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Exposing the Elusive Exocyst Structure

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Abstract

A major challenge for a molecular understanding of membrane trafficking has been the elucidation of high resolution structures of large, multisubunit tethering complexes (MTCs) that spatially and temporally control intracellular membrane fusion. Exocyst is a large hetero-octameric protein complex, proposed to tether secretory vesicles at the plasma membrane to provide quality control of SNARE-mediated membrane fusion. Breakthroughs in methodologies, including sample preparation, biochemical characterization, fluorescence and single-particle cryo-electron microscopy (cryoEM), are providing critical insights into the structure and function of the exocyst. These studies now pose more questions than answers, for understanding fundamental functional mechanisms, and open wide the door for future studies to elucidate interactions with protein and membrane partners, potential conformational changes, and molecular insights into tethering reactions.

Keywords

Exocyst; tethering complex; membrane trafficking; exocytosis; SNARE; membrane fusion; Rab GTPase; Rho GTPase

Tethering complexes regulate intracellular membrane trafficking

Eukaryotic cells are divided into numerous membrane-bound compartments, which require specific transport and delivery of cargo proteins and lipids to maintain cellular homeostasis, growth, signaling and division. The prevailing dogma is that both the multisubunit tethering complexes (MTCs) and coiled coil tethering proteins function to **tether** (see Glossary) two membranes together prior to fusion [1–3]. Specific MTCs are present at the sites of every major fusion step in the secretory and endocytic pathways, contributing to the exquisite specificity of each reaction: exocyst at the plasma membrane; Dsl at the ER; COG in the Golgi; GARP/EARP in the early endosomal retrograde pathway; the related HOPS and CORVET complexes at late endosomal-lysosomal and endosomal trafficking pathways, respectively; and the TRAPP complex in several pathways [2, 4, 5]. A common property of

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these tethering proteins is their interactions with both proteins and lipids on opposing membranes; however, direct experimental evidence for tethering has yet to be reported for most of these complexes [2, 6]. Tethering has been most convincingly demonstrated for the vacuolar/lysosomal HOPS complex [7] and for a number of the coiled coil Golgi and endosomal tethers [3, 8]. For the yeast exocyst, ectopic targeting of Sec3 to the mitochondrial membrane appeared to recruit not only the other exocyst subunits, but also vesicles, suggesting a tethering function [9].

Although not all MTCs are homologous to one another at the sequence or structural level (see below), most share common features consistent with membrane tethering and regulation of fusion—they bind specific Rab GTPases, SNAREs and Sec1/Munc18 (SM) proteins [2, 10, 11]. Each MTC has a distinct location in the cell (unlike the SNARE proteins), indicating a role in determining the specificity of vesicle fusion, while their interactions with SNAREs and SM proteins reveal direct roles in driving SNARE-mediated membrane fusion. Together, the data strongly suggest that MTCs provide a critical quality control mechanism to ensure proper delivery of cargo to the correct destination. The keys to understanding tethering and regulation of SNARE-mediated fusion by MTCs lie in obtaining high resolution structural information, complemented by functional analyses *in vitro* and *in vivo*. Until recently, the available structural data for the MTCs was limited to crystal structures of individual subunits, small subcomplexes, the three-subunit Dsl1 complex, the TRAPP complex, and low resolution negative stain EM structures [12–16]. Mei et al., have now determined a ground-breaking moderate-resolution cryoEM structure of the intact yeast exocyst complex ([17]; Key Figure 1).

The exocyst complex controls exocytosis and cell division

Six out of the eight subunits in the exocyst were originally discovered in the budding yeast *Saccharomyces cerevisiae* in the Novick & Schekman secretory pathway screen [18]. The complex consists of the subunits Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 [19, 20]. Decades of genetic, cell biological, and biochemical experiments determined that exocyst is required for fusion of secretory vesicles with the plasma membrane in most, if not all, eukaryotes [21–25]. The exocyst subunits are predominantly concentrated at sites of polarized exocytosis on the plasma membrane, but they have also been observed at additional locations in other cell types. All exocyst subunits are essential in cells (with the exception of Sec3; [26]); deletion of Sec3, acute degradation (e.g. with an auxin degron tag [16, 27]), or temperature sensitive alleles of any of the subunits leads to loss of exocytosis, cessation of cell growth and division. [26] Exocyst has been associated with a number of additional specialized fusion processes, including endocytosis, ciliogenesis, autophagy, invasion, cell motility, and bacterial pathogenesis; not surprisingly, mutations in exocyst have been implicated in various human diseases (these functions have been reviewed in detail elsewhere, see [21, 24, 25]).

The ~850 kDa exocyst is well conserved in Opisthokonta, Amoebozoa and plants [28, 29]. Remarkably, a dramatic expansion of several exocyst subunits occurred in plants; there are for example, 23 isoforms of Exo70 in *Arabidopsis thaliana* and 13 isoforms in the moss *Physcomitrella patens* [30, 31]. Organisms such as *Giardia intestinalis* (Excavata),

Toxoplasma gondii and *Plasmodium falciparum* (Apicomplexa) have few if any recognizable exocyst subunits [28, 29], suggesting either that exocyst function is absent or that the sequences are substantially diverged. Caution is perhaps warranted, however, since while bioinformatic analyses had suggested that several of the exocyst subunits were missing from *Trypanosoma brucei* (Excavata), biochemical studies later revealed that *T. brucei* not only contains the known eight subunits, but also a ninth Trypanosomid-specific Exo99 subunit [32].

Protein-protein interactions

A mechanistic understanding of exocyst function necessitates biochemical and structural studies, in combination with a cell biological approach. The structure of the complex was challenging to elucidate using recombinant proteins and crystallography, for reasons that are obvious in retrospect (discussed below). Many protein-protein interactions were identified between the subunits, and with binding partners, using a variety of methods, including: yeast two-hybrid assays, pullouts using recombinant proteins or *in vitro* translated polypeptides, and pullouts from cell lysates under destabilizing conditions [21, 24, 33]. Interactions with other partners (discussed below) have also been characterized. These include the Rab GTPase on secretory vesicles (Sec4 in yeast), SNARE proteins on both the vesicle (the paralogs Snc1/2) and the plasma membrane (Sso1/2 and Sec9), the Rab exchange factor Sec2, lipids and PI(4,5)P₂, Rho family GTPases including Cdc42 and RalA/B (in mammalian cells), the SNARE regulator Sec1, the regulator Sro7/77, the type V myosin Myo2, and a number of other factors [21, 24, 33, 34]. Many questions remain unanswered about which subunits and residues are used by exocyst to bind its many partners, as well as where and when they bind within the cell. Similarly, there is little understanding of the order of addition for any partners, e.g. does exocyst bind to the plasma membrane first, then tether incoming secretory vesicles? Or does exocyst need to associate with vesicles prior to tethering? Whether exocyst can interact with more than one binding partner at the same time (e.g. as a bridge to tether the vesicle to the plasma membrane) is, somewhat remarkably, still not known.

