Cryo-EM structure of the exocyst complex

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The exocyst is an evolutionarily conserved octameric protein complex that mediates the tethering of post-Golgi secretory vesicles to the plasma membrane during exocytosis and is implicated in many cellular processes such as cell polarization, cytokinesis, ciliogenesis, and tumor invasion. Using cryo-EM and chemical cross-linking MS (CXMS), we solved the structure of the Saccharomyces cerevisiae exocyst complex at an average resolution of 4.4 Å. Our model revealed the architecture of the exocyst and led to the identification of the helical bundles that mediate the assembly of the complex at its core. Sequence analysis suggests that these regions are evolutionarily conserved across eukaryotic systems. Additional cell biological data suggest a mechanism for exocyst assembly that leads to vesicle tethering at the plasma membrane.

Vesicular trafficking in eukaryotic cells is mediated by an elaborate network of molecular interactions that ensure the orderly transport, docking and fusion of secretory vesicles to their cognate target membrane. The initial contacts between the secretory vesicles and their target membrane are mediated by the tether family of proteins1–5. The multisubunit tethering complexes (MTCs) capture the vesicles to their specific target membranes before SNARE-mediated fusion at various stages of vesicular trafficking3,5,6. Elucidating the structure and assembly of the MTCs is essential to the understanding of the mechanisms of vesicle tethering and fusion.

The exocyst, first identified in the budding yeast S. cerevisiae, consists of the proteins Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 (refs 7,8). The exocyst mediates the tethering of post-Golgi secretory vesicles to the plasma membrane and promotes the assembly of the SNARE complex for membrane fusion2,9–11. Aside from exocytosis, the exocyst also plays pivotal roles in many cellular processes such as cell polarization, primary ciliogenesis, cytokinesis, pathogen invasion, tumorigenesis and metastasis10,12. Previous crystallography studies have revealed important structural features of a few exocyst subunits; these regions are mostly helical-bundle repeats1. Quick-freeze deep-etch EM and negative-staining EM have shown that the exocyst has an elongated shape consisting of packed long rods13,14. The rod structure seems to be a shared feature for complexes associated with tethering containing helical rods (CATCHR) proteins such as the conserved oligomeric Golgi (COG) and Golgi-associated retrograde protein (GARP) complexes15,16. Recently, a light microscopy approach, based on fluorescent protein tagging, provided new information on the exocyst complex in cells17. A topology regarding the connectivity of the exocyst subunits has also been depicted using biochemical analyses and negative-staining EM18. However, the structure model and mechanism of exocyst assembly remain unknown.

Using single-particle cryo-EM, we have solved the structure of the fully assembled yeast exocyst complex at an average resolution of 4.4 Å. The cryo-EM, together with chemical cross-linking mass spectrometry (CXMS) and cell biological assays, provides insights into the hierarchical assembly of the exocyst complex and mechanism of vesicle tethering.

Results

Structure determination of the exocyst complex. We tagged the chromosomal copy of individual exocyst subunits with TAP or ProA and purified the exocyst complex from yeast cell lysates using affinity chromatography and size-exclusion chromatography (Fig. 1a,b). The purification yielded a monodispersed intact exocyst complex comprising all of the eight subunits at equal stoichiometry (Fig. 1b). We next performed single-particle cryo-EM analysis of the complex and reconstituted the 3D structure of the intact complex at an average resolution of 4.4 Å (Fig. 1c,d and Supplementary Figs. 1 and 2). The holo-exocyst complex consists of long curved rods that pack against each other to form a hollow architecture measuring 320 Å long and 130 Å wide (Fig. 1d). The central body appears as a double-layer oval disk with a thickness of 60 Å. The front layer nestles into the concave side of the back layer, forming a prolate cavity between the two layers. From the top of the front layer, a neck-like structure stretches out, with two arms extending in opposite directions. One arm protrudes to the right edge of the front layer (henceforth termed arm I), and the other extends backward over the back layer (termed arm II). Stretching out from the bottom of the back layer is a tail-like structure (termed tail). Whereas the central portion of the reconstruction clearly shows bundles of α-helices with apparent helical pitches, the arm I, arm II and tail regions are less well resolved (Fig. 1d and Supplementary Fig. 2e), thus suggesting flexibility in these regions.

