The Exocyst Complex in Polarized Exocytosis

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Exocytosis is an essential membrane traffic event mediating the secretion of intracellular protein contents such as hormones and neurotransmitters as well as the incorporation of membrane proteins and lipids to specific domains of the plasma membrane. As a fundamental cell biological process, exocytosis is crucial for cell growth, cell–cell communication, and cell polarity establishment. For most eukaryotic cells exocytosis is polarized. A multiprotein complex, named the exocyst, is required for polarized exocytosis from yeast to mammals. The exocyst consists of eight components: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84. They are localized to sites of active exocytosis, where they mediate the targeting and tethering of post-Golgi secretory vesicles for subsequent membrane fusion. Here we review the progress made in the understanding of the exocyst and its role in polarized exocytosis.

KEY WORDS: Membrane traffic, Exocytosis, Exocyst, Vesicle targeting, Vesicle tethering, Rab, Rho, Ral, Cell polarity.

I. Introduction

Polarized exocytosis is essential for a wide range of biological processes from neuronal growth cone formation to epithelial asymmetry establishment. Polarized exocytosis consists of at least three stages. First, Golgi-derived secretory
vesicles are targeted to the vicinity of designated plasma membrane domains via microtubule- and/or actin-based transport systems. Second, after the vesicles arrive at their destinations, they are tethered to specific plasma membrane domains (Guo et al., 2000; Pfeffer, 1999). Finally, interactions between vesicle and plasma membrane integral membrane proteins, termed v-SNAREs and t-SNAREs, respectively (SNARE, soluble N-ethylmaleimide-sensitive fusion attachment protein receptors), lead to the fusion of the secretory vesicle with the plasma membrane. This fusion event allows the secretion of vesicle contents and the incorporation of membrane proteins at specific plasma membrane domains. Studies suggest that the membrane addition at the specific sites on the plasma membrane is mediated by the exocyst, an evolutionarily conserved multiprotein complex. Here, we review our current understanding of the exocyst complex and its role in polarized exocytosis.

II. Discovery of the Exocyst Complex

The origins of the exocyst can be traced to a single genetic screen performed in Saccharomyces cerevisiae in the late 1970s (Novick and Schekman, 1979; Novick et al., 1980). The screen identified 23 complementation groups of temperature-sensitive yeast mutants defective in secretion. These 23 genes could be divided into two basic groups. The first group consisted of 13 genes encoding proteins important for endoplasmic reticulum (ER)-to-Golgi and/or intra-Golgi membrane trafficking. The second group included 10 genes encoding proteins that function at a late stage in the exocytic pathway: after protein packaging into exocytic vesicles in Golgi but before vesicle fusion with the plasma membrane (Novick et al., 1980, 1981). These 10 genes are known as the “late-acting” secretory genes and are named SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC8, SEC9, SEC10, and SEC15. Yeast cells containing late-acting sec mutations share a common phenotype when examined by thin-section electron microscopy: they accumulate 80- to 100-nm post-Golgi vesicles at the nonpermissive temperature (Novick et al., 1980, 1981).

Almost all the late-acting SEC genes discovered in yeast now have homologs in higher organisms. Sec1 is homologous to fruitfly Drosophila melanogaster Rop (Salzberg et al., 1993), to nematode Caenorhabditis elegans UNC18 (Gengyo-Ando et al. 1993), and to mammalian neuronal Sec1 or Muc18 (Garcia et al. 1994; Pevsner et al., 1994). The Sec1 family proteins have been found to play a role in regulating SNARE protein interactions during membrane fusion (Jahn, 2002). Sec4 is the founding member of the Rab family of low molecular weight GTPases that function in many membrane-trafficking pathways (Salminen and Novick, 1987) and Sec2 is the guanine nucleotide exchange factor for Sec4 (Walch-Solimena et al.,...
Sec9 is the yeast homolog of SNAP-25, a neuronal t-SNARE (Brennwald et al., 1994). The remaining six late-acting SEC genes all function as part of the exocyst complex as described below.

The earliest data indicating that a late-acting SEC gene product may be present in a high molecular weight complex came from the analysis of the Sec15 protein in yeast. Sec15 was shown to associate with a 19.5S particle, of which 25% was associated with the plasma membrane while the remainder was cytoplasmic (Bowser and Novick, 1991). Interestingly, overexpression of Sec15 resulted in a block in the secretory pathway and caused clustering of exocytic vesicles. These vesicles, however, failed to cluster in the sec4-8 and sec2-41 mutant background (Salminen and Novick, 1989). These functional data are the first evidence showing a link between a component of the exocyst complex and Sec4, a Rab GTPase essential for post-Golgi secretion. In rapid succession, Sec8 and Sec6 proteins were shown to coisolate with Sec15 in a high molecular weight complex consisting of at least eight polypeptides (Bowser et al., 1992; TerBush and Novick, 1995). This complex was shown to be localized to sites of polarized exocytosis in yeast, indicating a possible role in vesicle targeting (TerBush and Novick, 1995). Importantly, the stability of this complex of eight subunits was found to be markedly disrupted in yeast strains containing sec3-2, sec5-24, sec6-4, sec10-2, and sec15-1 alleles (TerBush and Novick, 1995). This brings up the possibility that the protein products of the SEC3, SEC5, and SEC10 genes, the remaining members of the late-acting SEC genes, could be associated with Sec6, Sec8, and Sec15 in one multiprotein complex.