Live cell imaging of exocyst subunits in yeast suggested that subcomplexes may traffic to the daughter cell on actin cables with vesicles and assemble with other subunits (Sec3 and Exo70) on the plasma membrane [35]. In contrast, other imaging experiments observed that exocyst does not disassemble before fusion, or during recycling from the bud tip [36]. Moreover, various studies ruled out the presence of stable subcomplexes either *in vivo* or *in vitro*, and indicate that exocyst functions as a stable octamer [16, 17, 37, 38]. It is currently unknown if this is a yeast-specific attribute, however, as several experiments examining exocyst from other organisms suggested the presence of functional subcomplexes [25, 39–43]; these differences will likely be an active area of future study.

Structures of individual exocyst subunits and the intact complex

Obtaining high resolution structures of the exocyst and its subunits has been a major challenge and goal for the field for > 20 years. The first glimpse of the structure of the mammalian exocyst came from Hsu et al., using EM studies of purified samples that were

rotary shadowed with platinum ([44]; Figure 1A). Crystal structures of soluble recombinant domains from several individual exocyst subunits were subsequently determined at high resolution, however, no pairwise or higher order subcomplexes of exocyst yielded suitable crystals. The X-ray structures of individual exocyst structures include: C-terminal domains of yeast Exo84 [45], yeast Sec6 [46], fruit fly Sec15 [47]; and nearly full-length yeast Exo70 [45], zebrafish Sec10 ([48]; Figure 1B); and a related protein, M-Sec ([49]; not shown). These proteins are composed of long rods of helical bundles, and show structural similarity to one another, despite weak (<10%) sequence identity. Indeed, most of the subunits of known structure from the exocyst, COG, GARP, and Dsl complexes share a conserved fold [50, 51]; consequently, these four MTCs are collectively referred to as Complexes Associated with Tethering Containing Helical Rods, or the CATCHR family [2]. CATCHR family subunits also share structural homology with the cargo binding domain from the unconventional type V Myosin, Myo2, that carries yeast vesicles along actin cables [52]. The Myo2 cargo binding domain interacts directly with the Sec15 subunit of exocyst, although this interaction does not appear to be required for vesicle transport [53]. Rather, binding of Sec15 to Myo2 appears to have a role downstream of vesicle delivery. Another CATCHR domain was identified in the MUN region from Munc13, an important regulator of neuronal SNARE complex assembly and synaptic vesicle fusion [54]. Structures of a few non-CATCHR domains in exocyst have also been determined, including the Ral GTPase-binding domains from mammalian Exo84 ([55]; Figure 1B) and Sec5 [56, 57], and an N-terminal domain of Sec3 that contains a cryptic PH domain for PI(4,5)P₂, Cdc42 and Rho1 binding at the plasma membrane ([58, 59]; Figure 1B).

Lower resolution

Although the pioneering purification and platinum-shadowed EM of the mammalian exocyst ([44]; Figure 1A) was promising, additional structural information was slow to follow. When fixed with the crosslinker glutaraldehyde, the mammalian complex appeared as a “Y” shaped structure (see speculative model in [33]). In the absence of crosslinking, the Hsu *et al.*, particles were very heterogeneous, suggesting that the mammalian exocyst is unstable or dynamic (or both). Additional attempts to purify the complex from yeast or other organisms drew similar conclusions [21, 44, 60]. The challenge of purifying high concentrations of stable exocyst seemed insurmountable. Several groups therefore pioneered new methods for purifying exocyst or turned to approaches that could map subunit-subunit interactions without requiring purification of the complexes.

Katoh *et al.* mapped the pairwise connections between the mammalian exocyst subunits with a “visible immunoprecipitation assay” using EGFP-tagged and either mCherry- or tRFP-tagged exocyst proteins expressed in HEK293T cells ([61]; Figure 1C). In general, the interactions that they detected matched those observed in yeast two-hybrid studies of the mammalian proteins [62], and in studies of the yeast complex by Heider *et al.*, [16] and Mei *et al.*, [17], described in detail below. Curiously, Katoh *et al.*, also observed evidence for homodimerization of several subunits [61], but this has not been observed for the purified yeast complex by biochemical, negative stain, or cryoEM experiments (below). This finding may either represent a caveat of the overexpressed, transfected proteins used, recruitment of endogenous components, or a real difference between mammalian and yeast exocyst.

Homodimerization of recombinant yeast Sec6 had previously been observed, but was likely due to recombinant expression of Sec6 in the absence of its tightly associated partners, e.g. Sec8 [16, 17, 46, 63, 64].

A breakthrough in studying the yeast exocyst came from Heider *et al.*, who determined that the exocyst was actually quite stable if yeast cells were lysed with cryogenic milling and the complex pulled down quickly using an affinity tag [16]. These changes helped to prevent both proteolysis and destabilization of the complex. Using this purification strategy, the complex was shown to be fully octameric, stoichiometric and stable under a number of pH, salt and detergent conditions. No partial subcomplexes were observed, suggesting that complex assembly and disassembly did not occur to any detectable extent. The reason for the disagreement between our findings and those of microscopy studies of exocyst subunits in live yeast cells [35] is currently unclear, although the live cell tracking results could possibly be explained by the presence of a small fraction of individual subunits (newly synthesized?) or subcomplexes that were below the level of detection by biochemical methods.

Under partially denaturing conditions (e.g. high salt and/or pH), or when one of the subunits was degraded using an auxin-inducible degron system [27], the exocyst could be dissociated into two stable modules consisting of Sec3-5-6-8 and Sec10-15-Exo70-84 ([16]; Figure 1D). In contrast, degradation of factors known to interact with exocyst, including the Rab Sec4, Rho GTPases, the myosin Myo2 and various SNAREs, did not affect either the stability or stoichiometry of the complex. Together, these data indicated that the yeast exocyst exists as a single intact complex when it functions at sites of polarized secretion. Negative stain EM of the purified complex also demonstrated a single compact octameric structure, with no visible subcomplexes (Figure 1E). Similarly, negative stain EM of the mammalian exocyst, purified from baculovirus-expressed subunits, showed a structure comparable to the yeast complex [65].

