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CELL SCIENCE AT A GLANCE

Formation of COPI-coated vesicles at a glance

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ABSTRACT

The coat protein complex I (COPI) allows the precise sorting of lipids and proteins between Golgi cisternae and retrieval from the Golgi to the ER. This essential role maintains the identity of the early secretory pathway and impinges on key cellular processes, such as protein quality control. In this Cell Science at a Glance and accompanying poster, we illustrate the different stages of COPI-coated vesicle formation and revisit decades of research in the context of recent advances in the elucidation of COPI coat structure. By calling attention to an array of questions that have remained unresolved, this review attempts to refocus the perspectives of the field.

KEY WORDS: Arf1, ArfGAP, COPI, Coatomer, Golgi, Endoplasmic reticulum, Vesicle coat

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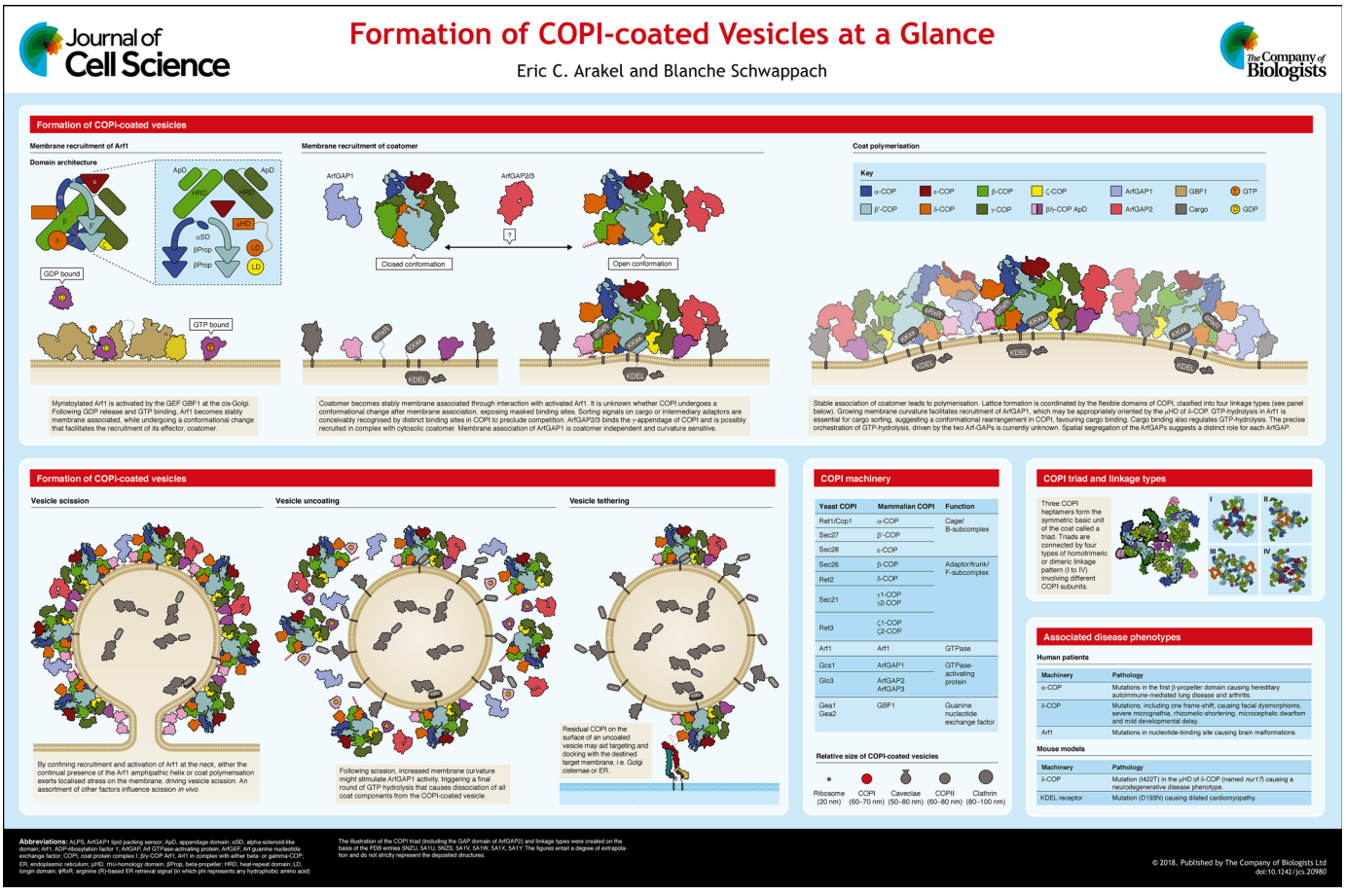
Introduction

