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The life cycle of a transport vesicle

A. Spang

Biozentrum, Growth and Development, University of Basel, Klingelbergstrasse 70, 4056 Basel (Switzerland), e-mail: anne.spang@unibas.ch, Fax: +41 61 267 2148

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Abstract. Vesicular transport is the basic communication mechanism between different compartments in a cell and with the environment. In this review I discuss the principles of vesicle generation and consumption with particular emphasis on the different types of coat proteins and the timing of the shedding of the coat proteins from transport containers. In recent years it has become clear that there are more coat complexes than the classical COPI, COPII and clathrin coats. These additional coats may generate vesicles that transport cargo in a temporally and/or spatially controlled manner. Work over the last years suggests that GTP hydrolysis occurs early during vesicle biogenesis, destabilizing the coat perhaps before fission of the vesicle from the donor membrane occurs. Recent findings imply, however, that tethers at the receiving compartment specifically detect the coat on vesicle. (Part of a Multi-author Review)

Keywords. Vesicle biogenesis, small GTPases, coat proteins, SNAREs, Arf1, coatomer, adaptor complex, clathrin, COPII, COPI, Sar1.

Why is vesicular transport important?

Communication between both diverse membrane-bounded organelles within a cell and between different cells is dependent on vesicle transport. Transport vesicles supply the various organelles with their appropriate protein and lipid composition, and deliver protein, lipids and signaling molecules to the plasma membrane and the intercellular space. The cell must tightly regulate the rate of delivery to, and removal from, individual organelles. For example, if there were more outgoing than incoming vesicles at a particular organelle, the compartment would disappear over time [1]. Likewise, if an organelle received too many vesicles, it would grow too big, most likely at the expense of other organelles. Furthermore, the inappropriate delivery of cargo and membranes may even change the organelle identity and interfere with its proper function. The importance of intracellular transport at the level of organ physiology is also highlighted by the fact that there are numerous mutations in components of the vesicular transport machinery that lead to disease in humans [2–8]. Significantly, most of these mutations only partially impair the function of the encoded protein, because a complete loss of function would lead to embryonic lethality.

A central dogma in vesicular transport is that the process is vectorial; a vesicle generated from a donor compartment will only fuse with the cognate acceptor membrane. This recognition process at the acceptor compartment involves SNAREs on the vesicle and the receiving membrane, small GTPases of the rab family, and tethering factors.

The common budding mechanism of a transport vesicle

How is a transport vesicle generated from a donor membrane? All the different players involved in the budding mechanism have to be present: an activated small GTPase of the ARF/SAR family; cargo which needs to be transported; coat proteins that are stabilized on the membrane via interaction with the small GTPase and cargo; and finally SNARE proteins, which ensure that the vesicle reaches the correct target compartment. Springer et al. [9] proposed the for-

mation of 'primers' that would serve as the initiation point for the vesicle budding process. These primers would consist of a v-SNARE, the small GTPase of the ARF/SAR family and coat components. The lifetime of such a primer would be limited, but if, during its lifetime, enough cargo were available for transport, primer stabilization and coat polymerization would occur. This model also provides an explanation for why SNAREs are included with high fidelity in each transport vesicle. The importance of cargo incorporation into nascent vesicles was for many years an unappreciated part of vesicle biogenesis. The finding that coated vesicles could be formed *in vitro* just by the addition of coat components plus Sar1 or Arf1 and guanine nucleotide [10–12], put cargo into the back seat, so to speak. Remarkably, these vesicles had the same size as those generated *in vivo*, and the appearance of the coat seemed to be indistinguishable from that of *in vivo* coats. Therefore, the coat proteins must intrinsically contain size information.

Nonetheless, in recent years it has become clear that cargo plays a rather active role in vesicle biogenesis. For example, in another *in vitro* system using synthetic liposomes, COPI vesicles were only formed when a lipopeptide exposing the tail of p24, which encodes a coatomer binding site, was included into the lipid mixture [13]. The founding member of the p24 family was identified as major component of Golgi-derived COPI vesicles [14]. Furthermore, recently evidence was provided that the p24 family members play an active role in the generation of COPI-coated vesicles at the cis-Golgi [15]. A mechanism was suggested by which the formation of p24 oligomers would enhance the binding of coatomer to membranes and hence facilitate the formation of primers [16]. Cargo may thus be important to stabilize coatomer on membranes during vesicle biogenesis. Moreover, overexpression of cargo proteins can rescue mutants in coat components [17]. Cargo proteins could, by stabilizing priming complexes, modulate ArfGAP activity [18,19]. Binding of coat components would deform the membrane, and this change in curvature could be sensed by ArfGAP1 [20,21]. As a result, Arf1 would hydrolyze GTP and increase the amount of cargo which can be included into vesicles [22,23]. Whether this scenario is generally true is still under debate, since only one of the ArfGAPs contains this curvature-sensing motif. Finally, cargo can influence the dynamic of coated pits [24]. Interestingly, the nature of the cargo can even change the morphology of transport carriers. Procollagen is too large to fit into a common COPII vesicle, so the nascent vesicle extends, and a 'rod' is formed that carries procollagen to the Golgi apparatus [25–27]. Hence, the coat is rigid enough to deform the membrane and flexible enough to accommodate a variety of cargo sizes [28–30].

