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The Exocyst Complex in Polarized Exocytosis

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Abstract

The exocyst is an octameric protein complex, which mediates the tethering of post-Golgi secretory vesicles to the plasma membrane prior to exocytic fusion. The exocyst assembles by side-by-side packing of rod-shaped subunits composed of helical bundles. The targeting of secretory vesicles to the plasma membrane involves direct interactions of the exocyst with PI(4,5)P₂. In addition, a number of small GTP-binding proteins interact with components of the exocyst and regulate the assembly, localization, and function of this complex. Here we review the recent advances in the field, focusing on the function of the exocyst in polarized exocytosis.

Polarized exocytosis is essential for a wide range of biological processes such as cell growth, morphogenesis, and cell migration. Exocytosis is accomplished by the fusion of secretory vesicles with the plasma membrane catalyzed by the assembly of the SNARE complex. Prior to membrane fusion, additional proteins mediate the initial interaction between the vesicles and the acceptor membrane, a process known as “vesicle tethering”. Tethering of secretory vesicles to the plasma membrane is thought to be mediated by the exocyst, an evolutionarily conserved octameric protein complex consisting of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 [[1**] and [2**]]. Recent studies from different disciplines have led to significant advance in our understanding of the exocyst in various eukaryotic systems and physiological processes. Here we review the most recent progresses in the field, focusing on the role of the exocyst in exocytosis.

The structure of the exocyst

Understanding the structure of the exocyst may provide important insight into the molecular mechanism of the exocyst function. So far, partial crystal structures of four exocyst components have been solved [3]. These include the near full-length yeast and mouse Exo70 [[4**], [5] and [6]], and the C-terminal domains of *Drosophila* Sec15 [7], yeast Exo84 [4**], and yeast Sec6 [8**]. While these exocyst components share little sequence homology, they all display rod-like structures composed of two or more consecutively packed helical bundles, with each bundle consisting of three to five α -helices linked by loops. The structural conservation among exocyst components suggests that they evolved from a common ancestor. In addition, several exocyst components have been found to share distant sequence homology with subunits of two other tethering complexes that function at the Golgi apparatus, the conserved oligomeric Golgi (COG) complex and the Golgi-associated retrograde protein (GARP) complex [9]. The crystal structure of the COG subunit COG2 shows a helical bundle structure similar to that of the exocyst components [10]. These findings suggest that the exocyst, the COG and the GARP

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complexes are structurally related and might have diverged from a common ancestor to mediate vesicle tethering at different membrane trafficking stages.

The structure of individual exocyst components also provides a clue to how the exocyst complex is assembled. For example, Exo70 has an extended rod-shaped structure. Mapping the binding sites of Sec8 and Sec10 on Exo70 indicates that they have an extended interface with Exo70, suggesting that Sec8 and Sec10 themselves also have extended rod-like structures [4**]. Therefore, the assembly of the exocyst complex likely involves the packing of at least some rod-shaped components in a side-to-side fashion. This is consistent with the previous structure study of the mammalian exocyst complex using quick-freeze/deep-etch electron microscopy which demonstrates that the exocyst displays a packed “Y”-shaped structure after fixation [11*]. To date, the subunit organization and the structure of the holo-exocyst complex remain unclear. However, the recent crystal structure data have provided valuable insights to the structure and molecular organization of the exocyst complex.

Polarized localization and activation of the exocyst at the plasma membrane

In order to understand how the exocyst tethers vesicles at the plasma membrane, it is important to elucidate how the exocyst itself is targeted to the plasma membrane. In yeast, although all the exocyst components are localized to the growing end of the daughter cells (“bud tip”), their targeting involves different mechanisms. Sec3 is localized to the bud tip independent of actin cables, along which the vesicles are transported [[12*] and [13**]]. Exo70 polarization seems to be partially actin-dependent [13**]. In contrast, the remaining exocyst components are associated with exocytic vesicles and depend on actin cables for their delivery to sites of exocytosis [[13**], [14] and [15]]. These results led to the hypothesis that Sec3 and Exo70 associate with the plasma membrane and interact with the rest of the exocyst components on the arriving vesicles. The assembly of the exocyst complex may tether the secretory vesicles to the plasma membrane.