Taking a different tack with an *in vivo* microscopy approach in live cells, Picco et al., applied a method they dubbed PICT (protein interactions from imaging complexes after translocation), with the goal of measuring distances between the N- and C-terminal ends of exocyst subunits in living yeast [38, 66]. Individual exocyst subunits were directed to a Sla2-RFP-based plasma membrane platform using a rapamycin-induced hetero-dimerization strategy. Other subunits were tagged with GFP at their N- or C-termini, and recruited to the platform through interactions with the Sla2-localized partner in the presence of latrunculin A (to disrupt the actin cytoskeleton and ongoing secretion). Distances were measured between the two centroids of the RFP and GFP fluorescent spots. Using the Integrative Modeling Platform [67, 68] and a total of 80 measured distances as restraints, a collection of potential models for the *in vivo* yeast exocyst were generated (Figure 1F; see discussion below). Additional measurements in the presence of labeled Sec2 (the Rab GEF, as a proxy for vesicles) suggested that the exocyst did not undergo substantial conformational changes or disassemble when bound to vesicles. Fluorescence recovery after photobleaching (FRAP) experiments and fluorescence cross-correlation spectroscopy (FCCS) experiments also indicated that the anchored exocyst complexes were stable. The authors' experiments estimated that an average of ~15 copies of exocyst were present at each exocytic site.

However, little is known about how many exocyst complexes might be required to tether and fuse a secretory vesicle, or if there is any coordination between multiple complexes to facilitate tethering and fusion.

Moving to higher resolution

Recent advances in cryoEM methodologies and sample preparation enabled Mei et al., to obtain higher resolution images for the yeast exocyst ([17]; Figure 1G). The overall shape of the complex is similar to that observed by negative stain EM ([16]; Figure 1E). The average resolution of the cryoEM structure, estimated by “gold standard” cryoEM criteria [69] is ~4.4Å, with the best regions (~3.2 to 5.6Å resolution) in the middle core subunits, and more dynamic regions (~7Å to ~9Å resolution) at both the top and bottom of the complex. At this range of resolutions, very few side chains are visible. In addition, many portions of the main chain, especially the loops, cannot be definitively placed into the cryoEM density. In a heroic effort, Mei et al., used the known crystal structures (Figure 1B), intra- and inter-molecular chemical crosslinking data, and iterative molecular modeling, to build a near-atomic model of each subunit within the complex ([17]; Figure 2).

Overall, the complex is ~32 nm long × ~13 nm wide. It is composed of two half complexes, or modules, of four subunits each, consistent with the modules identified using other methods (Figs. 1D and 2; [16, 61]). The modules pack side by side lengthwise, with the Sec15 C-terminal domain stretching out alone at the bottom, and the Sec6 C-terminal region lying lengthwise, as a cap across the top of both modules ([17]; Figure 2). Curiously, the N-terminal half (611 aa) of Sec3 is completely missing from the electron density (Figure 3); these residues are presumably substantially disordered and/or dynamic. Additional missing regions include a number of internal loops within subunits, and residues 1-168 of Exo84.

Each module is held together by an elongated bundle of four α-helical CorEx (Core of Exocyst) motifs (Figure 2). One bundle comprises the CorEx motifs of Sec3, Sec5, Sec6, and Sec8, while the other bundle comprises the CorEx motifs of Sec10, Sec15, Exo70, and Exo84. The typical CorEx motif is about 80 residues in length and is located somewhat near the N-terminus of each exocyst subunit. Notably, most of the CorEx motifs had been earlier predicted to participate in α-helical coiled coils and to underlie the ‘quatrafoil’ organization of the exocyst (and other CATCHR) complexes [70]. The intimate interaction among the four α-helical CorEx motifs that make up the two bundles rationalizes why the isolated N-terminal regions of exocyst subunits are generally poorly folded, aggregation prone, and refractory to crystallization. It is likely that the CorEx motif bundles are the critical starting point for assembling the modules, which would subsequently pack together to form the octameric complex. Consistent with a stabilizing role for the CorEx motif bundles, the C-terminal domains of most of the subunits are more exposed, where they can interact with non-exocyst binding partners (Figure 3). Additional stabilizing interactions, especially between the Sec3-5-6-8 and Sec10-15-Exo70-84 modules, involve other α-helical domains of the various subunits. None of these individual interactions are particularly tight; instead they appear to provide a multivalent network of interactions that hold exocyst together [16]. Consistent with a key role for the CorEx motifs in stabilizing exocyst, deletion of the CorEx motif section of Sec3 was shown to disrupt the function of exocyst in yeast [17]. Given the

remarkable and apparently unique architecture of exocyst, it will be very interesting to elucidate how it is able to assemble *in vivo* (e.g. with the assistance of chaperones) such that aggregation and misfolding of the CorEx motifs are prevented.

What have we learned about the exocyst architecture and function?

Overall, the various biochemical and structural studies described above agree on several features: the yeast exocyst is a compact, slightly elongated molecule made up of eight mostly helical subunits. These subunits pack into modules of four subunits each, which then stack upon each other. The tightest pairwise interactions are Sec6-8, Sec3-5, and Sec10-15, with lower affinity contributions between other pairs [16, 33]. No subcomplexes in yeast were detected in any of the biochemical or structural studies, in the absence of destabilizing conditions or mutations. Furthermore, no substantial interactions between multiple exocyst complexes have been detected in immunoprecipitation experiments or in characterization of the purified complexes [16, 17, 19]. Therefore, the exocyst in yeast cells is likely to function as a fully assembled, octameric complex and not in subcomplexes or cooperative assemblies. In other organisms, however, several experiments suggest that functional subcomplexes may exist and perhaps have additional functions in other pathways, such as autophagy [25, 39–43]. Therefore, it will be important to biochemically and structurally characterize exocyst from other organisms, to understand the similarities and differences compared with the yeast complex.

The critical subunits identified for interactions with non-exocyst partners include the Sec15 C-terminal end, which binds to the Rab GTPase and Myo2 on the vesicle [47, 53]; Exo84, which interacts with the regulator Sro7/77 [71]; and Sec6, which binds the v-SNARE Snc1/2 [64], the t-SNARE Sec9 [63, 72, 73], SNARE complexes [73] and the SNARE regulatory protein Sec1 [72] (Figure 3 and described below). The subunit Exo70 binds to the plasma membrane components PI(4,5)P₂, Cdc42 and Rho3; prenylation of both Cdc42 and Rho3 promotes their GTP-dependent interaction with Exo70. [24, 74]. Furthermore, the N-terminal domain of Sec3—which forms a PH domain not included in the cryo-EM-derived model [58, 59]—also binds to PI(4,5)P₂, Cdc42, Rho1 and the t-SNARE Sso1 [24, 75]. Additional interactors have been identified and are described elsewhere [21, 24, 33]. In mammalian cells, domains in Exo84 and Sec5 interact with the RalA and RalB GTPases [55–57]. Most of these interactions were identified in assays with recombinant partners and/or pullouts using cell lysates; it will be very interesting to see if these interactions can be recapitulated in binding studies with purified, intact exocyst, and what affect binding of these partners has on the exocyst structure.

