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Structure of Golgi Transport Proteins

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The function of the Golgi has long been recognized to critically depend on vesicular transport from, to, and within its cisternae, involving constant membrane fission and fusion. These processes are mediated by Arf GTPases and coat proteins, and Rabs, tethers and SNARE proteins, respectively. In this article, we describe structural studies of Golgi coats and tethers and their interactions with SNAREs and GTPases as well as insights regarding membrane traffic processes that these have provided.

GRASP PROTEINS

GRASP55 and GRASP65 (Golgi ReAssembly Stacking Protein) are required for proper cisternal stacking (Barr et al. 1997; Shorter et al. 1999), and their knockdown leads to fragmentation of the Golgi (Xiang and Wang 2010). Their phosphorylation during the cell cycle results in Golgi ribbon unstacking just prior to mitosis (Feinstein and Linstedt 2008; Sengupta and Linstedt 2010).

GRASP proteins are amino-terminally myristoylated for membrane attachment (Sengupta et al. 2009). They are composed of two tandem PDZ domains, followed by an extended region (~30 kDa) that is predicted to be unstructured. The crystal structure of GRASP55 reveals a tandem arrangement of two circularly permutated PDZ domains, each with a peptide binding groove (Truschel et al. 2011). Mutational data suggest that the peptide binding groove of PDZ2 mediates Golgi association via interactions with the golgin GM130 (Barr et al. 1998) (or golgin-45 for GRASP65) (Kuo et al. 2000; Short et al. 2001), whereas PDZ1 is important for multimerization to effect Golgi stacking (Wang et al. 2005; Sengupta et al. 2009). It was proposed that the stacking mechanism involves both PDZ domains and that a loop near the carboxyl terminus of PDZ2 binds in the peptide binding groove of PDZ1 of a second GRASP molecule (Truschel et al. 2011), allowing for oligomerization of the GRASP domains (Fig. 1A). The interaction between the PDZ2 loop and the PDZ1 domain was not observed in the crystal structures, but this may have been caused by crystal packing constraints (Truschel et al. 2011). The GRASP domain with a phospho-mimetic mutation known to disrupt stacking was also crystallized, and a small conformational change was observed in the PDZ2 loop, perhaps sufficient to prevent its binding to PDZ1. Thus, the findings provide a model that helps explain the molecular basis of cisternal stacking of the *cis* and medial Golgi by GRASPs and its disassembly during mitosis.

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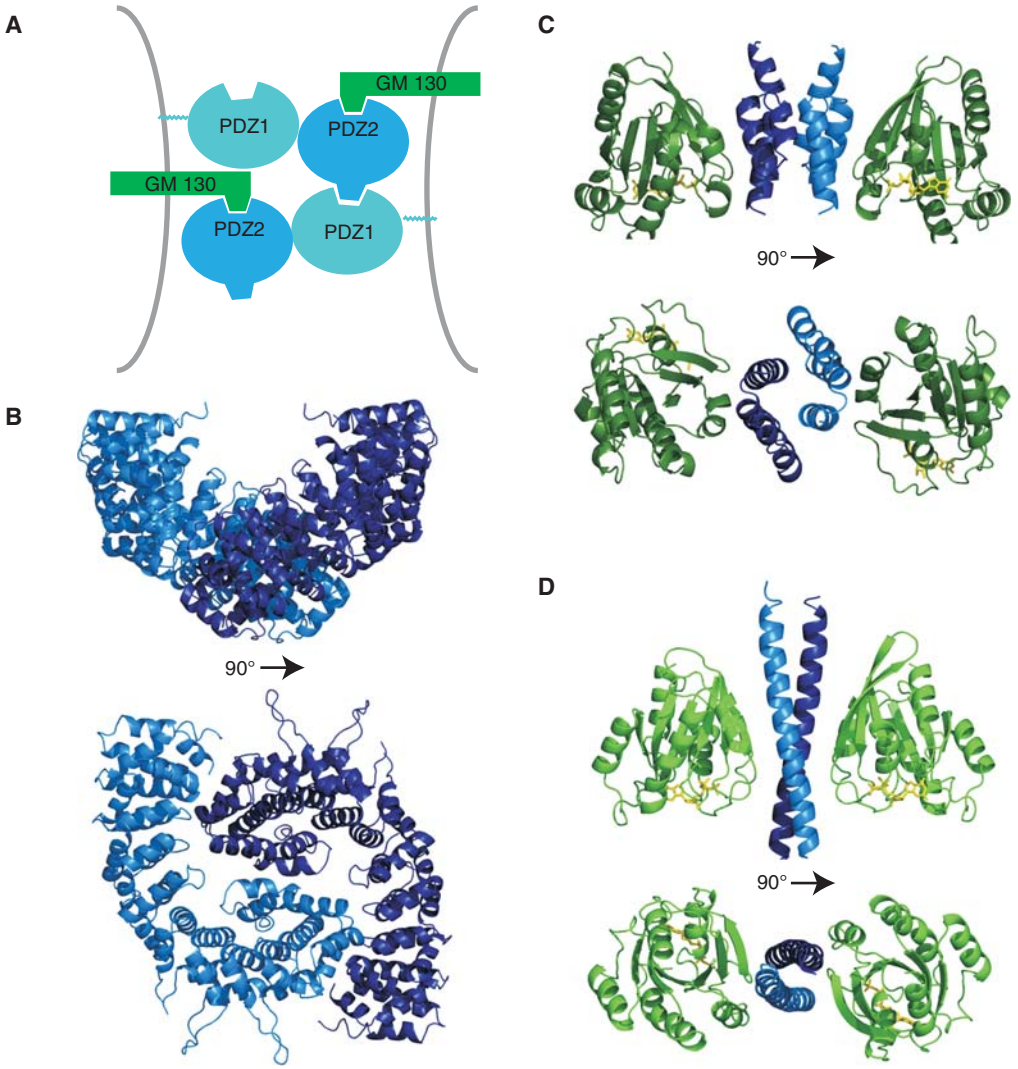

Figure 1. Structures of Golgi tethers. (*A*) The GRASP domain is composed of two tandem PDZ domains. An internal PDZ ligand in PDZ2 binds to PDZ1, suggesting a model for oligomerization of GRASPs. (*B*) The globular head domain of p115 adopts an armadillo-like fold. A crystallographic dimer, likely to resemble the configuration in solution, is shown (PDBID 2W3C). (*C*) The GRIP domain of golgin-245 (blue) in complex with the GTP (yellow) bound form of Arl1 (green) (PDBID 1R4A). (*D*) A short coiled-coil segment of GCC185 (blue) bound to GTP (yellow)·Rab6 (green) (PDBID 3BBP).