The assignment and structure of the exocyst subunits. The 4.4-Å resolution does not allow clear resolution of the side chains. To gain better structural information on the exocyst, we performed extensive intra- and intermolecular CXMS analyses. The combined approach allowed us to build a model of the exocyst complex consisting of the near full-length Sec5, Sec6, Sec8, Sec10, Sec15,
Exo70, Exo84 and the C-terminal half of Sec3 (Table 1, Fig. 2a, Supplementary Figs. 3–6 and Supplementary Table 1; details on model building are in Methods and Supplementary Video 1). When mapped onto the exocyst model, 86% of all cross-links fell within 24 Å (Supplementary Datasets 1–3 and Supplementary Video 2), which is comparable to X-ray crystallography studies showing that ~70% of cross-links are compatible with such a constraint19. Even though the subunits share less than 10% sequence identity among themselves, they all display an extended configuration with similar folds, consistent with previous predictions20 (Fig. 2b).

All subunits contain an N-terminal long coiled coil followed by a region of contiguous rod, which is composed of antiparallel short helical-bundle units (hereafter termed CAT domain for its conservation in CATCHR family proteins 5). Each of the seven subunits (except for Exo84) has four CAT domains (henceforth termed CAT-A to CAT-D) (Fig. 2b and Supplementary Table 1). For Exo84, there are only two CAT domains that follow its Pleckstrin homology (PH) domain. The N-terminal half of Sec3 could not be modeled into the cryo-EM map, probably owing to its flexibility. This finding is in agreement with the secondary structure prediction of this region, which contains a ~170-amino acid (aa) helix flanked by long unstructured sequences (Supplementary Fig. 7). The structure of the C terminus of Sec3 was less well defined, owing to its poor density (Supplementary Fig. 5c). This is consistent with a conformational change observed at the rod region of Sec3 that accounts for 8.6% of the particles in the 3D classifications (Supplementary Fig. 1, class 10).

**Hierarchical assembly of the exocyst complex.** The eight subunits assemble into the exocyst complex in a hierarchical manner. Rather than tiling together as discrete units, the eight subunits knit elaborately with each other through intertwined long coiled coils (Fig. 2a). The interactions can be divided into four pairs: Sec3–Sec5, Sec6–Sec8, Sec10–Sec15 and Exo70–Exo84 (Fig. 3 and Supplementary Video 3). Within each pair, the coiled-coil region of each subunit participates in the central pairwise interactions by forming an intact...
Fig. 2 | The structure of the holo-exocyst complex and individual subunits. 

(a) The model of the exocyst complex with subunits labeled. 

(b) Structures of individual exocyst subunits color coded by domain arrangement. Domains from N to C terminus: purple, N-terminal overhang; dark blue, CorEx motif; teal, CAT-A; light blue, CAT-B; green, CAT-C; yellow, CAT-D; orange, C terminus. In Exo84, teal indicates the PH domain, and green and yellow indicate CAT-A and CAT-B, respectively.

Fig. 3 | Pairwise interactions of the exocyst subunits through the CorEx motifs. The four pairs of exocyst subunits, Sec3–Sec5, Sec6–Sec8, Sec10–Sec15 and Exo70–Exo84, form zipper-like interactions through the CorEx motifs. The pairings of the CorEx motifs are indicated in boxes and shown close up in insets; the starting and ending residues of each CorEx motif are labeled.
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The four dimeric pairs further assemble into higher-order structures, in which the CorEx motifs of Sec3–Sec5 and Sec6–Sec8 form one four-helix bundle (named CorEx I) and those of the Sec10–Sec15 and Exo70–Exo84 dimers form another (named CorEx II) (Fig. 4b), resulting in the tetrameric subcomplex I and subcomplex II, respectively (Fig. 4c and Supplementary Video 3). Such molecular organization is consistent with previous speculative models of exocyst.

