surface, is not well understood. Our recent studies indicate the export of MHC I from the ER is regulated by binding to the putative cargo receptor B-cell associated protein (Bap31). Bap31 is a type III transmembrane protein and contains a cytoplasmic KEEK ER-retention/retrieval motif. Over-expression of wild-type enhances the rate of traffic and the stability of surface MHC I molecules; however, over-expression of a dominant negative Bap31 decreases surface level MHC I molecules. Quantitative immunofluorescence microscopy and electron microscopy show a significant fraction of Bap31 is colocalized with a marker for the ER to Golgi intermediate compartment, the ERGIC. Neutralization of ERGIC pH increases surface level MHC I molecules but decreases overall stability. This effect is not observed in Bap31 deficient cells. We propose that after Bap31 associates with the heavy chain of peptide-loaded MHC I (pMHC I), the Bap31-pMHC I complex is transported from ER exit sites to ERGIC. The acidity in the ERGIC may dissociate low affinity peptides from the MHC I; empty MHC I molecules could then recycle to the ER with Bap31. Stable MHC I molecules with a high affinity peptide would dissociate from Bap31 and continue to traffic to the cell surface. Therefore we hypothesize that Bap31 acts both as a transporter and a quality control protein for MHC I.

L75

Heterogeneous Distribution of VIP36 in the Endoplasmic Reticulum and Golgi Apparatus of Rat Hepatocytes

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Vesicular integral protein of 36 kDa (VIP36) is a intracellular protein lectin binding protein that is glycosylated with high-mannose glycans. We have found that VIP36 is highly expressed in rat hepatocytes and to further clarify its localization and function in these cells, we generated specific antibodies against three domains using synthetic peptides. NGSLSYSYDHSKDGRWS (amino acids 185-199, identified as a part of CRD of rat VIP36), produced specific antibodies from three out of ten immunized animals that only recognized VIP36 in the Golgi apparatus (VIP36-G). KQRQERNKRFFY (348-358, cytosolic domain of VIP36) and NFLKSPKDNVDDPTGNFR (299-316, stalk domain) generated antibodies that both recognized VIP36 in the Golgi and endoplasmic reticulum (VIP36-ER). Western blotting in non-reducing PAGE showed that the VIP36-ER species exists in a monomorphic form in the endoplasmic reticulum (ER) fraction and as a dimer in the Golgi fraction. However, VIP36-G remained in a monomorphic form in the Golgi. Immunoelectron microscopy showed that VIP36-ER is distributed diffusely throughout the cytoplasm, including the membrane of the rough ER (rER), the vesicular structure of the intermediate compartment between the rER and cis-Golgi apparatus, and in the cisternum of the Golgi apparatus. However, VIP36-G was mainly located in the cisternum of the Golgi apparatus. Our results showed that two types of VIP36 exist in the Golgi apparatus and localize in heterogeneous form in the ER and the Golgi apparatus. This strongly suggests that the heterogeneity of VIP36 may play some complex roles in the sorting of secretory proteins in cells.

L76

Phosphatidylinositol 4-kinase III betaRegulates the Transport of Ceramide between the Endoplasmic Reticulum and Golgi

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The bulk of the endogenously synthesized ceramide is delivered from the ER to the plasma membrane by the ceramide transporter protein, CERT. Ceramide transport requires the ability of CERT to bind phosphatidylsinoitol 4-phosphate (PtdIns4P) within its N-terminal pleckstrin homology domain suggesting that phosphatidylinositol 4-kinases (P4Ks) localized at the Golgi apparatus play a regulatory role in CERT-dependent ceramide transport. However, studies using fluorescent ceramide analogues, we showed that overexpression of PH-domains capable of binding PtdIns4P strongly inhibited the transport of the fluorescent ceramide analogue, BODIPY-ceramide, to the Golgi. In the present study we set out to identify which (if any) of the four mammalian P4Ks is involved in the regulation of ceramide transport. Here we show that either the selective inhibition of P4K type III beta by a newly described PI 3-kinase inhibitor, PIK33,
or knock-down of the same enzyme by siRNA-mediated gene silencing inhibited ceramide transport from the ER to the Golgi. Similarly, a defect of ceramide delivery to the Golgi was indicated by the inhibition of either Wortmannin or PIK93 of the conversion of [3H]-serine-labeled endogenous ceramide to sphingomyelin. Our data strongly suggest a pivotal role of PHK II beta in the CERT-mediated transport of ceramide to the Golgi.

**L77**
**Kinetic Switch for Vesicle Formation by Coat Protein Assembly**
F. Manolea,1 A. Claude, 2 J. Chun, 1 J. Rosas, 2 P. Melancon 1; 1Cell Dissection of GBF1 and BIGs Function through Overexpression L78

Membrane traffic in the cell often rely on the formation of coated vesicles (COPI, COPII and Clathrin) that proceed in well defined successive steps; recruitment of coat components to the membrane, coat polymerization and membrane deformation, vesicle fission, and coat release. The current molecular understanding of coat formation gives strong evidence that this succession of event is subjected to a tight kinetic regulation, possibly through the activity of GTPases recycling between the membrane and the cytosol. We propose a quantitative kinetic theory that combines the binding and unbinding of coat components to the membrane and vesicle fission. We show that metastable invaginated coats are expected to form at sufficiently high density of coat components, and that their growth into a fully formed vesicle is highly sensitive to the recycling rate of individual components to and from the membrane. We observe a sharp kinetic switch from a membrane shedding only coat and vesicle fission. We suggest that this metastable state is subjected to kinetic parameters, which are known to be affected, for instance, by the presence of a bound cargo.

**L80**
**Cell Spreading Increases Plasma Membrane Area and Drives an Exocytic Burst Proportional to Contact Area Expansion**
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When fibroblast cells in suspension contact a matrix-coated surface, they rapidly adhere and spread. This process induces changes in cell shape from a round-up cell in suspension to a flat, fully spread cell. The plasma membrane reflects the shape of the cell. When a mammalian erythrocyte undergoes a change in cell shape, the plasma membrane area is constant. By contrast, most cells, like fibroblasts, use endomembranes to constantly remodel the plasma membrane by exocytosis and endocytosis. In this study, we address the questions of whether the plasma membrane area is changing during fibroblast spreading and whether this change could be achieved through exocytosis. We pulled tethers from fibroblasts in the plasma membrane during their spreading and we observed a decrease in membrane-cytoskeleton tension through the different stages of cell spreading (from 16.2 to 6.8 pN). By measuring the surface area of fibroblasts using lipid dyes, we observed a linear increase between spread area onto a fibronectin-coated matrix and plasma membrane area. The area increase is then correlated to the decrease in membrane tension. By monitoring the exocytic rate using FM1-43, we observed a sharp increase in the exocytic rate during cell spreading. We propose that during fibroblast spreading, both increase in surface area and decrease in membrane tension are achieved by exocytosis.

**PHOSPHOINOSITIDES AND TRAFFICKING**

**L81**
**Mechanism for Phosphoregulation of Actin-driven Endocytosis in Yeast: the Role of the Type 1 Phosphatase Gc7p**
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Pan1p is a central component of yeast endocytic complex that couples actin assembly with vesicle internalization during endocytosis. It directs actin polymerization at endocytic sites by stimulating the activity of the Arp2/3 complex. Phosphorylation of Pan1p by the kinase Pk1p down-regulates its activity after the actin polymerization is no longer required. In this study, we focus on the molecular mechanism that acts to release Pan1p from the phosphorylation inhibition. We show that Pan1p can be dephosphorylated by the phosphatase Glc7p, and the dephosphorylation is dependent on the Glc7p-targeting protein Scd5p, which itself is a phosphorylation target of Pk1p. The dephosphorylation can take place because Scd5p, maintained constantly by Glc7p in an unphosphorylated state, links Glc7p to Pan1p in two ways, directly by interacting with Pan1p and indirectly by interacting with the Pan1p-binding protein End1p. These results significantly advance our understanding of the phosphoryregulation of the dynamic process of actin-driven endocytosis.

L82 Ultrastructure of the Rab11a-containing Apical Recycling Compartment in MDCK Cells

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Protein and protein localization and the recycling of receptors, pumps and channels to the plasma membrane are the critical to correct cell function. The trafficking of proteins to their respective domain is a dynamic event usually requiring various factors, including V- and T-SNAREs and small Rab-GTPases. Nascent or endocytosed proteins may first travel to the Golgi network or traffic to a common endosome, respectively. After sorting, the proteins travel one of three routes: (1) directly to the plasma membrane (2) transcytosed to the opposite membrane domain or (3) recycled to the apical or basal membrane domains. The Rab11a mediated apical recycling system has received extensive study in Madin-Darby Canine Kidney (MDCK) cells to determine the general and essential factors involved in apical recycling. Since the apical recycling system has received only limited ultrastructural work we have sought to examine the apical recycling compartment using conventional transmission electron microscopy (TEM) and immuno-gold labeling EM in polarized MDCK cells. Electron microscopic analyses of polarized MDCCK cells stably transfected with various EGFP constructs including, Rab11a and the Rab11a-family of interacting proteins 2 (FIP2) -- Rab11-FIP2WT, Rab11-FIP2 (129-512) and GFP-FIP2 (S229A/R413G) show that the apical recycling compartment can be visualized as a system of tubular elements in the subapical region. Additionally, dual labeled (anti-Rab11a and anti-gFP) immunogold postfixed with either secondary antibody. Moreover, Fungi display multiple lifestyles, allowing us to consider their cell biology in this context. Using sequence similarity searches we identified the complete Rab family in 29 Fungi, (Ascomycota, Basidiomycota and Microsporidia lineages). We used phylogenetic analysis and pairwise sequence comparisons to manually annotate all the identified Ypt proteins. We identified a core set of Ypt that likely represents the trafficking steps present on the last ancestor of the Fungi/Metazoan clade. True yeasts (Hemiascomycetes) are distinguished from other preferentially multicellular Ascomycota and from Basidiomycota by two groups of Ypt proteins with complementary phylogenetic prophylies. Duplications that give rise to isoforms are frequent, lineage specific and seldom change the size of the subfamily. For example the three isoforms Ypt51,2,3 in S.cerevisiae the three Rab5a,b,c in humans resulted from independent duplications. This suggests an ideal number of isoforms for each Rab function. The total number of Rab and Ypt proteins does not change as a function of genome size or lifestyle, suggesting there was little innovation in trafficking in the evolution of Fungi. We found evidence for a large-scale duplication of Rab proteins in the ancestor of Fungi and animal, which is consistent with a novel whole genome duplication.