Vesicle coat proteins, such as the archetypal clathrin and the coat protein complexes II and I (COPII and COPI, respectively) are molecular machines with two central roles: enabling vesicle formation, and selecting protein and lipid cargo to be packaged within them. Thus, coat proteins fulfil a central role in the homeostasis of the cell's endomembrane system and are the basis of functionally segregated compartments. COPI operates in retrieval from the Golgi to the endoplasmic reticulum (ER) and in intra-Golgi transport (Beck et al., 2009; Duden, 2003; Lee et al., 2004a; Spang, 2009), and maintains ER- and Golgi-resident chaperones and enzymes where they belong. Several reports have also highlighted a role for COPI in endosomal transport and function (Aniento et al., 1996; Daro et al., 1997; Gabriely et al., 2006; Gu et al., 1997;



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Guo et al., 1994; Razi et al., 2009; Whitney et al., 1995), regulating lipid droplet homeostasis (Beller et al., 2008; Soni et al., 2009; Thiam et al., 2013; Wilfling et al., 2013), mRNA transport (Bi et al., 2007; Todd et al., 2013; Trautwein et al., 2004; Zabezhinsky et al., 2016), and the breakdown of the nuclear envelope (Liu et al., 2003; Prunuske et al., 2006). Discussion of these roles is beyond the scope of this review (see textbox for some open questions). Based on its fundamental and diverse roles, COPI dysfunction culminates in disease (Bettayeb et al., 2016a,b; Ge et al., 2016; Hamada et al., 2004; Izumi et al., 2016; Watkin et al., 2015; Xu et al., 2010). Here, we address the different stages of COPI vesicle biogenesis and revisit them in the context of striking recent developments in elucidating the structure of the COPI coat.

Structural organization of coatomer

COPI consists of seven core subunits α-COP, β'-COP, ε-COP, β-COP, δ-COP, γ-COP and ζ-COP (see poster). A cytoplasmic heptamer of these subunits – termed coatomer – is recruited en bloc to the membrane bilayer to form the COPI coat (Hara-Kuge et al., 1994). However, mammalian coatomer can be biochemically dissected into a cage-like (B-) and an adaptor-like (F-) subcomplex, in analogy to the structure of the clathrin-adaptor complex, which is recruited to membranes in two stages (Fiedler et al., 1996; Lowe and Kreis, 1995, 1996; Pavel et al., 1998). Here, we provide evidence that in the baker's yeast *Saccharomyces cerevisiae*, coatomer also exists as a cytosolic heptamer (Fig. 1) that displays dissociation-resistant interactions within the B-complex (α-COP and ε-COP) and within the F-complex (β-COP and δ-COP as well as γ-COP and ζ-COP). This result further strengthens COPI models based on data from mammalian and yeast systems, and raises interesting questions with respect to COPI biogenesis (Box 1).

Subunits of the COPI coat share structural similarities to a wide range of proteins that compartmentalise diverse cellular functions, such as proteins of the nuclear pore-complex and other vesicular coats or protein-sorting machinery (Rout and Field, 2017), e.g. the longin domain (LD) in δ- and ζ-COP (De Franceschi et al., 2014); the β-propellers that comprise WD-40 repeats, i.e. ∼40 amino acids terminating in tryptophan (W) and glutamic acid (D); the α-solenoid-like domain in α- and β'-COP (Lee and Goldberg, 2010); heat-repeat domains in β- and γ-COP (Neuwald and Hirano, 2000); the β-sandwich in the appendage domains of β- and γ-COP (Watson et al., 2004); and the μ-homology domain (μHD) in δ-COP (Lahav et al., 2015; Suckling et al., 2014). Most of these domains occur as pairs due to gene duplication during evolution of the early ancestral coat complex (Hirst et al., 2014). Only one of the two longin-domain-containing subunits appears to have acquired or kept a μHD. All these domains co-ordinate the complex architecture of the COPI coat, making six of the seven subunits essential for viability in *S. cerevisiae*. Only ε-COP, emerging last during evolution, is non-essential (Duden, 1998). *S. cerevisiae* can also survive without the first of the two β-propellers in α- and β'-COP or without the μHD (Arakel et al., 2016; Eugster et al., 2004).

The en-bloc mode of membrane recruitment of coatomer contrasts with the stepwise membrane association of the other two archetypal coats, but the associated benefits or consequences of such concerted recruitment for COPI function are poorly understood.