Among their interactions, the contacts among CorEx motifs, the interactions of CAT domains further help to stabilize the subcomplexes. In subcomplex I, the bottom of Sec5’s CAT-B domain protrudes into the cradle formed by a long loop (aa 771–820) from the Sec8 CAT-C domain, and the N-terminal overhang (aa 1–30) of Sec5 clings to Sec6’s CAT-A domain. Sec3’s CAT-C domain inserts into the cleft formed between the Sec8 CAT-A and CAT-B domains. In subcomplex II, the two CAT domains of Exo84 form an extensive interaction interface with Sec15’s CAT-A domain, and the PH domain of Exo84 berths between the CorEx II and CAT-B and CAT-C regions of Sec10 (Fig. 4c).

Altogether, the four dimeric pairs further assemble into higher-order structures, in which the CorEx motifs of Sec3–Sec5 and Sec6–Sec8 form one four-helix bundle (named CorEx I) and those of the Sec10–Sec15 and Exo70–Exo84 dimers form another (named CorEx II) (Fig. 4b), resulting in the tetrameric subcomplex I and subcomplex II, respectively (Fig. 4c and Supplementary Video 3). Such molecular organization is consistent with previous speculative models of exocyst.

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At the highest hierarchy, subcomplex I and subcomplex II clasp into the holo-exocyst complex as two layers (Fig. 4d and Supplementary Video 3), which correspond to the front and back layers of the 3D cryo-EM assembly (Fig. 1d). Sec8 of subcomplex I connects the two layers via its multiple interaction interfaces with the rod regions of Sec10, Sec15 and Exo84 in subcomplex II (aa 460–550 of CAT-B of Sec8 with aa 570–780 of CAT-B and CAT-C of Sec10, aa 860–900 of CAT-D of Sec8 with aa 580–680 of CAT-B of Sec15, aa 870–970 of CAT-D of Sec8 with aa 620–720 of CAT-B of Exo84) (Fig. 4d). Sec5’s CAT-B and CAT-C domains from subcomplex I form a large binding interface with the CAT-C and CAT-D domains of Exo70 from subcomplex II. The C termini of Sec5 and Exo84 run very close to each other (Fig. 4d). Moreover, Sec3’s CAT-D domain binds to the joint of the CAT-B and CAT-C domains of Sec10. Lastly, Sec6’s CAT-C and CAT-D domains may also interact with the four-helix bundle in subcomplex II, which in turn helps stabilize the conformation of Sec6 in the exocyst complex (Fig. 4d). These intersubunit interactions yield the complicated architecture of the holocomplex. Together, the structure reveals detailed binding interfaces amid the exocyst subunits, with the CorEx motifs as the central assembly determinants.

The function of Sec3 CorEx motif in vesicle tethering. Among all of the exocyst subunits, Sec3 directly associates with the plasma membrane through its N-terminal PH domain23, whereas the other seven exocyst subunits are transported by the secretory vesicles to the plasma membrane marked by Sec3 (ref. 3). Sec3 also plays a critical role in the recruitment of other exocyst subunits during vesicle tethering. Genetic manipulation suggests that the loss of Sec3 has the least destabilizing effect on the assembly of the exocyst complex11. Consistently, the lack of density for the N-terminal half, the conformational change of the rod region (Supplementary Fig. 1, class 10) and the poor density at the C terminus of Sec3 (Fig. 1d and Supplementary Figs. 2e and 5c) all indicate its flexibility and suggest that Sec3 is the last subunit assembled into the exocyst complex.

Structural analysis shows that the CorEx motif of Sec3 (aa 640–710) mediates its interactions with the remaining subunits (Fig. 4). We thus tested the role of the Sec3 CorEx motif in subunit recruitment and vesicle tethering. Sec3, when tagged with Tom20, was targeted to mitochondria and was able to recruit the other exocyst subunits and tether secretory vesicles to the mitochondria3. We ectopically targeted various Sec3 truncation mutants to mitochondria (Fig. 5a–c). Thus, the shape of CorEx I resembles an upside-down Y (Fig. 4c, shown in magenta box). For CorEx II, the Sec10–Sec15 zipper is much longer than that of the Exo70–Exo84 pair (Fig. 4b). The interwintement of the four CorEx motifs is mainly located at the middle part of the Sec10–Sec15 zipper and takes about two-thirds of the length of the Exo70–Exo84 zipper from the N terminus of the Exo70 CorEx motif and the C terminus of the Exo84 CorEx motif. The two CorExes are different from the core of SNARE complex, which is assembled in a parallel fashion.