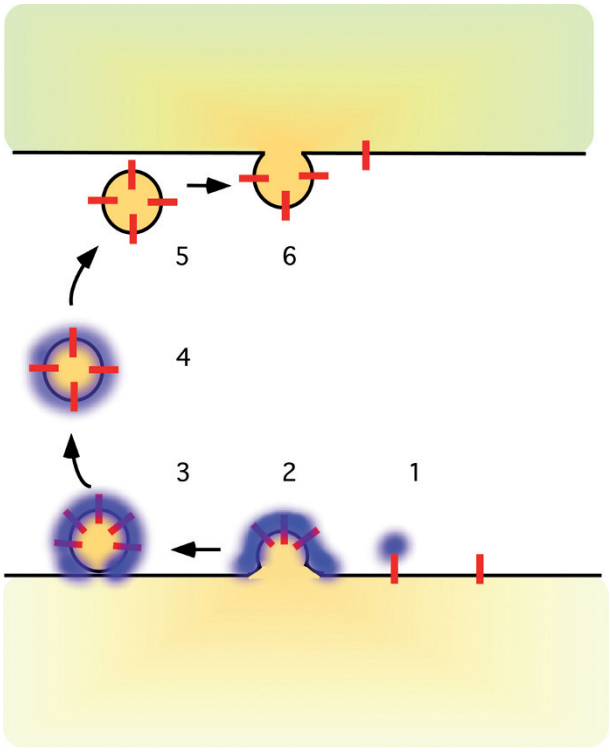

Figure 1. The life cycle of a transport vesicle. 1. Primer formation. Cargo or a SNARE protein interacts with coat components. 2. Deformation of the membrane and coat polymerization. If several of these primers can be formed, the coat can polymerize, more cargo is incorporated, and the membrane is deformed by the polymerizing coat. 3. Budding of the vesicle. The coat covers the entire transport vesicle and either with the help of the coat itself or an additional GTPase of the dynamin family, the connection between vesicle and donor membrane is severed. 4. Destabilization of the coat. Towards the end of the budding phase, during transport or prior to the arrival of the transport vesicle at the target membrane, the coat must be destabilized or probably partially lost. 5. Tethering and docking. The transport vesicle arrives at the target membrane. An initial recognition process involving tethers and coat proteins occurs, leading to the loss of the reminiscent coat complexes and the docking to the membrane. 6. Fusion. The second recognition step involves cognate vesicle SNARE and target membrane SNARE interaction. The SNAREs zipper up, and the vesicle membrane comes into close contact with the target membrane, yielding into spontaneous fusion of the lipid bilayers through a hemi-fusion intermediate. Content and lipid mixing occurs as the vesicle ceases to exist.

The major coats involved in vesicle biogenesis

COPII

The COPII coat is probably the best-studied, since the crystal structure all of its constituents are known and even complexes of different members of the COPII coat have been solved [29,31–35]. How is this coat formed? First, the small GTPase Sar1 is recruited to

the endoplasmic reticulum (ER) membrane by the guanine nucleotide exchange factor Sec12. Sar1 associates only with ER membranes because Sec12 is an ER-resident, transmembrane-anchored GEF. Unlike most other small GTPases, Sar1 does not possess a lipid modification that facilitates membrane association; instead, a hydrophobic patch, which is exposed in the Sar1-GTP form, suffices for membrane association. Sar1 itself has apparently a built-in membrane deformation device: the amphiphatic helix mediating membrane attachment is necessary and sufficient to deform liposomes into membrane tubes [36,37]. However, whether this ability to deform membranes is essential for vesicle biogenesis *in vivo* remains unknown.

After the binding of Sar1 to the ER membrane, the GTPase activating protein (GAP) Sec23, and the cargo recruiter Sec24, form a complex with Sar1, cargo, and the ER membrane. While there is only one gene for Sec23, four different Sec24 isoforms are present in mammals, and three different Sec24/Sec24-like genes are encoded in the yeast genome [38-41], suggesting that different types of cargo might associate with different Sec24 recruiters. However, in yeast no cargo has been identified to date which specifically requires only one of the two isoforms Lst1 or Iss1 for export of the ER, and no vesicles have been found that contain only Iss1 or Lst1 [40,41]. Still, the plasma membrane ATPase is a cargo that requires both Lst1 and Sec24 for efficient packaging into COPII vesicles [41,42]. In mammals the different isoforms mediate transport of subsets of cargo [5,38,43,44]. Finally, a second subcomplex, Sec13/31, binds to the membrane and to the cargo-associated Sec23/24 complex. The Sec13/31 complex helps to deform the membrane and to stabilize the polymerizing coat. Sec13/31 can even assemble into a cage-like form in the absence of the other components of the COPII coat [29]. The order of the assembly of the coat is critical, since lack of one of the components aborts vesicle formation at a distinct step [10,45,46].

COPII vesicle formation is restricted not only by the amount of available cargo (if this is a restriction at all at the ER) but also by spatial constraints. Vesicles cannot form randomly all over the ER; rather, they form at discrete locales – the ER exit sites (ERES) – and these sites are marked by Sec12. In *Pichia pastoris* and mammalian cells, the Golgi apparatus and the ER-Golgi intermediate compartment (ERGIC), respectively, are juxtaposed to the ERES, indicating that ER-derived COPII vesicles do not travel long distances to their acceptor compartment [47–51].

COPI

The COPI coat is organized by the Arf1 GTPase instead of Sar1. However, unlike the case of Sar1, Arf1 can induce vesicle formation at multiple distinct membranes along the secretory pathway, and it can recruit both coatomer and adaptor complexes of the clathrin coat. A variety of guanine nucleotide exchange factors (GEFs) are involved in the recruitment of Arf1 to different membranes. All GEFs have in common a specific domain, the Sec7 domain, where the exchange activity resides. GEFs come in two different flavors: those that are sensitive to the fungal metabolite brefeldin A (BFA) and those that are not [52,53]. Although the biological significance of the sensitivity to BFA remains elusive, it has allowed to study the consequences for a cell after loss of function of a subset of GEFs. Hence, in the case of Arf1 interaction with a particular GEF could determine both activation and identify the membrane to which attachment takes places. Whether localizing a GEF to a membrane is sufficient for catalyzing the exchange reaction remains unclear since at least some GEFs are phosphorylated [54,55]. Thus, phosphorylation may play a regulatory role for the exchange reaction or help to determine specificity, as activation of other Arf and Arf-like GTPases requires GEFs of the Sec7 family of exchange factors. Furthermore, some GEFs stimulate activation of Arf1 at different membranes [56–59]. Finally, numerous GEFs catalyze nucleotide exchange on different members of the ARF superfamily [60], which makes it unlikely that recruitment to membranes of Arf1 by GEFs is the major determining factor, which regulates Arf1 activation at organelles.

