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Yeast Sec1p Functions before and after Vesicle Docking

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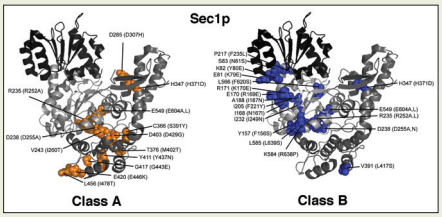
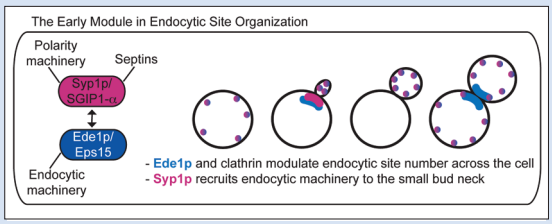


Macromolecules are sorted and transported intracellularly in membrane-bound vesicles that fuse to specific target membranes. This process requires SNARE proteins anchored in both membranes, plus a hydrophilic Sec1/Munc-18 (SM) protein. SNAREs assemble to draw the membranes together and drive fusion. Studies in different systems have suggested that SM proteins are required either upstream of SNARE assembly for vesicle docking, or later in membrane fusion. Thus a coherent model for SM protein function has yet to emerge. The authors used random and site-directed mutagenesis of the yeast Sec1p to identify two classes of mutants. Class A mutants establish a requirement for Sec1p in vesicle docking, whereas class B mutants impair Sec1p binding to SNARE complexes, which is required for fusion. The authors also discovered, unexpectedly, that the assembled SNAREs bind to Sec1p in a deep groove opposite the broad syntaxin (t-SNARE) binding cleft seen in Munc18-1. These findings in yeast help to unify our understanding of the mechanisms used by all SM proteins to help sort macromolecules.



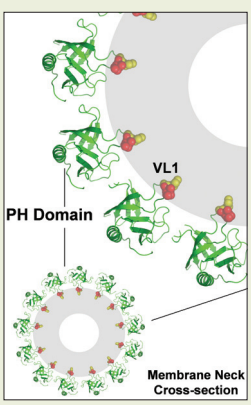
Early-Arriving Syp1p and Ede1p Function in Endocytic Site Placement and Formation in Budding Yeast

Helen E.M. Stimpson, Christopher P. Toret, Aaron T. Cheng, Barbara S. Pauly, and David G. Drubin

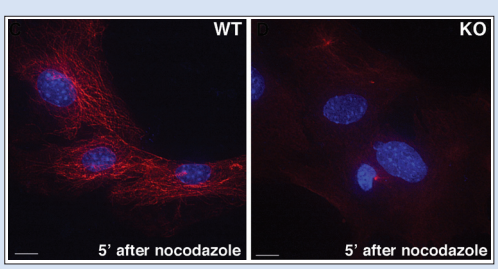
Clathrin-mediated endocytosis is essential for processes as diverse as nutrient uptake and neuronal signaling. The timing of events at individual endocytic sites is conserved from yeast to mammals, but little is understood about how sites are initiated. Here, and in another recent report (Reider *et al.* [2009]. *EMBO J* doi:10.1038/emboj.2009.248), it is shown that the septin-associated protein Syp1p is an endocytic component that associates with the endocytic adapter Ede1p. The two proteins mark the earliest stages of endocytosis. In this study, the authors show that cells lacking Syp1p or Ede1p have defects in the turnover of endocytic sites. Additionally, cells without Ede1p form fewer endocytic sites, while cells lacking Syp1p have a specific defect in site formation at the bud neck, a region to which Syp1p is concentrated by the septin ring. This study suggests that early-arriving endocytic proteins are key to endocytic site initiation and placement, and provides an important clue as to how the endocytic and cell polarity machineries are interlinked.

Membrane Insertion of the Pleckstrin Homology Domain Variable Loop 1 Is Critical for Dynamin-catalyzed Vesicle Scission

Rajesh Ramachandran, Thomas J. Pucadyil, Ya-Wen Liu, Sharmistha Acharya, Marilyn Leonard, Vasyl Lukiyanchuk, and Sandra L. Schmid



Efficient constriction of the membranous neck that connects a nascent vesicle to its parent membrane is essential for subsequent membrane destabilization events that lead to vesicle scission in intracellular membrane trafficking processes. The self-assembling GTPase dynamin mediates the scission of clathrin-coated vesicles from the plasma membrane. The authors previously showed that the hydrophobic variable loop 1 (VL1) in the pleckstrin homology (PH) domain of the neuronal dynamin-1 isoform inserts partially into the lipid bilayer upon dynamin self-assembly. Here, using a combination of genetic, biochemical, and biophysical approaches, including a newly developed system for in vitro reconstitution and imaging, the authors demonstrate that stable membrane insertion of this loop is critical for membrane constriction and fission both in vitro and in vivo. These findings demonstrate an active mechanical role for PH domain VL1 membrane insertion in dynamin-catalyzed membrane fission and vesicle release.



Murine CENP-F Regulates Centrosomal Microtubule Nucleation and Interacts with Hook2 at the Centrosome

Katherine Moynihan, Ryan Pooley, Paul Miller, Irina Kaverina, and David Bader

Several studies have linked CENP-F to microtubule (MT)-based activities because disruption of this protein leads to major changes in MT structure and function. Yet the basis of CENP-F regulation of the MT network remains elusive. Here, the authors reveal a novel localization and role for CENP-F at the centrosome, the major MT organizing center (MTOC) of the cell. They also identify Hook2, a linker protein essential for centrosomal MT network regulation, as a CENP-F binding partner. MT repolymerization from the centrosome after nocodazole treatment is disrupted in newly developed CENP-F$^{-/-}$ mouse embryo fibroblasts. Importantly, the regulation of MT repolymerization is centrosome-specific, because MT growth from the Golgi is readily observed. Further, analysis of partially reconstituted MTOC asters shows that disruption of CENP-F function impacts MT nucleation and potentially anchoring. These studies reveal a critical new localization and function of CENP-F that is likely to impact a broad array of MT-based actions in the cell. ■