ENDOCYTIC TRAFFICKING

L85 Probing Alix with Monoclonal Antibodies Reveals Autoinhibitory Functions of the Proline-Rich Domain of Alix

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Alix (ALG-2 interacting protein X) is the mammalian member of an evolutionarily conserved family of proteins that have in common an N-terminal Bro1 domain, a C-terminal Pro-rich domain (PRD), and a middle region. Studies of this protein and its yeast ortholog revealed that Alix functions as an adaptor protein in diverse cellular processes, such as apoptosis, cell proliferation, cell adhesion, endosomal sorting and retroviral budding. On the contrary to the intensive studies on the downstream effectors and functions of Alix, the regulation of this multifunctional protein is still poorly understood. Interestingly, data from the published literature suggested that the PRD of Alix plays a regulatory role in Alix's functions, as truncation of PRD promoted the interaction of Alix with some of its binding partners. This raises the possibility that the PRD may mediate a close conformation of Alix that prevents its protein-protein interactions. To test this hypothesis, we utilized multiple anti-Alix monoclonal antibodies to probe the effect of PRD on Alix's conformation. Our data show that the conformation sensitive 2H12 antibody that recognizes a central region of Alix does not immunoprecipitate the full-length Alix, but immunoprecipitates PRD-deleted Alix efficiently in crude cell lysates. Similar results were also obtained with purified full-length and the PRD deleted Alix recombinant proteins, eliminating the possibility that partner proteins of Alix covers the 2H12 recognition epitope. In addition, adding exogenous PRD fragment to compete with endogenous PRD could relieve the PRD-mediated inhibition of 2H12-epitope. Furthermore, the

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ASCB 2006 Late Abstracts
Sodium/Hydrogen Exchanger 7 (NHE7) Associates with Caveolae/Lipid Rafts

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Sodium/Hydrogen Exchanger 7 (NHE7) was identified as the first mammalian organelle-membrane type NHE. NHE7 predominantly localizes to the trans-Golgi network (TGN) and endosomes at steady state. Since organellar radioisotope tracer influx assays revealed that NHE7 transports both Na+ and K+ in exchange for H+, a model has been proposed in which NHE7 regulates mildly acidic luminal pH of the TGN and endosomes by an unique (Na+, K+)/H+ exchange mode. Previously, we showed that NHE7 is targeted from recycling endosomes to the TGN, and that this process is regulated by the interaction with SCAMPs. Here, we show that NHE7 interacts with caveolin-1 through a novel binding module. Heterologous expression of caveolin-1 dominant-negative mutants as well as drugs that disperse caveolae/lipid rafts diminished the association of NHE7 with caveolae/lipid rafts. Thus, caveolins appear to facilitate the caveola/lipid rafts association of NHE7. We further showed by cell-surface biotinylation/internalization assays that a small population of NHE7 is targeted to the cell-surface and then internalized. Endocytosis of NHE7 was efficiently inhibited by pharmacological maneuvers that block clathrin-dependent endocytosis, whereas dominant-negative caveolin-1 mutants or methyl β-cyclodextrin did not affect NHE7 internalization. These results indicate that NHE7 is internalized by a clathrin-dependent and caveola/lipid raft-independent pathway.

CHROMATIN REMODELING

Enhanced Differentiation of Hematopoietic Stem Cells by Suppression of DNMTs

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Epigenetic modification is important for regulating gene function. Numerous reports have shown that some demethylating agent such as TSA and 5-azacytidine can modify hematopoietic stem cell fate and induction of gene expression. We hypothesized that increase of globin gene expression associated with differentiation may be affected by DNA methyltransferase family. In human primary cells, siRNA of DNMT3b was expressed at levels lower than DNMT1 on 7 days. Moreover, DNMT1 was spontaneously expressed for erythropoiesis in hemin treated K562. DNA methylation was also increase during this period. In order to identify the function of DNMTs on erythocyte differentiation, we used specific siRNA as a tool to probe the relationship between DNA methyltransferase and differentiation into erythrocyte. When introduced of siRNA about DNMT1 and DNMT3 in both cells, siRNA transfected cells were more differentiation into erythrocyte that was confirmed by FACS analysis and expression of globin. Expression of GPA, which are marker of red blood cell, were more increased up to 20-30% and γ and ε-globin were also more increased than control vector transfected cells. Our data suggest that DNA methylation can affect positively the differentiation of hematopoietic stem cell and DNA methyltransferase may contribute to expression of erythrocyte lineage gene such as Glycerokin A and globins. This work was supported by grant No.R1F03-01-01 from the Regional Technology Innovation Program of the Ministry of Commerce, Industry and Energy (MOCIE), 1, 2 These authors are supported by the second stage of Brain Korea 21.

SUB-STRUCTURES OF THE NUCLEUS

Sun-1, a Regulator of the Nuclear Shape in Dictyostelium

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In mammals, flies and worms, the nucleus is attached to the cytoskeleton by binding of the SUN domain proteins to the KASH domain proteins. SUN domain proteins reside in the inner nuclear membrane (INM), which recruit KASH domain proteins, e.g. the Nesprins, to the outer nuclear membrane (ONM). The KASH domain proteins encompass a binding domain for either F-actin or microtubules, thus providing intracellular positioning and migration of the nucleus, respectively. Here, we report on Sun-1 and interaptin, homologues of SUN and KASH domain proteins in Dictyostelium discoideum. In general, SUN domain proteins are targeted to the INM by interaction with laminins, which are absent in D. discoideum; instead, Sun-1 appears to be immobilized in the INM by binding to chromatin. Further, we addressed the interaction of Sun-1 and interaptin for nuclear positioning. Surprisingly, Sun-1 and interaptin are displaced from the nuclear membranes if either one of the proteins is overexpressed, indicating a competition between SUN-1 and interaptin for a common yet unknown binding partner. Most interestingly, overexpression of Sun-1 causes nuclear deformation, i.e. nuclear blebs and enlargement of the nuclear and cellular size, additionally amplification of centrosomes.

MAMMALLIAN DEVELOPMENT

Gestational Lead Exposure Increases Cell Proliferation and Neurogenesis in the Developing Mouse Retina

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Gestational lead exposure (GLE) increases the scotopic electroretinogram (ERG) a- (rod photoreceptors) and b-wave (bipolar cells) amplitude in children, monkeys and rodents. These novel supernormal ERG findings suggest that GLE irreversibly increased rod pathway neurons. To test this hypothesis, a murine model of GLE was established. Adult retinas from control and GLE mice were immunostained with molecular markers that define different retinal cells and retinal immunoblots were performed. To assess kinetics of retinal cell proliferation, control and GLE dams or pups were injected with the S-phase cell marker BrdU. Fixed retinas were immunostained for BrdU uptake, the M-phase cell marker phosphohistone3 (PH3) and apoptotic cells (TUNEL). Retinal sections were examined by light or confocal microscopy and stereological techniques determined cell counts. Affymetrix mouse genome microarray studies were conducted on developing retinas from control and GLE mice. Real-time PCR validated the results. In adult mice, GLE produced a dose-dependent selective increase in rods and bipolar cells as horizontal, amacrine, ganglion and Müller glial cells counts were unchanged. Immunoblots confirmed these results. In retinas from GLE mice, the number of BrdU- and PH3-positive cells increased, retinal progenitor cell (RPC) proliferation was prolonged, and developmental apoptosis decreased. Microarray data analysis followed by functional classification revealed that genes associated with cell growth and cell fate determination were differentially expressed in GLE mice. Thus, GLE in mice produced a novel phenotype characterized by an increased proliferation and genesis of late-born neurons, but not glial cells. The persistent increase of these neurons likely produces the lead-induced supernormal ERGs. Uregulation of cell cycle and transcription factors genes appears to underlie the increased and prolonged RPC proliferation and rod/bipolar cell neurogenesis. These findings have relevance for retinal development and the permanent cognitive and behavioral deficits observed in GLE children. NIH Grants ES012482, EY007551, EY007024, EY11115 and Sramek Foundation.

The Primary Cilium in Planar Cell Polarity Signaling in the Organ of Corti

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The organ of Corti, the sensory organ of the mammalian ear, consists of four rows of precisely patterned sensory hair cells whose apical surfaces contain actin-filled stereocilia bundles. Each stereocilia bundle is invariably arranged in a V-shaped staircase formation with the vertex
of each V uniformly pointing toward the abneural side of the sensory epithelium. This polarity of the stereocilia within the plane of the epithelium is called Planar Cell Polarity (PCP). The uniform orientation of stereocilia is regulated by a conserved genetic pathway, the PCP pathway, and coupled to convergent extension of the cochlea. During development, a single primary cilium, the kinocilium, is closely positioned at the vertex of each V-shaped bundle transiently and appears to lead the polarization of stereocilia. This observation has led to the model in which the kinocilium orients the stereocilia bundle in PCP signaling. To test this model, we generated mice in which Polaris, an essential gene for ciliogenesis, has been conditionally knocked out (CKO) in the ear, thus effectively disrupting kinocilia formation. While general inner ear development and hair cell differentiation appear normal in these mice, they exhibit defects in convergent extension of the cochlea and stereocilia orientation. Moreover, both defects were enhanced in mice that were doubly mutant for Polaris and a known PCP gene in convergent extension of the cochlea and stereocilia orientation. A hallmark of PCP signaling involves the formation of polarized PCP complexes along the axis of planar polarization to direct cytoskeleton changes necessary to exhibit the morphological polarity. Two PCP components displayed normal polarized subcellular localization in Polaris CKO mice, indicating that Polaris or the kinocilium is downstream of the formation of polarized PCP complexes in PCP signaling. Altogether our data suggest a role for Polaris, or the kinocilium, in convergent extension and planar polarization in the cochlea.

**L91 Characterization of a Novel Gene, Jxc1, Which Is Associated with the Development of Hearing in Mice**

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Jackson circler (jc) is a recessive mutation. Homozygous mutants are deaf and show erratic circling behavior. Using a positional cloning approach, the novel Jxc1 gene has been cloned. Mouse genomic DNA was used as template to sequence the Jxc1 gene, and we detected a 10-bp deletion in the jc mutant mouse, causing a frame-shift mutation. We also detected a G to T transversion, causing an amino acid change from Gly to stop codon, in the new allele, jc2. These mutations result in truncated proteins and are predicted to inactivate the Jxc1 protein. Gross anatomy showed that the hearing loss in the jc mouse was associated with a cochlear malformation. Heterozygous mice had a normal development of the cochlea in both basal and apical turns, but homozygous mutants showed aplasia of the apical turn. In order to characterize the Jxc1 gene, RNA in situ hybridization was performed to determine the temporal and spatial expression pattern in the inner ear. At E15.5, Jxc1 expression was detected in the crista ampulla, maculae of utricle and sacculae, vestibular ganglion, cochlear duct and spiral ganglion. Jxc1 expression persisted from P0 through P6 in the vestibule and cochlea. The hybridization signals were spread across the sensory region. In addition, Jxc1 was expressed in the vestibular ganglion and spiral ganglion. Jxc1 expression in sensory cells of the spiral ganglion suggests that it may be expressed in the progenitor cells and thus is involved in the early development of the inner ear. We used the mouse Jxc1 sequence as a probe to BLAT-search the human genome. Seven exons were found in the human homolog. The sequence alignment showed 94.5% identity between the mouse and human homolog, and some regions were highly conserved. The jc mouse therefore may provide a valuable model to investigate molecular mechanisms underlying hearing in humans.

**L92 Using Transgenic Mice to Study the Function of Biglycan in Tooth Development**

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Biglycan belongs to small leucine-rich proteoglycans (SLRPs) family. The localization of SLRPs in mineralized tissues, such as bone, dentin, or cementum suggested that SLRPs might play a role in mineralization. In Biglycan knockout mice, the forming enamel of new born mouse molar was about three- to five-fold thicker than that of the wild-type, resulting from an up-regulation of amelogenin synthesis. The purpose of this study is to test whether biglycan is involved in enamel formation and acts as a suppressor of amelogenin expression. Transgenic mice over expressing biglycan driven by amelogenin promoter were generated. Because of the tissue-specific expression of amelogenin promoter, this transgenic model is ideal to determine the functions of biglycan during tooth formation. Using Immunohistochemistry of the incisors, two lines of transgenic mice were confirmed for the over expression of biglycan, with one having dramatic increase and the other slightly increase in biglycan expression level. The transgenic mice over expressing biglycan have been successfully established. The studies on the expression level of amelogenin and mineralization development in the transgenic mice and their enamel structure using Scanning electron microscopy are ongoing. The availability of two lines with different biglycan expression level can also be used in studies of dose dependent effect of biglycan.
**CELL POLARITY**

**L95  Studies of Interactions between Anterior PAR Proteins in C. elegans**
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par-3, par-6, and pkc-3 are important for establishment of C. elegans embryonic polarity. Loss of function of any one gives similar polarity defects suggesting that the proteins function in a common process. Indeed, biochemical interactions among their Drosophila and mammalian homologues indicate that these three proteins function together. By yeast two-hybrid and GST pull-down experiments, we verified that similar biochemical interactions take place among C. elegans PAR-3, PAR-6 and PKC-3. We find that PAR-6 B1 associates with PKC-3 B1; PAR-6 PDZ binds to PAR-3 PDZ and PKC-3 kinase domain directly interacts with PAR-3 C-terminal region. We also verified the in vivo interactions between these three proteins by co-immunoprecipitation. In addition, we find that the PAR-6-PKC-3 interaction is important for polarity establishment and PAR-6 cortical localization. We are in the process of determining the mechanism of binding of PAR-6 PDZ to PAR-3 PDZ.