Another way to localize Arf1 specifically to different membranes may be encoded in Arf1 itself, since a 16-amino acid sequence in Arf1 is necessary for interaction with the ER-Golgi SNARE membrin. Mutations in this localization motif still allow recruitment to the trans-Golgi, while association with the cis-Golgi is lost [61], indicating that SNARE/cargo may play an important role in recruiting Arf1 to membranes.

Once Arf1 is activated and membrane-bound, it is able to interact with ArfGAP and cargo/SNARE proteins. The GAP will not efficiently stimulate GTP hydrolysis at this point, but rather mediates the interaction with SNARE proteins by inducing a conformational change on the SNAREs that promotes the direct interaction with Arf to form a primer, which also includes the heptameric protein complex termed coatomer [62–64]. Interestingly, interaction of Arf1 with the SNAREs *in vitro* does not necessitate the activation of Arf1. Whether a conformational change in membrin is required for the recognition of the binding motif in Arf1 remains to be established.

Similarly to the case of Sec23 and the COPII coat, the ArfGAP is an intrinsic component of the COPI coat [65–67], and it is likely that GTP hydrolysis and coat stability are regulated in a similar manner. In fact, two lipid-packaging sensor motifs in mammalian ArfGAP1 have been identified, which couple COPI coat disassembly to membrane bilayer curvature [21,68]. Despite the ability of Arf1 to interact directly with SNAREs and mediate their inclusion into vesicles, the main cargo recruiter is coatomer, which acts to deform the membrane. At least three of the seven coatomer subunits are involved in cargo recognition (beta-, gamma-, delta-COP), and bind to different motifs [69–71]. Interestingly, some cargoes may negatively regulate coatomer-dependent GTP hydrolysis by Arf1 [18].

Hence, formation of COPI- and COPII-coated vesicles follows similar principles. Whether coatomer can form cages similar to the Sec13/31 complex remains to be shown. Perhaps this is not to be expected, since the overall structure (by modeling) of coatomer seems to be more closely related to the adaptor complexes of the clathrin coat than to Sec23/24 and Sec13/31.

Clathrin

Clathrin-coated vesicles can form at different compartments in the late secretory and in the endocytic pathway: the trans-Golgi network, endosomes and the plasma membrane. Cargo recognition and recruitment are mediated by so-called adaptor complexes (AP1–4) as well as adaptor-like complexes referred to as GGAs. Like COPI, the formation of clathrin-coated vesicles requires the small GTPase Arf1. Arf1 functions together with the adaptor complexes AP1, AP3, AP4 and the GGAs to generate clathrin-coated vesicles at the trans-Golgi [72]. A distinct adaptor, AP2, is involved in receptor-mediated endocytosis at the plasma membrane. AP1 and AP3 are also involved in the formation of clathrin-coated vesicles at endosomes. The use of different adaptor complexes from the same compartment suggests that there is a distinct cargo-sorting step perhaps at the trans-Golgi. AP1 may be responsible for a transport shuttle between the trans-Golgi and endosomes, while AP3 promotes the delivery of proteins to lysosomes, and AP4 may mark a direct pathway from the Golgi to the plasma membrane. Indeed, Harsay and co-workers [73,74] were the first to postulate that there is more than one transport route to the plasma membrane, because they could isolate vesicles of different buoyant densities and different cargo content from a mutant in which the fusion of transport vesicles with the plasma membrane was defective. The clathrin coat itself consists of an assembly of clathrin triskelions, which are formed from three clathrin heavy and clathrin light chains and which looks very reminiscent of a football (soccer ball).

How many coats does a cell need?

Three major coats have been identified to date. The clathrin coat can function in distinct vesicle transport steps by using various adaptors (AP-1 through AP-4 and the GGAs) [72], and there are two coatomer complexes – one at different levels of the Golgi and one on endosomes [75–77]. In addition, recent evidence points to the existence of other possible coats that mediate the transport of specific cargoes. In yeast, specialized vesicles deliver the chitin synthase Chs3 and possibly other proteins to the bud neck region. This transport is dependent on Arf1p and a protein complex called exomer [78–80]. Exomer components interact directly with Arf1 and with the cargo Chs3. Disruption of exomer function causes Chs3 to accumulate in the trans-Golgi [78,80]. However, the final proof that exomer is a coat is still missing. Yet, the identification of this novel potential coat raises the question how many vesicle coats are required in the cell. Clearly, the major traffic routes in various organisms have already been identified, and the coat proteins are well established. Furthermore, the components of the various major coats are well conserved in all eukaryotes. However, different cells have different needs and may require the delivery of specialized cargo at distinct times to a specific locale in the cell. The traffic of the chitin synthase Chs3 to the bud neck in *Saccharomyces cerevisiae* could serve as a model for this process [78–80]. Specialized transport is by no means a yeast specific problem, however: protein delivery to the tight junctions in epithelial cells is also likely to be regulated by a specialized transport route. Although in this case nothing is known about the coat, at least one of the cargoes – occludin – and part of the tethering machinery – Rab13 and its effector JRAB – are known [81–84]. Furthermore, it remains an unsolved issue which vesicle populations are required for membrane insertion during cytokinesis in various cells, and to spatially restricted areas during cellularization in *Drosophila*. Moreover, endocytosis is not only mediated by clathrin but also by at least two clathrin-independent pathways employing either the transmembrane protein caveolin or the soluble flotillins. Two flotillins, flotillin1 and flotillin2, can associate with each other and assemble into lipid microdomains and have then the ability to deform membranes [85]. Interestingly, the clathrin-independent pathways do not necessarily utilize small GTPases of the SAR/ARF family. The flotillin pathway seems

to be regulated by the Rho GTPase Cdc42 [86,87], while caveolin-dependent endocytosis employs Rab5 and dynamin. Still, it remains unclear how vesicle formation is orchestrated by in these cases. Taken together, these findings allow us to speculate that there could be a plenitude of different types of vesicles transporting special cargo in a temporally and spatially controlled manner. We propose that the COPI, COPII and clathrin coats would help to generate the vesicles for the major transport routes, the superhighways, while the specialized coats would use minor routes, the backroads.

