

# Spatial Regulation of Exocytosis: Lessons from Yeast

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**M**ANY types of cells maintain specialized plasma membrane domains to which different subsets of secretory vesicles are targeted, enabling the cells' performance of specific functions. The budding yeast *Saccharomyces cerevisiae* spatially and temporally regulates exocytosis, directing surface growth and secretion to different plasma membrane sites at different cell cycle stages (21). In very small buds, secretion occurs over the entire bud surface, but as the bud enlarges growth is directed to the bud tip. When the bud is two-thirds the size of the mother cell, secretion becomes isotropic over the entire bud surface. Late in the cell cycle, new material is inserted at the neck between mother and daughter cells, resulting in cytokinesis and septation.

The accurate delivery of vesicles to sites of exocytosis requires both actin-dependent vesicle transport and actin-independent establishment of a vesicle-receiving station. The first step, polarized vesicle transport, also involves a rab family small GTPase and its nucleotide exchange protein. The next step, docking of secretory vesicles at specific sites, involves a large protein complex peripherally associated with the plasma membrane at these sites. The final step, vesicle fusion, involves the integral membrane soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE)<sup>1</sup> proteins and their regulators (31). The target-SNAREs are distributed over the entire plasma membrane, not just at the regions of active exocytosis (7). Although the interaction of correctly paired vesicle- and target-SNAREs is proposed to specify the correct membrane fusion reaction (35), the restriction of exocytosis to particular regions of the plasma membrane is accomplished at the vesicle docking stage. We will consider the roles of the cytoskeletal and secretory machinery in the establishment of a polarized secretory pathway.

### *Polarized Transport of Vesicles to the Bud*

**The Cytoskeleton and Secretion.** In contrast to animal cells that use kinesin-dependent transport along microtubules to the cell periphery, followed by fast, actomyosin-depen-

dent transport near the cell membrane (4), polarized vesicle transport in yeast involves only the actin cytoskeleton (5). Temperature-sensitive (ts) mutants defective in actin and actin-associated proteins become enlarged, round, and multinucleate, and do not polarize secretion to the bud tip at the restrictive temperature (29, 39). Actin mutants accumulate post-Golgi secretory vesicles, and are partially blocked in invertase secretion, demonstrating that an intact actin cytoskeleton facilitates the exocytic process, in addition to polarizing secretion (29). Yeast lacking the Wiskott-Aldrich syndrome protein homologue, Las17/Bee1p, which regulates actin assembly, also accumulate post-Golgi secretory vesicles (22). Mutants in capping protein, which binds to the barbed end of actin filaments, preventing subunit addition and loss, show no block in invertase secretion, although secretion is depolarized (1). A ts mutant of profilin, which regulates actin assembly, does not accumulate vesicles after shift to the restrictive temperature (13), in spite of genetic interactions with mutations in several of the post-Golgi genes, and a depolarized phenotype (13, 15, 16).

Myo2p, a class V myosin, and tropomyosin, an actin filament-binding protein (encoded by *TPM1* and *TPM2*), have been implicated in polarized vesicle transport: mutants in either of these genes accumulate what appear to be post-Golgi vesicles by morphology (14, 19, 25); however, no defect in export of any cargo has been detected (14, 25). Both mutants grow isotropically at the restrictive temperature (19, 25), indicating that secretion continues, but is not correctly polarized. The *myo2* phenotype is epistatic to a ts mutation in the post-Golgi gene *sec6*; the *myo2* mutant and the *myo2 sec6* double-mutant accumulate vesicles randomly in both the mother cell and the bud, whereas the *sec6* mutant alone accumulates vesicles in the bud tip (14). Both *myo2* and *tpm1* genetically interact with several post-Golgi *sec* mutants (14, 25). These results, combined with the localization of Myo2p to sites of polarized growth (8, 24), are consistent with the hypothesis that Myo2p is a motor that brings secretory vesicles into the bud tip along actin cables.

The actin-sequestering drug, latrunculin-A (LAT-A), acts rapidly, specifically, and reversibly in yeast, results in complete loss of polymerized actin, and avoids the depolarizing effects of the temperature shifts necessary in studies of conditional actin alleles. Small unbudded stationary-phase cells lacking polarity can be released from G<sub>0</sub> in the presence of LAT-A to determine which proteins are able to assemble at the prebud site in the absence of F-actin.

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1. *Abbreviations used in this paper:* LAT-A, latrunculin-A; SNARE, soluble NSF attachment protein receptor; ts, temperature sensitive; t-SNARE, target SNARE; v-SNARE, vesicle SNARE.