Yeast was not the only organism in which genetics identified an exocyst component. In a promoter trap screen for mouse strains with embryonic lethal mutations, the SA bgeo4 gene (Friedrich and Soriano, 1991) was isolated and later shown to be homologous to exocyst SEC8 (Friedrich et al., 1997). SA bgeo4 is also known as spock because its in vivo expression in transgenic animals resulted in a bright staining pattern in the telencephalon resembling Mr. Spock’s facial expression. Mice homozygous for spock displayed markedly less mesoderm formation and showed arrest of development on embryonic day 6.5 (Friedrich and Soriano, 1991; Friedrich et al., 1997). Because it is known that secretion of growth factors is important for mesoderm formation, this result is consistent with spock playing a role in exocytosis. No direct data supporting this hypothesis, however, were available (Friedrich et al., 1997). While genetic screens identified genes coding for individual exocyst subunits, subsequent biochemical purification and characterization of these gene products enabled the discovery of these gene products as components of the exocyst complex as well as of additional exocyst subunits missed by the genetic methods.

Novick’s group, taking advantage of molecular biology and yeast genetics, generated a yeast strain containing a c-myc epitope-tagged SEC8 gene as the
sole copy of SEC8. With this strain, proteins associated with the Sec8 protein were isolated by large-scale coimmunoprecipitation from detergent extracts of yeast lysates. The exocyst complex proteins were then separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and individual complex-specific bands were excised and concentrated by funnel tube gel electrophoresis (TerBush et al., 2001). These bands were then separated for a second time by SDS–PAGE and concentrated. The protein present in each gel slice was then subjected to proteolysis, and the resulting peptides were separated by high-performance liquid chromatography. These peptides were then identified by Edman degradation peptide sequencing or by matching their mass against a peptide database of yeast proteins (TerBush et al., 1996). In the latter case, this was possible because the complete yeast genome was available to provide a complete data set against which to compare the exocyst peptide masses. This approach identified Sec3, Sec5, Sec10, and Exo70 as proteins that coisolated with the previously identified Sec6, Sec8, and Sec15. This seven-protein complex was named the exocyst as all of the known components of this complex are required for exocytosis in yeast (TerBush et al., 1996).

The search for mammalian exocyst complex was prompted by the timely realization of the conservation of the secretory pathway from yeast to mammals. It was observed that many proteins involved in the late stage of the yeast secretory pathway, such as the SNARE proteins, the SNARE-associated proteins, and the small GTPase rab family members, have homologs in mammals that participate in general exocytosis during membrane addition and in regulated exocytosis underlying neurotransmission (Bennet and Scheller, 1993; Bennet et al., 1993). Curiously, there were six secretory proteins (Sec3, Sec5, Sec6, Sec8, Sec10, and Sec15) required for the late stage of the yeast secretory pathway, which had no identified mammalian homologs (Novick and Schekman, 1980). In fact, yeast genetic studies showed that overexpression of Sso1, a yeast homolog of the mammalian t-SNARE protein syntaxin, can suppress mutations in two of these secretory proteins, Sec3 and Sec15 (Aalto et al., 1993). This observation suggests that at least two of these secretory proteins may act upstream of syntaxin.

By screening human genome expressed sequence tag (EST) databases with these yeast Sec proteins, Scheller’s group was able to identify human infant brain ESTs homologous to SEC6 and SEC8 (Ting, 1995). These ESTs were then used to clone rSec6 and rSec8 genes from rat brain cDNA libraries (Ting et al., 1995). Mammalian Sec6 and Sec8 comigrated on a continuous glycerol gradient at 17 svedberg, indicating that they are components of a 600- to 700-kDa complex. Subsequently, antibodies generated against rSec6p and rSec8p were used to monitor the purification of the rat brain rSec6/8 (exocyst) complex from rat brain lysate, using sequential column chromatography (Hsu et al., 1996). The purified mammalian exocyst complex contained
Sec6 and Sec8, as well as six other proteins. Peptide sequencing of the additional six exocyst subunits showed that they are mammalian homologs of the yeast secretory proteins Sec3, Sec5, Sec10, and Sec15, as well as of two additional yeast proteins Exo70 and Exo84 (Brymora et al., 2001; Hsu et al., 1996; Kee et al., 1997; Matern et al., 2001).

What about Exo84 in yeast? It turns out that in yeast Exo84 runs at almost exactly the same molecular weight as that of a prominent C-terminal breakdown product of Sec3 (91 kDa) in SDS–polyacrylamide gels. When the yeast exocyst complex was isolated, peptide sequence identified only the Sec3 fragment on the gel. Using a database search, Guo et al. was able to identify the yeast Exo84 on the basis of the rat Exo84 sequence. Genetic analysis indicates that the yeast Exo84, like the other exocyst proteins, is essential for exocytosis (Guo et al., 1999b; Zhang and Guo, unpublished data).

Although there is limited sequence homology between mammalian and yeast exocyst subunits (approximately 17 to 24% sequence identity) (Brymora et al., 2001; Hazuka et al., 1997; Guo et al., 1997; Kee et al., 1997; Matern et al., 2001; Ting et al., 1995), it is interesting to note that both complexes contain eight subunits of similar molecular weights ranging from 70 to 140 kDa (Hsu et al., 1996; TerBush et al., 1996).

The purified mammalian exocyst complex has been visualized by quick-freeze/deep etch electron microscopy (Hsu et al., 1999). In the absence of fixation with glutaraldehyde, the complex shows variable conformations, usually as a set of four or six arms radiating outward from a central point. The arms are about 4–6 nm in width and 10–30 nm in length. After a brief fixation with glutaraldehyde, the exocyst complex adopts a much more uniform conformation, resembling the letter “T” or “Y.” It has an elongated body about 30 nm long and 13 nm wide, with two arms approximately 15 nm long and 6 nm wide splaying outward from one end of this central body. The arms appear to attach to the body through a flexible hinge region, allowing the arms to extend from the body at varying angles. For comparison, the diameter of a synaptic vesicle is about 50 nm, demonstrating that the exocyst complex is a large macromolecule.