When does the coat come off?

Two unanswered questions in the field concern the time when GTP hydrolysis occurs, and when the coat is shed. Clearly the coat is held together by the GTPase in the active, membrane-associated, GTP-bound form, but what triggers GTP hydrolysis and coat destabilization? Work from the groups of Bruno Antonny and Jonathan Goldberg has provided good evidence that membrane curvature plays an essential role in stimulating GTP hydrolysis. They showed that ArfGAP would only efficiently act on Arf1 when the correct membrane curvature was presented and coatomer was bound to the complex, indicating that GTP hydrolysis may already occur during the formation of a transport carrier [20,21,88]. In support of these findings, several groups reported that GTP hydrolysis by Arf1 was also required to efficiently package cargo into COPI vesicles [19,23,89,90]. Vesicles generated in the presence of the non-hydrolyzable GTP-γ-S would contain less cargo than similar vesicles formed with Arf-GTP. Arf1 would leave the nascent vesicle, while coatomer would still remain associated with the vesicle stabilized by interaction with cargo proteins. This metastable coat would be shed during transport of the vesicle to the acceptor organelle or at the acceptor organelle triggered by the interaction with tethers. This model solves the problem about the timing of GTP hydrolysis and explains why one can isolate coated vesicles from cells.

But is this model generally applicable or is it only valid for COPI-coated vesicles? Like the COPI coat, COPII contains an intrinsic GAP, Sec23, and GTPase hydrolysis by Sar1 at the ER may also be required to incorporate more cargo into COPII vesicles. Therefore, a similar process may also be in place for COPII vesicles. Much less information is available about clathrin-coated vesicles or vesicles that use yet different coats or mechanisms for their biogenesis.

Interestingly, components of the COPI and of the COPII coat interact with tethering factors at the recipient membrane. The Sec23/24 complex binds to Uso1 and components of the TRAPP complex [91,92]. Coatomer interacts with the DSL complex at the ER [93,94], and Tip20, a component of the DSL complex, can discriminate between COPI- and COPII-coated vesicles [95]. Furthermore, Sec18/NSF is able to displace Arf1 from SNARE proteins *in vitro*, suggesting that Sec18/NSF checks whether the SNAREs are free to engage in a trans-SNARE complex at the target membrane [64]. This finding implies that at least some Arf1 is still present on COPI-coated vesicles once they reach the target organelle.

These results then lead to the question of how stable a meta-stable coat is. After budding off the donor compartment, the vesicle will encounter obstacles in the cytoplasm, because the cell is a crowded place. In the cytoplasm, 40 % of the total mass is contributed by dissolved macromolecules like proteins, lipids, nucleic acids and sugars [96,97]. Furthermore, the various membrane-bounded organelles represent large, relatively static obstacles to transport vesicles. And even when transported on microtubules by motor proteins, the coated vesicles will encounter friction, which the coat must be able to resist. A destabilized coat may not be able to preserve the coat structure in such an environment. As a consequence, the coat is either not destabilized by GTP hydrolysis, or at least some GTP hydrolysis occurs only at the target membrane upon interaction with tethering factors. We favor the latter possibility in which not all Arf-GTP is inactivated during the budding process. But how would only a part of the Arf1 be selectively inactivated during vesicle generation? Perhaps ArfGAP cannot always stimulate GTPase activity. Since ArfGAPs are phosphoproteins ([98,99] and Christina Schindler, Mark Trautwein and Anne Spang, unpublished observations), one possibility is that only one form, e.g., the dephosphorylated form, has a high GAP activity, while the other form – the phosphorylated form – may be less active. Another possibility is that ArfGAP in contact with cargo (as compared to being in a complex with Arf1 and coatomer) does not drive GTP hydrolysis.

An alternative mechanism is that all GTP is hydrolyzed and no GTPase is associated with the nascent vesicle. The coat would be stably bound to the vesicle by coat-cargo, coat-coat and coat-lipid interactions. Upon encountering the tethers at the target membrane, a conformational change would then be induced in the coat subunits, which would subsequently drive disassembly of the coat. A major function of tethers in this scenario would be not so much to tether the vesicle to the membrane but rather to induce shedding of the coat.

Some of these questions and possibilities are testable with currently available methods, and hopefully we will have soon a better understanding about the shedding of coats.

Coat-independent vesicle formation

Some endocytic events, like micropinocytosis and fluid phase uptake, do not depend on the classical coat assembly pathway to deform the membrane.

The exact mechanism of clathrin-independent endocytosis is not well understood. To date two clathrin-independent endocytosis pathways have been reported, one involving caveolin and the other flotillin. Flotillins may be able to associate to membranes similar to the classical coats. In contrast, caveolin contains a trans-membrane domain and is only 22 kD. Hence it seems unlikely that it represents a coat, though it could still be part of a yet unidentified larger complex. Caveolin enriches in special cholesterol-rich membrane domains and helps to drive invagination of these domains. Pinching off of these invaginations – so-called caveolae – is driven by dynamins. Dynamins are also essential in pinching off clathrin-coated vesicles from the plasma membrane. Recently, the endocytic pathway requiring caveolin was suggested to be controlled by integrin-mediated adhesion [100–102].

