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Making COPII Coats

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Newly synthesized proteins en route to the Golgi apparatus are exported from the endoplasmic reticulum by COPII coated vesicles. Fath et al. (2007) now reveal the structure of a large portion of the yeast Sec13/31 complex, which comprises the coat framework of COPII-coated vesicles. Their findings suggest a mechanism by which the COPII cage assembles and accommodates cargo of different sizes.

Vesicular traffic is essential for maintaining the identity of membrane-bound organelles. There is now wide agreement that fundamentally conserved principles underlie the way cargo is transported by vesicular carriers, which bud from a donor membrane and then fuse with the appropriate target membrane on an acceptor organelle. However, our molecular understanding of how these events occur is still in its infancy. Now, using X-ray crystallographic methods, Fath et al. (2007) have determined the composite atomic structure of a large portion of the yeast Sec13/31 complex, the coat framework of COPII-coated vesicles, which export newly synthesized proteins from the endoplasmic reticulum. They use their structural findings to fit a composite atomic model of Sec13/31 into the electron density map of Sec13/31 cages assembled in vitro, previously determined using cryoelectron microscopy (Stagg et al., 2006). In so doing, Fath et al. (2007) establish the organizing principle for the COPII coat at a molecular level. Moreover, comparison of the COPII coat model with the molecular model of the clathrin coat (Fotin et al., 2004), which is involved in trafficking between the plasma membrane and endosomal compartments, deepens our understanding of how coat assembly couples vesicle formation and the sorting of cargo.

Thirteen years ago, Barlowe, Scheckman, and colleagues first described the existence of vesicular carriers responsible for most, if not all, of the secretory traffic emanating from the endoplasmic reticulum (Barlowe et al., 1994). They designated these carriers "COPII-coated vesicles" to differentiate them from the COPI-coated vesicles involved in Golgi traffic. Based on a wealth of biochemical and genetic experiments, it is now believed that in the first step of COPII assembly a small GTPase Sar1p is recruited from the cytosol to the endoplasmic reticulum (ER), where it becomes activated upon GTP binding. Activated Sar1p can then recruit the heterodimer Sec23/24, an adaptor complex that recognizes sorting-peptide motifs on membrane-bound cargo proteins destined for recruitment into the budding COPII vesicle. Sec23/24 also binds Sec13/31, the scaffold ultimately responsible for the formation of the COPII coat. This process is analogous to the formation of clathrin coats, in which the AP-clathrin adaptor complexes and clathrin are functionally equivalent to Sec23/24 and Sec13/31, respectively.

In a tour de force of crystal "engineering," Fath and colleagues first used limited proteolysis to delineate the domain organization of the Sec13/31 complex. On this basis, they designed two sets of subcomplexes suitable for crystallographic analysis, which they designated the "edge" and "vertex" elements. The basic edge element comprises Sec13, a β-propeller, associated with the central section of Sec31. Sec31 folds into an extended α-helical zig-zag or α-solenoid, similar to the one that forms most of a clathrin leg (Fotin et al., 2004). In an unexpected twist, a small portion of Sec31 contributes to Sec13 providing the seventh blade of its propeller. Two such edge elements contribute to the full edge, forming a complex that is held together by antiparallel contacts contributed by the C-terminal ends of the α-helical zig-zags from two subunits of Sec31. These contacts are reminiscent of those between the α-helical zig-zags of the proximal legs of two clathrin triskelions (the three-legged complexes of clathrin light and heavy chains), centered on adjacent vertices in the cytosol-facing surface of a clathrin coat (Fotin et al., 2004). The second subcomplex, corresponding to the vertex element, comprises Sec13 and the N terminus of Sec31, itself a seven blade β-propeller. Four Sec13/Sec31 complexes associate in the crystal lattice, but with two different types of contacts, such that the junction has 2-fold rather than 4-fold symmetry.