Membrane recruitment of COPI and associated machinery

Formation of the COPI-coated vesicle begins with the recruitment of cytoplasmic coatomer to the membrane by the small GTPase Arf1 (see poster). Arf1 regulates the recruitment of both COPI and the clathrin-adaptor proteins AP-1, AP-3, AP-4 in addition to many other effectors

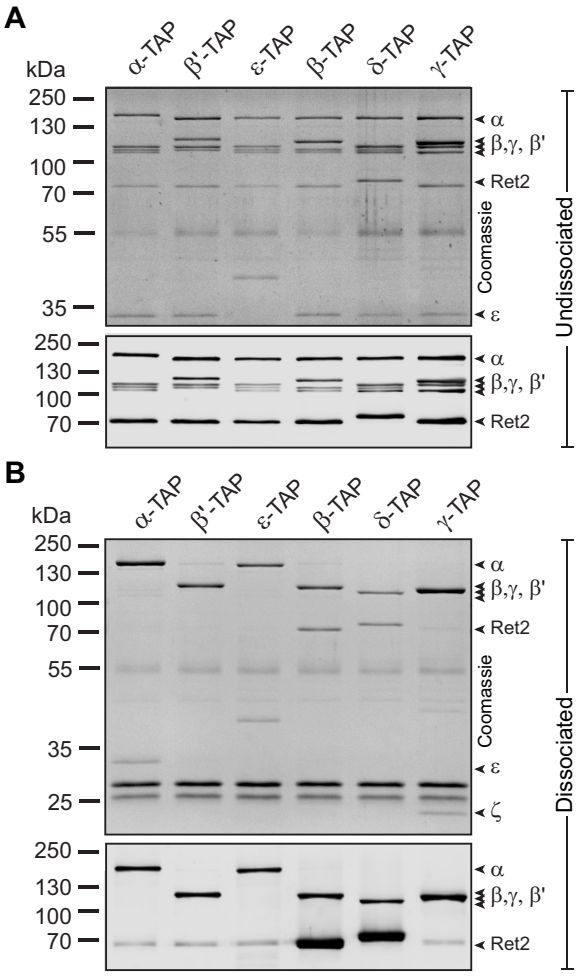

Fig. 1. The yeast coatomer is a stable heptamer in the cytosol. (A,B) Fusion of a tandem affinity purification (TAP) tag to a protein of interest enables the co-purification of interacting proteins. Here, six of seven coatomer subunits were C-terminally TAP-tagged to assess the co-purification of the other subunits within the heptamer in *S. cerevisiae*. TAP-tagging of ζ-COP is lethal in *S. cerevisiae*. Therefore, no purification using TAP-tagged ζ-COP is shown. The coatomer was TAP-purified from the cytosol. Matrix immobilized coatomer was incubated in (A) lysis buffer (10 mM sodium phosphate pH 7.4, 150 mM NaCl, 1 mM EDTA, 1.5 mM benzamidine, 1 mM PMSF/APMSF, complete protease inhibitor (Roche), and 5 μg/mL leupeptin/pepstatin) or (B) dissociation buffer (20 mM Tris pH 7.5, 250 mM $MgCl_2$, 2 mM EDTA, 1 mM DTT, 0.25% Triton X-100) for 1 h at 4°C. After 3 washes, proteins were eluted using tobacco etch virus (TEV) protease and analysed by SDS-PAGE. Proteins on the SDS gels were either stained directly using Coomassie Blue (top) or membrane-transferred and incubated with a coatomer-specific antibody (bottom). The residual calmodulin-binding domain of the TAP-tag causes an electrophoretic mobility shift of the tagged subunit. Purification followed by SDS-PAGE analysis of intact heptameric coatomer (A). Dissociation of purified coatomer followed by SDS-PAGE analysis (B). Following dissociation, coatomer exists as β'-COP and α-ε, β-δ and γ-ζ coatomer sub-complexes.

(Ren et al., 2013; Yu et al., 2012). Other members of the Arf-family have also been implicated in COPI function (Popoff et al., 2011).

cis- and trans-Golgi-localized guanine nucleotide exchange factors (GEFs) recruit and activate Arf1 by replacing GDP with GTP (Galindo et al., 2016; Gustafson and Fromme, 2017; Peyroche et al., 2001), which is catalysed by the conserved Sec7 domain (Mossessova et al., 1998). In its GTP-bound form, Arf1 inserts a myristoylated N-terminal amphipathic helix into the lipid bilayer (Antonny et al., 1997; Goldberg, 1998; Pasqualato et al., 2002). The

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Box 1. Open questions

The functional role of COPI within the cell has been dissected on the basis of both, *in vivo* approaches and rigorously controlled *in vitro* experiments. However, several interesting observations over the years demand a higher degree of scrutiny. For instance, fairly little is known regarding the biogenetic assembly and turnover of coatomer (i.e. how stoichiometric expression of the different subunits is achieved, which factors aid in the assembly and the turnover of the COPI heptamer).