III. Characteristics of the Exocyst Complex

A. Organization

To understand the function of the exocyst, it is important to understand the molecular organization of this complex. Guo et al. (1999a) have subcloned the cDNAs for individual yeast exocyst components into the pcDNA3 expression vector and translated these proteins in vitro in rabbit reticulocyte
lysates. Coimmunoprecipitation experiments were then carried out on all pairwise combinations of cosynthetized exocyst proteins in order to identify the interactions between these subunits. The individual components were tagged with specific epitope sequences such as hemagglutinin (HA) and FLAG. The tagged proteins were immunoprecipitated with monoclonal antibodies against the epitope sequences and the precipitates were analyzed for the presence of the nontagged subunit. The results of this experiment showed that Sec5 interacts with Exo70, Sec10 interacts with Sec15 (Roth et al., 1998), Sec5 interacts with Sec6, Sec5 interacts with Sec3, and Sec6 interacts with Sec8. Analysis using the yeast two-hybrid assay confirmed most of these results (Guo et al., 1999a). These binding results are also supported by genetics. For example, Sec10 is needed to link Sec15 to the remainder of the complex. In agreement, Sec15 was absent from the partial complex isolated from a sec10-2 mutant strain (TerBush and Novick, 1995). The binding of Sec10/Sec15 to the rest of the exocyst is mediated by the interaction of Sec10 with Sec5. Sec5 also makes critical links to Exo70, Sec6, and Sec3. Sec5 therefore appears to be at the core of the complex. As supporting evidence to this model, loss of Sec5 function leads to the failure of Sec10, Sec15, Exo70, and the mutant Sec5 protein to coprecipitate with Sec8 (TerBush and Novick, 1995; TerBush et al., 1996). The coprecipitation of Sec3 was reduced, suggesting that Sec5 also plays an important role linking Sec3 to Sec8, most likely through the association of Sec5 with Sec6 and Sec6 with Sec8.

Using a pairwise two-hybrid assay, Matern et al. (2001) studied the pairwise subunit–subunit interactions of the mammalian exocyst complex. Positive two-hybrid interactions were found between Sec15 and Sec10, Sec8 and Sec10, Sec5 and Sec6, and Sec6 and Exo70. There is also interaction between Sec3 and Sec5 and between Sec3 and Sec8. In addition to these strong two-hybrid interactions, numerous weak interactions were also detected. The existence of these weaker interactions brings up the possibility that the stability of the intact complex may be achieved through a series of higher order interactions not detectable by the pairwise protein interaction studies using the two-hybrid assay. While additional binding assays are still needed to support these data and identify new interaction partners, studies carried out so far have revealed similar subunit–subunit interaction pattern in both yeast and mammalian exocyst.

B. Subcellular Localization

In yeast, components of the exocyst complex are concentrated in subdomains of the plasma membrane that represent sites of active vesicle fusion (Finger et al., 1998; Mondesert et al., 1997; TerBush and Novick, 1995). These sites of active vesicle fusion relocate with the cell cycle. As cells enter the cycle,
exocyst subunits were found concentrated in the emerging bud. With further cell growth, the exocyst proteins were concentrated at the tip of the bud. When the daughter cell has grown to a certain size and their growth pattern has switched from apical to isotropic, the exocyst proteins were redistributed throughout the daughter plasma membrane. During cytokinesis, exocyst components reconcentrated first as one ring, and then as two rings at the neck that separates the mother cell and the bud. One of the components of the exocyst complex, Sec3, localized to these sites independently of ongoing secretion and of mutations in other components of the exocyst. These observations led to the proposal that Sec3 represents a spatial landmark for exocytosis and that it may be the component of the exocyst complex that is most proximal to the target membrane (Finger et al., 1998). It is interesting to note that during isotopic growth, disruption of septin affected the localization of Sec3 and Sec5 (Barral et al., 2000). Septins constitute a family of conserved GTPases implicated in many cell biological processes including cell division and cell polarization (Field and Kellog, 1999; Kartmann and Roth, 2001; Longtine et al., 1996; Mitchison and Field, 2002). This observation suggests that septins may play a role in restricting exocyst localization.

The subcellular localization of the mammalian exocyst complex seems to be much more complicated. With the cell biological approach, exocyst complex localization has been investigated by antibody detection of endogenous exocyst subunits and by green fluorescent protein (GFP) tagging of exogenously introduced exocyst subunits. Anti-exocyst subunit antibodies have detected two major exocyst subcellular distribution patterns in cells with no extensions or processes emanating from the cell body. In MDCK epithelial and pancreatic acinar cells, the Sec6 and Sec8 subunits were found to have both perinuclear and plasma membrane enrichment (Shin et al., 2000; Yeaman et al., 2001). In particular, plasma membrane–localized exocyst staining was enriched at the tight junction (Grindstaff et al., 1998; Yeaman et al., 2001) or the lateral membrane (Kreitzer et al., 2003) in MDCK cells. Sec10 also exhibited a similar tight junction enrichment (Lipschutz et al., 2000). When MDCK cells were treated with the calcium chelator EGTA to disrupt cell–cell contact among these cells, plasma membrane–localized Sec8 was found to redistribute into the cytoplasm (Grindstaff et al., 1998). Thus the plasma membrane localization of at least one exocyst subunit is dependent on cell polarity and/or cell-to-cell contact.