Sec4p, a marker for post-Golgi secretory vesicles, is unable to polarize in the presence of the drug (3), consistent with a role for actin in the polarized delivery of exocytic vesicles, and with the loss of Sec4p localization in the *act1-3* ts mutant (38). Myo2p is able to localize in cells treated with LAT-A (3), which could reflect an affinity of Myo2p for vesicle docking sites. A mammalian homologue of Myo2p, brain myosin V, localizes to synaptic vesicles (32), suggesting that Myo2p may associate with secretory vesicles. Consistent with this hypothesis, Sec4p cannot maintain a polarized distribution in the *myo2-66* ts mutant (38).

Another actin-associated protein that may act in polarized secretion is Aip3p/Bud6p, which localizes to sites of polarized cell growth, and is able to do so independent of actin and septins. Cells lacking Aip3p have abnormal septa and accumulate vesicles. Actin cytoskeletal polarity is initially normal in *aip3* cells, but is lost as the cells enlarge (2). The secretory and cytokinesis defects could result from a defect in actin cytoskeletal organization or function, or Aip3p could play a more direct role in linking secretory pathway and actin cytoskeletal polarity.

**Sec4p and Sec2p.** Sec4p is a rab family GTPase thought to act as a molecular switch to regulate exocytosis. It principally resides on post-Golgi secretory vesicles, resulting in the concentration of Sec4p that is normally seen at sites of polarized exocytosis, such as the bud tip (30, 38). Mutations in several genes cause loss of a polarized Sec4p distribution including *SEC2*, encoding a guanine nucleotide exchange protein (GEP) for Sec4p, *ACT1*, encoding actin, and *MYO2*. In *sec2* mutants, Sec4p remains associated with post-Golgi secretory vesicles, which accumulate randomly in both the mother cell and the bud (38). This suggests that Sec4p activation by Sec2p is coupled to polarized vesicle transport. Other post-Golgi *sec* mutants such as *sec6* initially accumulate vesicles only in the bud and maintain a polarized distribution of Sec4p (38).

### Establishment of the Docking Site

**Role of the Sec3/5/6/8/10/15–Exo70p Complex.** The second requirement for polarized exocytosis is the definition of the site at which vesicles dock and fuse. Recent evidence suggests that the docking site is defined by a large complex of proteins, comprising Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, and Exo70p, which is peripherally associated with the plasma membrane (6, 37). A homologous complex has been isolated from mammalian brain (17), implying that the role of this complex in exocytosis is conserved from yeast to mammals. Mutants defective in complex components maintain a polarized distribution of Sec4p (38), suggesting that the complex does not function in polarized vesicle transport.

The first link between these genes and vesicle docking came from studies on the dominant-negative effect on cell growth of *SEC15* overexpression. Phenotypic examination of these cells demonstrated accumulation of clustered vesicles and a patch of Sec15p, frequently near the mother–bud neck. Formation of the Sec15p patch requires functional Sec2p and Sec4p (36). The vesicle cluster might represent an aberrant docking event, in which Sec15p, working in response to activated Sec4p, may cause vesicles to adhere to each other rather than to the plasma membrane.

The localization of Sec8p to prebud sites and tips of small buds also suggested a role for the complex as a targeting patch (9, 37), but this distribution could result from recruitment to the target membrane in response to vesicle docking rather than stable association with the target membrane. Furthermore, different components could localize to this region by different mechanisms, forming the complex by assembly at the appropriate site.

A role for Sec3p as a spatial determinant for exocytosis was suggested by *sec3* mutant phenotypes including random budding in diploids (13, 16), depolarized chitin deposition and cytokinesis defects in addition to defective secretion (13). Since Sec3p is the only complex component to affect cell polarity (13), these results suggest that Sec3p could be a spatial landmark defining sites for polarized secretion. If this were the case, Sec3p would localize to sites of polarized secretion at each stage of the cell cycle independent of secretory pathway function.