What recruits Sec3 and Exo70 to the plasma membrane? Recent studies revealed that both components bind to phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) located at the inner leaflet of the plasma membrane [16**, 17 and 18**]. The exocyst components are no longer associated with the plasma membrane in yeast mutant cells in which the plasma membrane PI(4,5)P₂ level was reduced [16**]. Simultaneously disrupting the interaction of PI(4,5)P₂ with Sec3 and Exo70 blocks the plasma membrane association of the exocyst and results in severe secretion and growth defects or cell lethality [[16**] and [18**]]. Sec3 interacts with PI(4,5)P₂ through a polybasic region at its N-terminal domain [18**]. Exo70 interacts with PI(4,5)P₂ through a number of basic residues at its C-terminus [16**]. These basic residues, while sparsely distributed in the primary sequence, are clustered as a positively charged surface patch at the C-terminal end of the folded Exo70 [[4**], [5] and [6]]. Exo70 specifically binds to PI(4,5)P₂ and shows little or no interaction with the stereoisomeric PI(3,5)P₂ or mono-phosphorylated PI(3)P and PI(4)P, which are distributed to other membrane compartments [16**]. Similar to their yeast counterparts, mammalian Sec3 and Exo70 also interact with PI(4,5)P₂ through conserved regions [[17]; Guo lab unpublished data]. Other tethering factors, such as the HOPS complex that functions in yeast homotypic vacuolar fusion, have also been found to interact with phosphoinositides [19]. Phosphoinositides may function to recruit the tethering proteins to specific membrane compartments for vesicle tethering. A model illustrating the membrane association of the exocyst and its interaction with small GTP-binding proteins (see later) is shown in Figure 1.

In yeast, PI(4,5)P₂ is not restricted to the daughter cell membrane. Therefore there must be additional factors that contribute to the polarized localization of the exocyst. In budding yeast, the exocyst is diffused in several *cdc42* and *rho1* mutants [[20] and [21]]. Sec3 is a downstream

effector of both Cdc42 and Rho1. GTP-bound Rho1 and Cdc42 compete for their binding to the N-terminal region of Sec3 [20], leading to the speculation that they control Sec3 localization at different cell cycle stages or growth conditions. Sec3 with its N-terminus deleted shows synthetic growth defects with other exocyst mutants [[22], [18**] and [23]]. Cdc42 binds to Sec3 at a region adjacent to its PI(4,5)P₂-binding motif and controls Sec3 localization in synergy with PI(4,5)P₂ [18**]. Interestingly, a chimera in which the N-terminus of Sec3 was replaced by the N-terminus of Gic2, which binds PI(4,5)P₂ through a polybasic domain and binds GTP-bound Cdc42 through its CRIB domain, functions as well as the wild-type Sec3 in yeast cells [18**]. Cdc42 and PI(4,5)P₂ may regulate the recruitment of Sec3 to the bud tip. They may also be involved in the activation of the exocyst at the bud tip during polarized yeast cell growth ("budding"). The observed polarization of the exocyst may in fact be a result of both physical recruitment and kinetic reinforcement since polarity determinants such as Cdc42 itself rely on a functional secretory pathway [14,24].

While Sec3 and Exo70 directly associate with the plasma membrane, their stable polarization in cells also depends on the other members of the exocyst complex [15,22, and 23]. Future studies are needed for a more comprehensive understanding of exocyst polarization.

Communication with the SNAREs

Vesicle tethering precedes fusion. In exocyst mutants, assembly of the SNARE complex is blocked [25]. The exocyst is likely to interact with the SNAREs and regulate SNARE assembly. It was reported that the brain exocyst complex co-immunoprecipitated with syntaxin [2**]. Yeast Sec6 was shown to interact with the t-SNARE Sec9 *in vitro* and may inhibit Sec9 interaction with the other t-SNARE protein Sso1 [26]. The exocyst was also found to co-immunoprecipitate with Sec1 [27], a Sec1/Munc18 (SM) family protein, which binds to SNAREs and stimulates membrane fusion. The exocyst component Exo84 directly interacts with the yeast *lgl* homologues Sro7/77 [28], which bind to and activate Sec9 [[29] and [30]]. Sro7/77 is a downstream effector of Sec4, a Rab GTPase that regulates post-Golgi stage of exocytosis [31]. In mammalian cells, Exo70 interacts with the t-SNARE binding protein Snapin [32]. Similar to the exocyst, the majority of tethering complexes in different stages of membrane traffic were shown to interact with t-SNAREs at the corresponding acceptor membranes. For example, the HOPS complex was shown to interact with the SNAREs and promote SNARE-mediated fusion *in vitro* [33]. It may be a common theme that the tethering factors regulate SNARE assembly through multitude interactions to coordinate vesicle tethering and fusion.