In cells with processes or extensions emanating from the cell body, the exocyst complex subunits have been found at the perinuclear region, as well as throughout the processes with enrichment at the tip of the processes. In neuroendocrine PC12 cells Exo70 localization was dependent on the differentiation state of the cell (Vega and Hsu, 2001). In undifferentiated PC12 cells, Exo70 displayed perinuclear enrichment. On the addition of nerve growth factor to promote neurite outgrowth, Exo70 was found distributing from the
perinuclear region into the growing neurite and became enriched in the growth cone. This nerve growth factor-induced Exo70 redistribution was dependent on the activation of the mitogen-activated protein (MAP) kinase pathway. Antibodies against four other exocyst subunits, Sec6, Sec8, Sec15, and Exo84, showed similar localization (Wang and Hsu, 2003). In cultured hippocampal neurons, Sec6 and Sec8 were also found in the cell body, axons, and dendrites (Hazuka et al., 1999). In cultured kidney glomerular visceral epithelial cells known as podocytes, Sec6 and Sec8 were also found in the cell body, with enrichment at the terminus of podocyte processes (Simons et al., 1999).

Localization of the exocyst complex was also assessed by monitoring GFP-tagged exocyst subunits (Matern et al., 2001). When N- or C-terminal GFP fusion constructs of Sec3, Sec5, Sec8, Sec10, and Exo70 as well as the C-terminal GFP fusion construct of Sec15 were stably transfected into MDCK cells, all fusion proteins, except Exo70, displayed cytosolic distribution. This subcellular localization pattern may be due to the failure of GFP-tagged exocyst subunits to completely incorporate into the endogenous exocyst complex. Interestingly, GFP-tagged Exo70 showed plasma membrane enrichment. However, GFP–Exo70-expressing cells failed to establish stable cell-to-cell contact, suggesting that this construct may act as a dominant-negative construct. Overall, cell biological studies indicate that the exocyst complex is enriched at or recruited to distinct cellular locations and that this localization is dependent on cellular signals.

Localized exocyst enrichment in the cell is likely due to the anchoring or active recruitment of the exocyst complex by insoluble cellular structures. In agreement, sedimentation studies have shown that the majority of the exocyst complex cosedimented with an insoluble cellular fraction in rat brain (Hsu et al., 1996). The question now is: what is the cellular structure that associates with the exocyst complex. The perinuclear localization of the exocyst complex suggests that it is associated with either the Golgi apparatus/network or the microtubule-organizing center. Both structures have perinuclear localization.

In MDCK cells, pharmacological, biochemical, and cell biological studies showed that the exocyst complex is localized to the trans-Golgi network at the perinuclear region and to the plasma membrane, where it colocalized with adhesion junction proteins including ZO-1, cortical actin, E-cadherin, α-catenin, and occludin (Grindstaff et al., 1998; Yeaman et al., 2001). Exocyst complex Sec6 and Sec8 subunits colocalized with vesicles carrying exocytic cargoes. Blockage of exocytosis inhibited recruitment of the exocyst complex to the plasma membrane. Treatments that block exocytosis, such as low temperature and expression of kinase-inactive protein kinase D, also caused the accumulation of exocyst subunits at the perinuclear region. Thus it was hypothesized that there is a steady state distribution of the exocyst complex between the trans-Golgi network and the plasma membrane. In addition, the
introduction of various monoclonal antibodies against Sec6 and Sec8 into permeabilized MDCK cells resulted in protein cargo accumulation either at the perinuclear region or near the plasma membrane, implicating that the exocyst complex may function at multiple stages in Golgi-to-plasma membrane vesicle trafficking. These results show that exocyst Sec6 and Sec8 subunits are recruited to specific plasma membrane domains via vesicle trafficking. They are consistent with studies in yeast in which the localization of GFP-tagged exocyst was also dependent on vesicle trafficking (Finger et al., 1998; Guo et al., 1999b).

In neuroendocrine PC12 cells, three lines of evidence suggest that the exocyst complex is associated with cytoskeletal elements such as septins and microtubules (Vega and Hsu, 2001, 2003). First, density gradient centrifugation study of brain and PC12 cell lysates showed that the majority of exocyst subunits Sec6, Sec8, and Exo70 comigrated with septins and microtubules, but not with Golgi, endosome, or plasma membrane proteins. Consistent with this result, the complex coimmunoprecipitated with septins and tubulin from both brain and PC12 cell lysates. In addition, the exocyst complex copurified with five septins, including septin2, through a lengthy purification process involving multiple column chromatography (Hsu et al., 1999). Second, visualization of endogenous exocyst subunits and septin2 by monoclonal antibodies showed that both exocyst and septin2 have similar localization. They exhibited filamentous distribution emanating from the perinuclear region toward the plasma membrane (Vega and Hsu, 2003; Wang and Hsu, 2003). Septin2, like the exocyst complex, has also been shown to play a role in neurite outgrowth in PC12 cells (Vega and Hsu, 2003). Third, treatment of PC12 cells with microtubule-disrupting, but not Golgi-disrupting, drug dispersed both exocyst and septin2 perinuclear localization. These observations are consistent with findings in yeast in which exocyst subunits localization was also affected in septin-defective cells (Barral et al., 2000).

The above-described results suggest that exocyst localization is dependent on the cellular structures with which it associates. Identification and characterization of exocyst associated proteins/cellular structure should provide important insights into the molecular events underlying exocyst function.

IV. Function and Regulation of the Exocyst Complex

A. Role of the Exocyst Complex in Exocytosis

Most of the exocyst subunits (Sec3, Sec5, Sec6, Sec8, Sec10, and Sec15) in yeast were first identified as SEC gene products because of their involvement in secretion (Novick et al., 1980). Mutations in these genes resulted in
defective secretion as assayed by measuring the cell surface incorporation and intracellular accumulation of enzymes such as invertase. Furthermore, electron microscopic studies revealed the accumulation of 80-nm secretory vesicles in the mutant cells. Study of newly generated mutants in the other two genes, \textit{EXO70} and \textit{EXO84}, revealed similar phenotypes (TerBush and Guo, unpublished observation). In addition, genetic studies in yeast also demonstrated close genetic interactions among the exocyst components and other genes involved in secretion, such as Sso1 (Aalto et al., 1993).