**L96  Ceramide Synthase Is Required for Lipid Raft Polarization and Filamentous Growth in Yeast**
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Sphingolipids are enriched in lipid rafts, which are thought to be involved in polarity development in a wide range of cells. The core structure of sphingolipids is ceramide. During our search for genes responsible for the morphogenetic switch of Yarrowia lipolytica, we found that the Y. lipolytica LAG2 gene (YILAG2), a homologue of Saccharomyces cerevisiae LAG1 encoding ceramide synthase, is required for the hyphal growth of Y. lipolytica. In addition, the S. cerevisiae ceramide synthases (Lag1p and Lac1p) were shown to be essential for the filamentous growth of the Sigma28N peptide/BoxB tethering strain. Furthermore, we demonstrated that the Candida albicans genes (CaLAG1 and CaLAC1) homologous to S. cerevisiae LAG1 are important for the hyphal growth of the yeast. Interestingly, homozgyous CaLAG1A mutants could not polarize lipid rafts to growing hyphal tips, indicating that the lack of ceramide synthase lead to the disturbance of lipid raft polarization. We further investigated whether ceramide synthases from various organisms, including Aspergillus nidulans ceramide synthases (LagA and BarA), can functionally replace each other. Complementation tests revealed that CaLAG1, YILAG1 and lagA are functional homologues of S. cerevisiae LAG1 and LAC1, and BarA is functionally related with YILAG2. Taken together, our results suggest that ceramide synthases are required for the filamentous growth of yeast, but they may be different in substrate specificity or in their specific roles for the morphogenesis.

**L97  Investigation of the Function of the Exon Junction Complex in oskar mRNA Localization**
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Deposition of the Exon Junction Complex (EJC) on mRNA is a key consequence of splicing in vertebrates and in flies. Several core EJC components were first identified in Drosophila, based on their requirement in oskar mRNA localization in the oocyte, which is crucial for the establishment of the anterior-posterior axis of the fly. Splicing of the first intron in oskar is necessary for oskar mRNA localization, suggesting that EJC deposition at the first exon-exon junction is critical for properly modulating functional localization RNP. We are testing this hypothesis and determining the significance of EJC deposition on assembly of the oskar mRNP. To this end, we are directly recruiting individual EJC core components to the first exon-exon junction in oskar, in the absence of splicing, using the N-peptide/BoxB tethering system. We have generated oskar cDNA transgenes containing two copies of the BoxB sequence inserted at this position as well as individual EJC components fused to the N-peptide. This system is also adapted for GST-RNA chromatography, allowing isolation of the tagged mRNA and comparison of the content of mRNPs formed on spliced, versus unspliced oskar mRNA, for identification of EJC-associated molecules. Our first results show that, as do the native EJC core proteins, the N-tagged EJC proteins localize at the posterior pole of wild-type oocytes, indicating that they are incorporated normally into oskar localization mRNPs. Furthermore, intronless BoxB-tagged oskar mRNA is translated at posterior pole of oocytes, indicating that the mRNA is functional. We are now co-expressing the two components of the system, to identify the minimal EJC components required for oskar mRNA localization. While the N/BoxB system has been utilized in vivo in cell lines and in yeast, and in vitro in pull-down assays, our results demonstrate its applicability for in vivo analysis in a higher eukaryote.

**L98  Regulation of the Exchange Factor Cdc24 during Budding Yeast Polarity**
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Cell polarization is an essential step for processes such as cell motility and asymmetric cell division. Budding yeast is an excellent model organism for the study of polarity establishment, for two reasons: first, because the cells display a distinct cell and actin cytoskeleton morphology at each stage of the cell cycle, and second, because many proteins that regulate polarity, including the Rho family GTPase Cdc42, are conserved from yeast to mammals. Cdc42 and its guanine nucleotide exchange factor (GEF) Cdc24 are essential for yeast polarity establishment just before bud emergence. It was previously shown that a positive feedback loop mediated by the multidomain adaptor protein Bem1 was important for polarization (Butty AC, et al. 2002. EMBO J. 21(7):1565). We have recently shown that both an actin transport-mediated positive feedback loop and the Bem1-mediated feedback loop are required for robust and efficient cell polarization (Wedlich-Soldner R, et al. 2004. J Cell Biol. 166(6):889). Cdc42 is widely recognized to be a central regulator of cell polarity in most organisms, but the role and regulation of its Db1-homology GEF Cdc24 is relatively unclear. We aim to further elucidate the role of Cdc24 in polarity establishment by understanding the mechanism of Cdc24 regulation. Specifically, multisite phosphorylation of Cdc24 may regulate its localization or GEF activity. It may also play a role in lending cooperativity to the Bem1-mediated feedback loop. Through mass spectrometry analysis of purified Cdc24-TAP, we have identified a large number of in vivo phosphorylation sites. Currently, we are working to identify the sites that are most important for Cdc24 function. Understanding the molecular mechanism of Cdc24 activation will not only lead to a better understanding of yeast polarization and regulation of polarization processes in general, but it may also help to elucidate basic mechanisms for GEF regulation.

**STEM CELLS**

**L99  Characterization of miRNA Expression in the Differentiation of Human Embryonic Stem Cells into Cardiomyocytes**
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Although cardiomyocyte differentiation occurs spontaneously in human embryonic stem cell (hESC) cultures, generally fewer than 1% of the cells form cardiomyocytes. Furthermore, hESC-derived cardiomyocytes are heterogeneous and immature, with physiological properties that differ in important ways from those typically associated with adult cardiomyocytes. Therefore, large-scale clinical application for regenerative therapies await enabling technology to direct cardiomyocyte differentiation, maturation, and possibly replication of committed cardiomyogenic precursors to enhance the yield of transplantable cardiomyocytes. MicroRNAs (miRNAs) are 19-25 nt. non-coding RNAs that regulate gene expression by inhibiting translation or triggering degradation of specific mRNA targets. miRNAs appear to play a critical role in directing cellular
LPA on self-renewal and differentiation in mouse embryonic stem cells.

L102 Hedgehog Signalling Regulates Both Neurogenesis and Lamination in the Dorsal Forebrain and Midbrain

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During neurogenesis, the mammalian cerebrum develops as a gyrencephalic, laminated structure capable of higher order cognitive functions. The Sonic hedgehog (Shh) pathway is essential for patterning the developing spinal cord, cerebellum and ventral telencephalon. Here we show that neural-cell specific deletion of Patched1 and subsequent Shh pathway activation leads to an expansion of surface area in combination with a failure of early events in laminogenin in the dorsal nucleus tegmentum. Using a modification of the neurosphere assay, we determined that Shh pathway activation via Patched1 ablation regulates not only stem cells but also the more committed neural progenitor cell. Furthermore, we demonstrate that Patched1 regulates in vivo both cell cycle length and exit during neurogenesis. These data show that Shh signalling is responsible for controlling both the size and architecture of the developing cerebral cortex thereby providing a single molecular link for both of these key processes.

L103 Distinct Expression Profiles in Type III Collagen and α-Smooth Muscle Actin between Human Dental Pulp Stem Cells and Human Mesenchymal Stem Cells

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In this study, we characterized adult human dental pulp stem cells (hDPSC) in comparison with human mesenchymal stem cells (hMSC). hDPSCs revealed their capacity to form fibroblastic colony forming unit during the extended cell culture. Expression profile of surface markers of hDPSC was similar to that of hMSC. Both cells strongly expressed CD29 and CD44 but did not express CD 105, CD117, and CD140a. In contrast, CD34 and CD45. However their expression profiles of type III collagen and α-smooth muscle actin(α-SMA) were quite distinct between hMSC and hDPSC. HMSC were strongly positive in type III collagen expression but were weakly positive in α-SMA. In contrast, hDPSC were very weak in type III collagen expression and strongly positive in α-SMA expression. hDPSCs were also successfully differentiated into osteoblasts or adipocytes under differentiation into the specific conditions suggesting their multipotency. Therefore even with overall similarity in the surface marker expression, hDPSC may be clearly distinguished from hMSC based on the expression profiles of type III collagen and α-SMA. This work was supported by Musculoskeletal Bioregion Center of MOHW given to Dr Y Son

L104 Stimulating Effect of Substance-P on the Recovery from Radiation-induced Bone Marrow Suppression

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Substance-P (SP) is known as neurotransmitter as pain sensation as well as neurohormone involved in neuroinflammation. Recently its another role in the neuroimmunomodulation has been suggested. In this study, we investigate whether SP can affect the regeneration of bone marrow after suppression by radiation or not. Bone marrow suppression in C57BL/6 mouse was induced by 4.0 Gy radiation, which was confirmed as almost loss in the CFU of bone marrow cells as well as peripheral blood. Immediately after irradiation, SP was injected to mouse tail vein and repeated injection was performed at 48th post irradiation. At day 3 and 7 after irradiation, bone marrow and
MITOCHONDRIA AND CHLOROPLASTS

L107
The Saccharomyces cerevisiae ATP22 Gene Codes for the Mitochondrial ATPase Subunit 6 Specific Translation Factor

MITOCHONDRIA AND CHLOROPLASTS

L107
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In an earlier study, mutations in the Saccharomyces cerevisiae ATP22 gene were shown to block assembly of the F0 component of the mitochondrial proton-translocating ATP synthase (Helfenbein et al, J. Biol. Chem. 278, 19751-19756). The current study shows that Atp22p is essential for translation of subunit 6 of the mitochondrial ATP synthase. ATP22 mutants fail to synthesize subunit 6. To gain further functional information of Atp22p, three individual revertants from an ATP22 null mutant have been analyzed. The revertants are heteroplasmic cells containing both wild type mitochondrial DNA and a ρ deletion genome. In the ρ deletion genome, the 5'-untranslated region (UTR), first exon and first intron of cytochrome c oxidase subunit 1 (COX1) are fused to the forth codon of ATP6. The hybrid COX1/ATP6 gene is transcribed and processed to the mature mRNA by splicing of the COX1 intron from the promoter. The hybrid protein translated from the novel mRNA is proteolytically cleaved at the normal site between residues 10 and 11 of the subunit 6. The ability of the ρ suppressor genome to express subunit 6 in an atp22 null mutant constitutes strong evidence that translation of subunit 6 depends on the interaction of Atp22p with the 5'-UTR of the ATP6 mRNA.

L110
Sulfiredoxin Translocates to Mitochondria and Regulates Reduction of sulfiredoxin III Oxidase in Oxidative Stress

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Reactive oxygen species (ROS) containing H2O2 are continuously generated as a natural byproduct of cellular metabolism in the mitochondria. Peroxiredoxins (Prxs) are a family of peroxidases that are able to scavenge H2O2. While eliminating H2O2, sometimes Prx is overoxidized and reversibly inactivated by modification of the active site cysteine to cystine-like acid (Cys-SO2H). The 2-Cys Prx (Prx I–IV) are inactivated, sulfiredoxin (Srx) induces a reversion of the cysteine sulfonic acid to reduced cysteines of 2-Cys Prx in vitro. Whereas Srx is localized in the cytoplasm, Prx III is present in the mitochondria. Although Srx is able to reduce sulfenic Prx III in vitro, it remains unclear whether the reduction of Prx III in cells is actually mediated by Srx. Using RNA interference, we show that Srx is responsible for not only prevention of sulfenic acid but also sulfenic mitochondrial Prx III. We investigated further the translocation of Srx from the cytoplasm into the mitochondria for reaction of Prx III by combining subcellular fractionation and immunocytochemical analyses. We have shown that the translocation of Srx is initiated by oxidative stress in A549 cells. The data suggest that the mitochondrial translocation of Srx is an important mechanism for reactivation of mitochondrial sulfenic Prx III under oxidative stress.