A number of apparent discrepancies in subunit placement exist between the cryoEM structure and the Picco *et al.*, model (Figure 1F vs. 1G). Most notably, the C-terminal domains of Sec6 and Sec15 are near one another in the Picco *et al.*, model, while they are at opposite ends of the complex in the cryoEM structure; this difference profoundly affects hypotheses for how the complex could be interacting with vesicles (through Sec15) and SNAREs (through Sec6 and Sec3). Many other subunit-subunit interactions are altered as well; e.g., many of the N-terminal interactions that form the CorEx bundles are not observed in the Picco *et al.*, model. It is not particularly surprising that the structures differ, as the

number of *in vivo* fluorescence distances, and hence restraints for model building, are low and of limited precision, compared to the cryoEM data. However, the PICT method reports on the *in vivo* conformation, which may be different from that of the purified complex *in vitro*, a possibility that remains to be tested. Purified exocyst, when examined by either negative stain or cryoEM, does not display large conformational changes or dynamics; however, interactions with other proteins or with membrane lipids could affect its conformation *in vivo* (Figure 3).

Both the negative stain and cryoEM structures reveal substantial local dynamics in regions of the complex that are critical for function: the C-terminus of Sec15, most of the C-terminal half of Sec6, the C-terminal tip of Sec10, and the missing N-terminal half of Sec3 [16, 17]. These may be sites of regulated conformational changes to facilitate tethering and/or SNARE regulation. Indeed, flexibility could increase the “reach” of the complex, which seems useful for a tether. The major sites for exocyst phosphorylation also occur in several of the disordered regions: the N-terminal region of Sec3, several loops throughout Exo84, a few sites in Sec10 and Sec15, and the N-terminus of Sec8 [76–78]. More work will be needed to determine if phosphorylation at one or several of these sites regulates exocyst structure and function; several studies suggest a potential role in relocating exocyst from the sites of polarized secretion at the tips of daughter cells to other sites [21, 24, 79]. Similarly, interactions with the Rab, Rho and Ral GTPases and with membranes may play central roles in controlling exocyst function [21, 24, 74].

The idea that exocyst may undergo substantial conformational changes is also supported by negative stain EM studies of other MTCs, which differ markedly from the compact nature of the exocyst. Although most MTCs share structural homology with exocyst at the subunit level, the assembled COG [80], Dsl [81], GARP [15] and HOPS [15] complexes (with the exception of the crosslinked HOPS complex; [14]) are spread open and are quite dynamic (see COG in Figure 1H). It is possible that exocyst is triggered to form an open conformation by binding one or more of its partners, which could lead to tethering at a much longer distance. Moreover, if exocyst “opened” up to tether, then the transition to the “closed” conformation could be used to pull vesicles into close proximity with the plasma membrane, thus indirectly driving SNARE complex assembly and fusion.

Biochemical and genetic evidence suggests that exocyst also functions to directly facilitate SNARE complex assembly and membrane fusion. Exocyst subunits, in isolation, have been shown to bind to all three SNARE proteins on the vesicle and plasma membrane, as well as SNARE complexes and the SNARE regulator Sec1 (Figure 3). The molecular details of how exocyst regulates SNAREs are mostly unknown. The N-terminal domain of Sec3 binds the t-SNARE Sso1/2 and increases the rate of t-SNARE binary complex (Sso1/2:Sec9) formation *in vitro* [75]. Unlike the other subunits, Sec3 is not essential for the life of yeast [26], which suggests that its role is regulatory, rather than required. The interaction of Sec6 with the v-SNARE Snc1/2 was suggested to act as one of a series of multivalent interactions for stable vesicle binding [64]. Sec6 also interacts with the t-SNARE Sec9, assembled SNARE complexes and Sec1 [63, 72, 73]. The role(s) for these Sec6 interactions have been suggested to help drive SNARE assembly, proof-read incorrect SNARE complexes, and

perhaps stimulate fusion [11, 73]. Moreover, it is likely that other MTCs will function similarly with their cognate SNAREs, Rabs and SM partners [10, 11].

The current structural, biochemical and cell biological data have not exposed the molecular mechanism(s) of exocyst function yet. It is tempting to speculate that the initial interaction of exocyst with the Rab GTPase, myosin and/or v-SNARE on the vesicle triggers a conformational change(s) in exocyst that leads to exposure of the membrane and SNARE binding domains, to drive SNARE assembly and fusion at the correct sites on the plasma membrane. Alternatively, it is possible that interactions of exocyst with Rho GTPases and PI(4,5)P₂ on the plasma membrane at sites of secretion lead to a conformational change(s) that allows exocyst to bind and tether secretory vesicles as they arrive, followed by assembly of the proper SNARE fusion complexes. Or the answer could be a combination of both hypotheses. Further functional studies examining these various interactions in the context of assembled exocyst and membranes will be necessary to elucidate these mechanisms.

Concluding Remarks and Future Perspectives

The last few years have seen amazing progress in determining the structure of the exocyst, overcoming huge biochemical and structural challenges. Now is the time to push the complex to even higher resolution, alone and with its partners, so that the various interactions can be mapped at the level of specific residues. This information will be invaluable for the design and testing of functional hypotheses regarding exocyst in polarized secretion and other trafficking pathways. Ideally, subsequent biochemical, biophysical and structural analyses will examine exocyst in the presence of critical binding partners and membranes—perhaps catching exocyst in the act of tethering or driving membrane fusion. These types of studies will also elucidate important dynamics and conformational changes.