Aside from the contacts among CorEx motifs, the interactions of CAT domains further help to stabilize the subcomplexes. In subcomplex I, the bottom of Sec5’s CAT-B domain protrudes into the cradle formed by a long loop (aa 771–820) from the Sec8 CAT-C domain, and the N-terminal overhang (aa 1–30) of Sec5 clings to Sec6’s CAT-A domain. Sec3’s CAT-C domain inserts into the cleft formed between the Sec8 CAT-A and CAT-B domains. In subcomplex II, the two CAT domains of Exo84 form an extensive interaction interface with Sec15’s CAT-A domain, and the PH domain of Exo84 berths between the CorEx II and CAT-B and CAT-C regions of Sec10 (Fig. 4c).
mutant sec3(621–710) cells accumulated Bgl2, a cell-wall-modification enzyme, internally (Fig. 6a,b). Additionally, the secretion of the periplasmic enzyme invertase was also decreased (Fig. 6c). Thin-section EM showed that the sec3 cells accumulated electron-dense vesicles with diameters of 80–100 nm, which is characteristic of post-Golgi secretory vesicles (Fig. 6d). Taken together, the data suggest that disruption of the CorEx motif of Sec3 leads to defects in exocytosis.

A model of exocyst-mediated vesicle tethering. As elaborated above, a long linker region (aa 226–620) situates between the PH domain (aa 70–225) and the CorEx motif of Sec3 (Supplementary Fig. 7). The cross-linking of Sec3 (K119) to Sec15 (K278) suggests that the PH domain is located near the bottom of the back layer of the exocyst complex, far from Sec3’s CorEx motif (Supplementary Fig. 8). The linker, spanning ~150 nm with high solvent accessibility, is sufficient to bridge the PH domain and the CorEx motif. Notably, the PI(4,5)P2-binding site of Exo70 is located on the same face of the exocyst complex (Supplementary Fig. 8), suggesting that the exocyst associates with the plasma membrane through the rear face of its back layer. It is possible that Sec3 berths at the secretion sites through its PH domain and extends far into the cytoplasm through the long flexible linker as a preassembly state. As the vesicle arrives, Sec3 binds to the remaining exocyst subunits through the CorEx motif to form a stable holocomplex and bring the vesicle in proximity to the plasma membrane. Subsequently, the interaction between Exo70 and phospholipids completes the docking of the exocyst complex to the plasma membrane, allowing the exocyst to tether the secretory vesicle for subsequent SNARE-mediated membrane fusion.

Fig. 4 Assembly of the core subcomplexes and the holocomplex. a, The four-helix bundle formed by the CorEx motifs of Sec3, Sec5, Sec6 and Sec8. b, The four-helix bundle formed by the CorEx motifs of Sec10, Sec15, Exo70 and Exo84. c, Sec3, Sec5, Sec6 and Sec8 form subcomplex I. Sec10, Sec15, Exo70 and Exo84 form subcomplex II. Interactions of the subunits in subcomplex I and subcomplex II are shown in insets, with the four-helix bundles indicated by magenta boxes. d, Subcomplex I and subcomplex II assemble into the holo-exocyst complex. Interactions between subcomplex I and subcomplex II are indicated with dash lines (left) and close-up views (right).
Precise knowledge of the structure of MTCs is essential to the understanding of their assembly and vesicle tethering during various membrane trafficking processes. Our study, for the first time, provides a structural model of the fully assembled exocyst complex at high resolution. The model was built based on the 4.4-Å cryo-EM map, and 96% of cross-linked residue pairs have a Cα–Cα distance ≤35 Å when mapped onto the model (Supplementary Datasets 1–3). The only three intrasubunit cross-links with Cα–Cα space >35 Å are all located in Sec3's CAT-B and CAT-C domains. This finding is consistent with the conformational change of Sec3's rod region found in our 3D classifications (Supplementary Fig. 1). C-terminal GFP tagging further verified the subunit assignment in the model (Supplementary Fig. 2a).

