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Structures and mechanisms of vesicle coat components and multisubunit tethering complexes

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Abstract

Eukaryotic cells face a logistical challenge in ensuring prompt and precise delivery of vesicular cargo to specific organelles within the cell. Coat protein complexes select cargo and initiate vesicle formation, while multisubunit tethering complexes participate in the delivery of vesicles to target membranes. Understanding these macromolecular assemblies has greatly benefited from their structural characterization. Recent structural data highlight principles in coat recruitment and uncoating in both the endocytic and retrograde pathways, and studies on the architecture of tethering complexes provide a framework for how they might link vesicles to the respective acceptor compartments and the fusion machinery.

Introduction

The coordinated movement of proteins and lipids between distinct membrane-bound organelles is vitally important to eukaryotic cells. Defined trafficking pathways ensure accurate and timely delivery of cargo packaged within membrane-bound vesicular carriers. The formation, transport, and delivery of membrane-bound vesicles are controlled by a variety of protein and lipid components. Here we shall focus on two types of protein assemblies that play vital roles in vesicle formation and delivery: coat proteins and membrane tethering complexes.

Coat Proteins

Proteins involved in coat formation mediate a number of functions: they interact with specific membranes having a defined composition [1,2] they initiate, promote, and/or stabilize membrane curvature [3–6]; and they cluster and select the relevant cargo for incorporation [7,8]. Clathrin-based coats surround many vesicles in post-Golgi pathways, while COPI and COPII comprise the major coats in the retrograde and anterograde pathways, respectively, between the ER and Golgi. Additional protein complexes have been implicated in other pathways, including SNX/retromer from endosomes [9–13] and the

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BBSome in primary cilia [14,15], though whether these complexes function as canonical coats is not fully established.

The well-characterized clathrin coat [16,17] consists of two layers: an inner layer of clathrin adaptor proteins and the outer polyhedral clathrin scaffold. Clathrin cannot bind to the membrane directly, and thus clathrin adaptors [18] link clathrin to the vesicle membrane and its embedded cargo. Four sites on the surface of clathrin terminal domain (TD) can potentially recruit short linear motifs found in unstructured regions of clathrin adaptors: the clathrin-box site [19], W-box site [20], β -arrestin site [21], and a recently identified fourth site [22]. These four sites are thought to be functionally redundant [22], though recent work with small molecule inhibitors ('pitstops') suggests blocking the clathrin-box site alone inhibits endocytosis [23,24].

Like clathrin coats, the COPI coat consists of two layers, based on distant sequence and structural homology: the 'AP-like' $\beta/\gamma/\delta/\zeta$ subcomplex and 'clathrin-like' $\alpha/\beta'/\epsilon$ subcomplex. In contrast, COPII coats are distinct in both sequence and structure. We shall not focus on it here, as electron microscopy [25,26] and X-ray structures [27,28] have been reviewed elsewhere [29,30]. Here, we highlight recent advances in understanding clathrin- and COPI-based coats at the molecular level using structural techniques, including X-ray crystallography and electron microscopy (EM).

AP-like complexes couple membrane binding and cargo recognition

The adaptor protein complexes (APs) are a family of heterotetrameric clathrin adaptors (~300 kDa). Each AP localizes to a specific cellular compartment, where it recruits coat components and cargo [7,8]. AP2 ($\alpha/\beta_2/\mu_2/\sigma_2$ subunits), AP1 ($\gamma/\beta_1/\mu_1/\sigma_1$), and AP4 (μ_4) have proven amenable to structural studies and have provided mechanistic information about complex assembly [31,32]; interaction with accessory and regulatory proteins [33–36]; and cargo binding [37–41]. Both AP1 and AP2 have been observed in closed and locked conformations, in which the cargo binding sites on the μ subunits are blocked and inaccessible. A recent report shows AP2 in its open, active, and cargo-bound form for the first time (Figure 1A) [42].

The $\beta/\gamma/\delta/\zeta$ subcomplex of COPI also likely undergoes a large-scale conformational change (Figure 1B). Whereas AP2 is recruited to the plasma membrane by PtdIns(4,5) P_2 , small Arf GTPases play the central role in recruiting the AP1, AP3, AP4 and COPI coats to their respective membranes [43–45]. Yu and colleagues [46] have recently crystallized part of the $\gamma\zeta$ -COP heterodimer in complex with Arf1 in the presence of a non-hydrolyzable GTP analog. γ -COP adopts an α -solenoid conformation very similar to that found in the AP2 α and AP1 γ subunits. The interaction with Arf1 occurs through a number of hydrophobic contacts with α -helices in γ -COP and was confirmed by structure-based mutagenesis.

Biochemical studies revealed an unexpected second binding site for Arf1-GTP on the $\beta\delta$ -COP heterodimer. The authors propose a model in which membrane-bound Arf1-GTP is able to recruit COPI through a bivalent interaction with the $\gamma\zeta$ - and $\beta\delta$ -COP heterodimers (Figure 1B) [46], which restricts the orientation of COPI relative to the membrane. Therefore, $\gamma\zeta$ -COP exhibits structural similarity with α/σ_2 (AP2) and γ/σ_1 (AP1), and the spatial relationship between the Arf1-GTP [46] and PtdIns(4,5) P_2 binding sites [42] on COPI and AP2, respectively, are conserved. These data together suggest that the overall mechanism of conformational change triggered by membrane recruitment is conserved in all AP-like coats.

The role of auxilin in vesicle uncoating

In order to fuse with endosomes, clathrin-coated vesicles must undergo disassembly, driven by both hydrolysis of PtdIns(4,5)P₂ and depolymerization of the clathrin lattice. Recent structural [47,48] and biophysical studies [49] have further elucidated the role of auxilin in coat disassembly. Auxilin binds directly to both clathrin and the uncoating ATPase, Hsc70. The auxilin N-terminal PTEN-like domain structure [47] demonstrated a fold reminiscent of the PI3P phosphatase, PTEN, though auxilin lacks catalytic activity. Phosphoinositides possibly play a role in recruiting auxilin to newly budded vesicles through this PTEN-like domain. PI4P is the suggested candidate, since it is produced from PtdIns(4,5)P₂ by phosphatases such as synaptojanin, providing a direct link between the two steps of uncoating.