Another type of coat-independent budding is the generation of multivesicular bodies (MVBs). Endosomes sort cargo that has to go back to the plasma membrane from cargo that will be degraded in the lysosome. An entire machinery in the cell consisting of ESCRTI-III and ubiquitylation-deubiquitylation proteins is devoted to sorting proteins into the degradation pathway such that parts of the endosomes bud inwardly to form MVBs, which then fuse with lysosomes. The vesicles in MVBs are of roughly uniform size, and despite intensive efforts from various laboratories, no coat that promotes the inward deformation of the endosomal membranes has been identified. Thus, the mechanism by which the vesicles are formed in MVBs remains elusive.

The timing of vesicle generation

We have very little understanding of the time frame in which vesicles are formed. The major problem is that we still fail to detect single vesicles efficiently, and even if we can detect them, these methods usually employ cargo which is ectopically expressed and which as a result may interfere with the kinetics of vesicle generation. In addition we may only look at a subclass of vesicles in a certain pathway. Therefore, we will still need to develop more sensitive methods that would allow us to improve the spatial and temporal resolution under conditions where we can look at endogenous cargo. Receptor-mediated endocytosis is the event which is mostly studied at present [103,104] because it is more tractable then transport steps along the biosynthetic pathway.

Conclusions

A few years ago, the membrane traffic field seemed to have ended its exponential growth phase and entered into a stationary phase. Some of us really thought we understood the process of vesicle generation and its regulation, and that there would be nothing left to discover but the details. It is true that since the pioneering work of the Rothman and Schekman groups in the eighties available knowledge about vesicle transport has increased tremendously. However, we are still faced with important questions concerning the timing of vesicle formation, the number of different types of vesicles present in a cell, how vesicle targeting occurs, whether vesicle targeting is really a trial-and-error mechanism (with the vesicle wandering around until it finds the correct target) or whether all vesicles are transported in a directed manner.

The membrane traffic field has no reason to be depressed: there is plenty of work ahead of us, and new principles await discovery!

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1 Saraste, J. and Goud, B. (2007) Functional symmetry of endomembranes. Mol. Biol. Cell 18, 1430–1436.
2 Huizing, M., Anikster, Y. and Gahl, W. A. (2000) Hermansky-Pudlak syndrome and related disorders of organelle formation. Traffic 1, 823–835.
3 Jenkins, D., Seelow, D., Jehee, F. S., Perlyn, C. A., Alonso, L. G., Bueno, D. F., Donnai, D., Josifova, D., Mathijssen, I. M., Morton, J. E., Orstavik, K. H., Sweeney, E., Wall, S. A., Marsh, J. L., Nurnberg, P., Passos-Bueno, M. R. and Wilkie, A. O. (2007) RAB23 mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity. Am. J. Hum. Genet. 80, 1162–1170.
4 Kins, S., Lauther, N., Szodorai, A. and Beyreuther, K. (2006) Subcellular trafficking of the amyloid precursor protein gene family and its pathogenic role in Alzheimer's disease. Neurodegener. Dis. 3, 218–226.
5 Fromme, J. C., Ravazzola, M., Hamamoto, S., Al-Balwi, M., Eyaid, W., Boyadjiev, S. A., Cosson, P., Schekman, R. and Orci, L. (2007) The genetic basis of a craniofacial disease provides insight into COPII coat assembly. Dev. Cell 13, 623–634.
6 Schonthaler, H. B., Fleisch, V. C., Biehlmaier, O., Makhankov, Y., Rinner, O., Bahadori, R., Geisler, R., Schwarz, H.,