Insights into the biological functions of the exocyst complex in higher eukaryotes have been obtained by perturbing exocyst function in tissue culture cells or in whole organisms. In the MDCK epithelial cell line, the introduction of anti-exocyst Sec8 subunit monoclonal antibodies into streptolysin O-permeabilized cells perturbed protein targeting to the basolateral, but not to the apical, plasma membrane domain of these cells (Grindstaff et al., 1998). Similarly, the inhibition of exocyst function with anti-exocyst Sec8 subunit monoclonal antibodies in pancreatic acinar primary cells also disrupted the delivery of calcium signaling proteins to the apical plasma membrane domains of these cells (Shin et al., 2000). In the neuroendocrine PC12 cell line, the overexpression of an exocyst Sec10 deletion dominant-negative construct prevented neurite outgrowth (Vega and Hsu, 2001). Thus, perturbations in exocyst function affect protein targeting to specific plasma membrane domains to establish cell polarity and promote neurite outgrowth. In the mammalian system, the exocyst complex has also been shown to be required for vesicle trafficking. In fact, the exocyst complex is ubiquitously expressed, suggesting that it may be a core component of the vesicle-targeting machinery in every cell. Interestingly, the highest exocyst subunit expression is found in brain, an organ with highly polarized cells (Guo et al., 1997; Kee et al., 1997; Sjolinder et al., 2002; Ting et al., 1995). In mice with the exocyst subunit Sec8 knocked out, animals die shortly after the induction of gastrulation (Friedrich et al., 1997). The early embryonic lethality suggests that exocyst function is essential for development. However, it is not clear whether the induction of lethality at gastrulation is a result of maternal contribution depletion or of the dispensability of exocyst function until the induction of gastrulation. The first possibility is consistent with observations in yeast, in which exocyst null mutants are nonviable.

In the case of exocyst subunit Sec5 knockout in the fruit fly \textit{Drosophila melanogaster}, the organisms die as growth-arrested larva (EauClaire and Guo, 2003; Murthy et al., 2003). The null alleles failed to grow and the neuromuscular junctions failed to develop completely. Furthermore, neurite outgrowth in culture is inhibited in \textit{sec5} mutants once maternally supplied Sec5 is exhausted. Using a T cell transmembrane protein, CD8, as a plasma membrane protein marker, the authors showed that exocytosis at the plasma membrane was defective in \textit{sec5} mutants, whereas the synthesis of
CD8 protein and the generation of CD8-containing vesicles were normal. This is similar to what was observed in temperature-sensitive yeast exocyst mutants. After temperature shift, the mutant cells accumulated secretory vesicles; and the exocytosis of cell wall enzyme markers such as invertase was blocked. In sharp contrast to the arrest of neurite growth and plasma membrane protein addition, the authors found that synaptic transmission continued to be robust despite the decline of maternal Sec5 protein. These results indicate that the exocyst complex is required for constitutive secretion where secretory vesicles are delivered from Golgi to the plasma membrane. It is not, however, necessary for regulated secretion in the form of neurotransmission where synaptic vesicles are tethered near the plasma membrane and undergo repeated local exocytosis and endocytosis cycles. Similarly, the depletion of exocyst Sec10 subunit in *Drosophila* by RNA interference (RNAi) also resulted in early postembryonic lethality (Andrews et al., 2002). However, tissue-specific Sec10 RNAi did not cause defect in neuromuscular junction formation. Neurotransmission in this organism was not perturbed. Instead, the ring gland, an organ specialized in hormone secretion, was affected. These results suggest that fruit fly Sec10 is required for hormone secretion but not for constitutive or regulated secretion underlying neurogenesis or neurotransmission. This discrepancy in exocyst subunit deletion studies brings up at least two possibilities. First, the Sec10 subunit is not essential for exocyst complex function in membrane addition or neurotransmission. However, it is necessary, on its own or as part of the exocyst complex, in mediating hormone secretion. Alternatively, there is more than one Sec10 isoform in *Drosophila*. The Sec10 isoform that is deleted in this RNAi study is not the isoform that is part of the exocyst complex. In fact, Northern blot analysis in mammalian tissues suggests that there may be different isoforms of Sec10 (Guo et al., 1997). Coimmunoprecipitation experiments using antibodies against the Sec10 isoforms may help verify this possibility.

It was discovered that the last four amino acids of exocyst subunits Sec6 and Sec8 contain consensus sequence for type 1 PDZ domain protein binding (Riefler et al., 2003; Sans et al., 2003). Through this consensus sequence Sec8 was found to interact with the postsynaptic density protein PSD-95 (Riefler et al., 2003), a member of the membrane-associated guanylate kinase (MAGUK) family that has been shown to participate in the assembly of synaptic signaling complexes at excitatory synapses (Fang and Zhang, 2002; Hung and Sheng, 2002). In addition, Sec8 has also been found to interact with the NMDA (*N*-methyl-∗d*-aspartate) receptor through synapse-associated protein SAP-102 (Sans et al., 2003). NMDA receptor is a member of the ionotropic glutamate receptors that mediate most of the excitatory neurotransmission in the central nervous system (Dingledine et al., 1999). The Sec8/SAP-102/NMDA receptor interaction appears to have taken place at an early...
stage in the vesicle trafficking pathway, possibly at the endoplasmic reticulum or the Golgi apparatus. Overexpression of a sec8 mutant with deletion of its last four amino acids decreased NMDA-mediated current and surface expression, indicating that the Sec8/SAP-102/NMDA receptor interaction plays a role in regulating the delivery of the NMDA receptor to the cell surface. These results bring up the possibility that the exocyst complex may target a subset of proteins from the Golgi to the plasma membrane, using PDZ domain proteins as adapters (Hoogenraad and Sheng, 2003).