GOLGINS

The golgins constitute a diverse group of Golgi associated proteins that play a role in Golgi stacking, vesicle tethering, and constitution of the Golgi matrix (Short et al. 2005). Golgins are characterized by an extended coiled-coil region that mediates homodimerization. They also interact with one another and GTPases of the Rab, Arf, and Arl families, both through binding sites in the coiled-coil or via additional domains. Based on these specialized domains, golgins can be grouped into different classes.

p115 (Uso1 in yeast), which tethers ER-derived vesicles to the *cis*-Golgi, is one of the best studied golgins (Sapperstein et al. 1995;

Levine et al. 1996; Nakamura et al. 1997). Electron microscopy studies revealed that the amino terminus of p115 forms a globular domain and that the carboxyl terminus dimerizes to form a ~45 nm long parallel coiled-coil tail (Sapperstein et al. 1995; Yamakawa et al. 1996). The tail is extended, but has several kinks, indicating that the coiled-coil packing is not continuous along its entire length (Yamakawa et al. 1996). An important implication of this finding is that p115, and golgins in general, are not necessarily rigid rods but might bend.

Several crystal structures have been determined for the p115 amino-terminal head domain (An et al. 2009; Striegl et al. 2009). It has an armadillo-like fold, in which 11 triple-helical motifs, named tether-repeats (TRs), are arranged into a solenoid (Fig. 1B). The amino-terminal TR contains four rather than three α helices and mediates dimerization within the crystal (Striegl et al. 2009). The p115 constructs that were crystallized lacked the coiled-coil regions and behave as monomers in solution, so that the dimer interface may represent a crystallization artifact. However, the presence of such an interface is consistent with the formation of a parallel coiled-coil tail and may also reflect how the head domains associate in dimers of full-length p115.

Interactions have been identified between p115 and a number of other key proteins involved in traffic to the Golgi. The amino-terminal TR in the p115 head domains interacts with active, membrane-bound Rab1, likely serving to anchor p115 to the membrane (Allan et al. 2000; An et al. 2009). This interaction has been proposed to tether COP II vesicles to each other in homotypic vesicle fusion (Bentley et al. 2006). The amino-terminal head domain also interacts with the COG membrane tethering complex (Sohda et al. 2007), and with the β-COP subunit in coatomer (Guo et al. 2008). The coiled-coil tail interacts the v-SNARE GOS28 and the t-SNARE syntaxin-5 (Bentley et al. 2006; Shorter et al. 2002), and an acidic region carboxy-terminal to the coiled-coil binds to GM130 and giantin, two further Golgi-localized coiled-coil tethers, to link p115 to the *cis*-Golgi (Beard et al. 2005).

The GRIP domains present at the carboxyl terminus of many golgins mediate binding to the Arf-like GTPase Arl1 and function in recruiting these golgins to the *trans*-Golgi (Setty et al. 2003). GRIP-domain golgins include GCC185, GCC88, golgin-97, and golgin-245 (Short et al. 2005). The GRIP domain of golgin-245 was crystallized in complex with GTP-bound Arl1 (Panic et al. 2003a; Wu et al. 2004). The GRIP domain comprises three anti-parallel α-helices and forms a homodimer (Fig. 1C). Each GRIP domain binds one Arl1 molecule via interactions of the two amino-terminal helices with the switch1-interswitch-switch2 surface of the GTPase, where the switches are the regions that differ in the GDP- and GTP-bound forms of a GTPase. A highly conserved tyrosine residue in the GRIP domain is inserted into a selectivity pocket present only in the GTP-bound form of Arl1. Arl1 contains a myristoylation site and an amphipathic helix and GRIP domains have aromatic surface residues, which may all serve for membrane association (Panic et al. 2003a). In the tetrameric Arl1/GRIP complex, these interactions likely cooperate to mediate stable Golgi binding.

The golgin GCC185 is recruited to the Golgi via its GRIP domain but independently of Arl1, suggesting that a different GTPase is the physiological binding partner of GCC185 in vivo (Derby et al. 2007; Houghton et al. 2009). Given that residues at the GTPase interaction surfaces of different GRIP domains are not absolutely conserved, it would not be surprising if GRIP domains were able to interact with Arf or Arl GTPases other than Arl1.

In this context, it is interesting to note that golgin GMAP210 contains a GRAB domain, which is closely related in sequence to the GRIP domain but mediates binding to Arf1. In GRAB domains, the conserved tyrosine in GRIP, which mediates interactions with Arl1, is replaced by leucine or a branched, apolar residue, apparently shifting substrate specificity to Arf GTPases (Gillingham et al. 2004). Another interesting feature of GMAP210 in particular is the presence of an amino-terminal ALPS (amphipathic lipid-packing sensor) motif,

which intercalates into curved but not flat membranes (Drin et al. 2007). Thus, GMAP210 is thought to interact with Arf1 marked Golgi membranes via its carboxyl terminus while binding vesicles with its amino terminus, with its coiled-coil spanning ~200 nm between (Drin et al. 2008).

Some golgins are also directly bound to the Golgi membrane through single *trans*-membrane helices. These include Giantin and golgin-84 (Linstedt and Hauri 1993; Linstedt et al. 2000; Diao et al. 2003), which contribute to the complex Golgi matrix network by interacting with Rab GTPases and other golgins.

Even after extensive research on the golgins, the role, or maybe multiple roles, of their extended coiled-coil domains remains unresolved. The coiled-coil domains have generally been thought of as rigid rods that extend away from the Golgi, surveying the Golgi periphery for binding partners. However, the predicted coiled-coil propensity in these regions varies, with coiled-coil segments interrupted by unstructured regions that would allow the proteins to bend or even collapse like an accordion. This idea is supported by observations in the electron micrographs of p115, as discussed above (Yamakawa et al. 1996). Based on weak sequence similarity, it has also been suggested that the coiled-coil domains of several golgins might be interrupted by tether repeats related to the armadillo-like repeats in the globular head domain of p115 (An et al. 2009). The length of the coiled-coil domains of many golgins is enormous: in both giantin and Uso1, for example, they are ~150 nm long (Yamakawa et al. 1996; An et al. 2009). Thus, these golgins would be able to tether incoming vesicles at very long distances.