Function of the exocyst in exocytosis and beyond

In yeast, the exocyst mutants exhibit blockages of secretion and show intracellular accumulation of secretory vesicles [34**,35,15 and 36*]. In animal cells, the role of the exocyst in exocytosis and cell surface expansion has been demonstrated in a variety of cell types, such as protein targeting in epithelial cells [37** and 38*], dendritic delivery of NMDA receptors at postsynaptic membranes [39], and insulin-induced exocytosis of glucose transporter in adipocytes [40*]. It is worth noting that the exocyst is not required for all types of exocytic events in animal cells. For example, the exocyst does not seem to play a major role during neurotransmitter release at mature synapses, a specialized form of regulated exocytosis [41].

In animal cells, the exocyst components were found at the TGN and the perinuclear recycling endosomes [42*,43*,44*,45 and 38*]. Recycling endosomes mediate the transport of the internalized plasma membrane receptors back to the cell surface and are major sources of cargos destined to the plasma membrane in many types of cells. Loss of exocyst function blocks the recycling of internalized cargos and results in their accumulation in recycling endosomes [43*,45 and 38*]. Since in animal cells the exocyst is localized to both the plasma membrane

and perinuclear membrane compartments, it is possible that different pools of the exocyst function in different vesicular trafficking stages and that disruption of a specific pool of the exocyst leads to defect at the corresponding vesicular trafficking stage [42*].

The exocyst has been implicated in a variety of cellular processes including cytokinesis, cell migration, tumor invasion, and ciliogenesis. Furthermore, the exocyst has been shown to play important roles in a multitude of developmental processes, such as the establishment or remodeling of epithelial polarity, cell fate determination, neural and eye development, and oogenesis. In plants, the exocyst has been implicated in polarized cell growth such as pollen tube growth and hypocotyls elongation. Together, these *in vivo* studies have revealed various functions of the exocyst at the tissue and organ level. Due to the focus on the basic mechanisms of exocytosis in this review, these important works are not discussed and referenced here.

Regulation of the exocyst by small GTP-binding proteins

Functioning at a step before SNARE-mediated fusion, the exocyst is a target of a number of regulators that spatially and kinetically regulate exocytosis in cells. In particular, several small GTP-binding proteins directly interact with the exocyst. In yeast, Sec15 is a downstream effector of the Rab GTPase Sec4, which regulates the assembly of the exocyst complex [46**]. In higher eukaryotes, Sec15 is a downstream effector of the Rab GTPase Rab11, which regulates vesicle transport to the plasma membrane from recycling endosomes [7,38*,45 and 47*]. Several other tethering factors have also been shown to interact with specific Rab GTPases that regulate the corresponding membrane trafficking stages. The regulation of specific tethering proteins by distinct sets of Rab GTPases may be a common mechanism that assures membrane traffic fidelity. In mammalian cells, Sec10 is a downstream effector of the small GTPase Arf6, which promotes the translocation of Sec10 from recycling endosomes to the plasma membrane during cell surface remodeling and spreading [43*].