What questions remain to be answered? At a mechanistic level, we actually know very little about how exocyst functions. We can say with confidence that exocyst is required for a process (or processes) that follow vesicle arrival and precede SNARE-mediated fusion. Exocyst appears to be a fundamental quality control hub and/or platform for many interactions to take place; these specific interactions would control *which* vesicles would fuse, and *where*. Currently, there is little understanding of how exocyst integrates its different inputs, of the order of addition, or of which factors are able to interact with exocyst simultaneously. Answering such questions will require more than just structural studies; *in vitro* functional reconstitution assays will be necessary to tease apart the details of the tethering and SNARE assembly and fusion reactions. Complementary microscopy and mutagenesis experiments will be needed to examine the precise localization, dynamics and functions of exocyst complexes and their partners *in vivo*. Luckily, for studies of exocyst in other organisms, the advances in CRISPR technologies make mutagenesis and tagging feasible. These studies will help elucidate the evolutionary underpinnings of exocyst function and highlight similarities and differences across diverse organisms and cell types. Moreover, the ability to purify stable exocyst complexes suggests that they will soon be used in high throughput screens for small molecules that inhibit or disrupt the complex and/or interactions with its partners; recently, Exo70 was shown to be the target of the endocytosis inhibitor endosidin2, in *Arabidopsis thaliana* [82]. Further studies will also no doubt reveal

how exocyst-mediated trafficking is corrupted in cancer cells, or by pathogens for cell entry and/or replication. The new cryoEM structure [17] is a critical first step towards higher resolution mapping of many disease-associated mutations [23, 25] and gaining a molecular understanding of how the mutants disrupt exocyst function, as well as suggesting means by which these defects might be corrected.

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Glossary

Exocyst:

a conserved multisubunit tethering complex (MTC) consisting of the subunits Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, Exo84 (EXOC1-EXOC8 in mammals) that is required for secretory vesicle fusion at sites of polarized cell growth and division. Also shown to be involved in endocytosis, ciliogenesis, cell motility, invasion, bacterial pathogenesis, and autophagy

Membrane fusion:

the merging of two membranes to deliver protein and lipid cargo. Usually the fusion is between a cargo-containing vesicle or tubule and a target organelle or the plasma membrane. Most fusion reactions in eukaryotic cells are carried out by SNARE proteins, with a few exceptions, including cell-cell fusion, nuclear fusion and mitochondrial fusion

Myo2:

the unconventional type V myosin that transports post-Golgi vesicles to sites of secretion in budding yeast. Bind to the Rab GTPase on vesicles as well as the exocyst

Rab GTPase:

small Ras-family GTPases that serve as master regulators of the secretory pathway. The yeast Rab, Sec4, is present on secretory vesicles and its GTP-bound conformation is recognized by the exocyst

Resolution:

in a cryoEM structure, resolution refers to the separation of well-resolved (i.e. above a certain signal/noise threshold) points in a macromolecule, so the smaller the number the better (higher) the resolution. At atomic resolutions (up to 3.5 Å), detailed features can often be resolved, such as atoms of the protein side chains and nucleotides. Between 4 and 6 Å, secondary structure elements are resolved (most notably, α -helices), but not the positions of side chains or loop conformations. Visualization of the α -helical turns allows building a backbone model at this resolution; however, determination of the identity of the helix or modeling the connections between secondary structure elements may be difficult without additional information, such as crosslinking and atomic or near-atomic resolution crystal structures and homology models. Due to molecular flexibility and/or dynamics, resolution is

rarely uniform and may range from near-atomic in the core to worse than 10 Å at the periphery, making it nearly impossible to build detailed models of the peripheral regions

Rho GTPase:

small Ras-family GTPases, including Cdc42, that control actin cytoskeleton dynamics and cell polarity. Several Rho GTPases bind to exocyst on the plasma membrane at sites of exocytosis. The related Ral family of small GTPases also interact with exocyst in mammalian cells

Sec1/Munc18 proteins:

a family of regulatory proteins that control SNARE-mediated membrane fusion

SNARE proteins:

three to four SNARE proteins form an extremely tight parallel four-helix-bundle structure that is the minimal machinery for membrane fusion. In yeast, the SNARE on the vesicle (v-SNARE) is Snc1/2 (VAMP or synaptobrevin in mammals). The SNAREs on the plasma membrane (t-SNAREs) are Sso1/2 (syntaxin) and Sec9 (SNAP-25)

Tether:

long coiled-coil proteins or multisubunit tethering complexes (MTC) interact reversibly with proteins and lipids on both the vesicle and target membranes to bridge the two membranes at a distance, bring them closer together, and (directly or indirectly) facilitate SNARE-mediated membrane fusion

References

1. Pfeffer SR (1999) Transport-vesicle targeting: tethers before SNAREs. *Nat Cell Biol* 1 (1), E17–22. [PubMed: 10559876]
2. Yu IM and Hughson FM (2010) Tethering factors as organizers of intracellular vesicular traffic. *Annu Rev Cell Dev Biol* 26, 137–56. [PubMed: 19575650]
3. Gillingham AK and Munro S (2016) Finding the Golgi: Golgin Coiled-Coil Proteins Show the Way. *Trends Cell Biol* 26 (6), 399–408. [PubMed: 26972448]
4. Spang A (2016) Membrane Tethering Complexes in the Endosomal System. *Front Cell Dev Biol* 4, 35. [PubMed: 27243003]
5. Kim JJ et al. (2016) TRAPP Complexes in Secretion and Autophagy. *Front Cell Dev Biol* 4, 20. [PubMed: 27066478]
6. Brunet S and Sacher M (2014) Are all multisubunit tethering complexes bona fide tethers? *Traffic* 15 (11), 1282–7. [PubMed: 25048641]
7. Wickner W (2010) Membrane fusion: five lipids, four SNAREs, three chaperones, two nucleotides, and a Rab, all dancing in a ring on yeast vacuoles. *Annual review of cell and developmental biology* 26, 115–36.
8. Murray DH et al. (2016) An endosomal tether undergoes an entropic collapse to bring vesicles together. *Nature* 537 (7618), 107–111. [PubMed: 27556945]
9. Luo G et al. (2014) The role of Sec3p in secretory vesicle targeting and exocyst complex assembly. *Molecular biology of the cell* 25 (23), 3813–22. [PubMed: 25232005]
10. Hong W and Lev S (2014) Tethering the assembly of SNARE complexes. *Trends in cell biology* 24 (1), 35–43. [PubMed: 24119662]
11. Dubuke ML and Munson M (2016) The Secret Life of Tethers: The Role of Tethering Factors in SNARE Complex Regulation. *Front Cell Dev Biol* 4, 42. [PubMed: 27243006]