B. Exocyst Regulation by Ras Superfamily of Small GTPases

Genetic and cell biological experiments showed that the exocyst complex is required for a multitude of cellular processes, all requiring protein targeting to specific plasma membrane domains. The question now is, what are the molecular mechanisms controlling the exocyst in protein targeting? The exocyst functions in mediating protein targeting, and is likely to be tightly regulated to promote precise protein/membrane addition to the plasma membrane. Identification of proteins that interact with the exocyst will help us to understand the regulation of this complex as well as of exocytosis. Research from both yeast and mammalian cells identified several members of the Ras family of small GTPases that interact and regulate the exocyst (for review, see Lipschutz and Mostov, 2002; Novick and Guo, 2002). Because the small GTPases have multiple effectors, exocyst function could be coordinated with other cellular processes also controlled by these G proteins.

1. Rab

The first GTP-binding protein found to associate with the exocyst was the yeast Sec4 protein, a member of the Rab branch of the Ras superfamily. In general, Rab proteins associate with specific membrane compartments during both exocytic and endocytic pathways and regulate vesicular transport at distinct stages along these pathways (Lazar et al., 1997; Novick and Zerial, 1997). Sec4 is associated with secretory vesicles and is essential for vesicle transport from the Golgi apparatus to the cell surface. Exocyst subunit Sec15 was found to interact with the GTP-bound form of Sec4, but not with other yeast Rab proteins such as Ypt1. In addition, Sec15 interacts with Sec4ΔC (non prenylated) and Sec4L79 (GTP bound), but not Sec4I133 (nucleotide-free state) or Sec4V29 (probable GDP-bound state), suggesting that it prefers the GTP-bound form of Sec4 and that Sec4 prenylation is not required for its binding. Furthermore, the interaction with Sec15 with Sec4 requires the effector domain of Sec4p, because replacement of four amino
acids in this domain with the corresponding region of Ypt1, the closest homolog for Sec4 in yeast, abolished the interaction.

In addition to its interaction with Sec4, Sec15 also associates with secretory vesicles (Guo et al., 1999). Subcellular fractionation of yeast lysates demonstrated that Sec15 cofractionated with Sec4 and Snc (vesicle-bound v-SNAREs). Second, immunoelectron microscopy studies showed that both Sec4 and Sec15 colocalized with clusters of secretory vesicles that accumulate in response to Sec15 overproduction (Guo et al., 1999; Salminen and Novick, 1989). Sec4-GTP:Sec15 interaction may trigger further interactions between Sec15 and other exocyst components, eventually leading to docking and fusion of secretory vesicles with specific domains of the plasma membrane. Loss of Sec4 function left the exocyst in a partially assembled state (Guo et al., 1999b). Overproduction of Sec4, on the other hand, compensated for partial loss-of-function mutations in exocyst subunits. On the other hand, loss of either Sec4 function or Sec4 exchange protein Sec2 function prevented Sec15 overproduction-induced vesicle clustering (Salminen and Novick, 1989). These observations all support the hypothesis that the exocyst is a downstream effector of Sec4, possibly functioning in tethering vesicles that carry Sec4 in its GTP-bound state to the plasma membrane. In mammalian cells, there are several Rab proteins that, like Sec4, regulate exocytosis. It will be interesting to investigate whether any of these Rab proteins interact with the mammalian exocyst.

2. Rho

Given the proposed role of the exocyst in vesicle tethering, it is critical that this complex be properly positioned so that exocytosis will take place at the right site. In budding yeast, the exocyst was specifically localized to the bud tip or the mother/daughter connection, regions of active membrane addition during its life cycle. This polarized localization was lost in several rho1 and cdc42 mutant alleles (Guo et al., 2001; Zhang et al., 2001). The Rho GTP-binding proteins are master regulators of a wide range of cellular processes including cytoskeleton organization, cell polarization, gene transcription, and membrane traffic. The yeast Rho family consists of six members: Cdc42, Rho1, Rho2, Rho3, Rho4, and Rho5. The effect of Rho1 and Cdc42 on exocyst localization was not mediated through organization of the actin. Rather, the GTP-bound form of Rho1 and Cdc42 directly interacted with Sec3. Truncation of the Rho1/Cdc42-binding domain of Sec3 led to its depolarized localization in the cell. The availability of various cdc42 mutant alleles has identified various cellular processes controlled by Cdc42. While GFP-tagged exocyst components were depolarized in several cdc42 mutant alleles resulting in the mislocalization of GFP-tagged exocyst subunits (Zhang et al., 2001), one cdc42 mutant allele also exhibited
accumulation of secretory vesicles in the cell (Adamo et al., 2001). These
mutant phenotypes implicate a role for Cdc42 in regulating exocytosis, both
spatially and kinetically. Both Rho1 and Cdc42 interact with the N terminus
of Sec3. In fact, these two proteins compete in their binding to Sec3 (Zhang
et al., 2001). Cdc42 is essential for the establishment of yeast polarity and
Rho1 may be important for the maintenance of polarized growth (Yamochi
et al., 1994). Their interactions with the exocyst may help to restrict exocy-
tosis to the site of polarized cell growth, where new plasma membrane
components are added.

While Rho1 and Cdc42 interact with Sec3, Rho3, in its GTP-bound form,
directly interacts with the exocyst protein Exo70 (Adamo et al., 1999;
Robinson et al., 1999). rho3 mutants exhibited depolarized actin as well as
defects in exocytosis (Adamo et al., 1999). However, there is no report of
exocyst localization defects in these mutants. The role of Rho3 in exocytosis
is probably kinetic in nature. Although different Rho proteins talk to the
exocyst in different ways, all of these interactions may allow the Rho proteins
to coordinate exocytosis with other Rho-dependent processes, such as actin
organization, to achieve polarized yeast cell growth.

