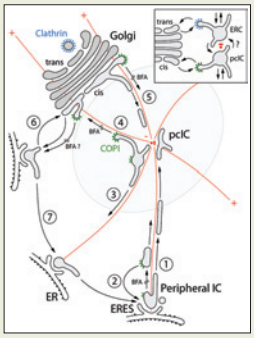


The Function of the Intermediate Compartment in Pre-Golgi Trafficking Involves Its Stable Connection with the Centrosome

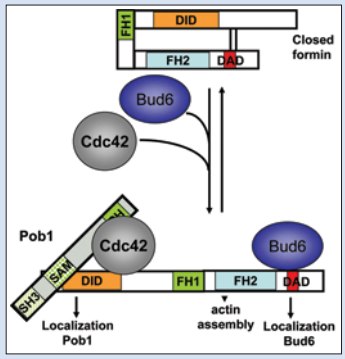
Michaël Marie, Hege A. Dale, Ragna Sannerud, and Jaakko Saraste

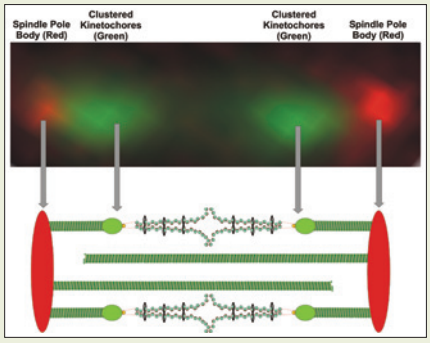
The intermediate compartment (IC) is thought to consist of pleiomorphic transport carriers that form at endoplasmic reticulum (ER) exit sites, move along microtubules to the cell center, and transfer ER-derived cargo to the Golgi apparatus by fusing with or transforming into cis-Golgi cisternae. Using live cell imaging the authors show that a pericentrosomal domain of IC elements (pcIC), defined by the GTPase Rab1, is stably anchored next to the centrosome. Together with the Rab11-containing endocytic recycling compartment (ERC), the pcIC establishes a pericentrosomal membrane system. Separation of the pcIC and ERC from the Golgi due to centrosome motility revealed that they communicate not only with the Golgi stacks, but also with each other, operating as way stations in unconventional trafficking of the cystic fibrosis transmembrane regulator to the cell surface. These results suggest that the biosynthetic-secretory pathway bifurcates at the level of the IC, allowing certain plasma membrane–destined cargo to bypass the Golgi apparatus.

Pob1 Participates in the Cdc42 Regulation of Fission Yeast Actin Cytoskeleton

Sergio A. Rincón, Yanfang Ye, M. Antonia Villar-Tajadura, Beatriz Santos, Sophie G Martin, and Pilar Pérez

Cdc42 plays an essential role in establishing polarized cell growth, in part through controlling actin cytoskeleton organization. *Schizosaccharomyces pombe* For3 formin, which nucleates assembly of linear actin filaments, is localized to cell tips and activated in a Cdc42-dependent manner. The authors identified Pob1 in a screen for multicopy suppressors of the thermosensitivity of *cdc42-879,* a mutant strain defective in For3 localization and actin cable assembly. Cdc42 directly binds to Pob1 and regulates its localization and/or stability. Pob1 overexpression partially restores For3 localization and the actin cables that are nearly absent in the *cdc42-879* strain. For3 docking at cell tips relies on two independent localization domains, at its N- and C-termini. While For3 C-terminal domain localization depends on Bud6, this study identifies Pob1 as the cortical tether that localizes the For3 N-terminal region to the cell tips and facilitates Cdc42-mediated relief of For3 autoinhibition to stimulate actin cable formation.





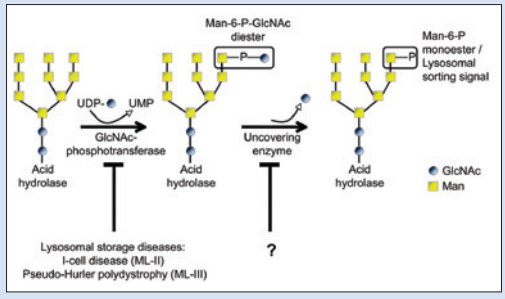
Function and Assembly of DNA Looping, Clustering, and Microtubule Attachment Complexes within a Eukaryotic Kinetochore

Marybeth Anderson, Julian Haase, Elaine Yeh, and Kerry Bloom

The kinetochore is the protein-DNA complex that mediates chromosome attachment to dynamic microtubules. How the kinetochore is physically connected to the centromere DNA and the mechanism by which the centromere is displayed on the outer surface of the chromosome are poorly understood. Using an inverse PCR assay to capture chromosome conformation, these authors demonstrate that kinetochore proteins proximal to the DNA (inner kinetochore) promote bending of centromere DNA. Kinetochore proteins bound to microtubule plus-ends (outer kinetochore) are required for the geometric organization of pericentric DNA loops and kinetochore clustering around the spindle microtubules. Analyses of the dependency relationships support the idea that the kinetochore comprises three autonomous complexes that mediate DNA looping, clustering, and microtubule binding. The linkage of microtubule-binding to centromere DNA-looping complexes positions the pericentric chromatin loops and stabilizes the dynamic properties of individual kinetochore complexes in mitosis. DNA loops are integral to the function of the mitotic segregation apparatus.

Mice Lacking Mannose 6-Phosphate Uncovering Enzyme Activity Have a Milder Phenotype than Mice Deficient for GlcNAc-1-Phosphotransferase Activity

Marielle Boonen, Peter Vogel, Kenneth A. Platt, Nancy Dahms, and Stuart Kornfeld



Acid hydrolases acquire mannose 6-phosphate (Man-6-P) residue(s) that serve as lysosomal targeting signals. These residues are synthesized by the sequential action of GlcNAc-phosphotransferase, which transfers GlcNAc-P from UDP-GlcNAc onto mannoses of the N-glycans, and "uncovering enzyme" (UCE), which removes the GlcNAc to expose the Man-6-P signal. Mutations in the two genes that encode GlcNAc-phosphotransferase underlie two lysosomal storage diseases (mucolipidosis type II and III), while no pathologic disorders have been attributed to UCE deficiency. Using insertional mutagenesis, the authors disrupt UCE and show that it accounts for virtually all of the uncovering activity in the mouse. Unlike mice with mucolipidosis type II, mice lacking UCE do not exhibit tissue abnormalities that result from acid hydrolase mistargeting because the acid hydrolases produced by the UCE-deficient cells still bind to the cation-independent Man-6-P receptor via Man-6-P-GlcNAc diesters, albeit with decreased affinity. Although this weak binding results in some acid hydrolase hypersecretion, sufficient sorting to the lysosome occurs to prevent the pathologic alterations seen with GlcNAc-phosphotransferase deficiency. ■