Neuhauss, S. C. and Dahm, R. (2008) The zebrafish mutant lbk/vam6 resembles human multisystemic disorders caused by aberrant trafficking of endosomal vesicles. Development 135, 387–399.
7 Shields, D. and Arvan, P. (1999) Disease models provide insights into post-golgi protein trafficking, localization and processing. Curr. Opin. Cell Biol. 11, 489–494.
8 Smith, R., Klein, P., Koc-Schmitz, Y., Waldvogel, H. J., Faull, R. L., Brundin, P., Plomann, M. and Li, J. Y. (2007) Loss of SNAP-25 and rabphilin 3a in sensory-motor cortex in Huntington's disease. J. Neurochem. 103, 115–123.
9 Springer, S., Spang, A. and Schekman, R. (1999) A primer on vesicle budding. Cell 97, 145–148.
10 Matsuoka, K., Orci, L., Amherdt, M., Bednarek, S. Y., Hamamoto, S., Schekman, R. and Yeung, T. (1998) COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. Cell 93, 263–275.
11 Spang, A., Matsuoka, K., Hamamoto, S., Schekman, R. and Orci, L. (1998) Coatomer, Arf1p, and nucleotide are required to bud coat protein complex I-coated vesicles from large synthetic liposomes. Proc. Natl. Acad. Sci. USA 95, 11199–11204.
12 Takei, K., Haucke, V., Slepnev, V., Farsad, K., Salazar, M., Chen, H. and De Camilli, P. (1998) Generation of coated intermediates of clathrin-mediated endocytosis on protein-free liposomes. Cell 94, 131–141.
13 Bremser, M., Nickel, W., Schweikert, M., Ravazzola, M., Amherdt, M., Hughes, C. A., Sollner, T. H., Rothman, J. E. and Wieland, F. T. (1999) Coupling of coat assembly and vesicle budding to packaging of putative cargo receptors. Cell 96, 495–506.
14 Sohn, K., Orci, L., Ravazzola, M., Amherdt, M., Bremser, M., Lottspeich, F., Fiedler, K., Helms, J. B. and Wieland, F. T. (1996) A major transmembrane protein of Golgi-derived COPI-coated vesicles involved in coatomer binding. J. Cell Biol. 135, 1239–1248.
15 Aguilera-Romero, A., Kaminska, J., Spang, A., Riezman, H. and Muniz, M. (2008) The yeast p24 complex is required for the formation of COPI retrograde transport vesicles from the Golgi apparatus. J. Cell Biol. 180, 713–720.
16 Bethune, J., Kol, M., Hoffmann, J., Reckmann, I., Brugger, B. and Wieland, F. (2006) Coatomer, the coat protein of COPI transport vesicles, discriminates endoplasmic reticulum residents from p24 proteins. Mol. Cell. Biol. 26, 8011–8021.
17 Sandmann, T., Herrmann, J. M., Dengjel, J., Schwarz, H. and Spang, A. (2003) Suppression of coatomer mutants by a new protein family with COPI and COPII binding motifs in Saccharomyces cerevisiae. Mol. Biol. Cell 14, 3097–3113.
18 Goldberg, J. (2000) Decoding of sorting signals by coatomer through a GTPase switch in the COPI coat complex. Cell 100, 671–679.
19 Lanoix, J., Ouwendijk, J., Lin, C. C., Stark, A., Love, H. D., Ostermann, J. and Nilsson, T. (1999) GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles. EMBO J. 18, 4935–4948.
20 Bigay, J., Casella, J. F., Drin, G., Mesmin, B. and Antonny, B. (2005) ArfGAP1 responds to membrane curvature through the folding of a lipid packing sensor motif. EMBO J. 24, 2244–2253.
21 Bigay, J., Gounon, P., Robineau, S. and Antonny, B. (2003) Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature. Nature 426, 563–566.
22 Gommel, D. U., Memon, A. R., Heiss, A., Lottspeich, F., Pfannstiel, J., Lechner, J., Reinhard, C., Helms, J. B., Nickel, W. and Wieland, F. T. (2001) Recruitment to Golgi membranes of ADP-ribosylation factor 1 is mediated by the cytoplasmic domain of p23. EMBO J. 20, 6751–6760.
23 Lanoix, J., Ouwendijk, J., Stark, A., Szafer, E., Cassel, D., Dejgaard, K., Weiss, M. and Nilsson, T. (2001) Sorting of Golgi resident proteins into different subpopulations of COPI vesicles: a role for ArfGAP1. J. Cell Biol. 155, 1199–1212.
24 Puthenveedu, M. A. and von Zastrow, M. (2006) Cargo regulates clathrin-coated pit dynamics. Cell 127, 113–124.
25 Mironov, A. A., Mironov, A. A., Jr., Beznoussenko, G. V., Trucco, A., Lupetti, P., Smith, J. D., Geerts, W. J., Koster, A. J., Burger, K. N., Martone, M. E., Deerinck, T. J., Ellisman, M. H. and Luini, A. (2003) ER-to-Golgi carriers arise through direct en bloc protrusion and multistage maturation of specialized ER exit domains. Dev. Cell 5, 583–594.
26 Stagg, S. M., LaPointe, P. and Balch, W. E. (2007) Structural design of cage and coat scaffolds that direct membrane traffic. Curr. Opin. Struct. Biol. 17, 221–228.
27 Fath, S., Mancias, J. D., Bi, X. and Goldberg, J. (2007) Structure and organization of coat proteins in the COPII cage. Cell 129, 1325–1336.
28 Lee, M. C., Miller, E. A., Goldberg, J., Orci, L. and Schekman, R. (2004) Bi-directional protein transport between the ER and Golgi. Annu. Rev. Cell Dev. Biol. 20, 87–123.
29 Stagg, S. M., Gurkan, C., Fowler, D. M., LaPointe, P., Foss, T. R., Potter, C. S., Carragher, B. and Balch, W. E. (2006) Structure of the Sec13/31 COPII coat cage. Nature 439, 234–238.
30 Gurkan, C., Stagg, S. M., Lapointe, P. and Balch, W. E. (2006) The COPII cage: unifying principles of vesicle coat assembly. Nat. Rev. Mol. Cell Biol. 7, 727–738.
31 Rao, Y., Bian, C., Yuan, C., Li, Y., Chen, L., Ye, X., Huang, Z. and Huang, M. (2006) An open conformation of switch I revealed by Sar1-GDP crystal structure at low Mg2+. Biochem. Biophys. Res. Commun. 348, 908–915.
32 Huang, M., Weissman, J. T., Beraud-Dufour, S., Luan, P., Wang, C., Chen, W., Aridor, M., Wilson, I. A. and Balch, W. E. (2001) Crystal structure of Sar1-GDP at 1.7 A resolution and the role of the NH2 terminus in ER export. J. Cell Biol. 155, 937–948.
33 Bi, X., Corpina, R. A. and Goldberg, J. (2002) Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat. Nature 419, 271–277.
34 Bi, X., Mancias, J. D. and Goldberg, J. (2007) Insights into COPII coat nucleation from the structure of Sec23.Sar1 complexed with the active fragment of Sec31. Dev. Cell 13, 635–645.
35 Lederkremer, G. Z., Cheng, Y., Petre, B. M., Vogan, E., Springer, S., Schekman, R., Walz, T. and Kirchhausen, T. (2001) Structure of the Sec23p/24p and Sec13p/31p complexes of COPII. Proc. Natl. Acad. Sci. USA 98, 10704–10709.
36 Bielli, A., Haney, C. J., Gabreski, G., Watkins, S. C., Bannykh, S. I. and Aridor, M. (2005) Regulation of Sar1 NH2 terminus by GTP binding and hydrolysis promotes membrane deformation to control COPII vesicle fission. J. Cell Biol. 171, 919–924.
37 Lee, M. C., Orci, L., Hamamoto, S., Futai, E., Ravazzola, M. and Schekman, R. (2005) Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. Cell 122, 605–617.
38 Wendeler, M. W., Paccaud, J. P. and Hauri, H. P. (2007) Role of Sec24 isoforms in selective export of membrane proteins from the endoplasmic reticulum. EMBO Rep. 8, 258–264.
39 Shimoni, Y. and Schekman, R. (2002) Vesicle budding from endoplasmic reticulum Methods. Enzymol. 351, 258–278.
40 Kurihara, T., Hamamoto, S., Gimeno, R. E., Kaiser, C. A., Schekman, R. and Yoshihisa, T. (2000) Sec24p and Iss1p function interchangeably in transport vesicle formation from the endoplasmic reticulum in Saccharomyces cerevisiae. Mol. Biol. Cell 11, 983–998.
41 Shimoni, Y., Kurihara, T., Ravazzola, M., Amherdt, M., Orci, L. and Schekman, R. (2000) Lst1p and Sec24p cooperate in sorting of the plasma membrane ATPase into COPII vesicles in Saccharomyces cerevisiae. J. Cell Biol. 151, 973–984.
42 Roberg, K. J., Crotwell, M., Espenshade, P., Gimeno, R. and Kaiser, C. A. (1999) LST1 is a SEC24 homologue used for selective export of the plasma membrane ATPase from the endoplasmic reticulum. J. Cell Biol. 145, 659–672.