Interesting discoveries, such as phosphorylation of the β- and δ- subunits of cytosolic coatomer (Sheff et al., 1996) have been overlooked. Whether they play a role in the reshaping of the COPI coat in a manner akin to AP2 is currently unknown (Höning et al., 2005; Ricotta et al., 2002). Future work investigating the role of phosphorylation, the kinases mediating such modification and, possibly, other mechanisms regulating accessory proteins that are instrumental in the biogenesis of COPI-coated vesicles, such as the ArfGEFs and ArfGAPs, are key in understanding the regulation of COPI (Miyamoto et al., 2008). Rab-GTPases have been shown to associate directly with the COPI coat (Arai et al., 2008). However, the precise role of this interaction also awaits further investigation. Another difficult-to-address, yet alluring, topic is the role of COPI in the maintenance of scattered Golgi complex observed in cells, such as cardiomyocytes (Arakel et al., 2014) and Golgi outposts – i.e. Golgi complex present in the dendrites of neurones in addition to the secretory pathway found in the main cell body – observed in neurons (Pierce et al., 2001). Even the nature of COPI-coated vesicles in the extensively characterized *S. cerevisiae* needs to be revisited in the context of the 'hug-and-kiss' model of cis-Golgi cargo capture and ER arrival sites (Kurokawa et al., 2014; Schröter et al., 2016).

The γ- and ζ-subunits of mammalian COPI are encoded by two genes, each of which are expressed in an overlapping manner in different tissues (Blagitko et al., 1999; Futatsumori et al., 2000). In evolutionary terms, it is puzzling why only the γ and ζ subunits would have been subjected to possibly the same selective pressure to diverge. Differential combinations of these isoforms create four isoforms of the COPI coat in mammals (Moelleken et al., 2007; Sahlmüller et al., 2011; Wegmann et al., 2004). Fairly little is known regarding whether these coats perform specialized roles within mammalian cells and whether ArfGAP2 and ArfGAP3 are coupled to and regulated in a differential manner by these coatomer isoforms. Scyl1, a protein that regulates Golgi complex morphology (Burman et al., 2010), specifically binds the γ2 appendage domain of COPI (Hamlin et al., 2014). Scyl1 also selectively links Arf4 to γ2-containing coatomer isoforms. Such isoform-specific interactions could assign specialized coatomer isoforms to unique tasks within the cell.

Elucidating how COPI function is adapted to changes in cellular homeostasis such as those during metabolic stress, to the unfolded protein response and to intracellular signaling will prove an important step towards understanding the physiological and pathological scope of COPI.

cis-Golgi-localized ArfGEF GBF1 interacts directly with the appendage domain of γ-COP, thereby spatially restricting coatomer in the vicinity of activated Arf1 (Deng et al., 2009).

Once activated, Arf1 remains membrane-associated. GTP-hydrolysis is critical for its dissociation from membranes. Therefore, GTPase-activating proteins (GAPs) play a pivotal role in its membrane release since Arf1 lacks inherent GTPase activity. Three mammalian ArfGAPs (ArfGAP1, ArfGAP2 and ArfGAP3) have been implicated in the regulation of COPI function (Weimer et al., 2008) and have been shown to interact with COPI-coated vesicles produced *in vitro* (Weimer et al., 2008; Yang et al., 2002). COPI catalyses the ArfGAP-dependent GTP hydrolysis of Arf1 (Ahmadian et al., 1997; Dodonova et al., 2017; Goldberg, 1999; Luo et al., 2009).

In *in vitro* reconstituted COPI-coated vesicles, ArfGAP2 was positioned near Arf1 that was in complex with γ-COP (hence termed γ-Arf) (Dodonova et al., 2017). In contrast, Arf1 bound to β-COP (β-Arf) does not recruit ArfGAP2. This structural elucidation of the two distinct environments occupied by Arf1 within the architecture of the COPI coat has major functional implications when viewed in conjunction with previous work on the ArfGAPs:

ArfGAP1 and its *S. cerevisiae* homologue Gcs1 contain an ArfGAP1 lipid-packing sensor (ALPS) motif (Bigay et al., 2005). The ALPS motif forms an amphipathic helix on curved membranes by sensing lipid packing and also stimulates ArfGAP1 activity, coupling GTP hydrolysis to increasing membrane curvature (Bigay et al., 2003; Mesmin et al., 2007). ArfGAP1 is recruited to liposomes in the absence of coatomer, whereas membrane-binding of ArfGAP2 and -3 is COPI-dependent (Kliouchnikov et al., 2008; Schindler et al., 2009; Weimer et al., 2008). The tryptophan-based δL-motif (Cosson et al., 1998) in ArfGAP1 binds δ-COP with low affinity (Rawet et al., 2010; Suckling et al., 2014). At the membrane, this interaction with δ-COP might help to orient ArfGAP1/Gcs1 within the assembled coat and stabilize it in the vicinity of the β-Arf.