Rho proteins are involved in polarized exocytosis in mammalian cells
(Kroschewski et al., 1999; Musch et al., 2001; Rogers et al., 2003). One
study demonstrated that the exocyst is a downstream effector of TC10, a
Rho protein sharing sequence similarity with Cdc42. A yeast two-hybrid
screen using constitutively active mutant of TC10 identified the exocyst
component, Exo70 (Inoue et al., 2003). Coimmunoprecipitation experiment
further showed that TC10, but not other Rho proteins, preferably binds to
the exocyst complex. Importantly, expression of the active form of TC10 in
3T3 cells promoted the recruitment of Exo70 to the plasma membrane, where
the exocyst carries out its physiological function. Overexpression of full-
length Exo70 in 3T3 adipocytes enhanced insulin-mediated glucose uptake.
On the other hand, overexpression of the N-terminal portion of Exo70
(Exo70N) blocked glucose uptake. Exo70N did not affect the translocation
of Glut4. Instead, it probably inhibited a step after Glut4-containing vesicles
were transported to the plasma membrane. This study not only reveals a
molecular connection between the exocyst and Rho protein in mammalian
cells, but also provides evidence for the role of the exocyst in exocytosis at the
vicinity of the plasma membrane. It was proposed that Glut4 exocytosis
takes place in the lipid raft domains of the plasma membrane in 3T3 adipocytes
(Saltiel and Pessin, 2002). On the other hand, it has also been shown that
exocysts are spatially limited to the basolateral domain, which is not
enriched with lipid rafts in MDCK cells (Grindstaff et al., 1998; Kreitzer
et al., 2003; Yeaman et al., 2001). While the plasma membrane is a primary
site for exocyst function, more experiments are needed to further investigate
the nature of the plasma membrane domains where the exocyst functions.
3. Ral

Yeast two-hybrid screens and in vitro protein-binding studies have revealed that exocyst subunit Sec5 interacts with a Ras family member, Ral, in mammalian cells (Brymora et al., 2001; Moskalenko et al., 2002; Polzin et al., 2002; Sugihara et al., 2002). Sec5 preferentially binds to the GTP-bound form of RalA, and the interaction domain has been mapped to the N terminus of Sec5p. The structure of the RalA-binding domain of Sec5 has been analyzed by crystallography and was shown to display an immunoglobulin-like β-sandwich structure that represents a novel interaction domain for an effecter of a GTP-binding protein (Fukai et al., 2003; Mott et al., 2003). The Sec5–RalA interaction may modulate exocyst function by either regulating the formation of the complex or modulating the exocyst activity. In this regard, the disruption of Ral function has been shown to perturb exocyst complex assembly (Moskalenko et al., 2002), an effect similar to that seen on the loss of Sec4 function in yeast (Guo et al., 1999). The involvement of Ral in protein targeting and the secretory pathway may be, at least partially, through their regulation of exocyst function. Both expression of constitutively active RalA and inhibition of either the function or synthesis of RalA had a similar effect on protein export in epithelial cells. Proteins normally targeted to the basolateral surface of epithelial cells became randomly distributed between the apical and basolateral surfaces, while proteins normally targeted to the apical surface were unaffected (Moskalenko et al., 2002). RalA has been shown to associate with regulated secretory compartments such as synaptic vesicles (Ngsee et al., 1991) and secretory granules (Mark et al., 1996). The RalA–exocyst interaction is also important in regulated exocytosis. Both expression of activated RalA and inhibition of RalA function were found to block the stimulated release of human growth hormone by the neuroendocrine PC12 cell line (Moskalenko et al., 2002). In another study, expression of a dominant-negative form of RalA was shown to diminish the readily releasable pool of synaptic vesicles (Polzin et al., 2002). Interestingly, Sugihara et al. (2002) found that injection of antibodies against Sec5 inhibited RalA and Cdc42-induced filopodia formation by a mechanism that is independent of the secretory pathway.

C. Role of the Exocyst in Protein Synthesis

When the human exocyst Sec10 subunit was overexpressed in MDCK cells, there was increased protein delivery to the basolateral, but not to the apical plasma membrane (Lipschutz et al., 2000). Curiously, however, the transfected cells also exhibited an increase in protein synthesis for both basolateral and apical secretory proteins, with a concomitant increase in intracellular
vesicles. In addition, these cells showed morphological changes and are more prone to form cysts and tubules, a process reminiscent of the situation in autosomal dominant polycystic kidney disease (ADPKD) (Charron et al., 2000a,b). These results bring to attention that exocyst function may be coordinated with the protein synthesis process, providing a link between protein synthesis and protein targeting to the plasma membrane.

In support of the above observations, an interaction between the exocyst and the β subunit of Sec61 (Seb1/Sbh1) was found in both yeast and mammalian cells (Lipschutz et al., 2003; Toikkanen et al., 2003). Sec61 complex is the main component of the endoplasmic reticulum translocon. Overexpression of subunits of Sec61 suppressed mutant alleles of the exocyst components in yeast. The interaction between these proteins suggests that there is a regulatory mechanism for exocyst function at both end points of the secretory pathway.

V. Conclusion and Future Directions

Although genetic, cell biological, and biochemical studies in various systems have shown that proper exocyst localization and function are important for protein and membrane trafficking from Golgi to the plasma membrane, it is still not clear how the exocyst carries out its function. Despite its large size, there are few recognizable sequence motifs on exocyst subunits to provide clues to the molecular mechanisms of this complex’s function. The subcellular localization and molecular associations of the exocyst complex have been extensively studied. The results from these studies have given us valuable information about this complex. On the basis of these results, we have the following two speculations.