L109
Replication of Hepatitis C Virus Occurs on Detergent Resistant Membrane Compartments Associated with Mitochondria

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The picornavirus genome is transported into the cell as a single-stranded positive sense RNA virus. A replication complex (RC) consisting of viral RNA-dependent RNA polymerase (RdRp) associated with viral nonstructural proteins and/or cellular proteins. A membrane structure formed with cellular proteins, which might also play a structural and organizational role in the viral replication complex formation, can provide a microenvironment for efficient RNA replication by concentrating and compartmentalizing RC and by preventing the activation of host defenses mediated by a replication intermediate, double-stranded RNA. The RNA replicase of hepatitis C virus (HCV), which has a single-stranded positive sense RNA genome, is known to be tightly bound to membranes, or is surrounded by a detergent-resistant membrane (DRM). However, there is considerable uncertainty on whether HCV nonstructural proteins form the RC. By subcellular fractionation, immunofluorescence, and immunogold staining studies, we demonstrate that each of the HCV nonstructural proteins and its...
replicating RNA are present in the mitochondrial fraction devoid of endoplasmic reticulum (ER) contaminants. In vitro RdRp assay revealed that HCV nonstructural proteins, including the RdRp and NS3 RNA helicase that are essential for HCV replication, and endogenous viral RNA form an active RC on the mitochondria. Proteasome K accessibility and membrane flotation assays along with alkaline extraction assay using the mitochondria fraction demonstrated that both the viral RdRp and helicase exist in a DRM compartment or lipid raft. Collectively, our results support a hypothesis that HCV nonstructural proteins and viral RNA may be recruited onto the mitochondria to form an active viral RNA RC within DRM after HCV nonstructural proteins are translated on the ER.

L110
X-ray Crystallographic Study of Mitochondrial Fission Complex
Y. Zhang, D. C. Chan; Division of Biology, California Institute of Technology, Pasadena, CA
Mitochondrial dynamics is regulated by coordinated fusion and fission, which play a critical role in maintaining the normal structure and function of mitochondria. An imbalance of mitochondrial fusion/fission results in severe cellular consequences. The mitochondrial fission machinery in Saccharomyces cerevisiae includes Dnm1p, Fis1p, Mdv1p, and Caf4p. The recruitment of enzymes from cytosol to dynamin, to the mitochondria surface is essential for mitochondrial division and depends on the outer membrane protein Fis1p. Recent studies have suggested that Fis1p-dependent Dnm1p recruitment is mediated by Mdv1p and Caf4p. These two adaptor proteins are able to bind Fis1p with an N-terminal region and to Dnm1p through a C-terminal WD40 repeat region. In order to understand the molecular mechanism of the assembly of the mitochondrial fission complex, we studied the Fis1p/Mdv1p complex using X-ray crystallography. We crystallized the complex of Fis1p bound to a fragment of Mdv1p and solved its structure using molecular replacement. The crystal structure shows that Mdv1p binds to a hydrophobic groove on the surface of Fis1p, but in a manner that is different from previous models. This study advances our current understanding of the interactions between Fis1p and Mdv1p, which are crucial for assembly of mitochondrial fission complexes.

L111
Reproducible Organelle Purification Facilitates Analysis and Discovery of Organelle Proteins
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The preparative dissection of cells into their substructure reduces sample complexity and facilitates functional analysis of proteins in a physiological context. Typical methods for separating organelles from other cellular components results in insufficient purity by cross contamination of other sub-cellular components and require ultracentrifugation steps. We have developed a new 12-step fractionation of subcellular organelles using BD™ Free Flow Electrophoresis System (FFE) that does not include ultracentrifugation. Cross contamination was observed to be a very low degree using this procedure. The FFE methodology relies on the net charge of the particles. According to their surface charge and size, particles are deflected differently in an electric field and are separated through a buffer flow perpendicular to the electric field. With this technique, we were able to achieve high purity and successful isolation of various cellular structures have been obtained. In case of organelle separation, standard procedures were developed for a serial sample preparation of higher eukaryotes e.g. animal tissue and yeast. Subcellular fractions were separated by SDS-PAGE, visualized and analyzed in more detail by immunoblotting using organelle specific antibodies. The respective subcellular fractions were also assayed for the activity of the indicated marker enzymes. Electron microscopy of the organelle fractions extracted using the FFE system indicated that the purification was selective on the organelle level, a finding that was confirmed on the protein level by immunoblotting with antibodies directed against appropriate organelle marker proteins. Furthermore, the protein patterns of the respective fractions were clearly distinct. Activity profiling showed the compatibility of the extraction procedure with enzyme assays and demonstrated the separation of the cellular components according to their subcellular localization in their native and functional state.

L112
Gol12 Is Targeted to the Mitochondria and Affects Mitochondrial Morphology and Motility
A. V. Andreeva, M. A. Kutuzov, T. A. Voyno-Yasenetskaya; Pharmacology, University of Illinois at Chicago, Chicago, IL
Mitochondria are involved in supplying the cell with energy, in apoptosis and Ca2+ homeostasis. Mitochondrial dysfunction has been implicated in premature aging, Alzheimer's and Parkinson's diseases, diabetes, and cardiovascular diseases. It has recently been realized that mitochondria also function as signaling platforms. Alpha subunit of heterotrimeric G protein G12 (Gol12) constitutes, along with Go13, one of the four families of heterotrimeric G proteins, which transduce signals from variety of heptahelical G protein-coupled receptors. Using algorithms that predict protein subcellular location, we found that N-terminus of Gol12, but not those of other Ga subunits, contains a potential mitochondrial targeting peptide. Using confocal laser scanning microscopy and cell fractionation followed by Western blotting, we show that up to 20-40% endogenous Gol12 in human umbilical vein endothelial cells (HUVEC) and NIH3T3 colocalize with mitochondrial markers. N-terminal sequence of Gol12 fused to GFP efficiently targeted the fusion protein (Nα12:GFP) to mitochondria, however no cleavage of the targeting peptide could be detected, possibly due to palmitoylation. We demonstrated that large Gol12-mutant flies, Male mitochondria, apparently unconnected to each other. These findings are the first demonstration of a heterotrimeric G protein α subunit specifically targeted to mitochondria by a signal peptide and involved in the control of mitochondrial morphology and dynamics.

L113
Roles for Mitoshell in Mitochondrial Aggregation and Meiotic Cytokinesis in Drosophila Spermatogenesis
A. C. Aldridge, S. E. Coffey, B. C. English, S. E. Favors, K. G. Hales; Biology, Davidson College, Davidson, NC
Mitochondrial aggregation and fusion beside the spermatid nucleus are hallmarks of early post-meiotic morphogenesis during wild type Drosophila spermatogenesis. To determine molecular mechanisms underlying these events, we have developed a protocol to induce mitochondrial motility in male flies homozygous for the mitoshell mutation are sterile and show dramatic defects in the timing and nature of mitochondrial aggregation. Mitochondria in mitoshell mutants prematurely aggregate in primary spermatocytes shortly before meiotic entry. Instead of gathering beside the nucleus, these mitochondria surround the spermatocyte nucleus in a shell-like configuration. Spermatocyte nuclei subsequently undergo meiotic karyokinesis without condensation of mitochondria. Heterologous mitochondria from the fourth cestheral post-meiotic nuclear remain together in the mitochondrial shell unless subjected to the pressure of a cover slip in live squashed testis preparations. Meiotic cytokinesis is not initiated. Microtubule staining indicates that a flagellar axoneme still forms for each post-meiotic nucleus. The mitochondria in the perinuclear shell eventually dissociate and elongate beside the axoneme; spermatids appear to contain many scattered mitochondrial organelles. With this model system, we have developed a protocol to induce mitochondrial fusion did not occur. We mapped the mitoshell mutation by deficiency to a narrow chromosomal region containing twenty-three genes, of which five are represented among tests expressed sequence tags. We will present our analysis of candidate genes. Identification and characterization of the mitoshell gene will allow better understanding of regulatory or structural mechanisms underlying directed mitochondrial movement in cells. This work was supported by the National Science Foundation under grant 0133286 to K. G. Hales.

GOLGI COMPLEX

L114
Zinc Finger Protein-like 1 (ZFPFL1), a Novel Regulator of cis-Golgi Dynamics
Pharmacology, University of Illinois at Chicago, Chicago, IL
Mitochondrial aggregation and fusion beside the spermatid nucleus are hallmarks of early post-meiotic morphogenesis during wild type Drosophila spermatogenesis. To determine molecular mechanisms underlying these events, we have developed a protocol to induce mitochondrial motility in male flies homozygous for the mitoshell mutation are sterile and show dramatic defects in the timing and nature of mitochondrial aggregation. Mitochondria in mitoshell mutants prematurely aggregate in primary spermatocytes shortly before meiotic entry. Instead of gathering beside the nucleus, these mitochondria surround the spermatocyte nucleus in a shell-like configuration. Spermatocyte nuclei subsequently undergo meiotic karyokinesis without condensation of mitochondria. Heterologous mitochondria from the fourth cestheral post-meiotic nuclear remain together in the mitochondrial shell unless subjected to the pressure of a cover slip in live squashed testis preparations. Meiotic cytokinesis is not initiated. Microtubule staining indicates that a flagellar axoneme still forms for each post-meiotic nucleus. The mitochondria in the perinuclear shell eventually dissociate and elongate beside the axoneme; spermatids appear to contain many scattered mitochondrial organelles. With this model system, we have developed a protocol to induce mitochondrial fusion did not occur. We mapped the mitoshell mutation by deficiency to a narrow chromosomal region containing twenty-three genes, of which five are represented among tests expressed sequence tags. We will present our analysis of candidate genes. Identification and characterization of the mitoshell gene will allow better understanding of regulatory or structural mechanisms underlying directed mitochondrial movement in cells. This work was supported by the National Science Foundation under grant 0133286 to K. G. Hales.

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GOLGI COMPLEX
endoplasmic reticulum is inhibited. The Golgi dispersal induced by dispergo was studied by three-dimensional live cell imaging and may resemble an intermediate Golgi state during its reversible fragmentation into small vesicles during mitosis. Our observations using dispergo suggest that the Golgi can self-organize from its cisternal substructures. The use of dispergo provides a novel approach for identifying the molecular interactions that guide the process of Golgi self-organization.