Cryo electron microscopy has been used to determine the placement of auxilin and Hsc70 in the clathrin cage at 11 Å [48,50]. These results indicate that approximately one copy of Hsc70 is found per vertex, with three copies of auxilin nearby. The authors propose that auxilin both recruits the ATPase (Hsc70) to its substrate (clathrin) and introduces strain within the cage, which facilitates uncoating.

Kinetic studies of cage disassembly [49] further explored the stoichiometries of auxilin and Hsc70. These results suggest however that a single auxilin per triskelion recruits three Hsc70:ATP molecules in series. Once the first ATP is hydrolyzed, the Hsc70:ADP complex has a higher affinity for clathrin and re-positions itself such that the J domain of auxilin can recruit a second Hsc70:ATP. Binding of all three Hsc70:ADP molecules and one auxilin introduces strain in the vertex, prompting uncoating.

Structural studies of the COPI coat

Several groups have recently advanced our molecular understanding of COPI coat architecture. Single-particle EM studies of native yeast coatamer [51] revealed that the complex is composed of two domains, as expected, of which one is 'globular' (assigned as $\beta/\gamma/\delta/\zeta$) and one 'extended'. Cryo electron tomography data obtained from COPI vesicles reconstituted *in vitro* [52] further indicated that COPI coats exhibit greater conformational heterogeneity than do clathrin- and COPII-based coats.

Two groups have published crystal structures of portions of the outer cage (Figure 2) [53,54], providing information on ~65% of the $\alpha/\beta'/\epsilon$ subcomplex. Crystal structures of an α - and ϵ -COP complex indicate that the C-terminal domain of α -COP interacts with the tetratricopeptide (TPR) superhelix formed by ϵ -COP [53,54]. The position of this complex in the context of cage assembly is currently unknown.

A second structure of the α -COP α -solenoid together with both β -propeller domains and the α -solenoid of β' -COP [54] suggested a possible mechanism for cage assembly. This structure reveals that the α - and β' -COP solenoids curve around one another, perhaps forming the 'legs' of a cage. The N-terminal β -propeller domains of β' -COP (residues 1-300) form an approximately trimeric interface, which may constitute the vertex of the COPI cage. However, the authors did not observe evidence of trimer formation in solution [54]. Attempts to dock this specific conformation into density from EM studies were unsuccessful [51], possibly as a result of conformational heterogeneity. Thus, a single particle cryo EM reconstruction at sufficient resolution will be required in order to understand cage formation more fully.

Multisubunit tethering complexes

Multisubunit tethering complexes (MTCs) act in the last stages of most membrane trafficking processes, as the vesicle docks and fuses. Most known MTCs belong to the so-called CATCHR (Complex Associated with Tethering Containing Helical Rod) family [55] and consist of multiple subunits, each comprising several four-helical bundle motifs arranged in tandem (Fig 3A) [56–66]. The simplest CATCHR family member was only recently identified and is not, in fact, a complex, but a monomer. It is Munc13, a neuronal protein that regulates secretion of synaptic vesicles [67]. CATCHR complexes are DSL1, GARP (Golgi-associated retrograde protein), the exocyst, and COG (conserved oligomeric golgi) (Table 1). The class C Vps tethering complexes HOPS (homotypic fusion and vacuole protein sorting) and CORVET (class C core vacuole/endosome tethering), which are involved in the fusion of vacuoles and endosomes, respectively, have several common components, but they belong to a family distinct from CATCHR [68]. Structure predictions indicate that their subunits contain β -propeller, α -solenoid, and RING ubiquitin ligase domains and SM proteins (see also [69,70]).

What is “tethering”?

Although these complexes are essential for transport, their molecular function and the meaning of “tethering” remains largely elusive. The best clues for how they might work come from their reported binding partners (Table 1). These include determinants of organelle identity, such as small GTPases, coat proteins, and lipids, as well as core components of the fusion machinery, the SM (Sec1/Munc18) and SNARE proteins.

MTCs were initially thought to play a role in vesicle tethering or docking, explaining their name. They were thought to function in recognition between an incoming vesicle and its acceptor compartment and to act as a physical bridge between them [68]. “Receptors” for MTCs are indeed located on both membranes; however, the receptors are diverse in their nature. DSL1 and COG both interact with subunits of the COPI vesicle coat [53,71,72] whereas GARP, the exocyst and indirectly Munc13 bind GTPases anchored either to the vesicle or acceptor compartment [73–79], and the exocyst additionally interacts specifically with PI4P at the plasma membrane [80,81]. HOPS and CORVET also specifically interact with the GTPases Ypt7 [82] and Vps21 [83], respectively. And, as discussed below, all of these complexes interact with SNARE proteins. A more unified picture as to how MTCs might tether may emerge as further MTC interaction partners are identified.

A number of recently identified interactions between MTCs and the core fusion machinery support the notion that MTCs might also act as fusion chaperones. Thus, most CATCHR complexes are known to interact with SM proteins: the exocyst interacts with Sec1p [84], COG interacts with Sly1 [85], and Munc13 interacts with Munc18 [86]. No SM protein has so far been identified as a GARP interactor, but likely this is only a matter of time. The HOPS and CORVET complexes both include the SM protein Vps33, which promotes fusogenic activity of vacuolar SNAREs [87].

