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Structure and Mechanism in Membrane Trafficking

Frederick M. Hughson^a and Karin M. Reinisch^b

^aDepartment of Molecular Biology, Princeton University, Princeton, NJ 08544, United States

^bDepartment of Cell Biology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, United States

Abstract

Cell biologists have long been interested in understanding the machinery that mediates movement of proteins and lipids between intracellular compartments. Much of this traffic is accomplished by vesicles (or other membranous carriers) that bud from one compartment and fuse with another. Given the pivotal roles that large protein complexes play in vesicular trafficking, many recent advances have relied on the combined use of X-ray crystallography and electron microscopy. Here, we discuss integrated structural studies of proteins whose assembly shapes membranes into vesicles and tubules, before turning to the so-called tethering factors that appear to orchestrate vesicle docking and fusion.

Introduction

A fundamental question in cell biology is how proteins and other materials are distributed amongst the intracellular compartments of a eukaryotic cell, or (at the plasma membrane) released by exocytosis or internalized by endocytosis [1]. Central to these transactions are proteins that interact with membranes, reshaping them, for example, to create vesicles laden with cargo. Vesicles are captured, and perhaps uncoated, by other proteins that serve to ensure that this cargo is delivered to the correct destination. Still other proteins, functioning in collaboration with these tethering factors, are essential for the fusion of the vesicle and target membranes.

For many cell biological problems, structural methods have proven to be especially effective tools for gaining mechanistic understanding. Intracellular trafficking is no exception, with early successes including, for example, the crystal structure of the neuronal SNARE complex essential for the fusion of synaptic vesicles with the axonal plasma membrane [2]. Nonetheless, many components of the vesicle trafficking machinery pose challenges for structural biologists, not only because these components interact – directly or indirectly – with membranes, but also because they often function as part of large multi-subunit assemblies. In this review, we seek to highlight a handful of recent successes, many of them employing a combination of electron microscopy (EM) and X-ray crystallography.

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Corresponding author: Hughson, F.M. (hughson@princeton.edu) or Reinisch, K.M. (karin.reinisch@yale.edu).

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COPII vesicle coats

Vesicle formation *in vivo* entails the assembly of vesicle coat proteins [3,4]. A major contribution to our understanding of these coats has come from the discovery of conditions that promote coat assembly *in vitro*. Combining cryo-EM studies of reassembled coats with X-ray crystal structures of coat components has led to dramatic progress, most recently with respect to the COPII coat implicated in vesicle traffic from the endoplasmic reticulum to the Golgi apparatus [5,6].

Like the long-studied clathrin coat [7], the COPII coat contains two layers [8]. The inner layer is responsible for cargo recruitment, while the outer layer makes up a “cage” that organizes the inner-layer elements into a regular lattice. For COPII, the inner layer of the coat comprises a bowtie-shaped heterodimer of Sec23 and Sec24 subunits, together with the small GTPase Sar1. The outer layer is made up of Sec13 and Sec31, one heterotetramer of which constitutes each edge of the cage lattice [9]. Both layers are clearly seen in cryo-EM images of coats reconstituted from recombinant Sec23–24 and Sec13–31 [10] (Figure 1a). The distinctive shape of the Sec13–31 heterotetramer allowed the known X-ray structure to be fitted unambiguously into the cryo-EM density [10,11] (Figure 1b–d). Uncertainty remains with respect to the Sec23–24 heterodimer which, owing to its symmetrical overall shape, could be fitted into the density in either of two non-identical orientations [10,12]. Also uncertain at present is the structural basis for the interaction between the inner and outer coats. Nonetheless, these structural studies have yielded striking insights.

One functional consequence of the structural work is a mechanism whereby cargo could influence the size of the COPII vesicle that carries it. This would, for example, be important for ensuring that large cargo molecules are enclosed within vesicles sufficiently large to accommodate them. How might this be accomplished? A key observation is that the addition of Sec23–24 to reconstitution reactions influences the size distribution of the resulting particles [10]. Specifically, 60-nm cuboctahedrons (Figure 1b) predominate in the absence of Sec23–24, while 100-nm icosidodecahedrons (Figure 1c) predominate in the presence of Sec23–24. This finding is consistent with the idea that the inner layer subunits Sec23–24, which bind to cargo either directly (for transmembrane cargo) or indirectly via transmembrane cargo adapters (for luminal cargo), transmit information about the cargo to the cage subunits Sec13–31. This is structurally plausible because Sec23–24 heterodimers are positioned directly underneath the four-way junctions that represent the vertices of the COPII cage (Fig. 1a). By influencing the geometry of these vertices, Sec23–24 could control curvature of the cage and thereby the size of the resulting coat [10].