Previous crystallographic studies have provided structural insights of several exocyst subunits. The solved regions were mostly helical-bundle repeats and GTPase-binding domains that assume structural autonomy. Our study revealed the near full-length structures of the exocyst subunits in the assembled state. All subunits display an extended configuration with their CorEx motifs interacting at the core of the complex. Cryo-EM provides us with the unique opportunity to determine the high-resolution structure of the intact exocyst complex. Moreover, it is only in the fully assembled state that we were able to identify the CorExes and, furthermore, the hierarchical assembly of the holocomplex. It is also important to note that the CorEx motif sequence in individual exocyst subunits is evolutionarily conserved (Supplementary Note 1). It is likely that the CorEx motifs mediate the core exocyst interactions in higher eukaryotes, including humans. Notably, the CorEx motifs in the exocyst have also been previously noted to share structural similarities with the N-terminal sequences of subunits of the COG and GARP complexes, which all share a secondary structure prediction of coiled coil, thereby suggesting the conservation of this structure across the MTCs.

Sec3 directly associates with the plasma membrane, and the assembly of other exocyst components with Sec3 may serve as a mechanism for vesicle tethering. Using the ectopic targeting strategy, we demonstrated that the CorEx motif of Sec3 plays an important role in this process. It is also interesting to note that a long linker region connects Sec3's PH domain and its CorEx motif, similar to the long coiled-coil tethers such as EEA1. Recently, it was reported that the conformational changes in EEA1 bring endosomal vesicles in proximity for membrane fusion. It would be interesting to examine whether this linker region of Sec3 undergoes similar conformational changes during vesicle tethering at the plasma membrane.

Because vesicle tethering precedes SNARE-mediated membrane fusion, subunits of the exocyst are direct targets of signaling

Fig. 5 | The CorEx motif of Sec3 is crucial for exocyst complex assembly and vesicle tethering. a, Schematic diagram of Sec3 full-length and truncation constructs for ectopic targeting to mitochondria through fusion with Tom20-mCherry. The black block indicates the CorEx motif. b, Colocalization of all of the Tom20-mCherry-tagged Sec3 proteins with Cit1-GFP, a marker protein of yeast mitochondria, in yeast cells. Scale bar, 2 μm. DIC, differential interference contrast. c, Colocalization of Sec5-GFP and the Tom20-mCherry-tagged Sec3 proteins in yeast cells. Scale bar, 2 μm. d, Colocalization of Sec4 (green), a marker of post-Golgi secretory vesicles, and the Tom20-mCherry-tagged Sec3 proteins. Scale bar, 2 μm. At least five images were taken for each sample, with all cells bearing both green and red fluorescence showing similar colocalization patterns to those displayed here.
proteins such as small GTPases and kinases, which spatially or kinetically regulate exocytosis. Different regulators could promote or inhibit the assembly of the exocyst complex, thereby coordinating exocytosis with other cellular activities, such as mitosis and tumor invasion. For example, it has been shown that mitotic phosphorylation of Exo84 by CDK1 disrupts the assembly of the exocyst complex and blocks exocytosis, thereby arresting cell surface expansion before the metaphase–anaphase transition. Our structural data suggest that the phosphorylated residues are located at a region that mediates the interactions of Exo84 with other subunits. CDK1 phosphorylation of these residues may thus disrupt the assembly of the complex. Future studies based on the high-resolution structure may help elucidate the molecular mechanisms of vesicular trafficking.

To our knowledge, this is the first cryo-EM structure of a tethering complex. Although different MTCs function at different stages of vesicular trafficking, they often share similar features in vesicle tethering. The exocyst cryo-EM structure may shed light on the molecular mechanisms of vesicular trafficking.

**Methods**

Methods, including statements of data availability and any associated accession codes and references, are available at [https://doi.org/10.1038/s41594-017-0016-2](https://doi.org/10.1038/s41594-017-0016-2).

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9. Author contributions

8. Additional information

7. Competing interests

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Methods
Yeast strains and plasmids. The budding yeast S. cerevisiae strains and plasmids used in this study are listed in Supplementary Table 2. To integrate SEC3-GFP and sec3-GFP into the TRP1 locus with the SEC3 promoter, pG213 and pG1980 were digested with SnaBI and transformed into the sec1 strain (GY2624).