The one binding partner common to all MTCs are SNARE proteins (Table 1). The interactions with SNAREs may play a role in tethering, but additionally, they could be important for stimulating or otherwise regulating SNARE complex assembly. *In vitro* studies have shown that the DSL1 complex accelerates SNARE assembly [61,88], albeit only modestly under the experimental conditions used; and GARP is required for efficient SNARE assembly *in vivo* [89]. Munc13 also catalyses fusion, binding the syntaxin1 SNARE motif and enhancing the transition of syntaxin from its closed to the open, fusion-competent conformation [86]. And in HOPS and CORVET, the SM subunit Vps33 interacts with SNAREs [87]. In detail, the MTC-SNARE interactions differ, even for the CATCHR

complexes. DSL1 and GARP both interact with the Habc motifs of their respective t-SNARE partners [61,90], whereas the exocyst interacts with the linker region between the two SNARE motifs of its Sec9 binding partner [91], and Munc13 interacts with the syntaxin1 SNARE motif itself [67]. As more MTC-SNARE interactions are characterized, it may well emerge that each MTC can interact with multiple SNARE proteins in one or more ways. Alternatively, MTC-SNARE interactions may simply be different in different transport pathways.

A structural framework

Only DSL1 has been characterized in its entirety at the atomic level (Fig 3B) [61,63]. It is an outlier among the CATCHR complexes in that only two of its subunits, Dsl1 and Tip20, exhibit the typical CATCHR fold, whereas the third, Sec39, does not. The three subunits associate into a conformationally flexible assembly, with multiple long legs extending from a junction. Dsl1 contains a hinge region that confers flexibility to the complex; and it has been proposed that the complex may, in fact, undergo conformational changes between a more closed conformation, in which Tip20 and Sec39 could make contact (as in Fig 3B) and an open one, where this interaction would be broken. Dsl1 and Tip20 bind each other through their N-terminal helices, which interact in an antiparallel fashion [61]. A propensity to form coiled-coil motifs has been identified in the N-terminus of a number of CATCHR subunits [92], and it is tempting to speculate that antiparallel association of single helices as observed for Dsl1/Tip20 might represent a common mechanism of CATCHR assembly. DSL1 interacts with t-SNARE proteins by regions in the Sec39 and Tip20 N-termini and with the vesicle coat via an extended loop in Dsl1 (arrows in Fig 3B). It remains unclear whether DSL1 is extended lengthwise between the vesicle and acceptor membrane, as one might imagine if it acts as a physical tether; whether it lies flat between the membranes, almost like a vesicle cage, as a fusion regulator; or whether it assumes multiple conformations consistent with multiple functions.

For COG, a tetrameric subcomplex (subunits Cog1-4) was visualized by negative staining electron microscopy [70] (Fig 3C). The subunits also form a flexible multi-legged arrangement, which is apparently held together by interactions of the predicted coiled-coil motifs at the N-termini of all four subunits. These form a portion of one leg closest to the junction, whereas the rest of the legs presumably comprise rods of tandem alpha-helical bundles, the CATCHR fold. Mapping studies reveal that the coiled-coil portion near the leg-junction interacts with the SM protein Sly1 and the SNARE syntaxin5 [70]. How COG interacts with either the COPI coat and Ypt1 is not known.

The common features between COG1-4 and DSL1 are that the subunits form extended arms that are linked at their N-termini and can move flexibly, and it seems reasonable to expect that the second half of COG, GARP and the exocyst may form similar leggy assemblies. Given that DSL1 may be an outlier in terms of overall architecture, we would speculate that COG5-8 and GARP will look more similar to COG1-4, and that the exocyst might also comprise two COG1-4-like subassemblies.

Our understanding of MTC function currently lags behind that for the coat proteins. Hopefully further progress in characterizing the structure and architecture of MTCs will provide a helpful framework for understanding their function, especially as further interactions with elements on the vesicle and acceptor compartments are identified and mapped to specific locations on the complexes.

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References

1. Höning S, Ricotta D, Krauss M, Späte K, Spolaore B, Motley A, Robinson M, Robinson C, Haucke V, Owen DJ. Phosphatidylinositol-(4,5)-bisphosphate regulates sorting signal recognition by the clathrin-associated adaptor complex AP2. *Mol Cell*. 2005; 18:519–31. [PubMed: 15916959]
2. Hurley JH, Wendland B. Endocytosis: Driving Membranes around the Bend. *Cell*. 2002; 111:143–146. [PubMed: 12408856]
3. Ford MGJ, Mills IG, Peter BJ, Vallis Y, Praefcke GJK, Evans PR, McMahon HT. Curvature of clathrin-coated pits driven by epsin. *Nature*. 2002; 419:361–6. [PubMed: 12353027]
4. Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJG, Evans PR, McMahon HT. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science*. 2004; 303:495–9. [PubMed: 14645856]
5. Gallop JL, Jao CC, Kent HM, Butler PJG, Evans PR, Langen R, McMahon HT. Mechanism of endophilin N-BAR domain-mediated membrane curvature. *EMBO J*. 2006; 25:2898–910. [PubMed: 16763559]
6. Zimmerberg J, Kozlov MM. How proteins produce cellular membrane curvature. *Nat Rev Mol Cell Biol*. 2006; 7:9–19. [PubMed: 16365634]
7. Kelly BT, Owen DJ. Endocytic sorting of transmembrane protein cargo. *Curr Opin Cell Bio*. 2011 doi:10.1016/j.ceb.2011.03.004.
8. Traub LM. Tickets to ride: selecting cargo for clathrin-regulated internalization. *Nat Rev Mol Cell Biol*. 2009; 10:583–96. [PubMed: 19696796]
9. Kurten RC, Cadena DL, Gill GN. Enhanced degradation of EGF receptors by a sorting nexin, SNX1. *Science*. 1996; 272:1008–10. [PubMed: 8638121]
10. Haft CR, de la Luz Sierra M, Barr VA, Haft DH, Taylor SI. Identification of a Family of Sorting Nexin Molecules and Characterization of Their Association with Receptors. *Mol Cell Biol*. 1998; 18:7278–87. [PubMed: 9819414]
11. Seaman MN, McCaffery JM, Emr SD. A Membrane Coat Complex Essential for Endosome-to-Golgi Retrograde Transport in Yeast. *J Cell Biol*. 1998; 142:665–81. [PubMed: 9700157]
12. McGough IJ, Cullen PJ. Recent Advances in Retromer Biology. *Traffic*. 2011 doi:10.1111/j.1600-0854.2011.01201.x.
13. Teasdale RD, Collins BM. Insights into the PX (phox-homology) domain and SNX (sorting nexin) protein families: structures, functions and roles in disease. *Biochem J*. 2012; 441:39–59. [PubMed: 22168438]
14. Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peränen J, Merdes A, Slusarski DC, Scheller RH, Bazan JF, Sheffield VC, et al. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell*. 2007; 129:1201–13. [PubMed: 17574030]
- 15*. Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, Bazan JF, Nachury MV. The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell*. 2010; 141:1208–19. [PubMed: 20603001] [The authors postulate that proteins forming a highly conserved octameric complex called the BBSome might constitute a novel coat for vesicular trafficking within primary cilia.]
16. Pearse BMF. Coated Vesicles from Pig Brain: Purification and Biochemical Characterization. *J Mol Biol*. 1975; 97:93–98. [PubMed: 1177317]
17. Kirchhausen T. Clathrin. *Ann Rev Biochem*. 2000; 69:699–727. [PubMed: 10966473]
18. Owen DJ, Collins BM, Evans PR. Adaptors for clathrin coats: structure and function. *Ann Rev Cell Dev Biol*. 2004; 20:153–91. [PubMed: 15473838]
19. ter Haar E, Harrison SC, Kirchhausen T. Peptide-in-groove interactions link target proteins to the beta-propeller of clathrin. *Proc Natl Acad Sci U S A*. 2000; 97:1096–100. [PubMed: 10655490]