Another consequence of the structural results is that the COPII coat has large “windows” [10] (Figure 1a–c). One therefore expects that, at least for COPII-coated vesicles, proteins embedded in the vesicle membrane would be accessible to cytosolic proteins, even large ones. This may be particularly relevant for tethering factors (discussed below) that interact with proteins or phospholipids on vesicle surfaces [13,14]; the COPII coat structure implies that uncoating is not necessarily a prerequisite for tethering factor recruitment. Similarly, the large windows in the COPII cage would provide access for those tethering factors that bind to inner-layer subunits of COPII or COPI coats [15,16].

Membrane deformation by BAR-domain proteins

Fundamental to many membrane transactions, including budding and fission, is the manipulation of membrane shape. Various mechanisms have been proposed for protein-induced membrane deformation (reviewed in [3]). One of these is a “scaffolding” mechanism, where membranes conform to a positively charged surface proffered by a protein. In a second mechanism, a hydrophobic wedge is inserted into one leaflet of the lipid

bilayer to induce curvature. In both cases, it is thought that sufficient force to drive membrane deformation can be generated only through the cooperative actions of many subunits.

A recent study provides the first direct evidence for cooperative deformation by the scaffolding mechanism [17]. The BAR superfamily of proteins includes classical BAR domains as well as F-BAR and I-BAR domains, which all function in membrane tubulation prior to vesicle scission (reviewed in [18]). These proteins are elongated dimers consisting of antiparallel coiled-coil α -helices. The dimers are gently curved, with conserved positively charged residues lining the concave face [19,20]. The mechanism by which the F-BAR proteins interact with and deform membranes was revealed by docking the X-ray structure into cryo-EM reconstructions of F-BAR domains bound to both flat and curved lipid bilayers [17] (Figure 1e,f). On flat membranes (not shown), F-BAR proteins assembled in a tip-to-tip manner and with their basic concave surfaces oblique to the membrane, so that maximum curvature was not imposed. By contrast, F-BAR proteins on tubules formed helical filaments that wound tightly around the membrane, with the entire basic, concave surface of each dimer in contact with the lipid bilayer. While basic residues on this surface were important for tubulation, hydrophobic residues that could function as wedges were not. The protein dimers on the tubules were found to interact via tip-to-tip interactions in a manner reminiscent of that observed for dimers bound to flat membranes, but in addition the tubule-bound dimers exhibited extensive lateral interactions (Figure 1f). The lateral interactions are unavailable to F-BAR proteins arrayed on flat membranes, and their formation was proposed to be critical in driving polymerization and concomitant membrane deformation. According to this model, individual dimers, partially arranged in tip-to-tip arrays, cause local membrane curvature. As the dimers transition to impose their full curvature on the lipid bilayer, the lateral interaction surfaces are exposed, leading to F-BAR polymerization and membrane tubulation.

Other proteins in the BAR superfamily likely work by similar, or slightly modified, mechanisms. I-BAR proteins differ from classical BAR and F-BAR proteins in that their convex and not their concave surface is positively charged [21,22]. The I-BAR domains associate with the inner leaflet of membranes and drive membrane protrusion in a direction opposite that of BAR and F-BAR domains [23]. However, as with F-BAR proteins, the interaction with membranes appears to occur via a cooperative scaffolding mechanism [24]. Further, some I-BAR domains have N-terminal amphipathic helices that insert into the membrane bilayer, affecting tubulation efficiency and tubule diameter [24].

It is tempting to speculate that ESCRT-III proteins, which drive vesicle budding into multivesicular bodies [25–27], may mediate membrane deformation by a similar mechanism. These proteins all contain a basic, α -helical domain similar in structure to a BAR domain [28,29]. Like BAR domains, these domains contain five α -helices, including a helical hairpin and two shorter helices that pack against it. In the crystal structure of the CHMP3 protein, elongated rod-like dimers were observed [29]. Two groups have combined cryo-EM of protein-coated tubules with crystallographic studies to formulate models for ESCRT-III-induced membrane deformation [25,27]. As the proposed mechanisms differ, however, this remains an active field of research.