Purification of the yeast exocyst complex. 33L of yeast culture was grown in YPD medium at 30°C for 10–14 h to an OD600 of ~2.5. Cells were pelleted by centrifugation at 6,600g for 8 min and washed once with double-distilled H2O. Normally 150 × 10^6 cells were obtained for cryo-EM. The yeast cells were resuspended in 40 ml (~25% v/v) of the 5x lysis buffer containing 50 mM HEPES, pH 7.4, 750 mM NaCl, 0.5% NP-40, 5 mM EDTA and 50% glycerol and drop frozen in liquid nitrogen. The cells were then disrupted into powders by SPEX 8670 Freezer Mill. The frozen powder was melted by stirring under a cold water bath. Protease inhibitors and 2 mM DTT was added during the melting process. The cell lysate was then subjected to centrifugation at 35,000 for 30 min, yielding ~100 ml supernatant. IgG Dynabeads were prepared as previously described.

1 ml Dynabeads suspension was added to the supernatant and incubated at 4°C for 3–6 h. After extensive wash by a buffer containing 10 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% NP-40, 1 mM EDTA, 10% glycerol and 2 mM DTT, proteins were released byTrypsin protease cleavage for 1.5 h at 4°C in 0.5 ml wash buffer containing 150 mM NaCl. The eluent was subsequently concentrated to a total volume of 45 μl and applied to a Superose 6 (3.2/300) column (GE Healthcare) with running buffer containing 10 mM HEPES, pH 7.4, 150 mM NaCl and 2 mM DTT. Peak fractions were collected for cross-linking MS and cryo-EM sample preparation.

Chemical cross-linking mass spectrometry (CXMS). For CXMS, approximately 10 μg of purified exocyst complex was cross-linked at 25°C with 0.25, 0.5, 1 mM DSS and BS3 for 1 h or with 2 mM EDC plus 5 mM sulfo-NHS for 2 h. The reactions were quenched with 20 mM NH4HCO3 or 10 mM hydroxylamine, respectively. Proteins were precipitated with ice-cold acetone, resuspended in 8 M urea and 100 mM Tris, pH 8.5, then digested by trypsin (Promega) in 2 M urea and 100 mM Tris, pH 8.5. The LC-MS/MS analysis was performed on an Easy-4k CCD camera.

Electron microscopy data acquisition and processing. The budding yeast strains used in this study are listed in Supplementary Table 2. To integrate SEC3-GFP and sec3-GFP into the TRP1 locus with the SEC3 promoter, pG213 and pG1980 were digested with SnaBI and transformed into the sec1 strain (GY2624).

1 μl Dynabeads suspension was added to the supernatant and incubated at 4°C for 3–6 h. After extensive wash by a buffer containing 10 mM HEPES, pH 7.4, 500 mM NaCl, 0.1% NP-40, 1 mM EDTA, 10% glycerol and 2 mM DTT, proteins were released by Trypsin protease cleavage for 1.5 h at 4°C in 0.5 ml wash buffer containing 150 mM NaCl. The eluent was subsequently concentrated to a total volume of 45 μl and applied to a Superose 6 (3.2/300) column (GE Healthcare) with running buffer containing 10 mM HEPES, pH 7.4, 150 mM NaCl and 2 mM DTT. Peak fractions were collected for cross-linking MS and cryo-EM sample preparation.

Model building and refinement. We used a combination approach with the available crystal structures of some of the subunits and their homologs, the secondary and tertiary structural prediction and the CXMS results to build models of the cryo-EM maps (Supplementary Fig. 3 and Supplementary Video 1). Model building was initiated with the crystal structures of yeast exocyst subunits Sec6 (aa 411–805, PDB 2B1E), Exo70 (aa 67–632, PDB 2B1I) and Exo84 (aa 525–753, PDB 2FJI). Exo84 (aa 525–753) was fit into the map unambiguously as a rigid body by Situs (Supplementary Fig. 4a). Exo70 (aa 67–632) is an extended helical bundle that couldn't be well docked into the map as a whole body. Because the Exo70 helical bundle has the tendency to bend in the middle, we split it into two halves: Exo70 (aa 67–344) and Exo70 (aa 345–632). The two halves fit into the map with a high degree of agreement as rigid bodies, with the break sites close enough to form a peptide bond (Supplementary Fig. 4b). Based on the intermolecular cross-linking pairs between Exo70 and Sec6, we depicted Sec6 (aa 411–805) proximal to the N terminus of Exo70 (aa 67–632) in the cryo-EM map (Supplementary Fig. 4c). The Sec6 model fit in the corresponding density very well. At this point, we have docked all of the known crystal structures of the yeast exocyst subunits into the map. Next, we fit the map with predicted tertiary structures obtained from homolog modeling. The tertiary structure of Sec10 (aa 234–867) was predicted with Swiss-Model based on the atomic model of Zebrafish Sec10 (aa 195–708, PDB 5H11). After the loops of aa 279–319 and aa 456–568 were pruned, the protein was fit into the right edge of the back layer (Supplementary Fig. 4d). For better fitting, it was split into two halves between aa 32 and 363 and docked into the map separately. The PH domain of Exo84 (aa 345–632) was predicted using PHYRE2 (ref. 68) based on the crystal structures of rat Exo84 (PDB 1ZC4) and Dro sophila Sec15 (PDB 2A2F) and docked into the map (Supplementary Fig. 4e,f).