In budding yeast, Sec3 is a downstream effector of Cdc42 and Rho1 (see above). In addition, Exo70 was shown to interact with the GTP-bound form of Rho3, which is implicated in exocytosis [4**,48,49]. However, mutations in Exo70 that disrupt its interaction with Rho3 did not affect exocyst polarization or exocytosis [16** and 50]. It was speculated that in addition to Rho3, other factor(s) interact with Exo70 and regulate its activity [16** and 50]. Studies in yeast demonstrate that the *exo70* mutants, unlike other exocyst mutants, are primarily defective in the secretion of a subset of exocytic vesicles [36*]. These vesicles carry cargos such as the endoglucanase Bgl2 that are needed for cell wall remodeling and plasma membrane expansion. In addition, the secretion defect in the *exo70* mutants is most prominent at the early stage of asymmetric daughter cell growth. It has been previously reported that a particular mutant allele of Cdc42, *cdc42-6*, specifically accumulates Bgl2 vesicles in small-budded cells [51*]. The phenotypical similarity between *cdc42-6* and the *exo70* mutants suggests that Exo70 functions downstream of Cdc42. In mammalian cells, Exo70 interacts with TC10 (a homolog of Cdc42) and this interaction was shown to be important for the fusion, rather than the delivery, of Glut4 vesicles to the plasma membrane in adipocytes in response to insulin [40*]. Consistent with this observation, HeLa cells with Exo70 knocked down by RNAi blocked the fusion of post-Golgi vesicles to the plasma membrane without significant effect on vesicle delivery [17].

A number of papers from different labs have demonstrated that the exocyst components Sec5 and Exo84 are downstream effectors of the small GTPase Ral. Ral regulates exocyst assembly; disruption of the Ral-exocyst interaction by overexpressing the Ral-binding domain of Sec5 inhibited protein targeting to the basolateral domain in polarized epithelial cells and secretion of secretory granules in neuroendocrine cells [52**]. Besides its role in exocytosis, the Ral-

exocyst interaction has also been implicated in actin-based membrane protrusion, cytokinesis, and neurite branching.

Overall, the exocyst seems to function as a hub for receiving regulatory information from various signaling pathways for precise spatiotemporal control of exocytosis. Studies of the exocyst and other tethering complexes imply that these tethering complexes not only physically tether the vesicles to the acceptor membrane, but may also regulate the assembly of the SNARE complex for membrane fusion. In addition, the interactions of specific tethering proteins with distinct sets of Rab GTPases and phosphoinositides may be crucial for membrane traffic fidelity and organelle identity.

Future perspectives

Recent decade saw exciting progresses towards our understanding of the exocyst. However, a number of important questions remain unanswered: What are the kinetics of exocyst assembly and disassembly? How are the exocyst components associated with secretory vesicles? While it is thought that the exocyst serves as a vesicle tether, can the tethering step be observed and characterized by microscopy? Is the exocyst just a physical tether or does it also activate the assembly of the SNARE complex? Is the exocyst also involved in the early steps of vesicular trafficking such as vesicle budding and cargo sorting at the donor compartments? Recent studies demonstrate the roles of the exocyst in a number of developmental and physiological processes. Future studies are needed to elucidate how the cellular functions of the exocyst are manifested at the multicellular organismal level. Combined efforts from cell biologists, structural biologists, and developmental biologists will not only elucidate the functions of this fascinating complex, but also help us better understand exocytosis and cell polarity in general.

Acknowledgments

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Figure 1. A model for the function of the exocyst complex in tethering the secretory vesicles to the plasma membrane in yeast

Sec3 and Exo70 directly associate with PI(4,5)P₂ (shown in red) in the plasma membrane via their positively charged residues (+++). Exo70 and Sec3 interact with the rest of the exocyst subunits riding on the arriving secretory vesicles. Another exocyst subunit Sec15 directly binds to the GTP-bound form of Rab GTPase Sec4, which is peripherally associated with the secretory vesicle and regulates the assembly of the exocyst complex. The assembly of the exocyst serves to tether the vesicles to the plasma membrane site specified by Sec3 and Exo70. The exocyst may also regulate the assembly of the SNARE complex through direct or indirect interactions. The exocyst is also regulated by the Rho GTPases Cdc42, Rho1, and Rho3. Cdc42 and Rho1 interact with Sec3 and control the polarized localization of the exocyst. Rho3 interacts with Exo70; however, the functional implication of this interaction is not clear. "3": Sec3; "5": Sec5; "6": Sec6; "8": Sec8; "10": Sec10; "15": Sec15; "70": Exo70; "84": Exo84. Sso1/2 and Sec9 are the t-SNAREs and Snc1/2 are the v-SNAREs for exocytosis at the plasma membrane in yeast.