12. Hughson FM and Reinisch KM (2010) Structure and mechanism in membrane trafficking. *Current opinion in cell biology* 22 (4), 454–60. [PubMed: 20418086]
13. Lees JA et al. (2010) Molecular organization of the COG vesicle tethering complex. *Nature structural & molecular biology* 17 (11), 1292–7.
14. Bocker C et al. (2012) Molecular architecture of the multisubunit homotypic fusion and vacuole protein sorting (HOPS) tethering complex. *Proceedings of the National Academy of Sciences of the United States of America* 109 (6), 1991–6. [PubMed: 22308417]
15. Chou HT et al. (2016) CATCHR, HOPS and CORVET tethering complexes share a similar architecture. *Nat Struct Mol Biol* 23 (8), 761–3. [PubMed: 27428774]
16. Heider MR et al. (2016) Subunit connectivity, assembly determinants and architecture of the yeast exocyst complex. *Nat Struct Mol Biol* 23 (1), 59–66. [PubMed: 26656853]
17. Mei K et al. (2018) Cryo-EM structure of the exocyst complex. *Nat Struct Mol Biol* 25 (2), 139–146. [PubMed: 29335562]
18. Novick P et al. (1980) Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* 21 (1), 205–15. [PubMed: 6996832]
19. TerBush DR et al. (1996) The Exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO J* 15 (23), 6483–94. [PubMed: 8978675]
20. Guo W et al. (1999) Exo84p is an exocyst protein essential for secretion. *J Biol Chem* 274 (33), 23558–64. [PubMed: 10438536]
21. Heider MR and Munson M (2012) Exorcising the exocyst complex. *Traffic* 13 (7), 898–907. [PubMed: 22420621]
22. Zarsky V et al. (2013) Exocyst complexes multiple functions in plant cells secretory pathways. *Current opinion in plant biology* 16 (6), 726–33. [PubMed: 24246229]
23. Tanaka T et al. (2017) Diverse Functions and Signal Transduction of the Exocyst Complex in Tumor Cells. *J Cell Physiol* 232 (5), 939–957. [PubMed: 27669116]
24. Zeng J et al. (2017) Polarized Exocytosis. *Cold Spring Harb Perspect Biol* 9 (12).
25. Martin-Urdiroz M et al. (2016) The Exocyst Complex in Health and Disease. *Front Cell Dev Biol* 4, 24. [PubMed: 27148529]
26. Wiederkehr A et al. (2003) Sec3p is needed for the spatial regulation of secretion and for the inheritance of the cortical endoplasmic reticulum. *Mol Biol Cell* 14 (12), 4770–82. [PubMed: 12960429]
27. Nishimura K and Kanemaki MT (2014) Rapid Depletion of Budding Yeast Proteins via the Fusion of an Auxin-Inducible Degron (AID). *Current protocols in cell biology* 64, 20 9 1–20 9 16.
28. Koumandou VL et al. (2007) Control systems for membrane fusion in the ancestral eukaryote; evolution of tethering complexes and SM proteins. *BMC Evol Biol* 7, 29. [PubMed: 17319956]
29. Klinger CM et al. (2016) Tracing the Archaeal Origins of Eukaryotic Membrane-Trafficking System Building Blocks. *Mol Biol Evol* 33 (6), 1528–41. [PubMed: 26893300]
30. Cvrckova F et al. (2012) Evolution of the land plant exocyst complexes. *Frontiers in plant science* 3, 159. [PubMed: 22826714]
31. Vukasinovic N and Zarsky V (2016) Tethering Complexes in the Arabidopsis Endomembrane System. *Front Cell Dev Biol* 4, 46. [PubMed: 27243010]
32. Boehm CM et al. (2017) The Trypanosome Exocyst: A Conserved Structure Revealing a New Role in Endocytosis. *PLoS Pathog* 13 (1), e1006063. [PubMed: 28114397]
33. Munson M and Novick P (2006) The exocyst defrocked, a framework of rods revealed. *Nat Struct Mol Biol* 13 (7), 577–81. [PubMed: 16826234]
34. Songer JA and Munson M (2009) Sec6p anchors the assembled exocyst complex at sites of secretion. *Mol Biol Cell* 20 (3), 973–82. [PubMed: 19073882]
35. Boyd C et al. (2004) Vesicles carry most exocyst subunits to exocytic sites marked by the remaining two subunits, Sec3p and Exo70p. *J Cell Biol* 167 (5), 889–901. [PubMed: 15583031]
36. Donovan KW and Bretscher A (2015) Tracking individual secretory vesicles during exocytosis reveals an ordered and regulated process. *The Journal of cell biology* 210 (2), 181–9. [PubMed: 26169352]