From the docked atomic models described above, we performed manual extension of the Cα peptide of the corresponding proteins following the density connectivity by Coot. We started with helix assignment based on the secondary structure predictions (Supplementary Fig. 7) and then modeled the connecting loops guided by a lower threshold of the map showing the densities. Wherein a loop was too long and not enough density was visualized, the corresponding region was left unmodeled. Using such a strategy, full-length Sec6, Sec10, Sec15 and Exo70 and Exo84 (aa 170–753) were modeled in the map (Supplementary Fig. 4g–k).

To build the models of Sec5 and Sec8, we first identified their densities based on several cross-linking derived residue pairs. Pairs Exo84(725–731)=Sec5(961) and Exo84(731–735)=Sec5(961) revealed that the C-terminal residues of Sec5 were adjacent to the C-terminal end of Exo84. This information, together with the pair Exo70(167–170)=Sec5(228), assigned the left branch of the front layer to Sec5 (Supplementary Fig. 5a). Similarly, pairs Exo84(707–710)=Sec6(893), Exo84(534)=Sec8(238) and Sec6(237–238)=Sec5(125) indicated that the long axis of the front layer of Sec8 (Supplementary Fig. 5b). All of these residues from Sec5 and Sec8 from their C to N termini. After the above modeling, the remaining density corresponding to arm I was assigned to Sec3 with one end of the density located near Exo70 and the other end reaching to the middle of Sec10. The cross-link pairs Exo70(167–206)=Sec6(313) and Exo70(167–206)=Sec5(1323) suggested that Sec3 extended from the first end with Ly613 to the second one with Ly613. Based on the above information and the density shape, we manually modeled Sec6(121–1322) into the map (Supplementary Fig. 5c). The very C terminus of Sec3 starting from Phe1040 cannot be modeled precisely because of the poor density.
Because the resolution of 4.4 Å is not sufficient for clear side chain modeling, all of the docked tertiary structures derived from homolog structure prediction and manually built models consist of polyalanines to trace the main chains. Only side chains derived from the crystal structures of yeast exocyst subunits were kept in the docked structures. To facilitate the CXMS analysis, all of the models are presented with their original sequences. The exocyst model was then validated with independent CXMS analyses, in which different cross-linking chemicals were used (Supplementary Datasets 1–3 and Supplementary Video 2). The Cα-Cα distance restraint of BS3 and DSS as well as EDC cross-links is normally within a range of 24 Å (refs 19,51), and approximately 70% of the DSS and BS3 crosslinks are compatible with the X-ray structures6. As a common practice in protein structure modeling, the distance restraints are often relaxed to 30 Å or 35 Å (refs 70–72). When mapped onto the exocyst model, 96% of all crosslinks fell within 35 Å, 94% within 30 Å, 91% within 27 Å and 86% within 24 Å, thus confirming the overall accuracy of the assignment, fold and chain tracing of the exocyst model.

After the validation, the exocyst model was subject to 100 iterations of Phenix.real_space_refine73 against the 4.4-Å map with global geometry and secondary structure restraints. Sec6 was also auto-replaced separately against the refined map of the head region (Supplementary Fig. 1). The quality of the models was evaluated with MolProbity74, Chimera75 was used for figure presentation.