Dsl1 Vesicle Tethering Complex

Another area in which X-ray crystallography and EM have been fruitfully combined is in the study of vesicle tethering factors, and especially the so-called multisubunit tethering factors (MTCs). MTCs are believed to mediate the initial attachment between an intracellular trafficking vesicle and its membrane target, and they probably help to coordinate vesicle

capture with vesicle uncoating and the assembly of membrane-bridging trans-SNARE complexes [14,30]. A notable feature of MTCs is their large size and architectural complexity: the known MTCs are hetero-oligomers containing 3–10 subunits, with total molecular weights ranging from 250 to 800 kDa. The past several years has seen substantial progress in elucidating the structures of MTC subunits, and these structures divide the MTCs into at least two classes. In this section, we discuss the Dsl1 complex, a relatively simple example of the class of MTCs that also includes the conserved oligomeric Golgi (COG), exocyst, and Golgi-associated retrograde protein (GARP) complexes [31]. Structures or partial structures of two Dsl1 subunits, two COG subunits, and four exocyst subunits provide evidence of a common fold, indicating that they are derived from a common evolutionary progenitor [32–38]. Whether these complexes also share quaternary structural features remains to be established.

The yeast Dsl1 complex functions in Golgi-to-ER trafficking by tethering Golgi-derived vesicles to the ER membrane [39,40]. It has only three subunits, each of them 80–90 kDa and encoded by an essential gene. A structural model for the Dsl1 complex, based on four overlapping crystal structures, was reported recently [38] (Figure 1g). The three subunits fit together to form a 200 Å-tall inverted “U” structure, with the two legs anchored via interactions with SNARE proteins to the ER membrane. The membrane-distal tip of the Dsl1 complex, meanwhile, contains a flexible “lasso” about 110 residues in length. Several lines of evidence suggest that the lasso functions to capture COPI-coated vesicles. First, sequences within the lasso bind directly to COPI subunits; second, shutting off expression of the lasso-containing subunit causes a reversible accumulation of COPI-coated membranes [16,41,42]. Thus, the structure is consistent with the model that the Dsl1 complex functions as a physical tether for COPI vesicle capture. Moreover, negative stain EM showed that the Dsl1 complex contains several hinges, suggestive of a dynamic complex that may adopt different conformations during the course of a functional cycle [38].

Many tethering factors appear to subserve functions well beyond that of tethering per se. For the Dsl1 complex, it has been suggested that the interaction with the COPI coat may play a role in coat disassembly [16]. Some but not all of the other known tethering factors appear, like the Dsl1 complex, to interact with the relevant vesicle coat proteins [14,30], although to our knowledge there is not yet experimental evidence that these interactions facilitate uncoating. A third potential role for tethering complexes is to regulate assembly of the trans-SNARE complexes responsible for membrane fusion [43]. In vitro experiments demonstrated a relatively modest Dsl1 complex-dependent enhancement of SNARE complex assembly [38]; other tethering complexes also appear to regulate SNARE complex assembly and/or stability [14,30]. It will be very interesting to determine the extent to which tethering complexes, as a class, have taken on additional roles (those enumerated here or others) in coordinating membrane trafficking reactions.

TRAPP Tethering Complexes

Several of the known membrane tethering complexes are unrelated to the Dsl1 complex in structure and hence also mechanism. One of the best characterized of these is the TRAPP I complex which functions in traffic from the ER to the Golgi [44,45]. Like Dsl1, TRAPP I is involved in vesicle recognition, interacting with a component in the inner layer of the COPII coat [15,46], presumably as the vesicle arrives at the target membrane. Unlike Dsl1-related complexes, TRAPP I is also a guanine exchange factor (GEF) and activates the Rab GTPase Ypt1 [47], a prerequisite for the downstream membrane fusion event. In yeast, this complex has six essential subunits (Bet5, Trs20, Trs23, Trs31, Trs33 and two copies of Bet3). All of these subunits are relatively small (17–33 kDa). Despite limited sequence similarity, Bet3, Trs31, and Trs33 display related folds, as do Bet5, Trs20, and Trs23; in all cases, the folds

consist of central β -sheets surrounded by α -helices [48–52] (Figure 1h). The overall architecture of the TRAPP I complex was elucidated using crystal structures of two subcomplexes that were fitted together based on a structural envelope determined by EM [49]. The subunits are arranged into a flattened ellipsoid ~180 Å long, with one copy of Bet3 near each end (Figure 1h).

How TRAPP I facilitates guanine nucleotide exchange was illuminated by a crystal structure containing four different TRAPP subunits (Bet5, Trs23, Trs31, and two copies of Bet3) and the Rab GTPase Ypt1 [53]. Trs23 and Bet5 form most of the Ypt1 interaction site, but the C-terminus of Bet3 (red arrow in Figure 1h) is critical for activity, invading the Ypt1 nucleotide binding pocket and precipitating a rearrangement in Ypt1 that opens the pocket for nucleotide exchange.