37. Roumanie O et al. (2005) Rho GTPase regulation of exocytosis in yeast is independent of GTP hydrolysis and polarization of the exocyst complex. *J Cell Biol* 170 (4), 583–94. [PubMed: 16103227]
38. Picco A et al. (2017) The In Vivo Architecture of the Exocyst Provides Structural Basis for Exocytosis. *Cell* 168 (3), 400–412 e18. [PubMed: 28129539]
39. Murthy M et al. (2005) Sec6 mutations and the Drosophila exocyst complex. *J Cell Sci* 118 (Pt 6), 1139–50. [PubMed: 15728258]
40. Bodemann BO et al. (2011) RalB and the exocyst mediate the cellular starvation response by direct activation of autophagosome assembly. *Cell* 144 (2), 253–67. [PubMed: 21241894]
41. Kulich I et al. (2013) Arabidopsis exocyst subcomplex containing subunit EXO70B1 is involved in autophagy-related transport to the vacuole. *Traffic* 14 (11), 1155–65. [PubMed: 23944713]
42. Riquelme M et al. (2014) The Neurospora crassa exocyst complex tethers Spitzenkorper vesicles to the apical plasma membrane during polarized growth. *Molecular biology of the cell* 25 (8), 1312–26. [PubMed: 24523289]
43. Inamdar SM et al. (2016) Probing Functional Changes in Exocyst Configuration with Monoclonal Antibodies. *Front Cell Dev Biol* 4, 51. [PubMed: 27376061]
44. Hsu SC et al. (1998) Subunit composition, protein interactions, and structures of the mammalian brain sec6/8 complex and septin filaments. *Neuron* 20 (6), 1111–22. [PubMed: 9655500]
45. Dong G et al. (2005) The structures of exocyst subunit Exo70p and the Exo84p C-terminal domains reveal a common motif. *Nat Struct Mol Biol* 12, 1094–1100. [PubMed: 16249794]
46. Sivaram MV et al. (2006) The structure of the exocyst subunit Sec6p defines a conserved architecture with diverse roles. *Nat Struct Mol Biol* 13 (6), 555–6. [PubMed: 16699513]
47. Wu S et al. (2005) Sec15 interacts with Rab11 via a novel domain and affects Rab11 localization in vivo. *Nat Struct Mol Biol* 12, 879–885. [PubMed: 16155582]
48. Chen J et al. (2017) Crystal structure of Sec10, a subunit of the exocyst complex. *Sci Rep* 7, 40909. [PubMed: 28098232]
49. Kimura S et al. (2016) Distinct Roles for the N- and C-terminal Regions of M-Sec in Plasma Membrane Deformation during Tunneling Nanotube Formation. *Sci Rep* 6, 33548. [PubMed: 27629377]
50. Richardson BC et al. (2009) Structural basis for a human glycosylation disorder caused by mutation of the COG4 gene. *Proc Natl Acad Sci U S A* 106 (32), 13329–34. [PubMed: 19651599]
51. Munson M (2009) Tip20p reaches out to Dsl1p to tether membranes. *Nat Struct Mol Biol* 16 (2), 100–2. [PubMed: 19190660]
52. Pashkova N et al. (2006) Structural basis for myosin V discrimination between distinct cargoes. *EMBO J* 25 (4), 693–700. [PubMed: 16437158]
53. Jin Y et al. (2011) Myosin V transports secretory vesicles via a Rab GTPase cascade and interaction with the exocyst complex. *Developmental Cell* 21 (6), 1156–70. [PubMed: 22172676]
54. Li W et al. (2011) The Crystal Structure of a Munc13 C-terminal Module Exhibits a Remarkable Similarity to Vesicle Tethering Factors. *Structure* 19 (10), 1443–55. [PubMed: 22000513]
55. Jin R et al. (2005) Exo84 and Sec5 are competitive regulatory Sec6/8 effectors to the RalA GTPase. *EMBO J* 24 (12), 2064–74. [PubMed: 15920473]
56. Mott HR et al. (2003) Structure of the GTPase-binding domain of Sec5 and elucidation of its Ral binding site. *J Biol Chem* 278 (19), 17053–9. [PubMed: 12624092]
57. Fukai S et al. (2003) Structural basis of the interaction between RalA and Sec5, a subunit of the sec6/8 complex. *EMBO J* 22 (13), 3267–78. [PubMed: 12839989]
58. Baek K et al. (2010) Structure-function study of the N-terminal domain of exocyst subunit Sec3. *J Biol Chem* 285 (14), 10424–33. [PubMed: 20139078]
59. Yamashita M et al. (2010) Structural basis for the Rho- and phosphoinositide-dependent localization of the exocyst subunit Sec3. *Nat Struct Mol Biol* 17 (2), 180–6. [PubMed: 20062059]
60. Terbush DR et al. (2001) Purification and characterization of yeast exocyst complex. *Methods Enzymol* 329, 100–10. [PubMed: 11210526]

61. Katoh Y et al. (2015) Architectures of multisubunit complexes revealed by a visible immunoprecipitation assay using fluorescent fusion proteins. *Journal of cell science* 128 (12), 2351–62. [PubMed: 25964651]
62. Matern HT et al. (2001) The Sec6/8 complex in mammalian cells: characterization of mammalian Sec3, subunit interactions, and expression of subunits in polarized cells. *Proc Natl Acad Sci U S A* 98 (17), 9648–53. [PubMed: 11493706]
63. Sivaram MV et al. (2005) Dimerization of the exocyst protein Sec6p and its interaction with the t-SNARE Sec9p. *Biochemistry* 44 (16), 6302–11. [PubMed: 15835919]
64. Shen D et al. (2013) The synaptobrevin homologue Snc2p recruits the exocyst to secretory vesicles by binding to Sec6p. *The Journal of cell biology* 202 (3), 509–26. [PubMed: 23897890]
65. Zhai Y et al. (2017) Large protein complex production using the SmartBac System - Strategies and Applications. *bioRxiv*.
66. Gallego O et al. (2013) Detection and characterization of protein interactions in vivo by a simple live-cell imaging method. *PLoS One* 8 (5), e62195. [PubMed: 23658712]
67. Webb B et al. (2011) Modeling of proteins and their assemblies with the integrative modeling platform. *Methods in molecular biology* 781, 377–97. [PubMed: 21877292]
68. Yang Z et al. (2012) UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *Journal of structural biology* 179 (3), 269–78. [PubMed: 21963794]
69. Scheres SH and Chen S (2012) Prevention of overfitting in cryo-EM structure determination. *Nature Methods* 9 (9), 853–4. [PubMed: 22842542]
70. Whyte JR and Munro S (2002) Vesicle tethering complexes in membrane traffic. *J Cell Sci* 115 (Pt 13), 2627–37. [PubMed: 12077354]
71. Zhang X et al. (2005) Lethal giant larvae proteins interact with the exocyst complex and are involved in polarized exocytosis. *J Cell Biol* 170 (2), 273–83. [PubMed: 16027223]
72. Morgera F et al. (2012) Regulation of exocytosis by the exocyst subunit Sec6 and the SM protein Sec1. *Molecular biology of the cell* 23 (2), 337–46. [PubMed: 22114349]
73. Dubuke ML et al. (2015) The Exocyst Subunit Sec6 Interacts with Assembled Exocytic SNARE Complexes. *J Biol Chem* 290 (47), 28245–56. [PubMed: 26446795]
74. Wu H et al. (2010) The Exo70 subunit of the exocyst is an effector for both Cdc42 and Rho3 function in polarized exocytosis. *Mol Biol Cell* 21 (3), 430–42. [PubMed: 19955214]
75. Yue P et al. (2017) Sec3 promotes the initial binary t-SNARE complex assembly and membrane fusion. *Nat Commun* 8, 14236. [PubMed: 28112172]
76. Albuquerque CP et al. (2008) A multidimensional chromatography technology for in-depth phosphoproteome analysis. *Molecular & cellular proteomics : MCP* 7 (7), 1389–96. [PubMed: 18407956]
77. Holt LJ et al. (2009) Global analysis of Cdk1 substrate phosphorylation sites provides insights into evolution. *Science* 325 (5948), 1682–6. [PubMed: 19779198]
78. Luo G et al. (2013) Mitotic phosphorylation of Exo84 disrupts exocyst assembly and arrests cell growth. *The Journal of cell biology* 202 (1), 97–111. [PubMed: 23836930]
79. Kono K et al. (2012) Proteasomal degradation resolves competition between cell polarization and cellular wound healing. *Cell* 150 (1), 151–64. [PubMed: 22727045]
80. Ha JY et al. (2016) Molecular architecture of the complete COG tethering complex. *Nat Struct Mol Biol* 23 (8), 758–60. [PubMed: 27428773]
81. Ren Y et al. (2009) A structure-based mechanism for vesicle capture by the multisubunit tethering complex Dsl1. *Cell* 139 (6), 1119–29. [PubMed: 20005805]
82. Zhang C et al. (2016) Endosidin2 targets conserved exocyst complex subunit EXO70 to inhibit exocytosis. *Proc Natl Acad Sci U S A* 113 (1), E41–50. [PubMed: 26607451]