TARGETING TO LYSOSOMES

L117
Polyunsaturated Omega-3 Fatty Acid Mediates Hepatic Lysosomal-degradative Activities Towards Intracellular Triacylglycerides

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Polyunsaturated omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) lower the secretion of triglycerides (TG) from the liver without causing steatosis. This suggests that omega-3 fatty acids enhance intracellular degradation of TG in hepatic cells. We examined the localization of triglyceride-rich lipid droplets within lysosomal and autophagolysosomal compartments in living rat (McA-RH7777) and human (HePG2) cell lines; cells were treated with either 0.4 μM of omega-3 fatty acids or mono-unsaturated fatty acids (such as oleic acid and myristic acid). Lipid droplets were microinjected using Coherem anti-Stokes Raman scattering (CARS) microscopy, while lysosomes/autophagolysosomes were followed using two-photon fluorescence microscopy (through monodansylcadaverine labeling). Localization of lipid droplets within lysosomal/autophagolysosomal compartments were 4-50 fold higher in cells treated with omega-3 fatty acids as compared those treated with mono-unsaturated fatty acids. Transmission electron microscopy (TEM) on fixed cells also revealed an enhanced localization of lipid droplets (ranging from <100 nm to several microns) within degradative vacuoles near cytosolic lipid droplets and Golgi in omega-3 fatty acid treated cells. Pulse-chase experiments using [3H]glycerol or [14C]oleic acid showed that the turnover of 3H- and 14C-TG was increased in cells treated with EPA as compared with OA. These three approaches combine to suggest an enhanced degradative activity toward intracellular TG when cells are supplied with omega-3 fatty acids.
A variety of cellular functions in eukaryotes, including receptor downregulation, require the formation of multivesicular bodies (MVBs), which provides a mechanism for delivery of transmembrane proteins into the lumen of the lysosome/vacuole. Entry of cargo into the MVB pathway is highly regulated, depending on both cis- and trans-acting factors. The covalent addition of ubiquitin to cargo is a characterized cis-signal to allow for recognition and entry into the MVB pathway, however cargoes that can enter the MVB pathway independent of ubiquitination have also been described. To understand ubiquitin-independent mechanisms of MVB cargo selection, we examined Sn3a, a reported ubiquitin-independent cargo, to determine alternative motifs for MVB sorting. Surprisingly, Sn3a is ubiquitinated, but can enter the MVB pathway in both ubiquitin-dependent and -independent methods. We found two alternative motifs that are important for Sn3a MVB sorting: a tyrosine-based motif and a PPAY motif, which is known to interact with WW protein-protein interaction domains. Unexpectedly, Rsp5, a WW domain-containing HECT ubiquitin E3 ligase, binds to the PPAY motif and mediates Sn3a ubiquitination. In addition, although Sn3a does not require ubiquitination for entry into the MVB pathway, Sn3a is mislocalized in Rsp5 HECT mutants, indicating a role for Rsp5 in both the ubiquitin-independent MVB sorting of Sn3a as well as directly ubiquitinating MVB cargo.

**BLOOD VESSELS AND ENDOTHELIAL CELLS**

**L120**

**The Suppression of Zfpm-1 Gene Enhances Erythropoiesis in Human CD34\(^+\) Cell Differentiation**

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Erythropoiesis is a complex multistage process for the differentiation of hematopoietic stem cells to mature erythrocytes. However, the molecular basis governing the functional behavior of erythropoiesis is still unclear. This study examined the differential expression profiles using a cDNA microarray technique with bone marrow cells in Prx\(^+\) and Prx\(^-\) mice to determine the regulatory factors in the erythropoiesis of CD34\(^+\) hematopoietic stem cells. 136 genes including 66 EST genes were differentially expressed in the bone marrow cells of the Prx\(^+\) mice and the Prx\(^-\) mice. We pointed to Zfpm-1 among differential expressed 136 genes. In order to verify functions of these interested candidates to erythropoiesis, we established human CD34\(^+\) cell culture system by using human umbilical cord blood. At day 7 of in vitro erythropoiesis, Zfpm-1 were initially up-regulated and then dramatically down-regulated. The Zfpm-1 siRNA treated cells contained 20% more GPA\(^+\) cells than the unrelated siRNA treated cells, and showed reduced expression of the hematopoietic transcription factors, c-myc and c-myb, but did not affect L/LTA-1 expression. Zfpm-1 overexpression was the opposite of loss-of-function results. But, expression of Zfpm-2, which is highly related with Zfpm-1, did not compensate in Zfpm-1 siRNA treated cells or overexpressed cells. These results suggest that the repression of Zfpm-1 expression accelerates erythropoiesis in on in vitro culture system. These authors are supported by the second stage of Brain Korea 21.

**L121**

**Plant Extract R-518 Enhances Wounded Repair via Promoting Angiogenesis**

T. Lee, G. Lee, J. Kim; Graduate School of Biotechnology, Life Sciences & Resources, Yongin, Republic of Korea

Numerous bioactive chemical compounds of plant origin may influence the cell behavior and biological activity of various cell types. In the course of screening of angiogenesis effectors, we demonstrated that methanol extract R-518 from Theacea promoted migration of human umbilical vein endothelial cells using Boyden chamber assays. The extract R-518 also induced endothelial cells proliferation in the ranges of 50 μg/ml without cytotoxicity. Treatment of HUVECs resulted in the activation of the p42/p44 mitogen-activated protein kinase (MAPK, ERK1/2) that was correlated with endothelial cells proliferation and migration. R-518 stimulated angiogenesis in the chick chorioallantoic membrane assay. It was also demonstrated that wounded areas in mice skin were recovered by treatment of R-518, as efficient as epithelial growth factor (EGF) and that the number blood vessels in the wounded area were increased by treatment of R-518. Our results indicate that R-518 extracts may enhance wound healing via promoting proliferation of endothelial cells and angiogenesis.

**L122**

**Role of Phosphatases in the Time-dependent Regulation of Tie2 Activity by Angiopoietin-2**

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**Introduction:** Essential to the processes of blood vessel development, maintenance and repair is the endothelial cell (EC)-selective receptor tyrosine kinase, Tie2, and its ligands angiopoietin (Ang) 1 and 2. While Ang1 is a full agonist that activates the Tie2 receptor, Ang2 is a context-dependent antagonist of Tie2 activation. To characterize the time and phosphatase dependent action of Ang2, human umbilical vein endothelial cells (HUVEC) were treated with Ang1 or Ang2 for varying lengths of time (10 minutes to 24 hrs) before lysis and immunoprecipitation of Tie2. Phosphorylation of the receptor was quantified by Western blot densitometry. Results: Ang1-induced Tie2 phosphorylation was rapid and robust, increasing at 10 minutes to 4.08±1.33 and remaining high for up to 24 hrs (3.50±0.69). In contrast, Ang2-induced activation was delayed and less intense, increasing from 0.38±0.08 at 10 minutes to 2.57±0.50 at 4 hrs and lasting for up to 24 hrs (2.34±0.48). Prolonged exposure (8 to 24 hrs) to Ang1 and Ang2 resulted in a similar degree of Tie2 phosphorylation, indicating a time-dependent regulation of Ang2 activity at the Tie2 receptor. In addition, pretreatment with the phosphatase inhibitor orthovanadate increased Ang2-induced Tie2 phosphorylation at 10 minutes from 0.42±0.08 to 2.88±0.47 (P<0.05), a level similar to Ang1 (2.38±0.51) in the absence of orthovanadate. Furthermore, silencing of the EC-selective phosphatase HTPP1 with siRNA also enhanced an Ang2-induced Tie2 autophosphorylation at 10 minutes. **Conclusion:** Our results demonstrate that the inhibitory activity of Ang2 is regulated in a time-dependent manner and indicate that phosphatases are in part responsible for the antagonistic effects of Ang2 during brief exposure times.
**CANCER**

**L125**

**Adenosine 2,3-dialdehyde (AdOx) Causes Reactivation of p53 and Induction of the G2/M Checkpoint Resulting in Programmed Cell Death in HTLV-1 Transformed Cells**

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Adenosine 2,3-dialdehyde (AdOx) an adenosine analog inhibited Tax transactivation and potently inhibited the growth of HTLV-1 leukemic cells. In this study we looked for novel inhibitors for the ATL are largely ineffective due to the intrinsic resistance of the suppressor activity. Most conventional chemotherapeutic treatments for transformation by deregulating transcription, cell-cycle and tumor suppressor activity. Most conventional chemotherapeutic treatments for ATL are largely ineffective due to the intrinsic resistance of the leukemic cells. In this study we looked for novel inhibitors for the growth of HTLV-1 infected cell lines. Recent results from the lab have shown that co-activated receptor arginine methyltransferase 1 (CARM1) which methylates histone H3 and other proteins is important in regulation of Tax transactivation. Methyltransferase inhibitor, adenosine 2, 3 dialdehyde (AdOx) an adenosine analog inhibited Tax transactivation and potently inhibited the growth of HTLV-1 transformed cells when compared to control lymphocytes. We found that AdOx inhibited Tax expression and Tax activated NF-kB pathway resulting in reactivation of p53 and induction of p53 target genes. Our data further demonstrated that AdOx induced G2/M cell-cycle arrest and apoptotic cell death in HTLV-1 transformed but not control lymphocytes. These findings indicate that AdOx and its derivatives could be used as potential candidates for developing drugs for the treatment of ATL.

**L126**

**Selection Of Agonistic Antibodies to a Receptor Tyrosine Kinase Using Flow Cytometry-based Assays for Detection of Total Tyrosine Phosphorylation and Receptor Internalization**

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To identify agonistic antibodies targeted to one particular RTK of interest, we developed two flow cytometry (FACS)-based assays to monitor downstream effects of receptor activation: (1) total cellular phospho-tyrosine (pY), as a measure of activation of signaling pathways; and (2) receptor internalization, to measure down-regulation of activated receptor. The total cellular pY assay employed a suspension-adapted, stably transfected CHO cell line expressing high levels of the receptor. This assay was used to screen hybridoma supernatants, purified hybridoma-derived antibodies, and purified whole IgG reformatted phage display-derived antibodies. Approximately 24% of the antibodies tested showed agonistic activity in the pY assay. Immunoprecipitation followed by Western analysis was then performed on lysates from antibody-treated cells (RTK over-expressing CHO and tumor cell lines), and the data confirmed that the pY-inducing antibodies triggered phosphorylation of the target RTK. Top candidates identified using the total cellular pY assay were further characterized for cell surface RTK down-regulation and degradation. Epitope competition studies were first conducted using FACS and Biacore to identify strong FACS-positive “detection” antibodies that showed minimal competition with the top pY-inducing antibodies. These antibodies were employed to develop a FACS-based assay monitoring cell surface RTK levels from 2 to 72 hours after addition of pY-inducing antibodies. A subset of the antibodies showed dramatic down-regulation of cell surface receptor by 2 hours that was maintained through 72 hours. Western analysis confirmed that RTK down-regulation observed in the FACS assay was associated with degradation of the RTK protein, and also indicated that the drop in total cellular levels of the RTK persisted for a greater period of time in response to the pY-inducing antibodies than in response to the natural ligand.

**L127**

**Matrix Metalloproteinase-7 Modulates the Transition of Normal Pancreatic Acinar Cells to Metaplastic Ducts via the Notch Pathway**

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Pancreatic ducal adenocarcinoma (PDAC) is a disease that affects approximately 52,000 per year. Even though it affects a small population, it is the fourth leading cause of cancer death in the U.S, demonstrating the lowest five-year survival rate of any cancer. Late diagnosis results in resistance to conventional treatments, making pancreatic cancer a pressing disease requiring new drug development. An important observation made in cases of PDAC is the consistent presence of putative preneoplastic lesions, known as metaplastic ducts. Here we found that matrix metalloproteinase-7 (MMP-7) is necessary and sufficient to convert normal pancreatic acinar cells to metaplastic ducts. As its name implies, MMP-7 degrades components of the extracellular matrix, but its contributions to tumor development are more commonly associated with release of bioactive molecules from the cell surface. Notch receptors and its ligands have been shown to be up-regulated in PDAC. Like MMP-7, Notch is sufficient to cause acinar-to-ductal metaplasia and remains active throughout PDAC progression. We show that an active Notch pathway is required for this transition. When a pharmacological inhibitor of Notch is added to the acinar cells, metaplastic duct formation is blocked. Notch activation requires extracellular processing by a metalloproteinase, supporting our hypothesis that MMP-7 directly cleaves Notch leading to its activation. Here we show Notch processing by MMP-7 using an in vitro cleavage assay. These data suggest both MMP-7 and Notch activity are critical for early pancreatic cancer development.