43 Tang, B. L., Kausalya, J., Low, D. Y., Lock, M. L. and Hong, W. (1999) A family of mammalian proteins homologous to yeast Sec24p. Biochem. Biophys. Res. Commun. 258, 679–684.
44 Boyadjiev, S. A., Fromme, J. C., Ben, J., Chong, S. S., Nauta, C., Hur, D. J., Zhang, G., Hamamoto, S., Schekman, R., Ravazzola, M., Orci, L. and Eyaid, W. (2006) Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. Nat. Genet. 38, 1192–1197.
45 Campbell, J. L. and Schekman, R. (1997) Selective packaging of cargo molecules into endoplasmic reticulum-derived COPII vesicles. Proc. Natl. Acad. Sci. USA 94, 837–842.
46 Springer, S. and Schekman, R. (1998) Nucleation of COPII vesicular coat complex by endoplasmic reticulum to Golgi vesicle SNAREs. Science 281, 698–700.
47 Bevis, B. J., Hammond, A. T., Reinke, C. A. and Glick, B. S. (2002) De novo formation of transitional ER sites and Golgi structures in Pichia pastoris. Nat. Cell. Biol. 4, 750–756.
48 Soderholm, J., Bhattacharyya, D., Strongin, D., Markovitz, V., Connerly, P. L., Reinke, C. A. and Glick, B. S. (2004) The transitional ER localization mechanism of Pichia pastoris Sec12. Dev. Cell 6, 649–659.
49 Bannykh, S. I., Nishimura, N. and Balch, W. E. (1998) Getting into the Golgi Trends. Cell Biol. 8, 21–25.
50 Nickel, W., Brugger, B. and Wieland, F. T. (1998) Protein and lipid sorting between the endoplasmic reticulum and the Golgi complex Semin. Cell Dev. Biol. 9, 493–501.
51 Appenzeller-Herzog, C. and Hauri, H. P. (2006) The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function. J. Cell Sci. 119, 2173–2183.
52 Peyroche, A., Paris, S. and Jackson, C. L. (1996) Nucleotide exchange on ARF mediated by yeast Gea1 protein. Nature 384, 479–481.
53 Jackson, C. L. and Casanova, J. E. (2000) Turning on ARF: the Sec7 family of guanine-nucleotide-exchange factors. Trends Cell Biol. 10, 60–67.
54 Franzusoff, A., Redding, K., Crosby, J., Fuller, R. S. and Schekman, R. (1991) Localization of components involved in protein transport and processing through the yeast Golgi apparatus. J. Cell Biol. 112, 27–37.
55 Dierks, S. (2001) Investigation of copper adsorption to peat using the simple metal sorption model Water. Sci. Technol. 44, 477–483.
56 Richter, S., Geldner, N., Schrader, J., Wolters, H., Stierhof, Y. D., Rios, G., Koncz, C., Robinson, D. G. and Jurgens, G. (2007) Functional diversification of closely related ARF-GEFs in protein secretion and recycling. Nature 448, 488–492.
57 Spang, A., Herrmann, J. M., Hamamoto, S. and Schekman, R. (2001) The ADP ribosylation factor-nucleotide exchange factors Gea1p and Gea2p have overlapping, but not redundant functions in retrograde transport from the Golgi to the endoplasmic reticulum. Mol. Biol. Cell 12, 1035–1045.
58 Chantalat, S., Courbeyrette, R., Senic-Matuglia, F., Jackson, C. L., Goud, B. and Peyroche, A. (2003) A novel Golgi membrane protein is a partner of the ARF exchange factors Gea1p and Gea2p. Mol. Biol. Cell 14, 2357–2371.
59 Chantalat, S., Park, S. K., Hua, Z., Liu, K., Gobin, R., Peyroche, A., Rambourg, A., Graham, T. R. and Jackson, C. L. (2004) The Arf activator Gea2p and the P-type ATPase Drs2p interact at the Golgi in Saccharomyces cerevisiae. J. Cell Sci. 117, 711–722.
60 Casanova, J. E. (2007) Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. Traffic 8, 1476–1485.
61 Honda, A., Al-Awar, O. S., Hay, J. C. and Donaldson, J. G. (2005) Targeting of Arf-1 to the early Golgi by membrin, an ER-Golgi SNARE. J. Cell Biol. 168, 1039–1051.
62 Spang, A. (2002) ARF1 regulatory factors and COPI vesicle formation. Curr. Opin. Cell Biol. 14, 423–427.
63 Rein, U., Andag, U., Duden, R., Schmitt, H. D. and Spang, A. (2002) ARF-GAP-mediated interaction between the ER-Golgi v-SNAREs and the COPI coat. J. Cell Biol. 157, 395–404.
64 Schindler, C. and Spang, A. (2007) Interaction of SNAREs with ArfGAPs precedes recruitment of Sec18p/NSF. Mol. Biol. Cell 18, 2852–2863.
65 Lewis, S. M., Poon, P. P., Singer, R. A., Johnston, G. C. and Spang, A. (2004) The ArfGAP Glo3 is required for the generation of COPI vesicles. Mol. Biol. Cell 15, 4064–4072.
66 Yang, J. S., Lee, S. Y., Gao, M., Bourgoin, S., Randazzo, P. A., Premont, R. T. and Hsu, V. W. (2002) ARFGAP1 promotes the formation of COPI vesicles, suggesting function as a component of the coat. J. Cell Biol. 159, 69–78.
67 Lee, S. Y., Yang, J. S., Hong, W., Premont, R. T. and Hsu, V. W. (2005) ARFGAP1 plays a central role in coupling COPI cargo sorting with vesicle formation. J. Cell Biol. 168, 281–290.
68 Mesmin, B., Drin, G., Levi, S., Rawet, M., Cassel, D., Bigay, J. and Antonny, B. (2007) Two lipid-packing sensor motifs contribute to the sensitivity of ArfGAP1 to membrane curvature. Biochemistry 46, 1779–1790.
69 Michelsen, K., Schmid, V., Metz, J., Heusser, K., Liebel, U., Schwede, T., Spang, A. and Schwappach, B. (2007) Novel cargo-binding site in the beta and delta subunits of coatomer. J. Cell Biol. 179, 209–217.
70 Cosson, P., Lefkir, Y., Demolliere, C. and Letourneur, F. (1998) New COP1-binding motifs involved in ER retrieval. EMBO J. 17, 6863–6870.
71 Harter, C. and Wieland, F. T. (1998) A single binding site for dilysine retrieval motifs and p23 within the gamma subunit of coatomer. Proc. Natl. Acad. Sci. USA 95, 11649–11654.
72 Robinson, M. S. (2004) Adaptable adaptors for coated vesicles. Trends Cell Biol. 14, 167–174.
73 Harsay, E. and Bretscher, A. (1995) Parallel secretory pathways to the cell surface in yeast. J. Cell Biol. 131, 297–310.
74 Harsay, E. and Schekman, R. (2002) A subset of yeast vacuolar protein sorting mutants is blocked in one branch of the exocytic pathway. J. Cell Biol. 156, 271–285.
75 Waters, M. G., Griff, I. C. and Rothman, J. E. (1991) Proteins involved in vesicular transport and membrane fusion. Curr. Opin. Cell Biol. 3, 615–620.
76 Gu, F., Aniento, F., Parton, R. G. and Gruenberg, J. (1997) Functional dissection of COP-I subunits in the biogenesis of multivesicular endosomes. J. Cell Biol. 139, 1183–1195.
77 Daro, E., Sheff, D., Gomez, M., Kreis, T. and Mellman, I. (1997) Inhibition of endosome function in CHO cells bearing a temperature-sensitive defect in the coatomer (COPI) component epsilon-COP. J. Cell Biol. 139, 1747–1759.
78 Trautwein, M., Schindler, C., Gauss, R., Dengjel, J., Hartmann, E. and Spang, A. (2006) Arf1p, Chs5p and the ChAPs are required for export of specialized cargo from the Golgi. EMBO J. 25, 943–954.
79 Wang, C. W., Hamamoto, S., Orci, L. and Schekman, R. (2006) Exomer: A coat complex for transport of select membrane proteins from the trans-Golgi network to the plasma membrane in yeast. J. Cell Biol. 174, 973–983.
80 Sanchatjate, S. and Schekman, R. (2006) Chs5/6 complex: a multiprotein complex that interacts with and conveys chitin synthase III from the trans-Golgi network to the cell surface. Mol. Biol. Cell 17, 4157–4166.
81 Wells, C. D., Fawcett, J. P., Traweger, A., Yamanaka, Y., Goudreault, M., Elder, K., Kulkarni, S., Gish, G., Virag, C., Lim, C., Colwill, K., Starostine, A., Metalnikov, P. and Pawson, T. (2006) A Rich1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells. Cell 125, 535–548.
82 Macara, I. G. and Spang, A. (2006) Closing the GAP between polarity and vesicle transport. Cell 125, 419–421.
83 Terai, T., Nishimura, N., Kanda, I., Yasui, N. and Sasaki, T. (2006) JRAB/MICAL-L2 is a junctional Rab13-binding protein mediating the endocytic recycling of occludin. Mol. Biol. Cell 17, 2465–2475.
84 Marzesco, A. M., Dunia, I., Pandjaitan, R., Recouvreur, M., Dauzonne, D., Benedetti, E. L., Louvard, D. and Zahraoui, A.