20. Miele AE, Watson PJ, Evans PR, Traub LM, Owen DJ. Two distinct interaction motifs in amphiphysin bind two independent sites on the clathrin terminal domain beta-propeller. *Nat Struct Mol Biol.* 2004; 11:242–8. [PubMed: 14981508]
21. Kang DS, Kern RC, Puthenveedu M a, von Zastrow M, Williams JC, Benovic JL. Structure of an arrestin2-clathrin complex reveals a novel clathrin binding domain that modulates receptor trafficking. *J Biol Chem.* 2009; 284:29860–72. [PubMed: 19710023]
22. Willox AK, Royle SJ. Functional Analysis of Interaction Sites on the N-Terminal Domain of Clathrin Heavy Chain. *Traffic.* 2011 doi:10.1111/j.1600-0854.2011.01289.x.
- 23*. von Kleist L, Stahlschmidt W, Bulut H, Gromova K, Puchkov D, Robertson MJ, Macgregor K a, Tomlin N, Pechstein A, Chau N, et al. Role of the clathrin terminal domain in regulating coated pit dynamics revealed by small molecule inhibition. *Cell.* 2011; 146:471–84. [PubMed: 21816279] [This report constitutes the first example of an effective and possibly specific small molecule inhibitor of clathrin-mediated endocytosis, which binds to the clathrin-box site on clathrin terminal domain (TD).]
24. Lemmon SK, Traub LM. Getting in Touch with the Clathrin Terminal Domain. *Traffic.* 2012 doi: 10.1111/j.1600-0854.2011.01321.x.
25. Stagg SM, Gurkan C, Fowler DM, LaPointe P, Foss TR, Potter CS, Carragher B, Balch WE. Structure of the Sec13/31 COPII coat cage. *Nature.* 2006; 439:234–38. [PubMed: 16407955]
26. Stagg SM, LaPointe P, Razvi A, Gürkan C, Potter CS, Carragher B, Balch WE. Structural basis for cargo regulation of COPII coat assembly. *Cell.* 2008; 134:474–84. [PubMed: 18692470]
27. Bi X, Mancias JD, Goldberg J. Insights into COPII coat nucleation from the structure of Sec23.Sar1 complexed with the active fragment of Sec31. *Dev Cell.* 2007; 13:635–45. [PubMed: 17981133]
28. Fath S, Mancias JD, Bi X, Goldberg J. Structure and organization of coat proteins in the COPII cage. *Cell.* 2007; 129:1325–36. [PubMed: 17604721]
29. Russell C, Stagg SM. New insights into the structural mechanisms of the COPII coat. *Traffic.* 2010; 11:303–10. [PubMed: 20070605]
30. Jensen D, Schekman R. COPII-mediated vesicle formation at a glance. *J Cell Sci.* 2011; 124:1–4. [PubMed: 21172817]
31. Collins BM, McCoy AJ, Kent HM, Evans PR, Owen DJ. Molecular architecture and functional model of the endocytic AP2 complex. *Cell.* 2002; 109:523–35. [PubMed: 12086608]
32. Heldwein EE, Macia E, Wang J, Yin HL, Kirchhausen T, Harrison SC. Crystal structure of the clathrin adaptor protein 1 core. *Proc Natl Acad Sci U S A.* 2004; 101:14108–13. [PubMed: 15377783]
33. Owen DJ, Vallis Y, Noble MEM, Hunter JB, Dafforn TR, Evans PR, McMahon HT. A Structural Explanation for the Binding of Multiple Ligands by the alpha-adaptin Appendage Domain. *Cell.* 1999; 97:805–815. [PubMed: 10380931]
34. Traub LM, Downs MA, Westrich JL, Fremont DH. Crystal structure of the alpha appendage of AP-2 reveals a recruitment platform for clathrin-coat assembly. *Proc Natl Acad Sci U S A.* 1999; 96:8907–12. [PubMed: 10430869]
35. Owen DJ, Vallis Y, Pearse BM, McMahon HT, Evans PR. The structure and function of the beta 2-adaptin appendage domain. *EMBO J.* 2000; 19:4216–27. [PubMed: 10944104]
36. Edeling MA, Mishra SK, Keyel PA, Steinhauser AL, Collins BM, Roth R, Heuser JE, Owen DJ, Traub LM. Molecular switches involving the AP-2 beta2 appendage regulate endocytic cargo selection and clathrin coat assembly. *Dev Cell.* 2006; 10:329–42. [PubMed: 16516836]
37. Owen DJ, Evans PR. A structural explanation for the recognition of tyrosine-based endocytotic signals. *Science.* 1998; 282:1327–32. [PubMed: 9812899]
38. Royle SJ, Qureshi OS, Bobanovi LK, Evans PR, Owen DJ, Murrell-Lagnado RD. Non-canonical YXXGPhi endocytic motifs: recognition by AP2 and preferential utilization in P2X4 receptors. *J Cell Sci.* 2005; 118:3073–80. [PubMed: 15985462]
39. Kelly BT, McCoy AJ, Späte K, Miller SE, Evans PR, Höning S, Owen DJ. A structural explanation for the binding of endocytic dileucine motifs by the AP2 complex. *Nature.* 2008; 456:976–79. [PubMed: 19140243]