Studies of Sec3p tagged with green fluorescent protein (GFP) established its localization to sites of polarized secretion, where it colocalizes with Sec4p and Sec8p. This localization is independent of the secretory machinery, including all other members of the Sec3/5/6/8/10/15–Exo70p complex for which mutants are available. Thus, Sec3p fits the criteria for a spatial landmark for exocytosis. Sec3–GFP maintains localization in actin and septin mutants, and does not colocalize with either type of filament. Furthermore, Sec3p is able to localize to prebud sites in LAT-A–treated cells released from G<sub>0</sub>. Sec3p maintains a polarized distribution after several hours at restrictive temperature in polarity establishment mutants such as *cdc24* and *cdc42*, although localization is lost in a mutant of the cyclin-dependent kinase Cdc28p. It is uncertain whether the localization seen in polarity establishment mutants is really independent of these gene products or if it is a remnant of a previous cytokinesis site, but clearly Sec3p is more upstream in the morphogenetic hierarchy than Sec proteins have been thought to be (12).

The roles of the remaining components of the Sec3/5/6/8/10/15–Exo70p complex are also being investigated. In contrast to the results obtained with Sec3p, small unbudded cells released from G<sub>0</sub> in the presence of LAT-A are unable to polarize Sec8p (3). Polarized localization of Sec8p is not maintained in any other *sec* mutant, implying that Sec8p is recruited to sites of exocytosis upon vesicle docking (12). The inability of Sec8p to polarize in LAT-A–treated cells could be a consequence of the lack of vectorial vesicle transport to the docking/fusion site. Sec5p also localizes to similar sites of polarized secretion as Sec3p, Sec8p, and Sec4p (27). Expression of the carboxyl-terminal third of Sec10p from a strong promoter inhibits growth and causes the formation of elongated cells, but does not block secretion, suggesting a possible role in morphogenesis in addition to its role in secretion (34). Sec10p may mediate the cell cycle-dependent reorientation of the complex.

### Regulation of the Polarity of Secretion during the Cell Cycle

**Spatial Regulation of Secretion by Cdc28p.** The interaction of Cdc28p, a cyclin-dependent protein kinase, with differ-

ent cyclins triggers changes in polarization of the actin cytoskeleton and the site of secretion. The mechanism by which Cdc28p effects these changes is unclear, since the substrates of the Cdc28p kinase that might regulate this pathway are unknown, but new clues are emerging. Genetic screens have identified mutants whose functions are essential when a morphogenesis checkpoint for bud emergence is bypassed by overproduction of the B-type cyclin, Clb2p. These screens, which eliminate proteins exerting their effects through depolarization of the actin cytoskeleton, have identified several gene products affecting N-glycosylation (27, 28), as well as the *SEC3*, *SEC5*, and *MCD4* genes (27). N-glycosylation could be involved in a signaling pathway for the polarization of secretion (27, 28), as has been proposed for sorting in polarized epithelial cells (20). The role of Sec5p in allowing efficient budding is less clear. Sec3p contains one or more consensus sites for Cdc28p-dependent phosphorylation. The putative Cdc28p phosphorylation site in Sec3p could regulate Sec3p's activity or localization through the cell cycle. The *MCD4* gene encodes a membrane protein of unknown function (27); future studies of the role of this gene may prove informative.

**Polarity Establishment.** The first step in yeast polarity establishment is the cell type-specific selection of the new bud site. A GTPase cycle operates downstream of bud site selection, involving the rho-family GTPase Cdc42p and its GEF, Cdc24p. These proteins are thought to be the most upstream components of the morphogenetic pathway for bud development. Polarity establishment mutants share common phenotypes: cells become large, round, and multinucleate, no buds are formed, chitin is found over the entire cell surface, and the actin cytoskeleton is depolarized (33). The activity of polarity establishment proteins in regulation of the cytoskeleton is thought to result in a polarized secretory pathway. The ts mutant, *cdc24-1*, secretes acid phosphatase at wild-type levels at the restrictive temperature, but secretion is directed over the entire cell surface (11). Cdc42p nucleates actin assembly (23) and localizes to sites of polarized growth. By immunoEM, Cdc42p concentrates in the vicinity of secretory vesicles, and those vesicles nearest to the cell surface are sometimes labeled with Cdc42p (40). Cdc42p could nucleate actin assembly near the plasma membrane for polarized vesicle delivery, or regulate actin rearrangements near the plasma membrane to allow exocytosis. The secretory machinery could also respond directly to Cdc42p to promote exocytosis at sites of polarized growth, independent of the effect of Cdc42p on actin.