Many golgins also have multiple GTPase binding sites along the length of their coiled-coil domains (Beard et al. 2005; Sinka et al. 2008; Hayes et al. 2009). This would be consistent with a tentacular model of the Golgi, as proposed by Munro and colleagues (Sinka et al. 2008), in which the golgins extend out from the Golgi in a dense network. The tethers would capture incoming vesicles via interactions with vesicle-bound GTPases at the periphery of this network, and the vesicles would then "hop" toward the Golgi by binding to successive GTPase binding sites. However, the finding of multiple GTPase binding sites per coiled-coil domain also fits the notion that golgins might be involved in stabilizing Golgi structure by linking Golgi cisternae (Hayes et al. 2009). In this model, the golgin is aligned between two cisternae and bridges them by binding GTPases on both their surfaces. The structure of Rab6 in complex with a short fragment of GCC185 may provide the molecular basis for these interactions (Burguete et al. 2008). The crystallized fragment of GCC185 is a helix that forms a parallel dimer, which binds two Rab6 molecules (Fig. 1D). The interaction is between a hydrophobic triad in the Rab6 switch1-switch2 region with two aliphatic residues in GCC185, conferring specificity for GTP-Rab/effector interaction. Recent in vivo data suggest that this interaction with Rab6 does not play a role for GCC185 function in cells (Houghton et al. 2009), but it is plausible that a different Rab might bind this site under physiological conditions. Likely, the mode of binding is representative for other Rab/golgin interactions.

CATCHR COMPLEXES

A number of multisubunit tethering complexes (MTCs) have been implicated in the regulation of membrane trafficking as tethering factors. The CATCHR (Complex Associated with Tethering Containing Helical Rod) family of tethers was first identified based on weak sequence homology in the amino terminus of their subunits, which contain an amphipathic helix that is predicted to form a coiled-coil (Whyte and Munro 2001, 2002). Structural studies revealed that CATCHR subunits also share a "helical rod"-fold, hinting at a divergent evolution of these complexes from a common progenitor (Yu and Hughson 2010). Four family members have been described: the hetero-octameric exocyst acts at the plasma membrane, the Dsl1 complex contains three subunits and is found at the endoplasmic reticulum, and another hetero-octameric complex COG (Conserved

Oligomeric Golgi) and the tetrameric GARP (Golgi-associated retrograde protein) complex are both localized to the Golgi.

The GARP complex (also named VFT, Vps fifty-three complex) is involved in retrograde transport from endosomes to the late Golgi (Conibear and Stevens 2000). GARP contains four subunits (Vps51, Vps52, Vps53, and Vps54). Carboxy-terminal fragments of the GARP subunits Vps53 and Vps54 have been crystallized, showing that these proteins consist of helical rods arranged in tandem, the fold characteristic for CATCHR subunits (Fig. 2A). A conserved surface patch that is essential for retrograde traffic was identified in Vps53 (Vasan et al. 2010), and the structure of Vps54 (Perez-Victoria et al. 2010a) provides the molecular basis of the wobbler mouse phenotype which causes spinal muscular atrophy and defective spermiogenesis (Schmitt-John et al. 2005). The mutation of leucine 967 to glutamine disrupts the hydrophobic core of the protein, leading to lower protein stability in vitro and reduced Vps54 levels, and consequently GARP levels, in vivo (Perez-Victoria et al. 2010a).

In contrast to the human ortholog Ang2 (Perez-Victoria et al. 2010b), yeast Vps51 is small, containing an amino-terminal coiled-coil region but lacking the helical rod domain found in other CATCHR subunits (Conibear et al. 2003). Ang2/Vps51 mediates the interaction of GARP with the regulatory Habc domain of the endosomal t-SNARE Syntaxin6 and Tlg1p, respectively (Perez-Victoria et al. 2010b; Siniossoglou and Pelham 2002), though this interaction is not essential for vesicle docking or fusion (Fridmann-Sirkis et al. 2006). The structure of a 20-residue peptide from the Vps51 amino terminus in complex with the Habc domain of Tgl1 is to date the only interaction of a tether with the fusion machinery characterized at an atomic level (Fig. 2B). The Vps51 fragment is partially α-helical and aligns alongside Tgl1 in a groove between two of the Habc helices distinct from the binding site for the SNARE motif. This binding mode may well be conserved in other SNARE-CATCHR interactions, as the Dsl1 and COG complexes also interact with the Habc domains of their SNARE partners (Shestakova et al. 2007; Ren et al. 2009).

Interaction partners of GARP also includes GTPases. In yeast, Vps53 is an effector of Arl1 (Panic et al. 2003b) and Vps52 is an effector of Ypt6 (Siniossoglou and Pelham 2001). This last interaction is conserved in mammals as

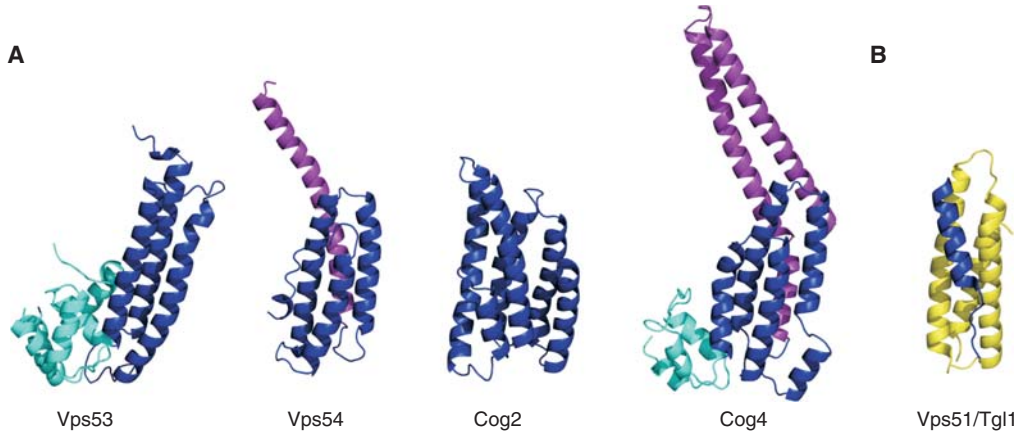

Figure 2. Structures of CATCHR subunit domains. (*A*) Domains of the GARP subunits Vps53 (PDBID 3NS4) and Vps54 (PDBID 3N1E), and of the COG subunits Cog2 (PDBID 2JQQ) and Cog4 (PDBID 3HR0). Proteins were aligned with their central helical-rod domain (blue), with amino-terminal (purple), and carboxy-terminal (cyan) extensions. (*B*) A short helical peptide from Vps51 of the GARP complex (blue) bound to the Habc domain of the t-SNARE Tgl1 (yellow) (PDBID 2C5K).

human Vps52 interacts with Rab6 (Liewen et al. 2005).