With these atomic models in hand, Fath and colleagues then sought to fit their structures into the electron density map corresponding to the cuboctahedron (a shape with eight triangular and six square surfaces) of the COPII coat assembled from intact Sec13/31 (Stagg et al., 2006). One of the contacts in the crystals of the vertex element fits neatly into the four-way junction seen in the vertices of the cuboctahedral lattice. Contrary to earlier speculation, it is the propellers of Sec31 and not of Sec13 that abut to establish the

contacts required for COPII vertex formation. In contrast, the vertex of a clathrin coat contains elements from more than the three clathrin chains that converge there. In addition to the 3-fold "hub" of a single triskelion, the clathrin vertex includes distal leg elements contributed by six other triskelions, centered two and three vertices away. One likely similarity between the COPII coat and the clathrin lattice involves the antiparallel contacts between the α zigzags of two Sec31 molecules in an edge. Subtle changes in its contacts probably provide the adaptability required to accommodate larger lattices of lower curvature. Fath et al. (2007) also note the absence in the cryo-electron microscopy map of electron density corresponding to the proline-rich C-terminal segment of Sec31, presumably because the orientation of this segment is not fixed with respect to the other parts of the coat. As this is the segment involved in the interactions with Sec23/24, it is possible that in a complete COPII coat the location of Sec23/24 is also not precise, perhaps as a way to accommodate the position of cargo of different sizes in the interior of a budding vesicle.

The molecular details unveiled in this study provide a glimpse of what may be discovered by further work. Take for example the question of how the COPII coat might form. The composite shape of Sec13/31 deduced from the crystal structure of the vertex and edge subcomplexes closely resembles the configurations it adopts when forming the different types of vertices and edges within the Sec13/31 coat. Although the current fitting is of low resolution, we can safely conclude that reorganization of a preassembled flat array into a curved structure is unfeasible. In other words, formation of a COPII bud must occur in sequential steps, in which an initiating set of Sar1p/Sec23/24 complexes already bound to the ER, perhaps in association with cargo, move laterally on the membrane surface until captured by soluble Sec13/31. The flexible link between Sec31 and Sec23/24 allows for relatively minimal stringency in the starting position of the Sec13/31 rods. The two-dimensional constraint coupled to the relative increase in local concentration would facilitate formation of the contacts required to create a tetrameric vertex; this process can propagate, with curvature mainly provided by the overall structure of the vertices and edges. The actual size and shape of the COPII vesicle may be strongly influenced by the size of the enclosed cargo, with the default size corresponding to the abundant 600–700 Å diameter profiles observed in sections of cells by standard electron microscopy (Barlowe et al., 1994). Larger coats have been suggested, in particular in association with collagen fibers and chylomicron particles as they exit the ER. Larger coats are also observed in the course of COPII-based budding reactions in vitro. By what mechanism are different sizes achieved? Is it a trial-and-error process? Or are the coats not always as regular as the current cage model, based on the in vitro assembly of Sec13/31, might lead us to believe? Another question begging an answer is why, up to now, has it not been possible to see the triangular and square facets of native COPII coats? Does the cage model not apply to native coats? Or do lots of other proteins fill in the facets? Clearly, these questions can now be approached because access to a detailed structural model allows for the directed mutagenesis of contacts.

Another fundamental question that can now be tackled concerns membrane pinching. This is the final step that separates the invaginating membrane from the donor membrane. What provides the driving force for this reaction? Perhaps the localized accumulation of Sar1/Sec23/24 on the neck connecting the budding vesicle with the ER membrane is sufficient to facilitate scission. Biochemical data suggest that membrane association of activated Sar1 has the potential to create local curvature (Bielli et al., 2005; Lee et al., 2005), and structural analysis of Sec23/24 reveals that its concave surface faces the membrane (Bi et al., 2002). Functional defects in Sec23A of humans or zebrafish result in defects in ER export with the accumulation of curved coated profiles budding from the ER (Boyadjiev et al., 2006; Lang et al., 2006). These observations suggest that there is a problem in completing late stages of coat assembly, which may involve a specialized form of Sec23/24 and or of Sec13/31 needed for closure and or pinching. The future is exciting. The molecular snapshot provided by Fath et al. (2007)—and there are surely more to come—can now be used to deduce molecular movies by thoughtfully combining these static images with future observations of COPII dynamics (for example from live-cell fluorescence microscopy and from structure-guided in vitro reconstitution assays).

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