40. Burgos PV, Mardones GA, Rojas AL, DaSilva LLP, Prabhu Y, Hurley JH, Bonifacino JS. Sorting of the Alzheimer's disease amyloid precursor protein mediated by the AP-4 complex. *Dev Cell*. 2010; 18:425–36. [PubMed: 20230749]
41. Yu A, Xing Y, Harrison SC, Kirchhausen T. Structural analysis of the interaction between Dishevelled2 and clathrin AP-2 adaptor, a critical step in noncanonical Wnt signaling. *Structure*. 2010; 18:1311–20. [PubMed: 20947020]
42. Jackson LP, Kelly BT, McCoy AJ, Gaffry T, James LC, Collins BM, Höning S, Evans PR, Owen DJ. A large-scale conformational change couples membrane recruitment to cargo binding in the AP2 clathrin adaptor complex. *Cell*. 2010; 141:1220–9. [PubMed: 20603002]
43. Austin C, Hanners I, Tooze S. Direct and GTP-dependent interaction of ADP-ribosylation factor 1 with clathrin adaptor protein AP-1 on immature secretory granules. *J Biol Chem*. 2000; 275:21862–9. [PubMed: 10807927]
44. Boehm M, Aguilar RC, Bonifacino JS. Functional and physical interactions of the adaptor protein complex AP-4 with ADP-ribosylation factors (ARFs). *EMBO J*. 2001; 20:6265–76. [PubMed: 11707398]
45. Spang A, Matsuoka K, Hamamoto S, Schekman R, Orci L. Coatamer, Arf1p, and nucleotide are required to bud coat protein complex I-coated vesicles from large synthetic liposomes. *Proc Natl Acad Sci U S A*. 1998; 95:11199–204. [PubMed: 9736713]
- 46*. Yu X, Breitman M, Goldberg J. A Structure-Based Mechanism for Arf1-Dependent Recruitment of Coatamer to Membranes. *Cell*. 2012; 148:530–542. [PubMed: 22304919] [The first structural example demonstrating how a coat complex binds a small Arf GTPase also implies a conserved mechanism between AP-like coats, in which membrane recruitment triggers a large-scale conformational change.]
- 47*. Guan R, Dai H, Han D, Harrison SC, Kirchhausen T. Structure of the PTEN-like region of auxilin, a detector of clathrin-coated vesicle budding. *Structure*. 2010; 18:1191–8. [PubMed: 20826345] [The structure of the PTEN-like region of auxilin reveals a PI3P phosphatase-like fold having no catalytic activity while maintaining the potential to bind specific phosphoinositides found in uncoating vesicles.]
- 48*. Xing Y, Böcking T, Wolf M, Grigorieff N, Kirchhausen T, Harrison SC. Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly. *EMBO J*. 2010; 29:655–65. [PubMed: 20033059] [Cryo EM maps of the clathrin cage, with and without Hsc70 and auxilin, suggested a two-fold role for auxilin during coated vesicle disassembly: Hsc70 recruitment and introduction of strain into the uncoating lattice.]
- 49*. Rothnie A, Clarke AR, Kuzmic P, Cameron A, Smith CJ. A sequential mechanism for clathrin cage disassembly by 70-kDa heat-shock cognate protein (Hsc70) and auxilin. *Proc Natl Acad Sci U S A*. 2011; 108:6927–32. [PubMed: 21482805] [Using stopped-flow techniques to measure kinetics, the authors report the required stoichiometries of auxilin and Hsc70, as well as a three-step model, for disassembly of clathrin-coated vesicles.]
50. Fotin A, Cheng Y, Sliz P, Grigorieff N, Harrison SC, Kirchhausen T, Walz T. Molecular model for a complete clathrin lattice from electron cryomicroscopy. *Nature*. 2004; 432:573–9. [PubMed: 15502812]
- 51*. Yip CK, Walz T. Molecular structure and flexibility of the yeast coatamer as revealed by electron microscopy. *J Mol Biol*. 2011; 408:825–31. [PubMed: 21435344] [Single particle EM data enable authors to assign density for the two subcomplexes of COPI within the heptameric coat.]
- 52*. Faini M, Prinz S, Beck R, Schorb M, Riches JD, Bacia K, Brügger B, Wieland FT, Briggs JAG. The Structures of COPI-Coated Vesicles Reveal Alternate Coatamer Conformations and Interactions. *Science*. 2012 doi:10.1126/science.1221443. [Cryo electron tomography reveals the conformational heterogeneity inherent in COPI coated vesicles reconstituted *in vitro*.]
53. Hsia K-C, Hoelz A. Crystal structure of alpha-COP in complex with epsilon-COP provides insight into the architecture of the COPI vesicular coat. *Proc Natl Acad Sci U S A*. 2010; 107:11271–6. [PubMed: 20534429]
- 54*. Lee C, Goldberg J. Structure of coatamer cage proteins and the relationship among COPI, COPII, and clathrin vesicle coats. *Cell*. 2010; 142:123–32. [PubMed: 20579721] [The first X-ray structure of components of the α and β' -COP subunits provides a possible mechanism for coatamer cage assembly.]