The COG complex is essential for proper retrograde transport to the early Golgi. COG contains eight subunits (Cog1-8) (Oka et al. 2005) and is localized to the tips and rims of the Golgi cisternae and also to vesicular and tubular structures at the *cis*- and *trans*-Golgi network. COG interacts with p115, which is required for Golgi integrity, Ypt1 in its GTP-bound form, the Golgi t-SNARE Syntaxin5a/Sed5, the v-SNAREs GS15 and GS28, as well as the γ-subunit of COP I (Suvorova et al. 2002; Oka et al. 2004; Zolov and Lupashin 2005; Vasile et al. 2006; Shestakova et al. 2007). Several mutations in *COG* genes lead to congenital disorder of glycosylation (CDG), in which COG is necessary for the proper recycling of Golgi glycosyltransferases required for normal glycosylation (see Foulquier 2009 for a review).

The crystal structures of two COG subunit domains, of Cog2 (Cavanaugh et al. 2007) and Cog4 (Richardson et al. 2009), unambiguously identify Cog as a member of the CATCHR family of tethers (Fig. 2A). Interestingly, the carboxy-terminal fragment of Cog4 that was crystallized contains the residue arginine 729 that is mutated to tryptophan in patients with CDG. R729 is part of a salt bridge interaction network that stabilizes the helical bundle structure of the protein. Because this domain of the protein is not required in complex formation, its role is probably in mediating association with binding partners of COG (Richardson et al. 2009).

Based on interaction studies of COG subunits in yeast and functional differences caused by the loss of COG components, the COG proteins form two subcomplexes, termed "lobes." Subunits Cog1–4 form one subcomplex, the "A lobe," and are all essential for cell growth (Oka et al. 2005; Lees et al. 2010); a second subcomplex, the "B lobe," consists of Cog5–8. Interestingly, mutations in genes from the two lobes lead to distinct phenotypes in yeast (Whyte and Munro 2001) and distinguishable effects on glycosylation in mammalian cells (Oka et al. 2005). The mapping of intracomplex interactions confirmed this model, placing Cog1 at the center of the complex where it connects both lobes (Fotso et al. 2005; Loh and Hong 2004). The two subcomplexes might be involved in the tethering of vesicles from different compartments, mainly from the Golgi but also from endosomes. The bilobal model for COG assembly was supported by cryo-EM studies of crosslinked, purified bovine COG (Ungar et al. 2002). However, more recent studies of reconstituted yeast lobe A (Cog1–4), in which the COG subunits were not cross-linked, suggest that the bilobal structure observed in the earlier work is probably an artifact of the crosslinking procedure (Lees et al. 2010). The EM class averages of reconstituted Cog1–4 show that the subcomplex adopts an extended Y-shape and is not globular (Fig. 3A). The position and orientation of the subunits within the complex were determined, showing that the proteins interact via their amino termini and that the carboxyl termini, probably folded into helical-rods, form the "legs" of the complex. The mode of interaction in the amino termini might be through coiled-coil interactions, where each subunit contributes at least one helix. This model is compelling because the CATCHR family was originally identified by the conserved coiled-coil motif putatively present in many of its proteins, tempting the speculation that these motifs mediate complex assembly in all CATCHR complexes. But it is still unclear whether the interactions in Cog1–4 are coiled-coil interactions and whether the other CATCHR complexes will be assembled similarly. In the Dsl1 complex, two of its three subunits belong to the CATCHR family, and these two subunits associate via their amino-terminal α-helices, which are antiparallel (Fig. 3B) but do not pack as coiled-coils (Tripathi et al. 2009). The overall organization of the Dsl1 subunits differs from that in Cog1–4, suggesting that at least Dsl1 and COG are assembled differently. Structural studies of other CATCHR assemblies are still ongoing.

Although the structural studies provided a great deal of information on the architecture of CATCHR complexes, our mechanistic

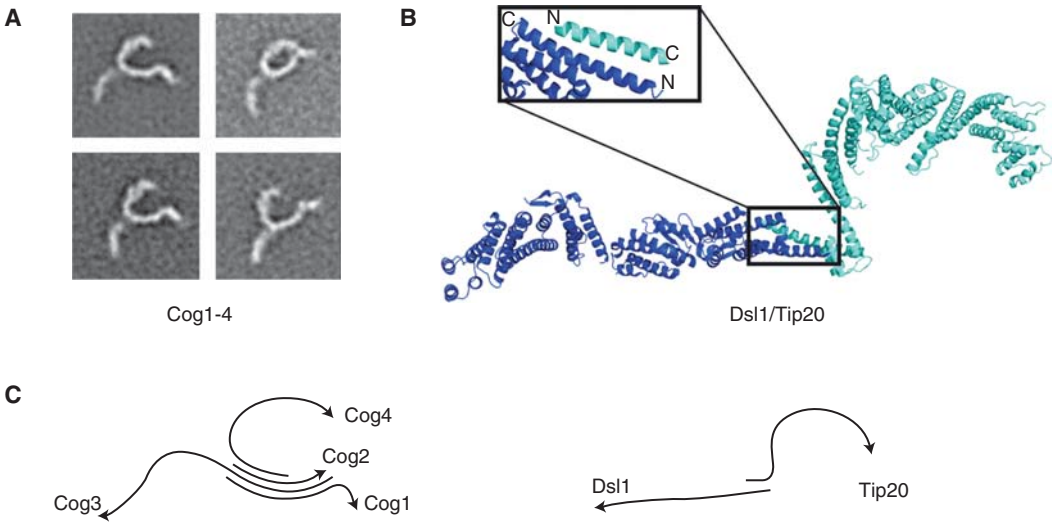

Figure 3. CATCHR subcomplexes. (*A*) EM class average of the COG1–4 subcomplex (courtesy of T. Walz). (*B*) The Dsl1/Tip20 subcomplex of the DSL complex (PDBIDs 3ETV, 3K8P, 3FHN). The inserts show the antiparallel association of the amino-terminal helices of the proteins that mediate heterodimerization. (*C*) Schematic representation of the subunit orientation within the complexes with arrows heads marking the carboxyl terminus.

understanding is still limited. The multilegged, extended tertiary arrangement of COG and presumably other CATCHR complexes (Dong et al. 2005; Munson and Novick 2006; Ren et al. 2009; Lees et al. 2010) suggests several models.