**L128**

**Evaluation of Cytotoxicity and Protective Effects against UV Radiation of Two Flavonoids: Chrysin and Quercitin**

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Studies show that flavonoids, compounds commonly found in vegetables, have protective activity against DNA-reactive environmental carcinogens. One flavonoid, chrysin (CH), is considered a potent chemopreventive agent against aflatoxinB1 and benzo(a)pyrene-induced mutagenicity. Another flavonoid, quercitin (QC), protects skin against UVA irradiating damage in rats, but it is found to induce DNA damage by prooxidative effects. Due to the usual intake of flavonoids by human beings, the present work aimed to compare the cytotoxic and genotoxic effects of QC and CH and their protective action against UV radiation on normal and tumor cell lines. Cell viability assay showed that both flavonoids reached IC50 just on the tumor cell line HTC. The minimum concentration to cell growth inhibition was 5.0μg/mL to CH and 16.0μg/mL to QC. To evaluate the genotoxic effects of the flavonoids, the micronucleus assay was performed in MDCK cell line. QC did not alter the frequency of micronuclei. On the other hand, CH increased the frequency of micronuclei from 3.67% in...
control cells to 25.67% in treated cell at 5.0 g/mL. Due to the low cytotoxicity of QC, we evaluated its protective potential against DNA damage caused by UV radiation in BRL3A cells. MTT assay revealed pronounced protective effect in BRL3A cells after irradiation for one minute and treatment for 48 hours. The concentration of 1.6 g/mL had the best effect since it showed low cytotoxicity (4.0%) and great protective effect (400.0%) on BRL3A cells and high cytotoxicity (30.0%) and none protective effect on HTC cells. The CH genotoxic activity observed in normal cells suggest that its protective effects against aflatoxin B1., and benzo(a)pyrene-induced mutagenicity should be further evaluated. On the other hand, QC presented a good potential in controlling tumor cell growth and in protecting normal cells against damages caused by UV.

### Quantitative Proteomics of Formalin Fixed Archival Tissue Using Mass Spectrometry

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Expression Pathology has developed methods and protocols for high resolution mass spectrometry-based proteomic profiling of formalin fixed paraffin embedded tissue. Using Liquid Tissue® reagents and protocols, we have been able to profile and validate known and novel cancer biomarkers across a wide variety of archival cancer tissue samples. Recent work includes quantitation of protein expression in formalin fixed oral cavity cancer tissue and pancreatic precursor lesions using the spectral count analysis method. Current work is focused on a novel mass spectrometry-based quantitative assay for Her2 in archival breast cancer tissue by the AQUA method. Diagnostic detection and quantification of Her2 in cancerous tissue is today routinely carried out by immunohistochemistry (IHC); however, IHC lacks sensitivity and relies on subjective data analysis. Using a stable isotope standard (SIS) derived from Her2 we have developed a method for direct detection and absolute quantitation by selected reaction monitoring (SRM) of Her2 directly in formalin fixed paraffin embedded breast cancer tissue. Protein extracts from cells microdissected from a collection of breast cancer tissues known to express a range of Her2 were prepared using Liquid Tissue® reagents (a series of reagents used to solubilize and extract the protein content in FFPE tissue), and the level of Her2 in each sample was determined. The results indicate the ability to quantitate Her2 expression in extracts from fixed tissue sections and they also demonstrate the ability to quantify Her2 in IHC negative cell lines. Results will be discussed within the context of a novel, highly sensitive technology designed to impart absolute quantitation of cancer protein biomarkers in formalin fixed cancer tissue.

### Sensitivity Detection of DNA Double Strand Breaks Induced by Low Doses of Ionizing Radiation

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This study investigates the level of DNA damage induced in healthy tissue proximal and distal to the cancerous tumor during Intensity Modulated Radiation Therapy (IMRT). IMRT is becoming the radiation treatment modality of choice because it allows for the escalation of the photon dose to the tumor while reducing the exposure to normal tissue. However, in order to achieve this higher dose, the accelerator must be energized for a longer period during a daily IMRT treatment. This increases the amount of exposure to leakage and scattered radiation for healthy tissue outside the treatment field, thus increasing the risk of radiation-induced secondary malignancies. It has been estimated that IMRT could double the incidence of secondary malignancies compared to conventional radiation therapy. To induce DNA damage, we will irradiate normal human diploid skin fibroblasts (HSF-55 cells) both within and at points peripheral and distal to the treatment field using 6 MV X-rays from a linear accelerator. Data from preliminary studies show that there are a significant number of DNA double strand breaks (DSBs) induced at a distance of 10 cm from the treatment field. To assess the level of radiation-induced DNA damage, we will use the Comet Assay and immunofluorescent staining of γH2AX. Our data indicate that the Comet Assay is not sensitive enough to detect damage induced by the very low levels of radiation present outside the treatment field during radiotherapy. The histone protein, H2AX, becomes phosphorylated in response to DNA damage and binds to DSBs. Immunofluorescent methods can be used to detect phosphorylated H2AX (γH2AX), and we are currently using flow cytometry to measure levels of DSBs by γH2AX detection.

### Hyperphosphorylation Prevents the Binding of Tau to the Golgi Membranes

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The neuronal microtubule-associated protein tau is known to stabilize the axonal microtubules. We recently reported that tau was enriched in a subfraction containing Golgi membranes (I2) isolated from rat brain (Cell Mol. Cyto. ’06). In an in vitro reconstitution assay, we showed that tau could act as a linker between Golgi membranes and microtubules. Phosphorylation regulates tau binding to microtubules. Here, we examined whether the association of tau with the Golgi membranes was regulated by phosphorylation. In Alzheimer disease (AD), tau becomes hyperphosphorylated and aggregates into insoluble filaments. Our subfractionation protocol was applied to AD and control brain. In AD brain, hyperphosphorylated tau was not detected in the subfraction enriched in Golgi markers but was rather found in the subfraction containing endoplasmic reticulum membranes (RM). Immunogold labeling revealed that nonfilamentous and filamentous tau were found to vesicles at the RM subfraction. The phosphorylated distribution of tau was also examined in JNPL3 mice overexpressing the mutant form of tau, P301L. In JNPL3 mice, tau was found in the Golgi subfraction but was also present in the RM subfraction. The phosphorylation state of tau was higher in the RM subfraction than in the Golgi subfraction. Collectively, the above results indicate that phosphorylation decreases the binding of tau to the Golgi membranes and favors its association with ER membranes. We lastly examined whether the redistribution of membranous tau was accompanied by the redistribution of other membranous proteins such as amyloid precursor protein (APP) linked to AD. In control mice, APP was found in the RM subfraction. In JNPL3 mice, in addition to its presence in the RM subfraction, an important amount of APP was detected in the I2 subfraction containing Golgi membranes. This redistribution of APP might be caused by a perturbation of its trafficking as suggested in neuronal cultures overexpressing tau.

### Skeletal Architecture and GPI-anchored Protein Complex in Neuronal Progenitor Cell as Revealed by Electron Structural Analysis

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In central nervous system (CNS), the molecular architecture of the skeletal filament as spectrin, actin, gial fibrillary acidic protein (GFAP), tubulin and the membrane protein-complex has an indispensable role to success for good health. The 14-3-3 protein, which is abundantly expressed in the CNS, recently detected in the cerebrospinal fluid of Creutzfeldt-Jacob disease (CJD) and used as a biochemical maker in the diagnosis of CJD. Currently, we have examined the cellular prion protein (PrPC), a glycophosphatidyl-inositol (GPI)-anchored protein, in the human neuronal progenitor cell line, by using of the structural analysis containing the conventional immunological electron microscopy and computed tomography (CT). The PrPC have known to localize on the external surface of the plasma membrane and the invagination like caveolea and clathrin-coated pits in some kinds of fibroblastoid cells. In our recent study, we showed that the PrPC could localize on the mitochondria membrane in the cytoplasm of the neuronal progenitor cell. The immunological labeling against the 14-3-3 protein was also detected on the mitochondria, which could construct a molecular complex with the PrPC in the human CNS under physiological conditions.
conditions. It was suggested that the mitochondria, which associated with the microtubule and some cytoskeleton, could coordinate the migration of the protein complex in the cytoplasm. The PrPC/14-3-3 protein complex on the mitochondria membrane might have a correlation with the conformation change of the PrPC in the pathology of CJD. These findings are consistent well with the previous biochemical analysis as protein overlay [Satoh et al., 2005].

L133 Ubiquitination Modulates Activity of the Parkinson’s Disease-associated Ubiquitin Hydrolase UCH-L1

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Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is a small deubiquitinating enzyme with unknown substrates and poorly defined cellular activities. Its high expression level in brain (1-2% of total protein) and association with cancers and neurodegenerative diseases suggest a critical role in cell function. Accordingly, recent studies suggest that UCH-L1 contributes to the regulation of apoptosis and modulation of free ubiquitin levels in neurons. However, little is known about the regulation of UCH-L1 activity itself. Several regulatory post-translational modifications target UCH-L1, including glycosylation and farnesylation. We have identified a ubiquitinated species of UCH-L1 and characterized the role of ubiquitination in the regulation of UCH-L1 activity using cell culture and in vitro biochemical techniques. Our results demonstrate that post-translational modification by ubiquitin alters enzymatic and cellular activities of UCH-L1 and may affect disease pathogenesis.

L134 Seizure Protection by Delphinium specie

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Epilepsy is a chronic neurological disorder effecting 50 million people globally. The available anticonvulsant only suppresses the seizure but do not cure the underlying cause of epilepsy, therefore search for better drug is going on. As an example, the epilepsy is in progress. Natural product based medicines provides better cure with minimal side effects. The Delphinium specie traditionally reported as anticonvulsant and selected in the present study for the routine anticonvulsant screening. Using the acute severe models the isolated fraction of Delphinium were tested in male mice. The animals groups (six each) were injected three different doses of DNS II (acetone fraction) i.e 60mg/kg, 65mg/kg and 70mg/kg respectively. We have identified a ubiquitinated species of UCH-L1 (3.15 mg/kg), animal were observed for the seizure development induced by sc PTZ and scPicrotoxin, but none of the animal of used three doses Whereas the positive control groups i.e Diazepam (7.5 mg/kg i.p.) also prevented the picrotoxin induced seizures but Phenytin (20 mg/kg, i.p.) group 50% animals showed the seizures. Based on these finding we may conclude that the isolated fraction of Delphinium may possess active anticonvulsant constituent that may be helpful in treating the partial seizure, absence seizure and generalized tonic convulsion.

OTHER DISEASES

L135 Baicalin Inhibits IGF-II-induced VEGF and HIF-1α Expressions in HaCaT Cells


Baicalin is major compound isolated from Scutellaria baikalensis. In this study, we demonstrated the inhibitory effect of IGF-II-induced HIF-1α and VEGF expression by baicalin in keratinocyte HaCaT cells. Cytotoxicity of baicalin in HaCaT cells was examined by MTS assay. We used RT-PCR for the VEGF mRNA levels and used Immunoblotting for the study specific protein expression in HaCaT cells. Our results showed that baicalin significantly down-regulated the expressions of IGF-II-induced HIF-1α protein levels in HaCaT cells. Reduction of HIF-1α protein by baicalin resulted in a decrease in VEGF mRNA and protein expression levels. The mechanisms of baicalin inhibition of IGF-II-induced protein level of HIF-1α via ERK1/2 pathway and AKT pathway. These results suggest that the baicalin is reduced in IGF-II-mediated HIF-1α and VEGF expressions via ERK1/2 and AKT pathways.

L136 The Study of the Effects on Mammalian Cells by Naphthalene Exposure

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The naphthalene is a bicyclic aromatic hydrocarbon that is widely used commercially in moth repellents, livery scent discs and soil fumigants. The naphthalene causes lens opacification (cataracts) and histopathological changes. The naphthalene decreased cell proliferation in diverse organ cells. The naphthalene effectively induces apoptosis in 293T cells in a cell-specific manner. Also, we studied to find the cellular factors that effect the apoptosis. So, we found that biomarkers candidates of the naphthalene signal transduction by ACP-based PCR method using the GeneFishing DEG kits. As a result, we found 16 candidates. Also, we reconfirm the specifically down- or up-regulates 16 candidates mRNA levels by RT-PCR and Real Time PCR. So, we are studying to prove the effect of 16 candidates by cloning. We analyzed the apoptosis signaling pathway by western blotting.