How TRAPP I interacts with membrane-bound organelles remains unclear. It has been proposed that palmitoyl groups tucked into a hydrophobic channel in the Bet3 proteins (yellow arrows in Figure 1h) may be extruded to aid in membrane attachment [52], or that TRAPP associates via a flattened side of the ellipsoid [49]. In a third model for homotypic tethering in higher eukaryotes, TRAPP I uses its two copies of Bet3 to bind simultaneously to Sec23 subunits in the coats of two different COPII vesicles [15,53].

A larger complex, TRAPP II, functions in traffic to the late Golgi [46]. TRAPP II includes all TRAPP I components and three additional large subunits (Trs120, Trs130, and Trs65). The role of the large subunits is under debate: it has been proposed that they might alter or mask the Ypt1 binding site of TRAPP I to convert TRAPP into a GEF for the Rabs Ypt31/Ypt32 [54]. Accumulating data from other groups, however, suggest that, like TRAPP I, TRAPP II is a Ypt1 GEF [53,55]. The Trs130 subunit of TRAPP II recognizes COPI-coated vesicles [55]. Thus, it is very likely that the additional subunits present in TRAPP II serve to target it to a different trafficking pathway than TRAPP I.

Perspective

An increasingly common approach in tackling the structures of large biological assemblies has been to combine high-resolution crystal structures of individual subunits or subassemblies with lower-resolution information pertaining to overall architecture. In many cases, the lower-resolution information has been supplied by electron microscopic techniques. But not all samples are suitable for EM, and in these cases hydrodynamic approaches such as size exclusion chromatography, ultracentrifugation, or small angle X-ray scattering can also provide restraints for crystal-structure-based models of intact assemblies. In very recent examples, such hydrodynamic methods have been used to model the ESCRT-0, -I, and -II complexes that function in multi-vesicular body budding [56–58]. We anticipate that hybrid approaches will play an increasingly important role in unraveling the molecular mechanisms that underlie membrane trafficking and other cell biological processes.

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Figure 1.

Macromolecular assemblies important for membrane trafficking. **(a–d)** Cryo-EM reconstructions of the COPII coat [10]. **(a)** Single-particle reconstruction of COPII coats reconstituted using Sec13–31 (green) and Sec23–24 (yellow) components. Additional EM density observed inside the inner cage, and attributed to non-specifically bound protein, is not shown. **(b)** Sec13–31 complexes self-assemble into cuboctahedrons 60 nm in diameter. The X-ray structure of Sec13–31 heterotetramers [11] is also shown, docked into the EM density [10]. **(c)** In the presence of Sec23–24, most particles display icosidodecahedral symmetry and a diameter of 100 nm. The Sec23–24 heterodimers (not shown; see panel (a)) are positioned under the vertices of the outer coat, where four Sec13–31 heterotetramers

interact. **(d)** Side view emphasizing the fit between the Sec13–31 heterotetramer crystal structure [11] and the EM density [10]. The curvature at the center of the heterotetramer was modified by normal modes flexible fitting, relative to the crystal structure, to optimize the agreement with the EM density. **(e–f)** F-BAR modules bound to membrane tubules [17]. These panels show crystal structures of F-BAR modules fitted into cryo-EM reconstructions. **(e)** View along the cylindrical axis of an F-BAR coated membrane tubule, with the subunits in an F-BAR dimer shown in yellow and orange. **(f)** Surfaces from two different reconstructions, in which the tubule diameters were 57 nm and 67 nm, respectively. In the top panel, several F-BAR dimer structures are docked into the cryo-EM map, showing both tip-to-tip and lateral contacts between dimers. The bottom reconstruction shows just one dimer docked into the density. **(g)** X-ray crystallography-based model of the Dsl1 tethering complex, assembled from four overlapping crystal structures [38]. The entire complex is ~30 nm in its longest dimension. At its base, the complex is anchored to the ER membrane through direct interactions between two different subunits (Sec39 and Tip20) and ER-localized SNARE proteins. At the top of the complex is a long, unstructured loop region that may “lasso” COPI-coated vesicles. **(h)** The structure of TRAPP I containing all the essential subunits. The overall architecture was initially determined by fitting crystal structures of two subcomplexes into a reconstruction of the whole complex obtained from EM [49]. The complex is ~18 nm long. Palmitoyl groups buried in a hydrophobic channel in Bet3 are labeled with yellow arrows. A red arrow indicates the position of the Bet3 C-terminus, which is critical in facilitating nucleotide exchange by the Rab GPTase Ypt1 [53]. How TRAPP I interacts with membranes is not well understood, although Bet3 is thought to play a role [15,52].