ArfGAP2 and -3, and its *S. cerevisiae* homologue Glo3 lack an ALPS motif and are unlikely to be regulated by membrane curvature (Yahara et al., 2006). The appendage domain of γ-COP binds to ArfGAP2 and -3 with differential affinities (Frigerio et al., 2007; Watson et al., 2004). The strong binding of ArfGAP2 to coatomer raises the possibility that ArfGAP2/Glo3 is stably associated with cytosolic coatomer and is, hence, co-recruited to membranes (Eugster et al., 2000; Lewis et al., 2004; Watson et al., 2004), where it can be observed in association with γ-Arf (Dodonova et al., 2017). In consequence, the two distinct molecular environments of Arf1 in the COPI coat might translate to specific interactions with distinct ArfGAPs.

COPI, Arf1 and its regulators the ArfGEFs and ArfGAPs, are the fundamental factors of the cycle of COPI-coated vesicles. In fact, COPI and Arf1 are sufficient to form coated vesicles *in vitro* (Bremser et al., 1999; Spang et al., 1998). As cargo sorting is an important physiological rationale for vesicle formation, cargo proteins conceivably regulate the vesicle cycle.

Cargo recognition by COPI

Cargo recognition by COPI is largely mediated by sorting signals present on the cytoplasmic domains of retrieved proteins. Of these, the di-lysine KKxx and KxKxx motifs presented by many ER residents are best characterized. These motifs contact the propeller domain of α- or β'-COP that are ideally positioned adjacent to the surface of the membrane (Eugster et al., 2004; Jackson et al., 2012; Ma and Goldberg, 2013; Schroder-Kohne et al., 1998). A second site in the propeller domain of β'-COP also regulates recycling of the exocytic SNARE Snc1 by recognizing polyubiquitylated Snc1 (Xu et al., 2017). Arginine (R)-based ER retrieval signals (ϕRxR; in which ϕ represents any hydrophobic amino acid) are another class of sorting signals on the cytoplasmic domains of heteromultimeric proteins, such as channels and receptors (Zerangue et al., 1999, 2001). COPI plays an important role in the assembly-dependent quality control of such proteins, by preventing the cell-surface expression of unassembled, dysfunctional complexes. Partially assembled subunits expose Arg-based signals that can be masked by correct assembly, regulatory post-translational modifications or the recruitment of accessory proteins (Arakel et al., 2014; Kilisch et al., 2016; Schwappach et al., 2000; Yuan et al., 2003).

The p24 family of proteins are a well-characterized, albeit poorly understood, class of proteins observed in COPI-coated vesicles. Several studies have clearly implicated p24 proteins as key players both in the recruitment of coatomer and Arf1 during vesicle formation and luminal cargo sorting (Aguilera-Romero et al., 2008; Bonnon et al., 2010; Bremser et al., 1999; Fiedler et al., 1996;

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Gommel et al., 2001; Lanoix et al., 2001; Muñiz et al., 2000; Sohn et al., 1996). Curiously, simultaneous deletion of all eight members of the p24 family in *S. cerevisiae* does not affect cell viability (Springer et al., 2000), while knockout of just one member in the mouse is embryonically lethal (Denzel et al., 2000). The $FFxxBB(x)_n$ motif (in which B is a basic amino acid) that is displayed by p24 proteins on short cytosolic tails, binds two distinct sites in γ-COP (Béthune et al., 2006).

Some proteins, such as Rer1, Vps74, the Erv41-46 complex (Shibuya et al., 2015) and the KDEL receptor, bind COPI and serve as adaptors, mediating sorting of cargo into COPI-coated vesicles. Rer1 recognizes retrieval signals within the transmembrane domain of many proteins, while Vps74 – a member of the GOLPH3 family – recognizes sorting signals in the cytoplasmic domain of many Golgi-localized glycosyltransferases (Eckert et al., 2014; Sato et al., 2001; Tu et al., 2008, 2012). COPI-dependent retrieval of many luminal ER-resident chaperones is governed by a distal C-terminal signal – KDEL – that is recognized by the KDEL receptor in a pH-dependent manner (Munro and Pelham, 1987; Semenza et al., 1990). How COPI recognizes the KDEL receptor is still a matter of debate (Cabrera et al., 2003; Dominguez et al., 1998; Lee et al., 2005; Townsley et al., 1993).

Segments of six of the seven COPI subunits are membrane proximal and it is, therefore, conceivable that different subunits of COPI engage in cargo recognition. The diversity of cargo proteins with distinct, yet COPI-dependent, steady-state localizations – i.e. ER, ER-to-Golgi shuttle, Golgi or plasma membrane – raises the fundamental and unanswered question whether recognition of different cargo proteins by different coat subunits contributes to their steady-state localization or physiological function.