L137 Scutellaria baikalensis Extracts Inhibit IGF-II-induced VEGF and HIF-1α Expressions in HaCaT Cells

H. J. Jo, M. H. Hong, Y. C. Shin, J. H. Park, C. Y. Jun, S. G. Ko; 1Biological Science, Sookmyung Women's University, Seoul, Republic of Korea, 2Korea Food Research Institute, Seoul, Republic of Korea

In previous study for the routine anticonvulsant screening. Using the acute severe models the isolated fraction of Delphinium were tested in male mice. The animals groups (six each) were injected three different doses of DNS II (acetone fraction) i.e 60mg/kg, 65mg/kg and 70mg/kg respectively. We have identified a ubiquitinated species of UCH-L1 (3.15 mg/kg), animal were observed for the seizure development induced by sc PTZ and scPicrotoxin, but none of the animal of used three doses Whereas the positive control groups i.e Diazepam (7.5 mg/kg i.p.) also prevented the picrotoxin induced seizures but Phenytin (20 mg/kg, i.p.) group 50% animals showed the seizures. Based on these finding we may conclude that the isolated fraction of Delphinium may possess active anticonvulsant constituent that may be helpful in treating the partial seizure, absence seizure and generalized tonic convulsion.

L138 Inversin Is Dependent on Bbs4 for Basal Body and Cilia Localization

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Bardet-Biedl Syndrome (BBS) is a largely autosomal recessive, pleiotropic disorder, involving retinal degeneration, cistic kidneys and situs inversus. These pathologies are also present in Nephropithiasis (NPHP). This overlap of disease features has been linked recently by one overlap and others to ciliary dysfunction in these conditions. So far, 11 different genes have been found to be mutated in BBS (BBS 1 - 11), all coding for proteins of unknown function (Bbs 1 - 11). Similarly,
Prostaglandin E2 from LPS-activated Raw 264.7 Cells, Reduces Plant Extract SKE Suppresses Production of Nitric Oxide, Fructus might be of therapeutic value in treating asthma. Suppression of the expression of eotaxin can be accomplished by cells. In addition, eosinophil migration caused significant inhibition αeotaxin expression upon stimulation with TNF-α. When the A549 cells had been prestimulated with TNF-α demonstrated whether Schizandrae Fructus affects eosinophil chemoattractant that is mobilized in the respiratory epithelial cells. Eotaxin is a potent and specific eosinophil accumulation at sites of allergic inflammation is largely hall mark of apoptosis by exposure of the PCBs in 293T cell. Also, we dispersed environmental pollutants and shows its toxic effects including neurotoxicity, hepatotoxicity, carcinogenicity, immunotoxicity and cardiotoxicity. These toxicities depend on the chemical structure of congeners, each of which is chlorinated to various degrees. The PCBs causes abnormalities in the developing central nervous system. The PCBs treatment decreased cell proliferation in diverse organ cells. So, we investigated DNA fragmentation which is a hall mark of apoptosis by exposure of the PCBs in 293T cell. Also, we studied to find the cellular factors that effect the apoptosis. As a result, we found that biomarker candidates of PCBs by two dimensional electrophoresis and Maldi-ToF analysis.

L139
The Study of the Effects on Mammalian Cell by PCBs Exposure
J. An, J. Youm, J. Seol, M. Yoo, S. Choi; Biological Science, Sookmyung Women's University, Seoul, Republic of Korea
The polychlorinated biphenyls (PCBs, Aroclor 1254) is widely dispersed environmental pollutants and shows its toxic effects including neurotoxicity, hepatotoxicity, carcinogenicity, immunotoxicity and cardiotoxicity. These toxicities depend on the chemical structure of congeners, each of which is chlorinated to various degrees. The PCBs causes abnormalities in the developing central nervous system. The PCBs treatment decreased cell proliferation in diverse organ cells. So, we investigated DNA fragmentation which is a hall mark of apoptosis by exposure of the PCBs in 293T cell. Also, we studied to find the cellular factors that effect the apoptosis. As a result, we found that biomarker candidates of PCBs by two dimensional electrophoresis and Maldi-ToF analysis.

L140
Eotaxin Inhibition Effect of Schizandrae Fructus in Cytokine-induced A549 Human Epithelial Cells
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Eosinophils accumulation at sites of allergic inflammation is largely regulated by chemokines and lipid mediators released by a variety of cells of the local microenvironment. Eotaxin is a potent and specific eosinophil chemoattractant that is mobilized in the respiratory epithelium after allergic stimulation. In this study the author demonstrated whether Schizandrae Fructus affects eosinophil chemotaxis and changes adhesion molecules in cytokine-induced A549 epithelial cells. When the A549 cells had been treated with Eotaxin (50ng/ml), IL-4 (50ng/ml) and IL-1β (10ng/ml), both eotaxin and other chemokines were costimulated. Schizandrae Fructus reduced eotaxin expression upon stimulation with TNF-α and IL-4 on A549 cells. In addition, eosinophil migration caused significant inhibition upon Schizandrae Fructus treatments. These findings indicate that the suppression of the expression of eotaxin can be accomplished by Schizandrae Fructus treatment, raising the possibility Schizandrae Fructus might be of therapeutic value in treating asthma.

L141
Plant Extract SKE Suppresses Production of Nitric Oxide, Prostaglandin E2 from LPS-activated Raw 264.7 Cells, Reduces Collagen-induced Arthritis in Mice and Osteoclast Differentiation
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2Dentistry, Seoul, Republic of Korea
Several plants and herbs have long been used to treat folk medicine in oriental countries. In this study, we aimed to screen extracts from plants for potential application to anti-inflammatory effect and to prevention of osteoporosis. We demonstrate that the plant extract SKE is a potent inhibitor of NF-κB, and also reduces release from macrophage RAW 264.7 cells. We investigated the effect of SKE on signaling pathways and found that SKE inhibited the LPS-induced phosphorylation of IkB and nuclear translocation of NF-κB p65 protein levels in the nucleus. Further investigation of the pathways revealed that SKE reduced levels of phospho-JNK and phospho-ERK in macrophage cells. SKE also showed inhibitory effects on osteoclast differentiation without cytotoxicity. We examined the effect of SKE on osteoclast signaling pathways, and found that SKE reduced levels of NFATc1 and c-Fos, crucial transcriptional regulators for osteoclastogenesis. SKE also downregulated mRNA levels of c-Fos and NFATc1 in RANKL-stimulated BMMs. In addition, the effect of SKE to regulate the RANKL-stimulated transcriptional induction of c-Fos was found to depend on p38 and ERK signals leading to the phosphorylation of CREB via the intermediate kinase MSK1. Finally, SKE showed great inhibitory effects on arthritis symptoms in a collagen-induced arthritis mouse model and on LPS-induced bone resorption in mouse model. Taken together, we demonstrated that SKE suppressed LPS-induced iNOS and COX-2 expression by blocking NF-κB activation and that it also strongly inhibited bone resorption in vitro and in vivo by blocking osteoclast differentiation signal. Our results suggest that the SKE extracts may be beneficial for prevention and treatment of inflammatory diseases and osteoporosis.

L142
Role of E-cadherin Endocytosis in the Internalisation of Listeria monocytogenes
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Listeria monocytogenes is a Gram positive invasive bacteria responsible for food-borne infections that affects immunocompromised subjects, pregnant women and newborns. Listeriosis results in gastroenteritis, fetoplacental and central nervous system infections. This wide range of symptoms is due to the ability of Listeria to cross multiple barriers during infections such as the intestinal, the blood-brain and the placental ones. Internalin (InlA) and Internalin B (InlB) are the main responsible for the internalisation of Listeria in host cells among bacterial surface proteins. Infections mediated by either of the two proteins follow two independent and distinctive pathways that depend on E-cadherin and on Met receptor signalling cascades respectively. It has been described how InlA interacts with E-cadherin to initiate the internalisation of this complex within host cells. Here we try to apply the knowledge on the dynamics of the adherence junction to bacterial infection in order to characterise the signalling cascade that mediates bacterial internalisation. Endocytosis of E-cadherin occurs upon signalling through ubiquitination by the ubiquitin-ligase Hakai. E-cadherin is then internalised via clathrin coated vesicles and destined either to recycling or degradation. During InlA-mediated Listeria infection Hakai is recruited at the bacterial entry site and its activity is required for efficient cell invasion. Following Hakai recruitment ubiquitinated proteins are recruited at the bacterial entry site and in parallel purified InlA stimulates the ubiquitination of E-cadherin. In addition clathrin and dynamin are present in the bacterial entry site whereas the role of p120 catenin, a protein responsible for the protection of E-cadherin from internalisation, is currently under investigation. These evidences suggest that Listeria hijacks the E-cadherin internalisation pathway described for the disruption of adherens junctions.

L143
Multistem™ (Multipotent Adult Progenitor Cells) Are Non-immunogenic and Display Immunosuppressive Properties on Activated T Cells
M. Kovacsics-Bankowski1, P. Streeter1, W. J. Van't Hof2, R. Deans2, R. Maziarz2; 1Hematologic Malignancies, OHSU, Portland, OR, 2Regenerative Medicine, Athersys, Inc., Cleveland, OH
Multistem™ are non-embryonic stem cells capable of differentiating in vitro and in vivo into mesodermal, endodermal, and ectodermal cell lineages (including neuronal lineages). (Jiang Y et al, Nature, 2002, 418:41-49). This capacity to differentiate into these diverse cell types distinguishes MultiStem from Mesenchymal Stem Cells (MSC), and suggests that these pluripotent adult stem cells may therefore be an ideal cell for in vivo therapies for tissue repair or regeneration in multiple organ systems. In support of this, in several preclinical disease models, these cells have demonstrated the potential for substantial therapeutic benefit in a variety of human diseases. Still, to optimize in vivo utilization, the understanding of immunologic properties of MultiStem is critical. PGE2, released in response to inflammatory cytokines, plays a role in PGE2-induced suppression of the immune response. As such, we hypothesize that these cells may represent an immunologically favorable scaffold for application in vivo. To investigate the immunologic properties of MultiStem, we have previously used a mouse model to demonstrate that these cells are not immunogenic, i.e., they do not stimulate immune responses. In this study we characterized the immunosuppressive potential of MultiStem in rat multi-lineage cultures. We report here that MultiStem releases significant levels of TGF-β and IL-10, cytokine profiles consistent with the immunosuppression and anti-inflammatory effects in vivo that we and others have previously described. These findings provide further evidence that human MultiStem is an immunologically favorable platform for cell-based therapy.
strains did not stimulate allogeneic T cells proliferation, while splenocytes of the same rat strains elicited strong proliferative responses in a mixed lymphocytic culture. Second, these pluripotent stem cells displayed immunesuppressive properties. Addition of stem cells at the initiation of a mixed lymphocyte reaction (MLR) suppressed T cell proliferation in a dose dependent manner. The inhibition was detectable when number as low as 3,000 stem cells/well where added to 1x10^5 T cell responder. Lymphocyte proliferation in stem cell-containing cultures was inhibited by up to 80% when compared to cultures without stem cells. The ability to inhibit a T cell alloresponse was independent of the MHC, predicting the use of third party stem cells as inhibitory cells. Taken together, these data indicate that MultiStem are non-immunogenic to T cells, suggesting that universal MultiStem donors may be used for tissue repair or regeneration. MultiStem exhibit potent immunesuppressive properties suggesting that these cells may be useful in the management and/or prevention of GVHD or other inflammatory pathologies.

L144
The Implant of Mononucleares Bone Marrow Cells (Stems Cells) after the AMI Improve and Maintain the Ventricular Function during Long Time
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Objectives: To evaluate the long time performance of the Left Ventricular function of Acute Myocardial Infarction treated with Stems cells implant. Material and Method: 32 patients that suffered AMI were treated with primary PTCA with Stents. The Ventriculography Fraction Ejection oscillated between the 21 and 32%. Between the 7 and 12 days after AMI were implanted the artery and with occlusion of the Coronary Sinus Veined, autologous mononucleares cells CD 34(+) and CD38 (-) in a quantity average of 22x10^6.