Fluorescence microscopy. Yeast cells were grown to an OD60 of 1.0–1.5 in synthetic complete medium without leucine at 25 °C and harvested by centrifugation at 3,000 r.p.m. for 1 min. 2μl of cell suspension was processed for fluorescence microscopy together with a Nikon ECLIPSE Ti confocal laser-scanning microscope equipped with an Apo TIRF 100 X 1.49 oil immersion objective lens. Images were collected with a digital camera (CSU-X1, Nikon) operating with NIS-Elements AR 4.06.00 software (Nikon). A rabbit anti-Sec4p polyclonal antibody diluted to 1:2,000 was used for immunofluorescence microscopy.

Bgl2 secretion assay. Yeast strains expressing SEC3-GFP and sec3(6721–710)-GFP under the SEC3 promoter as the sole copy of Sec3 were grown to an early log phase in YPD medium at 25 °C, followed by another 2 h of culturing at 25 °C and 37 °C, respectively. 10 mM NaN3 and 10 mM NaF were added into the culture, and a total of 10 OD60 units of cells were collected for each sample. Cells were washed by 50 mM Tris, pH 7.5, 10 mM NaN3, and 10 mM NaF and spheroplasted by Zymolase in spheroplast buffer containing 50 mM Tris, pH 7.5, 1.4M sorbitol, 10 mM NaN3, and 10 mM NaF. After pelleting, supernatant was taken for external Bgl2 measurement. The spheroplast was washed once by spheroplast buffer and lysed by 0.5% Triton X-100 and then fixed with 4% KMNO4 for 1 h at 25 °C. After extensive wash with double-distilled H2O, the pieces were treated with 0.5% sodium meta-periodate for 15 min to increase cell penetrability and then embedded in 2% low-melting-point agarose in a 1:1 ratio and cut into small pieces (~1 mm3). The samples were then fixed with 4% KMNO4, 1 h at 25 °C. After extensive wash with double-distilled H2O, the pieces were treated with 0.5% sodium meta-periodate for 15 min to increase cell penetrability and then placed into 2% uranyl acetate overnight at 25 °C after another wash. The next day, the samples were dehydrated through a graded series of ethanol (50–100%) and embedded in 100% Spurr resin and sectioned for EM. Cells were observed using a JEM-1011 JEOL transmission microscopy at 15,000x magnification.

Statistics. For invertase secretion assay analysis, the online GraphPad software was used to do the statistics. Unpaired student's t test was used to analyze the difference between invertase secretion rate of SEC3-GFP and sec3(6721–710)-GFP. For data collected at 25 °C, the two-tailed P value is 0.1376, t value is 1.8526 and degree of freedom is 4; for data collected at 37 °C, the two-tailed P value is <0.0001, t value is 53.7907, and degree of freedom is 4.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. EM structures and associated atomic model have been deposited into the Electron Microscopy Data Bank and Protein Data Bank with the accession codes EMD-6827 and PDB 5YFP, respectively. Source data for Fig. 6c are available online. Other data supporting this study are available upon reasonable request.

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   - Not applicable.

2. Data exclusions
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   - No data exclusion.

3. Replication
   - Describe whether the experimental findings were reliably reproduced.
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5. Blinding
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
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6. Statistical parameters
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
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   See the web collection on statistics for biologists for further resources and guidance.

7. **Software**

   Policy information about availability of computer code

   7. Software
      - Describe the software used to analyze the data in this study.
      - EMAN2 and SPIDER were used to analyze Negative staining EM data; MotionCorr.
MotionCor2, CTFFIND3, RELION1.4 and ResMap were used to analyze cryo-EM data; Predictprotein was used to predict the secondary structure of the exocyst subunits; PHYRE2 and Swiss-Model were used to predict tertiary structures based on known homology structure; Chimera was used for rigid body docking, structural analysis and figure presentation; Coot was used for manually model building; Phenix was used for model refinement; PROCHECK was used for model validation; PROMALS3D was used for multi-sequence alignment. Prism7 was used for statistical data presentation.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

**Materials and reagents**

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8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No restriction.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The Bgl2 antibody has been tested in yeast by several labs and has been used in many previous publications.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

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