In one model, to tether the vesicles to the Golgi, the complexes are anchored with at least one leg on the Golgi whereas other legs extend away to capture incoming vesicles. The reach of the Cog1–4 complex is ~30 nm, consistent with such a tethering function (Pfeffer 1999). Given the considerable flexibility observed for CATCHR assemblies (Hsu et al. 1998; Ren et al. 2009; Lees et al. 2010) it is also plausible that conformational changes in these complexes might aid the transition from vesicle capture to docking and/or fusion (Munson and Novick 2006; Ren et al. 2009).

It is worth noting that the Cog1–4 assembly binds the t-SNARE Syntaxin5 at its center where the legs meet, so that if the complex does extend from the Golgi, the Syntaxin5 SNARE motif would have to be in its unfolded state in order for the SNARE to interact with this binding site. In a second model, the CATCHR complexes would lie flat on the membrane, promoting fusion by organizing the fusion machinery but not as tethers per se.

GOLGI COATS

The generation of transport vesicles is mediated by coat proteins, which play a role in the recruitment of specific cargo proteins, vesicle budding, and vesicle recognition at the target membrane (McMahon and Mills 2004). At the Golgi, clathrin-coated vesicles mediate exit from the *trans*-Golgi network. COPI (or coatomer) vesicles are required for retrograde intra-Golgi transport as well as *cis*-Golgi-to-ER transport. Finally, COPII- coated ER-derived vesicles are recognized at the Golgi.

Vesicle coats have an inner and an outer layer. The inner layer consists of adaptor proteins, which are first recruited to the site of budding through interactions with active Arf GTPases, and subsequently concentrate cargo at these sites through specific interactions with sorting motifs. Cage proteins constitute the outer coat and their ability to oligomerize and thereby encapsulate budding vesicles drives formation of these vesicles and also supports their

fission. In all known vesicle coats, the cage proteins form a lattice rather than a solid coat, so that the inner coat as well as other proteins at the surface of the vesicle are solvent accessible.

Cage Proteins

The minimal building block for the clathrin cage consists of a curved 190 kDa heavy chain (CHC) and a 25 kDa light chain (CLC) (Fig. 4A) (Fotin et al. 2004). These heterodimers assemble into a trimer via a central hub region in the CHC carboxyl terminus, giving clathrin a triskelion shape (Fig. 4B). The clathrin heavy chain is composed of eight clathrin heavy-chain repeats (CHCR) in a right-handed superhelical coil (Ybe et al. 1999). Each CHCR consists of ~145 residues that form 10 helices arranged in a zig-zag of "helix-turn-helix-loop" hairpin units. Each α-hairpin is slightly twisted with respect to the preceding hairpin, and the protein is bent at two positions, termed ankle (see Fig. 4) (between CHCR 2 and 3) and knee (between CHCR 5 and 6). As a result, the CHC overall adopts a curved, slightly screwed conformation. To form a cage, clathrin triskelia interdigitate into a lattice of hexagons and pentagons (Fotin et al. 2004). The arrangement of the CHC is such that each vertex of the polygons consists of two hub-knee and two knee-ankle segments of CHC, contributed by four different triskelia. The amino terminus of the CHC is composed of a β-propeller of WD40 repeats (ter Haar et al. 1998), which mediates interactions with accessory factors and the inner coat layer. At the carboxyl