Interplay between cargo and the COPI coat

The importance of GTP-hydrolysis in cargo-sorting emerged from early *in vitro* reconstitution experiments using purified Golgi complex. Less cargo was packaged into vesicles generated by using non-hydrolysable GTP analogues or a GTP-locked Arf2, the constitutively active Arf1[Q71L], compared to those formed in the presence of GTP (Lanoix et al., 1999; Nickel et al., 1998; Pepperkok et al., 2000). Several models – in which cargo proteins have different effects on GAP activity – have been proposed to resolve this dynamic interplay of cargo-sorting and GTP hydrolysis.

Some sorting signals inhibit coatomer-dependent ArfGAP activity, invoking ‘discard’ or ‘productive’ states to explain the role of GTP hydrolysis in the formation of COPI-coated vesicles (Goldberg, 2000; Springer et al., 1999). This model proposes that, in the absence of sorting signals during the early stages of vesicle formation, the membrane association of COPI is volatile owing to the rapid GTP-hydrolysis of Arf1. However, GAP activity is decreased in the presence of sorting signals, affording COPI ample time to form polymers, thereby decreasing its dependence on Arf1-GTP for its stability. Such a kinetic-timer model delineates dedicated vesicle formation from unproductive cycles, in which coatomer binds to and dissociates from membranes. The model also explains the spatial specificity of COPI-coated vesicle formation by constraining it to regions where appropriate cargo is present (Goldberg, 2000; Springer et al., 1999). However, this model has been questioned on the basis that other sorting signals were observed to stimulate coatomer-dependent GAP activity (Luo and Randazzo, 2008; Luo et al., 2009).

Cargo proteins have also been shown to bind ArfGAPs, indicating that, in addition to their canonical role in GTP-hydrolysis, ArfGAPs operate as cargo-adaptors that sort proteins, such as SNARES, into coated vesicles (Aguilera-Romero et al., 2008; Aoe, 1997; Lee et al., 2005; Rein et al., 2002; Robinson et al., 2006; Schindler et al., 2009). Unsurprisingly, other models have been proposed to explain the complex interplay between ArfGAP activity, cargo sorting and vesicle formation (Bigay et al., 2005; Lee et al., 2005; Nie and Randazzo, 2006; Park et al., 2015; Spang et al., 2010).

Arf1 binds almost equivalent surfaces on both γ- and β-COP (Yu et al., 2012). This GTP-dependent interaction facilitates the membrane association of, otherwise cytoplasmic, coatomer and also confines COPI orientation relative to the membrane. In addition, a recently identified helix in δ-COP was shown to interact with activated Arf1 and to play a crucial role in cargo-retrieval (Arakel et al., 2016), suggesting that this interaction couples GTP-hydrolysis to cargo sorting through a conformational change in COPI (Dodonova et al., 2017). Whether this helix stabilizes COPI on membranes post-hydrolysis or regulates (either stimulates or inhibits) Arf1 GTPase activity is currently unknown.

Recruitment of cytoplasmic clathrin adaptor AP2 to membranes results in a large conformational change from a closed cytoplasmic to an open membrane-associated form. This transformation couples membrane recruitment to cargo sorting by freeing access to cargo-binding sites (Jackson et al., 2010). Membrane-associated COPI appears to be splayed out to a greater extent in a ‘hyper-open’ form, in comparison to AP2 (Dodonova et al., 2015). Recognition and binding to the peptide motifs present in the p24 family proteins is believed to induce a conformational change in COPI, suggesting conformational pliability (Langer et al., 2008; Reinhard et al., 1999). Early cryo-electron microscopy (cryo-EM) analysis of COPI purified from *S. cerevisiae*, demonstrated significant flexibility and characterized coatomer as consisting of a globular domain (B-subcomplex) and an extended domain (F-subcomplex) (Yip and Walz, 2011). However, recent work using recombinant human coatomer failed to observe any significant differences between membrane-associated and soluble coatomer (Wang et al., 2016).

It is currently unclear whether coatomer undergoes a conformational change upon membrane recruitment, whether binding to Arf1 effectuates such a rearrangement, and in which order Arf1 GTP hydrolysis and cargo-recognition are executed. Further work is needed to elucidate the hierarchy of COPI recruitment and productive membrane association that is coupled to the recognition of short peptide motifs presented by proteins, such as the p24 proteins.

Organization of the COPI lattice

COPI coat polymerization is distinct from the assembly of the COPII coat and the clathrin-adaptor coat that both occur in two stages – first being mediated by elements of the inner coat and then by the outer cage (Faini et al., 2013). COPI does not form an extensive cage-like lattice but, instead, creates clusters of flexibly linked units (Faini et al., 2012).