Results: It was done after 180 days a coronaryography and ventriculography and were observed improvements of all the Stents, in the improvement of the FEj. Among of them 76% in all the patients in relationship to the basal FEj. The patients were controlled during one period up to 2 years and its was verified by echocardiogram that deterioration of the contractile function didn't take place and there was not MACE, existing a single non related death. This group was compared with a Control Group of 26 patients that suffered a similarly characteristics AMI and that were subjected to the implant of a balloon PTCA and Stent and it was observed that the FEj of 35% in relationship with the basal FEj. 180 days after the acute episode it was observed an improvement of the restenosis of the Stents and in a years period after, episodes of MACE were verified in 32% of the patients and a mortality of 3 patients (1,10%) and it was verified a lost of the FEj. In 16% in relationship of the control Ventriculography done after 180 days. Conclusions: The implant of Mononucleares bone marrow Cells (Stems Cells) after the AMI improve the performance of the VI which is maintained in the time (2 years) and it seems to maintain the Coronary Stent restenosis at least in immediate form.

L145
The Genetic Analysis of Hepatitis C Virus Isolates Obtained from 70 Korean Blood Donators
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Hepatitis C virus (HCV) is a major factor of chronic hepatitis, is a family of Flaviviridae, enveloped RNA virus, and has a positive-strand. It has been classified into six classes by high genetic variability. Classification of HCV is based on phylogenetic analysis of the strains reported internationally. It is also reported that HCV may cause to the development of liver cirrhosis and hepatocellular carcinoma. Nearly 170 million persons are infected by HCV worldwide. In Korea, type 1b is most abundant and 2a is secondarily abundant. We analyzed 70 blood samples obtained from HCV carriers in Korea. Sixty-four HCV positive sera were offered from Red Cross and 6 were Gil Hospital in Incheon. All of these samples were tested by ELISA assay. 17 samples have low titer and 11 samples have high titer. We performed reverse transcriptase and hemi-nested polymerase chain reaction assays targeting the specific region of viral genome. In the RT-PCR result, unexpected 400 and 900 base pair bands were confirmed. Unknown genotypes were interpreted by selective cloning on X-gal and CDNA sequencing. These data suggest some variants of HCV are existed in Korea.
24.6 pg/ml with low sensitivity (50 %) and high specificity (90 %), whereas the cutoff value for urinary PDGF was ≥ 2.2 pg/mg creatinine with high sensitivity (100 %) and specificity (100%). The cutoff value for serum MCP-1 was ≥ 73.3 pg/ml creatinine with moderate sensitivity (73.3 %) and specificity (60 %), whereas the cutoff value for urinary MCP-1 was ≥ 76.7 pg/mg creatinine with moderate sensitivity (76.7 %) and high specificity (100 %). Ultrastructural studies of platelets revealed misshaping or absence of a granules with released internal contents and distension of surface connected canaliculer system in 100% of patients as well as absence of dense bodies in 50% of them. In conclusion, urinary PDGF and MCP-1 are sensitive markers in diagnosis and monitoring the diabetic nephrophy disease activity. So, the development of PDGF receptor is therefore highly warranted.

IMAGING TECHNOLOGY

L148
Semiconductor Quantum Dots for Probing Endocytosis, Molecular Motors, and Protein Translocation in Living Cells
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Due to their unique optical properties, semiconductor quantum dots (QDs) have emerged as a novel class of fluorescent probes for cellular imaging as well as other biomedical applications. However, to date few successful applications of QDs to probe intracellular events in living cells have been reported. We have conducted a systematic investigation on applying QDs for intracellular imaging. Using QD-Tat as a probe for endocytosis, we find that QDs are capable of monitoring molecular motors, as well as the QDs translocate through the membrane. Single QDs would also indicate that the QDs are free from endosome trapping. Streptolysin O toxin and microinjection were found to be able to deliver single QDs into cells. Part of the single QDs was associated with cellular motors. We found that spectrin is likely to be responsible for this association. This association also allowed us to image the dynamics of single motor proteins in real time. Furthermore, preliminary results showed that the part of the single QDs that were free from non-specific binding could be used to label protein kinase C alpha, which translocates from cytoplasm to plasma membrane and perinuclear upon stimulation. A novel labeling strategy based on targeting genetically-engineered tags of proteins with QD bioconjugates was used in the protein kinase C alpha targeting. In conclusion, our work not only paves the way for visualizing intracellular events at single molecule level, but provides guidance for designing other types of nanoparticles for intracellular applications.

L149
A High-Throughput Two-Color Mitotic Index Assay Using the IsoCye™ Laser Scanning Platform
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High throughput assays for adherent cells that quantify the number of cells in specific stages of the cell cycle are important for drug discovery efforts. Here we used paclitaxel (Taxol™) as a model drug treatment that prevents mitotic spindle assembly resulting in a late G2/M block. The percent of cells in mitosis (mitotic index) was determined with an assay that used a mitotic marker antibody to detect the phosphorylation of Ser10 of histone H3 (phospho-H3). An Alexa fluor-488 (AF488) labeled secondary antibody detected the primary antibody and propidium iodide (PI) was used to identify all nuclei. The IsoCye™ laser scanning platform (Blueshift Biotechnologies, Inc.) was configured with a 488 nm laser; AF488 fluorescence was measured in the green channel and the PI fluorescence in the red channel. Whole well images were acquired using an integrated image acquisition and analysis software module for the quantitation of mitotic cells. HeLa cells were seeded at 2500 cells/well in 96-well plates and cultured overnight. The medium was removed and dose-response treatments were done with 4 or 17 hour exposure to paclitaxel. After paclitaxel treatment, the cells were fixed, permeabilized, immunostained, and the nuclei were stained by PI/RNase A treatment. Whole well images were acquired on a confocal basis by identifying cells in the red channel and using the background corrected integrated fluorescence intensity for the green and red channels. Mitotic cells were characterized by their green/red intensity ratio. Cell-by-cell scatter plots showed excellent discrimination between the mitotic and non-mitotic cells. HeLa cells treated with paclitaxel showed a dose-dependent increase in the mitotic index as well as a time-dependent decrease in the number of cells per well. The scan & analysis time for this assay was measured at four minutes for either 96 or 384-well plates making it well-suited for a high throughput screen.

L150
Membrane Geometry and Forces
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Recent advances in cryo-electron tomography have made possible reconstructions of subcellular structures. In particular, tomography can be exploited to capture detailed information about complex membrane conformations. We propose that the observed membrane shapes may be modeled as an elastic material under external forces. Using results derived from a Helfrich elasticity model, we can determine the forces acting on a membrane directly from the observed shape. We compare these results with in vitro experiments performed on synthetic vesicles, where we apply known forces with optical tweezers. The outlook for applying these ideas to in vivo data from tomograms is also discussed.

L151
FRET and Multicolor Labeling Applications of eDHFR-fusion Proteins Labeled with Fluorescent TMP
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Due to limitations of fluorescent proteins, there has been efforts to develop small molecule fluorescent tags that will selectively label proteins in vivo. To this end trimethoprim (TMP) and E.coli DHFR (eDHFR) have been developed as a ligand-receptor pair for in vivo imaging (1). The TMP:eDHFR pair is particularly advantageous because the interaction is orthogonal to mammalian systems. When added to cell culture medium, fluorescent TMP derivatives bind selectively to eDHFR fusion proteins. TMP binds with a higher affinity to eDHFR (Ki = 10^9 M) than to mammalian forms of DHFR (Ki ≥ 10^6 M). Thus, the use of TMP:eDHFR does not require a knock-out or modified cell line. Here, we show that eDHFR fusion proteins labeled with TMP-Hexachlorofluorescein (HEX) complement green fluorescent protein (GFP) in two-color labeling and fluorescence resonance energy transfer (FRET) experiments in vivo (2). U2OS cells were co-transfected with DNA encoding nuclear localized GFP and plasma membrane localized eDHFR and labeled with TMP-HEX in two-color labeling experiments. The spectrally and spatially distinct fusion proteins were easily imaged simultaneously. To demonstrate the use of eDHFR fusion proteins in FRET applications, a fusion between GFP and eDHFR (GFP-eDHFR) was constructed. The GFP served as the donor (with excitation at 488 nm) and TMP-HEX-labeled eDHFR was the acceptor for intramolecular FRET. Labeling of pGFP-eDHFR expressed in U2OS cells with 2 µM HEX for 2 hours led to a 40% decrease in GFP donor emission at 510-540 nm, indicating FRET between GFP and HEX. Addition of 100 µM unlabeled TMP to the culture medium partially reversed the decrease in GFP emission. Furthermore, the initial decrease was due to FRET. FRET between GFP and HEX was also measured by the change in anisotropy of HEX after labeling of pGFP-DHFR protein.

L152
Fluorescence Methods for Tracking Adipocyte Differentiation
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Adipogenesis is an important step in several diseases such as obesity, diabetes, atherosclerosis, and steatosis (Gregoire et al, 1998). Stem cell research is another area that examines the ability of multipotent progenitors to undergo phenotypic changes upon differentiation. Researchers use in situ methods such as Fluorescently-labeled T34L fibroblasts and human mesenchymal stem cells to study adipogenic pathways as part of an effort to understand and regulate these complex phenomena. The
standard reagent for monitoring neutral lipid accumulation during adipogenesis in situ has been Nile red (Greenspan et al, 1985) but this dye has significant drawbacks. Nile red labels both phospholipids and neutral lipids and has a broad emission spectrum, compromising the specificity of adipocyte detection and hampering multiparametric analyses. The objective of this study was to evaluate LipidTOX™ neutral lipid stains, originally developed as tools for toxicological profiling of new drugs in HCS applications, for their efficacy in labeling adipocytes. Mouse 3T3-L1 fibroblasts and human mesenchymal stem cells were differentiated, labeled, and visualized either live or after fixation. To confirm the adipocyte phenotype, fixed and permeabilized cells were also labeled with an antibody against FABP4 and the observed neutral lipid staining correlated very strongly with this marker. Our results indicated that LipidTOX dyes were specific for neutral lipids, produced high signal to noise labeling of adipocytes, and were suitable for multiparametric analysis, directly addressing the limitations of current approaches in tracking adipogenesis.

L153
Determining Protein Number and Aggregation from High Resolution Images
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We developed a new tool for mapping the average number of fluorescent proteins and aggregation state from fluorescent images. The variations in fluorescence intensity at each pixel in an image time series were analyzed using the moment analysis. In solution, the average number of molecules <N> in a given focal volume is obtained from the average (first moment) and the variance (second moment) of the intensity. Calibration with a monomeric protein allows absolute numbers of proteins and their aggregation state to be reported. The moment analysis assumes that fluctuations are caused only by changes in the number of the fluorescent particles in a given volume. However, in cells the intensity at a pixel may be due to a combination of fluorescence from intense immobile structures, dim fast diffusing particles, and autofluorescence. If it is assumed that all sources of variance in the intensity are independent, then the overall variance for a pixel is simply the sum of the variance of each of the above components plus the variance of the detector. The variance due to the number fluctuations is proportional to the square of the particle fluorescence, while the variance of the immobile fraction and auto fluorescence is proportional to the intensity of these components. By changing the excitation intensity, we distinguish among these contributions. Based on this new analysis we present maps of the molecular brightness and number of particles of cell migration related proteins using TIRF imaging and one and two-photon scanning microscopy. The analysis reveals an interesting heterogeneity among adhesions across the cell that reflects their origin and fates during cell migration. Supported by U54 GM064346 Cell Migration Consortium and NIH-P41 P41-RRO3155 grants.
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