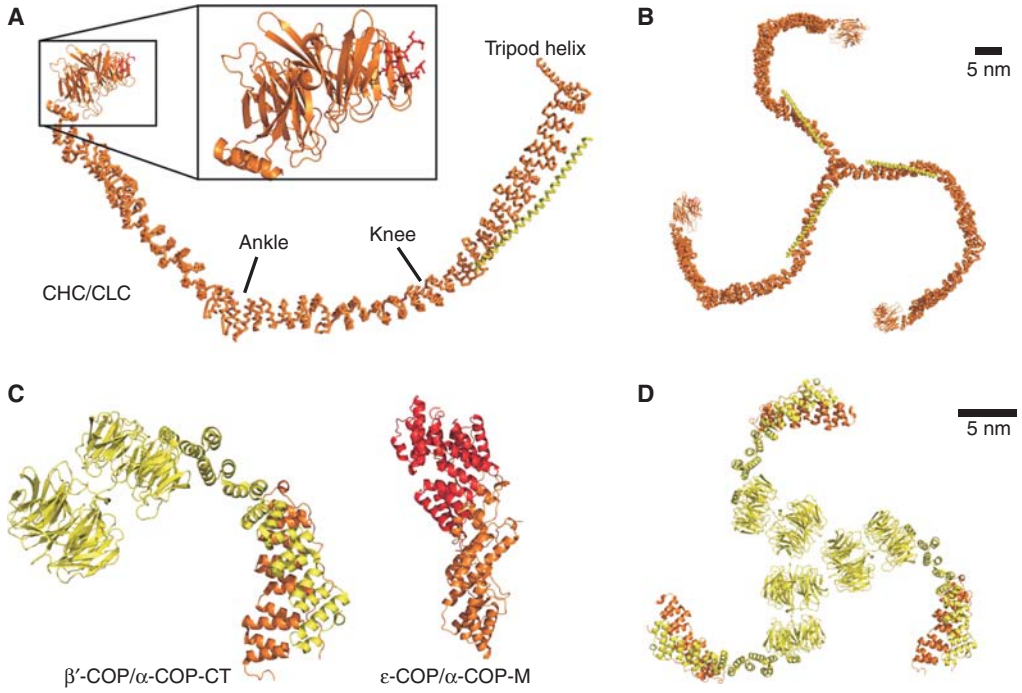

Figure 4. Coat cage protein structures. (*A*) Clathrin heavy chain (CHC, orange) and clathrin light chain (CLC, yellow) form an extended α-selenoid structure that is bend at a "knee" and "ankle" region (PDBID 1XI4). The amino-terminal WD40 propeller recruits cargo by interacting with the clathrin box motif Lϕxϕ[D/E] (red, PDBID 1UTC). (*B*) Triskelion arrangement formed by clathrin. (*C*) β′-COP (yellow) consists of amino-terminal tandem WD40 domains followed by an α-selenoid that interacts with α-COP carboxyl terminus (orange, PDBID 3MKQ). The middle portion of α-COP (orange) binds to ε-COP (red, PDBID 3MKR). (*D*) Trimeric crystal packing of α/β′-COP.

terminus, a long helix extends toward the center of the cage and forms a tripod-like arrangement. The tip of the tripod contacts the ankle regions of interdigitated triskelia, thus strengthening the coat assembly. The CLC aligns along the CHC near the central hub on the outside of the cage (Fotin et al. 2004; Wilbur et al. 2010), forming an extended helix with short interruptions. It can undergo conformational changes and thereby modulate bending in the CHC knee region (Wilbur et al. 2010). As a consequence, clathrin can switch between a straightened and a curved conformation, and only the curved conformer is capable of efficient coat assembly. This finding suggests a regulatory role for the CLC in coat formation.

The cage of the COPI coat is formed by the coatomer B-subcomplex containing three subunits: α-COP, β′-COP, and ε-COP. Recent structures of α/β′-COP (Lee and Goldberg 2010) and α/ε-COP (Hsia and Hoelz 2010; Lee and Goldberg 2010) subcomplexes have provided first insights regarding COPI cage assembly. The almost full-length construct of β′-COP used for crystallization contains two amino-terminal seven-blade WD40 β-propeller domains that are arranged in tandem. These domains are followed by an α-solenoid region, which binds to the α-solenoid domain in the center of α-COP (Fig. 4C). The carboxyl terminus of α-COP was crystallized in complex with ε-COP, which folds into tetratricopeptide repeat (TPR) superhelix that caps off α-COP. The α-COP carboxy-terminal fragment consists of another α-solenoid abutted by a four-stranded β-sheet. A long β-hairpin emanating from the α-solenoid portion is deeply inserted into ε-COP and was therefore termed domain invasion motif (DIM) (Hsia and Hoelz 2010).

The amino terminus of α-COP is the only part of the coatomer B-subcomplex that has not been structurally characterized. It is predicted to contain another WD40 propeller domain and an α-solenoid region and—in analogy to the Sec13/31 subunits of the COPII coat (Fath et al. 2007)—probably is a further extension of the legs of α/β′-COP.

Interestingly, the α/β′-COP complex trimerizes in the crystal via interactions of the β′-COP amino-terminal β-propeller domains (Fig. 4D). Although this interaction has not been observed outside the crystal, it has been speculated that the threefold interface might represent a vertex of the COPI cage and that, as for the clathrin cage, a triskelion may be the physiological assembly unit (Lee and Goldberg 2010). This notion is appealing because the inner coat layers of coatomer and clathrin are thought to be similar (discussed below). Several observations are of note in this context, however. First, the topology of the COPI cage complex more closely resembles that observed for the COPII proteins Sec13 and Sec31 (Fath et al. 2007) than for clathrin, in that the Sec13/31 heterodimer features two tandem β-propeller domains at one end abutting an α-solenoid. And second, the organization of the COPII cage is entirely distinct from that of clathrin, and does not rely on the formation of a triskelion (Stagg et al. 2008). In COPII, the physiological assembly unit is a heterotetramer, consisting of two Sec13/Sec31 complexes that form a rod, with β-propeller domains placed at the ends. Four, not three, of these rods are arranged around each coat vertex, associated via β-propeller domains rather than, as in clathrin, the α-solenoid domains. Given the similarity in the topology of the cage subunits, it would not be entirely surprising if the organization of the COPI cage turns out to be more similar to that of COPII rather than clathrin. Finally, α/β′-COP is also more similar to COPII than to clathrin in dimension (note the scale bars in Fig. 4).

Inner Coat Layer

The cage complexes are attached to the vesicle by inner coat protein complexes. Clathrin binds to large variety of clathrin adaptor protein complexes, more than 20 in total. Adaptor complexes of the *trans*-Golgi are AP-1 (Ahle et al. 1988), AP-4 (Dell'Angelica et al. 1999; Hirst et al. 1999) and the Golgi-localized γ-ear-containing Arf-binding proteins (GGA 1–3) (Boman et al. 2000; Dell'Angelica et al. 2000a; Hirst et al. 2000; Poussu et al. 2000; Takatsu et al. 2000). Like most clathrin adaptors, these

complexes bind the β-propeller domain of the CHC through short peptide motifs, the so-called Clathrin box, that are located in an extended disordered region. The clathrin box (Dell'Angelica et al. 1998) has the sequence requirement Lϕxϕ[D/E] (ϕ represents bulky hydrophobic residue L, I, M, F, or V). The crystal structures of the clathrin box from β-arrestin2 and the β-subunit of AP-3 show how this motif interacts with a groove between two blades of the CHC WD40 β-propeller (Fig. 4A) (ter Haar et al. 1998).