The COPI triad (see poster), consisting of three heptamers, represents the symmetric basic unit of the coat (Dodonova et al., 2015). Triads are linked by flexible domains forming either trimeric or dimeric interactions, depending on their position within the two-dimensional coat array. The corresponding COPI linkage patterns have been grouped into four types, I–IV (Dodonova et al., 2015, 2017). A combination of such three-fold and two-fold symmetries facilitates the assembly of a curved lattice. The flexible C-termini of α and ε-COP mediate the central contacts in linkage type I and IV, whereas the μHD of δ-COP mediates those in linkage type II (Dodonova et al., 2017). Curiously, simultaneous deletion of

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ε-COP and μHD did not adversely affect the viability of *S. cerevisiae*, suggesting that other subunits in addition mediate coat polymerization (Arakel et al., 2016). By altering the angle between adjacent triads, the COPI coat can accommodate vesicles of varying sizes and curvature to include diverse cargo (Faini et al., 2012).

The polymerizing coat generates positive membrane curvature. Membrane deformation is aided by all components of the COPI coat. Arf1-GTP alone (Beck et al., 2008; Krauss et al., 2008; Wang et al., 2016) or coatomer alone (Wang et al., 2016) tubulate synthetic liposomes. A combination of both results in vesicle formation (Beck et al., 2008; Krauss et al., 2008; Wang et al., 2016), with altering vesicle size depending on the presence or absence of ArfGAP1 (Wang et al., 2016). The properties of membrane lipids affect different stages in vesicle formation, such as membrane deformation, cargo sorting and fission. Lipids that form liquid-ordered phases, such as sphingomyelin and cholesterol, and are abundant at the plasma membrane, are excluded from COPI-coated vesicles (Brügger et al., 2000). In *in vitro* reconstituted systems, Arf1-GTP and COPI assemble on liquid-disordered domains, and protect them from undergoing phase separation, suggesting that COPI assists lipid sorting during coat polymerization (Manneville et al., 2008).

By playing a vital role in the sorting of proteins and, crucially, lipids, COPI alters the compositional characteristics of the different Golgi cisternae. This, in turn, is likely to affect the sorting and membrane deformation properties of COPI.

Scission of COPI-coated vesicles

COPI polymerization ultimately results in vesicle fission at the neck, where membrane curvature is negative. GTPases, such as dynamin and Sar1, play an important role in clathrin- and COPII-coated vesicle scission, respectively (Lee et al., 2004b; Sweitzer and Hinshaw, 1998). Likewise, Arf1 has been ascribed a key role in the fission of COPI-coated vesicles (Beck et al., 2011). Curiously, scission of COPI-coated vesicles from membranes depends on the ability of Arf1 to oligomerize (Beck et al., 2008, 2011). A dimerization-deficient mutant of Arf1 recruits coatomer to membranes *in vitro*, without inducing the liberation of free vesicles. The precise means by which Arf1 drives vesicle scission is poorly defined. Notably, vesicle release from the donor compartment is independent of GTP hydrolysis (Adolf et al., 2013). The observation of a bud scar would also suggest that the polymerization of the COPI coat need not proceed to completion in order to form a fully closed cage (Faini et al., 2012). Several other factors have additionally been implicated in the fission step of COPI-coated vesicle formation, such as acyl-COA, CtBP/BARS, PLD2 and its product phosphatidic acid, as well as diacyl-glycerol (DAG) – which can be phosphorylated by the DAG kinase to generate phosphatidic acid (Fernández-Ulibarri et al., 2007; Ostermann et al., 1993; Yang et al., 2005, 2008). Given the ability of the minimal machinery to enable scission, it is likely that this variety of auxiliary factors performs a regulatory role *in vivo*.

The role of the coat on a coated vesicle becomes superfluous post-scission, having achieved membrane deformation, cargo-sorting and separation from the donor compartment. Vesicle uncoating is typically considered a pre-requisite to engagement and fusion with the acceptor membrane, presumably by facilitating access to the fusion machinery, such as SNAREs, on the vesicle surface.

