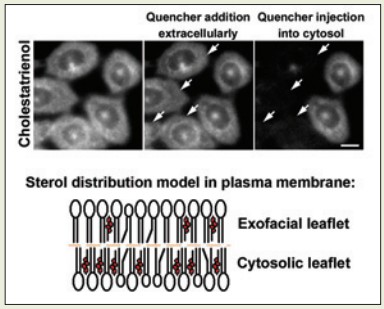
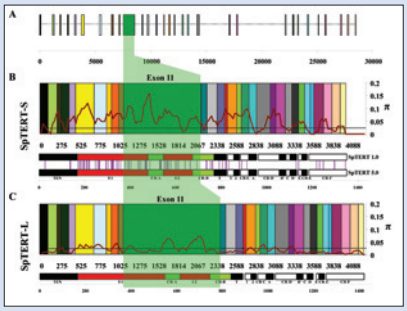
Sterols Are Mainly in the Cytoplasmic Leaflet of the Plasma Membrane and the Endocytic Recycling Compartment in CHO Cells

Mousumi Mondal, Bruno Mesmin, Sushmita Mukherjee, and Frederick R. Maxfield



Transbilayer asymmetry is a general feature of most lipids in the plasma membrane and other post–endoplasmic reticulum organelles. This asymmetry has important consequences for membrane physical properties and cell signaling. Although cholesterol is a major lipid in these membranes, its transbilayer distribution is not well understood. Using fluorescent sterols (dehydroergosterol and cholestatrienol) and a variety of fluorescence quenchers, the authors determined that the majority of sterol is in the cytoplasmic leaflet of the plasma membrane and endocytic recycling compartment of CHO cells. Quenchers that are restricted to the exofacial leaflet of the plasma membrane reduce the fluorescence intensity by about 20%–30%, whereas microinjection of quenchers into the cytosol quenched the fluorescent sterols associated with the plasma membrane and endocytic recycling compartment by about 60%. The presence of high amounts of cholesterol in the cytoplasmic leaflet might have important implications for intracellular cholesterol transport and for membrane domain formation.



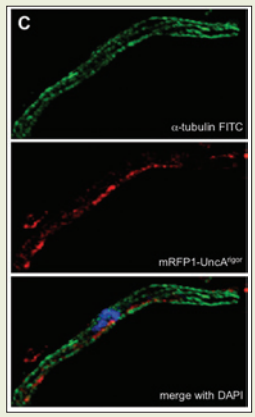
Genetic Hypervariability in Two Distinct Deuterostome Telomerase Reverse Transcriptase Genes and Their Early Embryonic Functions

Trystan B. Wells, Guanglei Zhang, Zenon Harley, and Homayoun Vaziri

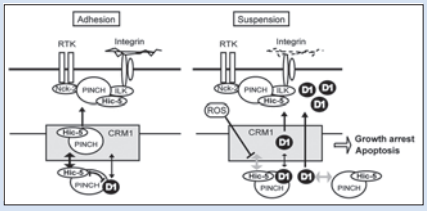
Within a species of complex animals, genes for functional proteins are rarely variant. This constancy is thought to be required for the function of essential proteins. One such crucial protein is telomerase reverse transcriptase catalytic subunit (TERT). To study the function of TERT during early development, the authors cloned *SpTERT* from purple sea urchin embryos. Unexpectedly, they discovered two distinct telomerase genes named *SpTERT-S* and *SpTERT-L.* By cloning SpTERT from several individuals, they further discovered regions, especially exon 11 of *SpTERT-S,* with intraspecific germline hypervariability. Although the variant enzymes remained catalytically active, there were significant amino acid variations in multiple regions, including those involved in binding of TERT to its RNA component. The authors also uncovered a noncanonical essential function for telomerase that is required for embryo polarity at the mesenchymal blastula stage. These results suggest the presence of an active diversity-generation mechanism that has neofunctionalized telomerase throughout evolution.

The *Aspergillus nidulans* Kinesin-3 UNCa Motor Moves Vesicles along a Subpopulation of Microtubules

Nadine Zekert and Reinhard Fischer



The microtubule cytoskeleton is not as rigid and uniform as the name implies, but is characterized by its dynamic instability. In addition, microtubules can be made up of different tubulin isoforms and—to make a eukaryotic cell even more complex—of different posttranslationally modified tubulins. Microtubule modifications, such as acetylation or polyglutamylation, are evolutionarily old "inventions" and occur in primitive eukaryotes such as *Giardia lamblia,* whereas detyrosination appeared later during evolution. Although many modifications were discovered more than 20 years ago, their cellular functions are not well understood. Here, the authors show that in the filamentous fungus *Aspergillus nidulans* at least two different microtubule populations exist. This discovery came from studies of an unc-104–related motor protein that preferentially moves along detyrosinated microtubules and transports vesicles. These microtubules are more stable than the tyrosinated ones and even remain intact during mitosis when other cytoplasmic microtubules are degraded.



Competitive Nuclear Export of Cyclin D1 and Hic-5 Regulates Anchorage Dependence of Cell Growth and Survival

Kazunori Mori, Etsuko Hirao, Yosuke Toya, Yukiko Oshima, Fumihiro Ishikawa, Kiyoshi Nose, and Motoko Shibanuma

Anchorage dependence of cell growth is a critical trait that distinguishes nontransformed from transformed cells. The authors report a novel mechanism whereby anchorage-independent cell growth and survival is prevented. Cyclin D1 is a proto-oncogene that exhibits cell cycle– dependent nuclear localization. Its nuclear export is dependent on CRM1. The authors report that the nuclear localization of cyclin D1 is adhesion-dependent and regulated by the focal adhesion protein Hic-5 and its binding partner PINCH, which also cycle in and out of the nucleus. Hic-5 binds to CRM1 with high affinity and is a competitive inhibitor of CRM1-dependent cyclin D1 export in adherent cells. PINCH interacts with both cyclin D1 and Hic-5 and enhances the Hic-5–dependent inhibition of cyclin D1 export. Under nonadherent conditions, the cellular level of reactive oxygen species increases and inhibits the nuclear export of Hic-5, resulting in the nuclear export of cyclin D1. Consequently cells undergo growth arrest and apoptosis. *Ras* overexpression led to the anchorage-independent nuclear localization of cyclin D, revealing an interesting interdependence of the oncogenic potential of two oncogenes. ■