55. Yu IM, Hughson FM. Tethering factors as organizers of intracellular vesicular traffic. *Annu Rev Cell Dev Biol.* 2010; 26:137–156. [PubMed: 19575650]
56. Cavanaugh LF, Chen X, Richardson BC, Ungar D, Pelczar I, Rizo J, Hughson FM. Structural analysis of conserved oligomeric golgi complex subunit 2. *J Biol Chem.* 2007 [no volume].
57. Dong G, Hutagalung AH, Fu C, Novick P, Reinisch KM. The structures of exocyst subunit Exo70p and the Exo84p C-terminal domains reveal a common motif. *Nat Struct Mol Biol.* 2005; 12:1094–1100. [PubMed: 16249794]
58. Hamburger ZA, Hamburger AE, West AP Jr. Weis WI. Crystal structure of the *S.cerevisiae* exocyst component Exo70p. *J Mol Biol.* 2006; 356:9–21. [PubMed: 16359701]
59. Moore BA, Robinson HH, Xu Z. The crystal structure of mouse exo70 reveals unique features of the Mammalian exocyst. *J Mol Biol.* 2007; 371:410–421. [PubMed: 17583731]
60. Perez-Victoria FJ, Abascal-Palacios G, Tascon I, Kajava A, Magadan JG, Pioro EP, Bonifacino JS, Hierro A. Structural basis for the wobbler mouse neurodegenerative disorder caused by mutation in the Vps54 subunit of the GARP complex. *Proc Natl Acad Sci U S A.* 2010; 107:12860–12865. [PubMed: 20615984]
- 61**. Ren Y, Yip CK, Tripathi A, Huie D, Jeffrey PD, Walz T, Hughson FM. A structure-based mechanism for vesicle capture by the multisubunit tethering complex Dsl1. *Cell.* 2009; 139:1119–1129. [PubMed: 20005805] [This paper presents a model of the entire DSL1 complex derived from the combination of several crystal structures. Additionally, DSL1 activity to promote the assembly of SNARE complexes is demonstrated in an *in vitro* assay.]
62. Sivaram MV, Furgason ML, Brewer DN, Munson M. The structure of the exocyst subunit Sec6p defines a conserved architecture with diverse roles. *Nat Struct Mol Biol.* 2006; 13:555–556. [PubMed: 16699513]
63. Tripathi A, Ren Y, Jeffrey PD, Hughson FM. Structural characterization of Tip20p and Dsl1p, subunits of the Dsl1p vesicle tethering complex. *Nat Struct Mol Biol.* 2009; 16:114–123. [PubMed: 19151722]
64. Vasan N, Hutagalung A, Novick P, Reinisch KM. Structure of a C-terminal fragment of its Vps53 subunit suggests similarity of Golgi-associated retrograde protein (GARP) complex to a family of tethering complexes. *Proc Natl Acad Sci U S A.* 2010; 107:14176–14181. [PubMed: 20660722]
65. Wu S, Mehta SQ, Pichaud F, Bellen HJ, Quiocho FA. Sec15 interacts with Rab11 via a novel domain and affects Rab11 localization in vivo. *Nat Struct Mol Biol.* 2005; 12:879–885. [PubMed: 16155582]
66. Richardson BC, Smith RD, Ungar D, Nakamura A, Jeffrey PD, Lupashin VV, Hughson FM. Structural basis for a human glycosylation disorder caused by mutation of the COG4 gene. *Proc Natl Acad Sci U S A.* 2009; 106:13329–13334. [PubMed: 19651599]
- 67*. Li W, Ma C, Guan R, Xu Y, Tomchick DR, Rizo J. The crystal structure of a Munc13 C-terminal module exhibits a remarkable similarity to vesicle tethering factors. *Structure.* 2011; 19:1443–1455. [PubMed: 22000513] [The crystal structure of a Munc13 subdomain shows its relationship with CATCHR complexes, indicating a common function of these proteins in tethering and SNARE regulation.]
68. Whyte JR, Munro S. Vesicle tethering complexes in membrane traffic. *J Cell Sci.* 2002; 115:2627–2637. [PubMed: 12077354]
- 69**. Brocker C, Kuhlee A, Gatsogiannis C, Kleine Balderhaar HJ, Honscher C, Engelbrecht-Vandre S, Ungermann C, Raunser S. Molecular architecture of the multisubunit homotypic fusion and vacuole protein sorting (HOPS) tethering complex. *Proc Natl Acad Sci U S A.* 2012 doi: 1117797109 [pii] 10.1073/pnas.1117797109. [This paper was published after preparation of this manuscript. The EM structure of the entire HOPS complex provides the first structural information on class C core tethering complexes, identifying two binding sites for Ypt7 at opposing ends of an elongated seahorse-like structure.]
- 70**. Lees JA, Yip CK, Walz T, Hughson FM. Molecular organization of the COG vesicle tethering complex. *Nat Struct Mol Biol.* 2010; 17:1292–1297. [PubMed: 20972446] [The structural characterization of a COG subcomplex by EM reveals a multi-legged, extended conformation that might represent the underlying organizational principle of all CATCHR complexes.]