Highlights

- Intracellular trafficking—the movement of protein and lipid cargos between different eukaryotic organelles and to the plasma membrane for secretion—is facilitated by small, membrane-bound vesicles and tubules. SNARE proteins provide the energy for membrane fusion to deliver cargos.
- Major mechanistic questions remain regarding how cargo-containing vesicles are specifically recognized, and how the SNARE proteins are activated for membrane fusion and cargo delivery.
- Tethers are large coiled coil dimers or multisubunit protein complexes that interact specifically with the vesicle and target membranes, as well as with associated proteins, including small GTPases, SNARE proteins and Sec1/Munc18 (SM) proteins. Tethers are also proposed to pull the membranes closer together, and activate SNARE proteins for fusion.
- Elucidation of the function of tethering complexes at the molecular level requires multidisciplinary approaches. These include high resolution structures, genetics, *in vivo* functional assays and *in vitro* reconstitution experiments.
- Here we describe recent progress in determining the structure (both *in vitro* and *in vivo*) of the yeast exocyst complex, which controls fusion of secretory vesicles at the plasma membrane to facilitate cellular growth, signaling and division.

Outstanding Questions

What does the exocyst structure from yeast or other organisms look like at atomic resolution?

How does the exocyst tether vesicles to the plasma membrane (is it really a tether)?

How does the exocyst (with or without collaboration with Sec1?) control SNARE complex assembly and membrane fusion?

How is the exocyst activated for function? What are the roles of the small GTPases that bind to it? What is the role of PI(4,5)P₂ binding?

What (if any) conformational changes are associated with tethering and/or SNARE complex assembly and fusion? Why is the conformation of exocyst so different from other CATCHR family tethering complexes?

How many exocyst complexes are necessary to tether and fuse a vesicle? Do they act cooperatively?

Why are there eight large subunits in the exocyst? What are their functions? What role(s) do the many paralogs of the exocyst subunits in plants play?

How does the exocyst switch between different aspects of its function, e.g. between exocytosis and cell division, or in autophagy?

How do post-translational modifications, such as phosphorylation, affect exocyst structure, stability and function?

Do exocyst complexes from other organisms have regulated subcomplexes that assemble and disassemble during the exocyst functional cycle?

If exocyst is not present in some Excavate or Apicomplexan species, how are regulation and quality control of membrane fusion achieved? If only a few exocyst subunits are expressed, do they assemble together into with different stoichiometries into oligomeric structures?

What are the locations and consequences of disease-associated mutations in exocyst? What happens when tethering and quality control go wrong?

Figure 1.

Timeline of the major exocyst structural studies. (A) Unfixed and glutaraldehyde-fixed mammalian exocyst complexes, visualized by platinum rotary shadowing (adapted from [44]). (B) High resolution crystal structures of various domains of exocyst subunits (residues as indicated): yeast Exo70 (PDB ID: 2B1E; [45]); yeast Exo84 C-terminal domain (PDB ID: 2D2S; [45]); mammalian Exo84 RalA-binding domain (PDB ID: 1ZC3; [55]); *Drosophila* Sec15 C-terminal domain (PDB ID 2A2F; [47]); yeast Sec3 N-terminal domain (PDB ID 3HIE; [58]); yeast Sec6 C-terminal domain (PDB ID 2FJI; [46]); and zebrafish Sec10 (PDB ID 5H11; [48]). Overall, high resolution structural information is available for <30% of yeast exocyst. (C) Network of protein-protein interactions between mammalian exocyst subunits, as mapped by a visible immunoprecipitation assay (from [61]). Identified subcomplexes are boxed and colored, subunits that showed homodimerization are outlined in red, and the thickness of the blue interaction lines indicates the strength of the interactions. (D) Network of protein-protein interactions between subunits, as mapped by auxin-induced degradation experiments plus *in vitro* binding experiments (adapted from [16]). (E) Negative stain EM of intact yeast exocyst (adapted from [16]). (F) Representative model for the yeast exocyst structure, built using distance restraints from *in vivo* fluorescence measurements (adapted from [38]). The flat ends of the beads represent the N-terminal ends of each subunit, while pointy beads are the C-terminal ends. (G) Moderate resolution model of the intact yeast exocyst structure determined using cryoEM, modeling and crosslinking/mass spectrometry (PDB ID 5YFP; [17]). Note that the subunit colors are not the same as in panel

F. (H) In contrast to the compact exocyst, negative stain EM of the COG complex shows an open, highly dynamic structure (adapted from [80]).

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Figure 2.

The architecture of the yeast exocyst is assembled from two modules containing four subunits each. The subunits within the Sec3-5-6-8 module and the Sec10-15-70-84 module each contribute a helix to one of two CorEx helical bundles (insets). The modules are stacked one on top of the other in this view, to form the octameric complex observed in the negative stain and cryoEM structures [16, 17]. The biochemical studies, PICT experiments and EM images of the yeast complex demonstrate that no detectable subcomplexes or stable modules are observed in the absence of destabilizing conditions [16, 17, 38]. The subunits are colored as indicated on the right. The most dynamic and/or flexible—and therefore lowest resolution—regions are the C-terminal end of Sec15 at the bottom (**red**), the C-terminal half of Sec6 across the top (**purple**) and the C-terminal end of Sec10 (**cyan**).

Figure 3.

Diagram indicating many of the known interactions between the yeast exocyst and various partners. This structure is rotated $\sim 180^\circ$ relative to that shown in Figure 2. Each of the partners is color-coded to match the subunit with which they interact. The dissociated yellow domain is the Sec3 N-terminal domain (residues 71-241; PDB ID 3HIE; [58]). The first 610 amino acids of Sec3 do not appear in the cryoEM electron density, likely due to flexibility/dynamics; if extended, the region between 241 and 611 could span a distance up to 15 nm. The exact binding site(s) on Exo70 for Rho3 and Cdc42 have not been determined; binding studies with the prenylated Rho-GTPases indicates that their GTP-dependent interaction requires both the N- and C-terminal regions of Exo70 [74]. Although the diagram suggests that these interactions may all take place simultaneously, the spatial constraints and overlapping binding sites suggest that stepwise interactions and/or conformational changes are likely to take place.