Uncoating of COPI-coated vesicles

Following vesicle scission, COPI-coated vesicles proceed to uncoat. Although the role of GTP-hydrolysis in this process is undisputed, the exact timing of GTP hydrolysis on Arf1 is ill-defined. A GTP-locked form of Arf1[Q71L] was observed to prevent release of COPI from the membrane (Presley et al., 2002). Likewise, *in vitro* reconstitution assays in the presence of non-hydrolysable GTP analogues result in a similar outcome (Tanigawa et al., 1993). This highlights the importance of the GTP-hydrolysis of Arf1 in vesicle uncoating. A number of studies investigating the dwell time of coatomer and Arf1 on membranes concluded that the half-lives of COPI and Arf1 on membranes are different (Liu et al., 2005; Presley et al., 2002; Yang et al., 2002). This culminated in a model where polymerized COPI remains, due to interactions with cargo and lipids, stable on membranes – even after GTP-hydrolysis and dissociation of Arf1 from membranes. However, a recent study elucidating the structure of the COPI coat *in situ* in *Chlamydomonas reinhardtii* cells (Bykov et al., 2017) determined that the stoichiometry of Arf1 and COPI was unaltered in vesicles of different stages of disassembly. This, essentially, suggests that vesicle uncoating is not cataclysmic, and that dissociation of Arf1 and COPI is linked and, possibly, synchronous.

With recent advances in elucidating the structure of COPI at the membrane and the opportunity to position distinct ArfGAPs within specific niches of the coat lattice (ArfGAP1-βArf1 and ArfGAP2-γArf1) (Dodonova et al., 2017), it is tempting to speculate that GTP hydrolysis occurs in two stages: (i) a curvature-independent manner at the center of a triad regulated by ArfGAP2 and, (ii) a curvature-dependent manner at the periphery of a triad regulated by ArfGAP1. Hence, GTP hydrolysis on all resident Arf1 molecules within the coat and subsequent coat disassembly occurs only after a critical threshold in membrane curvature is surpassed, possibly when a vesicle nears the state of scission. It has been proposed that ArfGAP2 activity is triggered only after it docks into its recess in the assembling coat (Dodonova et al., 2017). However, this does not preclude functionality early in coat assembly. Likewise, ArfGAP1 whose activity is linked to membrane curvature might be additionally regulated by cargo. Therefore, a key question to resolve is at which point and order in the vesicle cycle each ArfGAP exerts its activity.

Taken together, current results imply that the two ArfGAPs serve functionally unique roles. It is, therefore, surprising that in both yeast and mammalian cells, individual ArfGAPs are non-essential, demonstrating a certain degree of overlapping function. It is unclear how one ArfGAP would physically supplant the other, deleted subtype with regard to its specific targeting elements and niches within the framework of the coat. Combined deletion or knockdown of both sub-types (ArfGAP1, and ArfGAP2 and -3) critically affects cell viability of yeast and mammalian cells (Frigerio et al., 2007; Kartberg et al., 2010; Poon et al., 1999). Future studies on cargo-sorting and vesicle uncoating in the absence of either ArfGAP will prove instrumental in elucidating the true nature of their functional roles.

Tethers cooperating with COPI

Specificity in the final phase of vesicle trafficking is furnished by vesicle tethers localized on the target organelles. Tethers are either large proteins or multi-subunit assemblies that recognize and bind transport vesicles. Tethering can be mediated through direct interaction with the coat or through factors, such as SNAREs and Rab GTPases (Cai et al., 2007). Various tethers that mediate intra-Golgi trafficking, such as p115 (Uso1) (Guo et al., 2008), the trafficking protein particle II (TRAPPII) complex (Yamasaki et al., 2009) and the conserved oligomeric Golgi (COG) complex (Miller et al., 2013) have been shown to bind the COPI coat. By recognizing

specific elements that confer identity to these coated vesicles, possibly in combinations that allow for coincidence detection, these tethers target distinct types of COPI-coated vesicle for fusion with specific cisternae within the Golgi (Malsam et al., 2005). COPI-coated vesicles involved in the retrograde transport of cargo from the Golgi to the ER are catered to by the ER-associated Dsl1-tethering complex (Andag and Schmitt, 2003; Ren et al., 2009; Suckling et al., 2014; Zink et al., 2009).

Taken together, and if our current view of the function of tethers is accurate, vesicle uncoating is likely to be incomplete, leaving residual COPI on the vesicle surface to enable vesicle recognition and tethering. Such tethering of both partially COPI-coated and non-coated vesicles to Golgi cisternae has been observed by electron microscopy in Golgi fractions purified from Chinese hamster ovary cells (Orci et al., 1998).

Conclusions

Despite being the subject of exhaustive research, COPI has remained enigmatic since its discovery (Balch et al., 1984; Malhotra et al., 1989; Orci et al., 1986; Serafini et al., 1991; Waters et al., 1991), and insights into its structure have lagged behind those of clathrin and COPII. Methodological advances have, recently, enabled the field to explore the functional characterization of defined elements within the COPI coat on the basis of molecular structure. Such directed approaches may shed light on mechanisms fundamental to coat function, for instance the identification of subunit-specific cargo recognition sites and of binding sites for accessory components, or a redefinition of the interplay between GTP hydrolysis and specific stages of the vesicle cycle.

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Cell science at a glance

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