71. Andag U, Neumann T, Schmitt HD. The coatamer-interacting protein Dsl1p is required for Golgi-to-endoplasmic reticulum retrieval in yeast. *J Biol Chem.* 2001; 276:39150–39160. [PubMed: 11493604]
72. Oka T, Ungar D, Hughson FM, Krieger M. The COG and COPI complexes interact to control the abundance of GEARs, a subset of Golgi integral membrane proteins. *Mol Biol Cell.* 2004; 15:2423–2435. [PubMed: 15004235]
73. Guo W, Roth D, Walch-Solimena C, Novick P. The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *EMBO J.* 1999; 18:1071–1080. [PubMed: 10022848]
74. Dulubova I, Lou X, Lu J, Huryeva I, Alam A, Schneggenburger R, Sudhof TC, Rizo J. A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? *EMBO J.* 2005; 24:2839–2850. [PubMed: 16052212]
75. Panic B, Whyte JR, Munro S. The ARF-like GTPases Arl1p and Arl3p act in a pathway that interacts with vesicle-tethering factors at the Golgi apparatus. *Curr Biol.* 2003; 13:405–410. [PubMed: 12620189]
76. Siniosoglou S, Pelham HR. An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes. *EMBO J.* 2001; 20:5991–5998. [PubMed: 11689439]
77. Guo W, Tamanoi F, Novick P. Spatial regulation of the exocyst complex by Rho1 GTPase. *Nat Cell Biol.* 2001; 3:353–360. [PubMed: 11283608]
78. Robinson NG, Guo L, Imai J, Toh EA, Matsui Y, Tamanoi F. Rho3 of *Saccharomyces cerevisiae*, which regulates the actin cytoskeleton and exocytosis, is a GTPase which interacts with Myo2 and Exo70. *Mol Cell Biol.* 1999; 19:3580–3587. [PubMed: 10207081]
79. Zhang X, Bi E, Novick P, Du L, Kozminski KG, Lipschutz JH, Guo W. Cdc42 interacts with the exocyst and regulates polarized secretion. *J Biol Chem.* 2001; 276:46745–46750. [PubMed: 11595741]
80. Liu J, Zuo X, Yue P, Guo W. Phosphatidylinositol 4,5-bisphosphate mediates the targeting of the exocyst to the plasma membrane for exocytosis in mammalian cells. *Mol Biol Cell.* 2007; 18:4483–4492. [PubMed: 17761530]
81. Zhang X, Orlando K, He B, Xi F, Zhang J, Zajac A, Guo W. Membrane association and functional regulation of Sec3 by phospholipids and Cdc42. *J Cell Biol.* 2008; 180:145–158. [PubMed: 18195105]
82. Wurmser AE, Sato TK, Emr SD. New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. *J Cell Biol.* 2000; 151:551–562. [PubMed: 11062257]
83. Peplowska K, Markgraf DF, Ostrowicz CW, Bange G, Ungermann C. The CORVET tethering complex interacts with the yeast Rab5 homolog Vps21 and is involved in endo-lysosomal biogenesis. *Dev Cell.* 2007; 12:739–750. [PubMed: 17488625]
84. Morgera F, Sallah MR, Dubuke ML, Gandhi P, Brewer DN, Carr CM, Munson M. Regulation of exocytosis by the exocyst subunit Sec6 and the SM protein Sec1. *Mol Biol Cell.* 2012; 23:337–346. [PubMed: 22114349]
85. Laufman O, Kedan A, Hong W, Lev S. Direct interaction between the COG complex and the SM protein, Sly1, is required for Golgi SNARE pairing. *EMBO J.* 2009; 28:2006–2017. [PubMed: 19536132]
- 86*. Ma C, Li W, Xu Y, Rizo J. Munc13 mediates the transition from the closed syntaxin-Munc18 complex to the SNARE complex. *Nat Struct Mol Biol.* 2011; 18:542–549. [PubMed: 21499244]
[Munc13 is shown to interact with syntaxin1 and Munc18 to aid SNARE assembly which might provide the molecular basis of priming of vesicle fusion.]
87. Pieren M, Schmidt A, Mayer A. The SM protein Vps33 and the t-SNARE H(abc) domain promote fusion pore opening. *Nat Struct Mol Biol.* 2010; 17:710–717. [PubMed: 20453860]
88. Diefenbacher M, Thorsteinsdottir H, Spang A. The Dsl1 tethering complex actively participates in soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptor (SNARE) complex assembly at the endoplasmic reticulum in *Saccharomyces cerevisiae*. *J Biol Chem.* 2011; 286:25027–25038. [PubMed: 21482823]

- 89*. Perez-Victoria FJ, Bonifacino JS. Dual roles of the mammalian GARP complex in tethering and SNARE complex assembly at the trans-golgi network. *Mol Cell Biol.* 2009; 29:5251–5263. [PubMed: 19620288] [This report shows that GARP is required for proper localization and assembly of specific Golgi SNARE complexes.]
90. Fridmann-Sirkis Y, Kent HM, Lewis MJ, Evans PR, Pelham HR. Structural analysis of the interaction between the SNARE Tlg1 and Vps51. *Traffic.* 2006; 7:182–190. [PubMed: 16420526]
91. Sivaram MV, Saporita JA, Furgason ML, Boettcher AJ, Munson M. Dimerization of the exocyst protein Sec6p and its interaction with the t-SNARE Sec9p. *Biochemistry.* 2005; 44:6302–6311. [PubMed: 15835919]
92. Whyte JR, Munro S. The Sec34/35 Golgi transport complex is related to the exocyst, defining a family of complexes involved in multiple steps of membrane traffic. *Dev Cell.* 2001; 1:527–537. [PubMed: 11703943]
93. Darsow T, Katzmann DJ, Cowles CR, Emr SD. Vps41p function in the alkaline phosphatase pathway requires homo-oligomerization and interaction with AP-3 through two distinct domains. *Mol Biol Cell.* 2001; 12:37–51. [PubMed: 11160821]
94. Hickey CM, Stroupe C, Wickner W. The major role of the Rab Ypt7p in vacuole fusion is supporting HOPS membrane association. *J Biol Chem.* 2009; 284:16118–16125. [PubMed: 19386605]
95. Kraynack BA, Chan A, Rosenthal E, Essid M, Umansky B, Waters MG, Schmitt HD. Dsl1p, Tip20p, and the novel Dsl3(Sec39) protein are required for the stability of the Q/t-SNARE complex at the endoplasmic reticulum in yeast. *Mol Biol Cell.* 2005; 16:3963–3977. [PubMed: 15958492]
96. Siniosoglou S, Pelham HR. Vps51p links the VFT complex to the SNARE Tlg1p. *J Biol Chem.* 2002; 277:48318–48324. [PubMed: 12377769]
97. Perez-Victoria FJ, Schindler C, Magadan JG, Mardones GA, Delevoye C, Romao M, Raposo G, Bonifacino JS. Ang2/fat-free is a conserved subunit of the Golgi-associated retrograde protein complex. *Mol Biol Cell.* 2010; 21:3386–3395. [PubMed: 20685960]
98. Shestakova A, Suvorova ES, Pavliv O, Khaidakova G, Lupashin V. Interaction of the conserved oligomeric Golgi complex with t-SNARE Syntaxin5a/Sed5 enhances intra-Golgi SNARE complex stability. *J Cell Biol.* 2007; 179:1179–92. [PubMed: 18086915]
99. Stroupe C, Collins KM, Fratti RA, Wickner W. Purification of active HOPS complex reveals its affinities for phosphoinositides and the SNARE Vam7p. *EMBO J.* 2006; 25:1579–1589. [PubMed: 16601699]
100. Suvorova ES, Duden R, Lupashin VV. The Sec34/Sec35p complex, a Ypt1p effector required for retrograde intra-Golgi trafficking, interacts with Golgi SNAREs and COPI vesicle coat proteins. *J Cell Biol.* 2002; 157:631–643. [PubMed: 12011112]