The adaptor protein (AP) complexes share a common tetrameric architecture but differ in their composition, containing different subunit isoforms (adaptins). Four subunits interact to form a trunk, composed two large 110–130 kDa subunits β and α, γ, δ, or ε—which also contain γ-ear appendages flexibly linked to their trunk domains (see below)—one medium 50 kDa subunit μ, and one small 15–20 kDa subunit σ (Fig. 5A). The main differences between the isoforms that constitute the AP complexes are in their specificity for binding partners. The dileucine-based sorting signal ([D/E]xxxL[L/I]) for clathrin cargo (Aiken et al. 1994) is recognized by all AP complexes through a split binding pocket between the σ and large α/γ/δ/ε subunits. In addition, the μ subunits recognize an alternative tyrosine-based sorting signal Yxxϕ (ϕ represents bulky hydrophobic residue L, I, M, F, or V) (Ohno et al. 1995). Biochemical studies have shown that binding sites of distinct complexes provide specificity for different dileucine signals (Mattera et al. 2010). Further, the different adaptin complexes bind different phosphoinositide lipids as a prerequisite for their distinct localization. AP-2 binds to PtdIns(4,5)P_2 and PtdIns(3,4,5)P3 at the plasma membrane, whereas the AP1 complex binds to the Golgi-localized PtdIns(4)P (Ghosh and Kornfeld 2003). However, the overall architecture and location of phosphoinositide binding sites on the complexes are thought to be similar. Of all adaptin complexes, the structure of the endocytic AP-2 complex (Fig. 5A) has been studied most extensively (Collins et al. 2002; Kelly et al. 2008; Jackson et al. 2010). In the light of the structural similarity to AP-1 (Heldwein et al. 2004), the principles deducted from these studies and discussed below are thought to hold true for all APs.

The large adaptin subunits are structurally related, all forming an α-solenoid. σ-adaptin and the amino-terminal domain of μ-adaptin both adopt the longin fold, a domain that is found widely in transport-related proteins (Fig. 5A) (Collins et al. 2002). The α-solenoids in the two large subunits each contribute a half circle to wrap around the longin domains that are in the center of the complex, giving the complex the overall shape of a basket. In the closed, cytosolic from of AP-2, the carboxy-terminal domain of μ-adaptin, which forms an elongated β-sheet structure, lies in the shallow depression formed by the trunk subunits. The binding sites on AP-2 for the endocytic cargo motifs in the μ-adaptin carboxy-terminal domain (Yxxϕ) and in σ-adaptin ([E/D]xxxL[L/I]) are partially blocked. Also, the binding sites of α-adaptin and μ-adaptin are located on different faces of the complex, indicating that this conformation is not active in mediating cargo recruitment and budding.

By cocrystallizing the AP complex with a 70-fold molar access of endocytic tyrosine-based sorting peptide, it was possible to drive the complex into its open, sorting-active state (Fig. 5A) (Jackson et al. 2010). The complex undergoes large structural rearrangements; most prominently, the μ-adaptin carboxy-terminal domain moves about 130° and 40 Å, thereby revealing its Yxxϕ binding pocket. In addition, the α/β/σ-adaptin interface opens up to expose the binding site for dileucine motifs. Strikingly, both endocytic motif-binding sites and four PtdIns(4,5)P_2 pockets are located on the same face of the complex. This arrangement will allow the AP complexes to efficiently interact with membranes and cargo in a cooperative manner, providing an elegant explanation of how sorting and membrane association are coupled.

The large adaptin subunits as well as GGA adaptor proteins contain appendage domains that are connected to their trunk domains by a long flexible linker (Fig. 5B) and function in