The Rho3p GTPase, which functions either cooperatively with or downstream of Cdc42p (26), might also be involved in polarization of vesicle transport. The isolation of Sec4p as a high copy suppressor of a deletion of *rho3* (18), strongly suggests that Sec4p functions downstream of Rho3p, and that an important function of Rho3p may be to activate Sec4p. *RHO3* also suppresses an effector domain mutant of *sec4*, as well as suppressing *sec8* and *sec15* ts mutants (7; Brennwald, P., personal communication). Furthermore, *rho3* and *sec4* ts mutants are synthetically lethal, and the ts *sec4* mutant is able to suppress a dominant-negative *RHO3* mutant (18). A deletion of *RHO3* is suppressed by high copy expression of the target (t)-SNARE

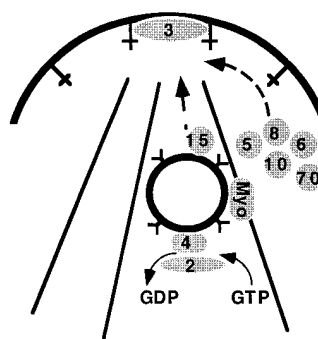
Sec9p (Brennwald, P., personal communication), and as *SEC9* is itself a suppressor of an effector domain mutant of *sec4* (7), these results suggest that Sec9p functions downstream of both Rho3p and Sec4p.

### A Model for Polarized Secretion in Yeast

These results suggest a model whereby the actin-dependent vectorial transport of vesicles is coupled to the actin-independent establishment of a site for vesicle docking and fusion (Fig. 1). First, vesicles with appropriate vesicle (v)-SNAREs bud from the Golgi, and Sec4p binds to them. Exchange of GDP for GTP, catalyzed by Sec2p, occurs on Sec4p, resulting in activated Sec4p on the post-Golgi vesicles. The vesicles move on actin tracks, perhaps by means of Myo2p, into the bud. The t-SNAREs are distributed over the entire plasma membrane, but Sec3p is associated with the plasma membrane specifically at exocytic sites. Sec15p may dock the vesicle in response to activated Sec4p at the appropriate site marked by Sec3p and recruit the other components of the Sec3/5/6/8/10/15-Exo70p complex. The v- and t-SNAREs could then interact, promoting fusion of the vesicle with the plasma membrane. The polarity of secretion would be established by the action of the Cdc28p kinase on a variety of substrates, including the polarity establishment machinery, which regulate polarization of the actin cytoskeleton, and thus polarization of vesicle transport into the bud. The site of vesicle fusion is established in an actin-independent manner, possibly under more direct control of the cell cycle machinery.

This model raises many questions regarding specific mechanisms. Is there a direct link between vesicles and actin? If so, is Myo2p a motor mediating polarized vesicle transport along actin cables? Is one function of Sec4p to regulate a motor, as has recently been demonstrated for Rab6 regulation of rabkinesin (10)? How does Sec3p (a hydrophilic protein), interact with the plasma membrane? How is Sec3p's localization regulated over the cell cycle? Changes in protein levels and/or phosphorylation state over the cell cycle would provide some indication of direct regulation of Sec3p by the cell cycle machinery.

Why would the cell require separate pathways for targeting of vesicles and establishment of the docking/fusion



**Figure 1.** Model for establishment of a polarized secretory pathway. Sec3p localizes to a domain of the plasma membrane independent of the actin cytoskeleton, defining the site of exocytosis. Vesicles are transported to the correct region of the cell on actin cables, perhaps using Myo2p as the motor. The activation of Sec4p, on vesicles, by Sec2p allows polarized delivery of vesicles. Sec15p may dock the correctly targeted vesicles at the docking/fusion site via interactions with other components of the Sec3/5/6/8/10/15-Exo70p complex. *Y shapes*, v-SNAREs on the vesicles; *crosses*, t-SNAREs on the plasma membrane.

site in order to achieve polarized exocytosis? Vesicle docking sites must exist before and independent of vesicle delivery, and of the machinery for such delivery. The alternative possibility, that vesicles are delivered to the bud tip, and fuse with the plasma membrane in this area solely by virtue of proximity, may not be sufficient for events such as cytokinesis when closure of the neck requires precise membrane addition. The independent establishment of the docking/fusion site provides an additional layer of specificity.

Similar mechanisms will certainly apply in other eukaryotes. Most of the components discussed here have clear homologues in mammals where they appear to play analogous roles in the context of more complex traffic systems. For example, epithelial cells concurrently transport different classes of vesicles to their apical and basolateral surfaces (20). Basolateral transport uses components similar to those of yeast, whereas apical transport is quite different (20). A complete definition of the machinery that spatially regulates exocytosis in yeast will certainly further our understanding of morphogenesis in all eukaryotic cells.

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