Figure 1. A large-scale conformational change driven by membrane recruitment is conserved between AP-like complexes

A: Prior to clathrin coat formation, AP2 ($\alpha/\beta_2/\mu_2/\sigma_2$) exists in the cytoplasm in a closed and locked conformation, in which cargo binding sites are obstructed (left, PDB ID: 2VGL, 1B9K, 1E42). Plasma membrane recruitment is driven by four PtdIns(4,5) P_2 binding sites on the α , β_2 , and μ_2 subunits (not shown). Once docked, AP2 undergoes a large-scale conformational change into its open form (right, PDB ID: 2XA7, 1B9K, 1E42), and both binding sites for transmembrane cargo become accessible. The dileucine binding site is shown in yellow, the Yxx Φ in orange.

B: In the retrograde pathway, the ‘AP-like’ subcomplex of COPI ($\gamma/\beta/\delta/\zeta$) may also undergo a conformational change upon membrane recruitment by Arf1-GTP. A recent X-ray crystal structure (PDB ID: 3TJZ) shows the molecular basis for binding of Arf1-GTP (red ribbons) by a portion of the $\gamma\zeta$ -COP heterodimer. Biochemical evidence and structure-based mutagenesis suggest a second binding site on the $\beta\delta$ -heterodimer (red circle, structure not yet determined). The spatial location of these sites are equivalent to PtdIns(4,5) P_2 binding sites on AP2, suggesting a similar conformational change by COPI upon membrane recruitment. All undetermined structures are modeled on AP2 (PDB ID: 2VGL, 2XA7) and shown in grey ribbons. The structure of the γ -COP appendage has been determined (PDB ID: 1R4X, 1PZD).

Figure 2. COPI coat architecture

Recent X-ray crystal structures provide the first molecular information regarding portions of the outer $\alpha/\beta'/\epsilon$ subcomplex of COPI (colored). The yeast α/β' structure (left, PDB ID: 3MKQ) indicates that the two α -solenoids of α - (green) and β' -COP (yellow) curve around one another, possibly representing the 'leg' of the cage. The N-terminal WD40 domain of β' -COP (gold) may constitute the vertex of this cage. The C-terminus of bovine α -COP interacts with TPR domain of ϵ -COP (right, PDB ID: 3MV2, 3MKR). The yeast sequence is shown in the schematic for reference.

Figure 3. Structure of Multisubunit tethering complexes

A: The structure of the C-terminal half of the Munc13 CATCHR domain is an example of the characteristic fold (PDB ID: 3SWH).

B: Structure of the intact DSL1 complex derived from individual crystal structures (PDB ID: 3ETV, 3K8P, 3FHN).

C: EM class average of the Cog1–4 subcomplex (courtesy of T. Walz) with a schematic representation of the subunit orientation within the complex. Arrows mark mapped interaction sites.

MTCs and their binding partners.

Table 1

		Munc13	DSL	GARP	COG	Exocyst	HOPS	CORVET
Subunits		Munc13	Dsl1 Tip20 Sec39	Vps51 Vps52 Vps53 Vps54	Cog1-8	Sec3, Sec5 Sec6, Sec8 Sec10 Sec15 Exo70 Exo84	Vps11 Vps16 Vps18 Vps33* Vps41 Vam6	Vps11 Vps16 Vps18 Vps33* Vps38 Vps41 Vps3
	Pathway							
Vesicle		Synaptic vesicle fusion	ER to Golgi	Endosome to Golgi	Intra Golgi	Secretory vesicle exocytosis	Vacuole fusion	Vacuole/ endosome fusion
	Coat		α -COP [53,71]		γ -COP[72]		δ -AP3[93]	
Target membrane	GTPase	Rab3[74]				Sec4[73]	Ypt7[80,94]	Vps21[83]
	t-SNARE	Syntaxin1 [84]	Sec20 [95] Use1[95] Ufe[95]	Tgl1[90,96]/syntaxin6[97] Syntaxin16 [89]	Sed5/syntaxin5a[98]	Sec9[91]	Vam7[99]	
	GTPases			Arf1[75] Ypt6[76]	Ypt1[100]	Rho1[75] Rho3[77] Cdc42[78]		
SM proteins		Munc18[84]			Sly1[83]	Sec1[82]	Vps33*	Vps33*

*The SM protein Vps33 is part of the class C tethers