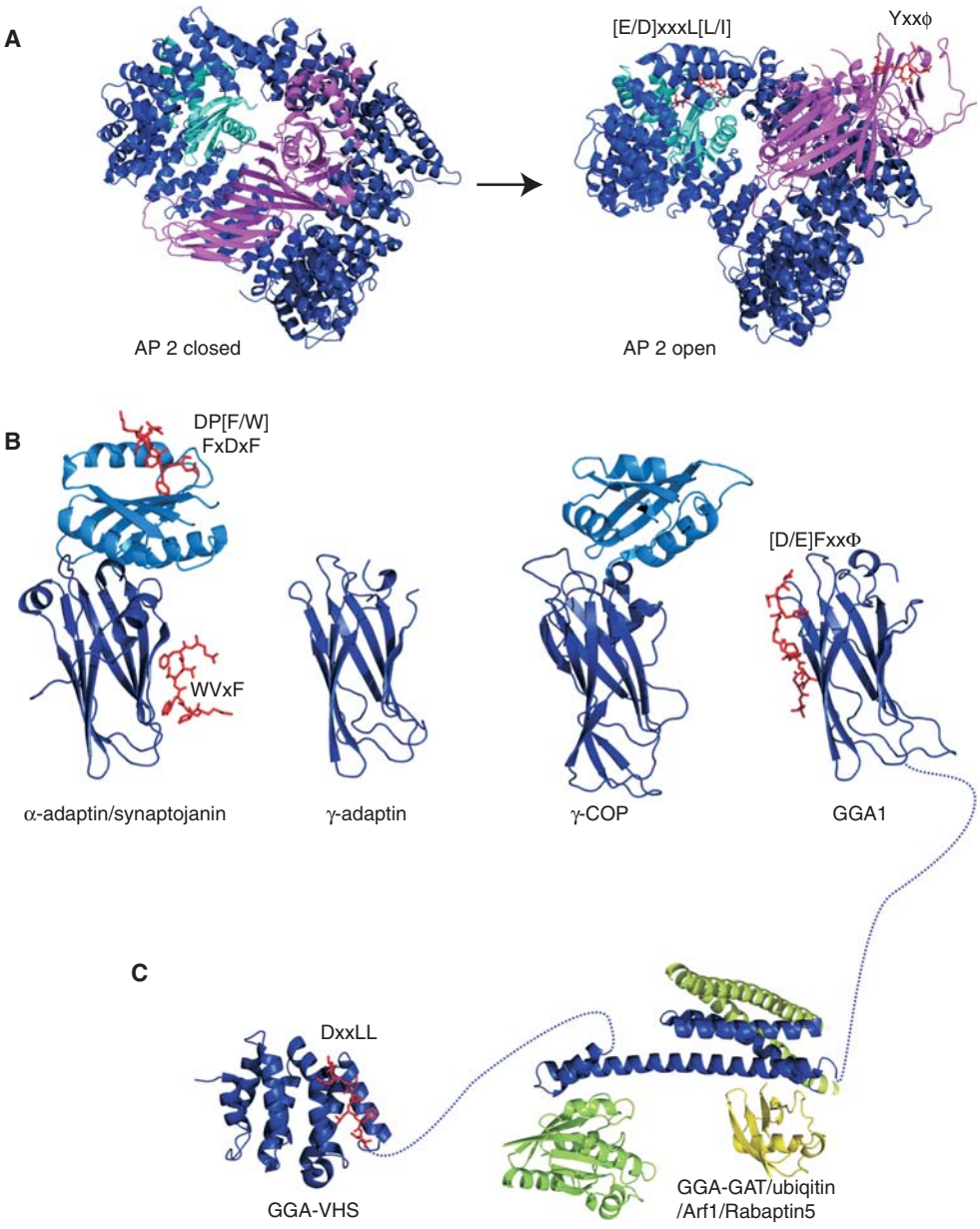

Figure 5. Structures of adaptor proteins. (*A*) The AP-2 complex, consisting of α- (light blue), β2- (dark blue), μ2- (magenta), and σ2- (cyan) adaptin, undergoes large-scale conformational changes during its transition from the closed cytosolic form (PDBID 2VGL) to the open, sorting-active form (PDBID 2XA7). (*B*) The appendage domains of α-adaptin (PDBID 1W80), γ-adaptin (PDBID 1GYU), γ-COP (PDBID 1PZD), and GGA1 (1OM9). (*C*) The VHS domain of GGA1 (PDBID 1JWG) and the GAT domain (PDBID 1NAF) in complex with Arf1 (light green, PDBID 1J2J), ubiquitin (yellow, PDBID 1WR6), and rabaptin5 (dark green, PDBID 1X79). Bound sorting peptides are shown in red as stick representation and are labeled with the corresponding consensus sequence.

recruiting auxiliary proteins. The structure of the α2-adaptin appendage (Owen et al. 1999; Traub et al. 1999; Brett et al. 2002) consists of an amino-terminal β-sandwich domain and a carboxy-terminal "platform" domain. The platform domain binds the DP[F/W] peptide motif and FxDxF motif with an overlapping binding site. In addition, the WVxF peptide motif is bound by the β-sandwich domain in a "side" binding pocket (Praefcke et al. 2004). Some appendages lack the platform domain, and only contain the β-sandwich domain. Members of this class include AP-1 γ-adaptin (Kent et al. 2002) and the GGA proteins (Miller et al. 2003). These sandwich-only appendages bind to a [D/E]Fxxφ motif (β represents L/F/W/M) at a site different from the WVxF pocket (Collins et al. 2003a).

The GGA adaptors contain, in addition to the carboxy-terminal γ-ear appendage domains, an amino-terminal VHS (Vps27/Hrs/Stam) domain and a central GAT (GGA and TOM) domain (Boman et al. 2000; Dell'Angelica et al. 2000a; Hirst et al. 2000; Poussu et al. 2000). The VHS and GAT domains are structurally related to the large trunk subunits of the AP adaptors only in their all α-helical fold, but they fulfill an equivalent function, to sort cargo and to mediate membrane association. The three domains of the GGA adaptor proteins are connected by flexible linkers, suggesting that they act as independent units. The hinge region between GAT domain and γ-ear appendage contains a clathrin box, another similarity to adaptin adaptor organization.

The VHS domain recognizes cargo with an acidic-cluster dileucine (DxxLL) motif, which is found in the cytoplasmic tails of cargo proteins trafficked from the *trans*-Golgi to the endosomal system (Puertollano et al. 2001a,b; Takatsu et al. 2001; Zhu et al. 2001). The domain consists of eight helices arranged into a right-handed superhelix (Kato et al. 2002; Shiba et al. 2002; Zhu et al. 2003a). The acidic-cluster dileucine motif binds in an extended conformation to a groove between two helices on the surface of the VHS domain (Fig. 5C). The [E/D]xxxL[L/I] motif that is recognized by σ-adaptin, in which the acidic residue is further separated from the dileucine sequence, cannot be accommodated in the binding groove. Interestingly, phosphorylation of a serine residue adjacent to the DxxLL motif can increase the binding affinity to GGA proteins, and GGa1 and 3 contain an internal acidic dileucine motif in their hinge region that can be phopshorylated and act as a regulatable, autoinhibitory ligand for the VHS domain (Doray et al. 2002).

The GAT (GGA and TOM) domain binds Arf1 and Arf3 and thereby mediates recruitment of GGAs to *trans*-Golgi membranes (Boman et al. 2000; Dell'Angelica et al. 2000b). The GAT domain consists of a three-helical bundle and a short fourth amino-terminal helix, which forms a so-called hook region (Fig. 5C) (Collins et al. 2003b; Shiba et al. 2003; Suer et al. 2003; Zhu et al. 2003b). The Arf GTPases interact with the hook region in a GTP-dependent manner. The three-helix bundle of the GAT domain interacts with the coiled-coil domain of Rabaptin5 (Stenmark et al. 1995; Zhu et al. 2005), an effector of Rab5 in the fusion of early endosomes, and via a different surface binds ubiquitin (Kawasaki et al. 2005; Prag et al. 2005). As with most ubiquitin effectors, the GAT domain interacts with the ubiquitin surface around hydrophobic residue I44. The interactions of GGAs with ubiquitin suggest that GGAs mediate transport of ubiquitinated cargo to endosomes (Puertollano and Bonifacino 2004; Scott et al. 2004).

Only limited structural information for the inner layer of coatomer is available, but sequence analysis suggests that the coatomer F-subcomplex (β-COP, γ-COP, δ-COP, and ε-COP subunits) is structurally related to the AP complexes (Schledzewski et al. 1999). It is likely that these COPI subunits form a similar trunk arrangement that serves as cargo adaptor, but will recruit B-coatomer instead of clathrin. β- and γ-COP are also similar to the AP complexes in that they have appendage domains that are connected to their trunk domains by a long flexible linker (Fig. 5B) (Hoffman et al. 2003; Watson et al. 2004). These similarities suggest that coatomer and the clathrin coats are evolutionarily related.

INTERACTIONS BETWEEN TETHERS AND COATS

There is a growing body of evidence for communication between the budding and fusion machinery, as evidenced by interactions of golgins, CATCHR complexes, and coats with one another. In particular, Golgin p115 binds to the CATCHR complex COG (Sohda et al. 2007) and the β-subunit of the COPI coat (Guo et al. 2008); the ε/α-subcomplex of COPI interacts with the Dsl1 complex (Hsia and Hoelz 2010); and γ-COPI binds to COG (Suvorova et al. 2002). The exact role of these interactions, however, remains elusive. Structural characterization of these interactions may be an important step forward as we improve our understanding of the mechanisms that underlie vesicular traffic to/within the Golgi and in general.

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