First, the exocyst may function as a member of the family of “tethering” proteins found in various stages of membrane trafficking ranging from ER to Golgi transport to endocytosis (reviews for “tethering” proteins are provided by Guo et al., 2000; Pfeffer, 1999; Short and Barr, 2002; Waters and Hughson, 2000; Whyte and Munro, 2002). This family of proteins may tether incoming vesicles to their targeting membrane and function before the docking and fusion events mediated by SNAREs. Although there is limited sequence homology among these proteins at different stages of membrane traffic, some similar mechanisms can be found. For example, most of these proteins interact (physically and/or functionally) with Rab proteins at specific stages. In essence, the exocyst can be regarded as a tethering complex that functions at the plasma membrane for tethering post-Golgi secretory vesicles. It interacts with the Rab protein Sec4 and may further interact with SNAREs for exocytosis. Ample genetic evidence links the exocyst to
SNARES; but what is the molecular basis for the connection between this vesicle-tethering complex and the downstream membrane-docking and fusion machinery? Answering this question is not only important for the studies of the exocyst, but also for our understanding of post-Golgi exocytosis. Because the exocyst functions upstream of “committed” docking and fusion, its vesicle-tethering function makes it an ideal candidate to spatially, temporally, and/or kinetically control exocytosis. The eight components of the exocyst complex are therefore excellent targets for regulatory proteins. Although small GTPases have been found to interact with the exocyst, it is likely that other proteins also interact and regulate exocyst proteins.

Second, we propose that the exocyst may regulate vesicle trafficking at the vicinity of the plasma membrane by modulating cytoskeletal dynamics and/or vesicle transfer from microtubules to actin and finally to the plasma membrane. Currently few proteins, in addition to the microtubule, actin, and their associated motors, have been identified to play a role in this process (Goode et al., 2000). In neuroendocrine PC12 cells, the exocyst showed association with microtubules and septin proteins, especially septin2. On nerve growth factor activation of the MAP kinase pathway, both exocyst and septin2 were recruited, possibly in coordination with a subset of microtubules, from the microtubule-organizing center to the plasma membrane via filament-like structures. Once the exocyst is recruited to the vicinity of the plasma membrane, the exocyst may mediate vesicle transfer, by its direct or indirect association with the cytoskeletons, from microtubules to cortical actin or from cortical actin to the plasma membrane. It is also interesting to note that septins have been shown to coordinate with microtubules and actin through microtubule- and actin-associated proteins (Kinoshita et al., 1997, 2002; Nagata et al., 2003; Surka et al., 2002; Vega and Hsu, 2003), making them suitable candidates for communicating between microtubules and actin. Overall, current findings strongly suggest a role for exocyst function at the vicinity of the plasma membrane to ultimately allow the docking and fusion of vesicles with the plasma membrane. The regulation of exocyst localization by cellular signals, such as members of the Ras family of small GTPases, plays an important role in regulating the site and kinetics of exocyst function.

The exocyst was originally discovered in the budding yeast Saccharomyces cerevisiae. Genetic analyses carried out in this organism have proved particularly useful in identifying and characterizing the role of many proteins involved in the vesicle trafficking pathway, including the exocyst complex, in the spatial regulation of secretion and cell polarization. The apparently “simple” budding process requires a sophisticated system to temporally and spatially coordinate membrane traffic to several cellular processes such as cell cycle progression and cell polarization. It will be important to study how these cellular processes are coordinated at the molecular level. The
realization that many proteins involved in the vesicle trafficking pathway are conserved from yeast to mammals has prompted the identification and isolation of the exocyst complex in higher eukaryotes such as the fruitfly *Drosophila melanogaster* and mammals. Although these two systems, especially the mammalian system, do not have the advanced genetic screening methodology developed in yeast, they allow the characterization of exocyst function in a wide variety of biological processes ranging from tight junction formation to neurite outgrowth. Elucidating the role of the exocyst in these processes should provide valuable insights into the molecular mechanisms of exocyst function as well as the molecular events that coordinate many complex biological processes.

While there are many reasons to believe that the basic mechanisms of exocyst function are conserved, it is also clear that different protein–protein interactions and regulatory strategies have been gained or lost during evolution. This specialization may also occur within the same organism, in which the exocyst participates in distinct biological processes in different cell types. It is important to appreciate that the specialization/variation may also be generated by some common molecular basis. For example, the exocyst subunit Sec8 can interact with different type 1 PDZ domain proteins in different cells. There are about 300 PDZ domain proteins in mammals. Thus the PDZ domain proteins can function as adaptors to allow the exocyst to bind different proteins and target different protein cargoes to the plasma membrane in different cells and/or at different developmental stages. Second, exocyst function has been shown to be regulated by different Ras family members in different organisms and cell types. Because these small GTPases have been shown to function as secondary messengers downstream of many signaling pathways, the regulation of exocyst function by various Ras family members may be dependent on the signaling cascades and the different Ras members present in each cell type. These two mechanisms may explain, in part, how exocyst can bind to different proteins and participate in a multitude of biological processes.

In conclusion, the exocyst is an essential component in the exocytosis pathway in all cell types and organisms studied so far. Although different experimental approaches in various systems have led to the proposal of distinct models on the molecular basis of exocyst function, it is clear that mechanisms regulating exocyst subcellular localization and molecular associations play a major role in modulating exocyst function. In addition, these studies also show that the function of this multisubunit complex is required for a multitude of biological processes and is both spatially and temporally regulated. Elucidation of the biological functions of the exocyst will not only contribute to our understanding of vesicle traffic, but will also provide insights into the mechanisms of many complex biological processes requiring polarized secretion.


