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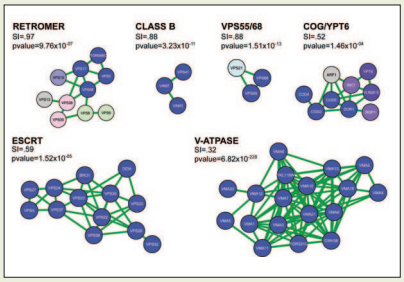


Global Analysis of Yeast Endosomal Transport Identifies the Vps55/68 Sorting Complex

Cayetana Schluter, Karen K. Y. Lam, Jochen Brumm, Bella W. Wu, Matthew Saunders, Tom H. Stevens, Jennifer Bryan, and Elizabeth Conibear

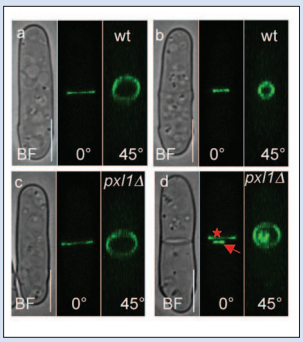
Sorting at multivesicular endosomes is coordinated by a number of distinct protein complexes. Many of these have conserved functions throughout evolution, including the **e**ndosomal **s**orting **c**omplexes **r**equired for **t**ransport (ESCRT) that control the formation of intralumenal vesicles and the retromer and Golgi-associated retrograde protein complexes that are required for recycling to the Golgi. To systematically characterize the endosomal sorting machinery, the authors quantified sorting defects in genome-wide yeast knockout collections and used phenotypic profiling and a novel computational approach to group genes into significant clusters. This not only identified known transport complexes, but also predicted the existence of a novel complex containing two small membrane proteins. Indeed, Vps55 and Vps68 were found to form a complex that acts with or downstream of the ESCRT machinery to regulate trafficking at endosomes. Because mammals have two sets of Vps55- and Vps68-related proteins, the authors suggest there may be two distinct versions of the Vps55/68 complex in higher cells.

Pxl1p, a Paxillin-related Protein, Stabilizes the Actomyosin Ring during Cytokinesis in Fission Yeast

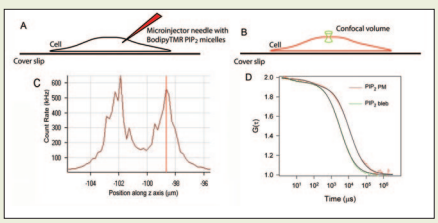
Wanzhong Ge and Mohan K. Balasubramanian

Schizosaccharomyces pombe Pxl1 Is a Paxillin Homologue that Modulates Rho1 Activity and Participates in Cytokinesis

Mario Pinar, Pedro M. Coll, Sergio A. Rincón, and Pilar Pérez



In the fission yeast *Schizosaccharomyces pombe*, as in animal cells, the assembly of a contractile ring with actin filaments and the motor protein myosin II is necessary to generate the constrictive force that separates the two daughter cells during cytokinesis. These reports converge on the discovery that *S. pombe* Pxl1p, a LIM domain–containing protein similar to animal cell paxillin, is part of the actomyosin ring and interacts with myosin II. Both groups report that *pxl1Δ* cells display a novel phenotype characterized by a splitting of the actomyosin ring in late anaphase into two rings, only one of which undergoes constriction. Moreover, the rate of actomyosin ring constriction is slower in the absence of Pxl1p. Pinar *et al.* demonstrate that Pxl1p interacts with Rho1p and negatively regulates this GTPase, and both groups demonstrate a genetic interaction with Rng2p-IQGAP and myosin II. Whether the role of Pxl1p in cytokinesis is related to modulation of Rho1p activity remains to be determined. Future studies will address the possible involvement of Pxl1p, Rho1p, Myo2p, and Rng2p in maintaining actomyosin ring integrity and regulating its assembly and contraction in *S. pombe.*



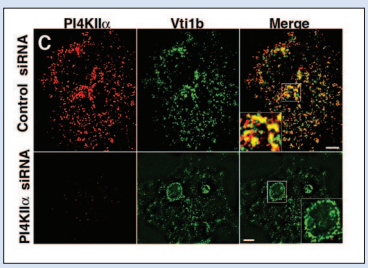
Diffusion Coefficient of Fluorescent Phosphatidylinositol 4,5-bisphosphate in the Plasma Membranes of Cells

Urszula Golebiewska, Marian Nyako, William Woturski, Irina Zaitseva, and Stuart McLaughlin

Phosphatidylinositol 4,5-bisphosphate (PIP_2) regulates ion channels, mediates endocytosis/exocytosis, and affects other processes at the inner leaflet of the plasma membrane; it is also the source of three second messengers. Several investigators have suggested there may be different pools of PIP_2 in the plasma membrane. If a significant fraction of PIP_2 is reversibly bound to proteins, its diffusion coefficient will be lowered. The authors used fluorescence correlation spectroscopy to measure the diffusion coefficient (D) of fluorescently labeled PIP_2 microinjected into living cells and obtained $D = 0.8 \pm 0.2\ \mu m^2/s$ on the inner leaflet. This is threefold lower than the D of PIP_2 in blebs. It is also lower than the D of PIP_2 in the outer leaflet or in phospholipid vesicles and the D of other lipids on the inner leaflet of cell membranes. The simplest interpretation is that approximately two-thirds of the PIP_2 on the inner leaflet is bound reversibly.

Phosphatidylinositol-4-kinase Type II α Contains an AP-3 Sorting Motif and a Kinase Domain that Are Both Required for Endosome Traffic

Branch Craige, Gloria Salazar, and Victor Faundez



Membrane-bound organelles communicate by vesicular transport. Cytosolic adaptor complexes, for example, adaptor protein 3 (AP-3), bind to membranes, where they recruit membrane proteins (cargoes) to be concentrated into vesicles and interact with cytosolic regulatory enzymes required for vesicle formation. Cargoes and enzymes that regulate adaptors are generally considered independent molecular entities. Here, the authors present evidence that phosphatidylinositol-4-kinase type II α (PI4KIIα), a lipid-modifying enzyme attached to membranes by palmitoylation, behaves both as a membrane protein cargo concentrated into AP-3 vesicles and as an enzymatic regulator of AP-3 function. Both a PI4KIIα sorting motif and PI4KIIα kinase activity are necessary to interact with AP-3, to properly localize PI4KIIα to endosomes, and to rescue endosomal PI4KIIα siRNA-induced mutant phenotypes. These data suggest a novel regulatory principle whereby adaptors use canonical sorting motifs to selectively recruit a regulatory enzymatic activity ("smart" cargoes) to discrete membrane domains. ■