(2002) The small GTPase Rab13 regulates assembly of functional tight junctions in epithelial cells. Mol. Biol. Cell 13, 1819–1831.
85 Frick, M., Schmidt, K. and Nichols, B. J. (2007) Modulation of lateral diffusion in the plasma membrane by protein density. Curr. Biol. 17, 462–467.
86 Chadda, R., Howes, M. T., Plowman, S. J., Hancock, J. F., Parton, R. G. and Mayor, S. (2007) Cholesterol-sensitive Cdc42 activation regulates actin polymerization for endocytosis via the GEEC pathway. Traffic 8, 702–717.
87 Mayor, S. and Pagano, R. E. (2007) Pathways of clathrin-independent endocytosis Nat Rev Mol Cell Biol 8, 603–612.
88 Goldberg, J. (1999) Structural and functional analysis of the ARF1-ARFGAP complex reveals a role for coatomer in GTP hydrolysis. Cell 96, 893–902.
89 Malsam, J., Gommel, D., Wieland, F. T. and Nickel, W. (1999) A role for ADP ribosylation factor in the control of cargo uptake during COPI-coated vesicle biogenesis. FEBS Lett. 462, 267–272.
90 Weiss, M. and Nilsson, T. (2003) A kinetic proof-reading mechanism for protein sorting. Traffic 4, 65–73.
91 Cai, H., Yu, S., Menon, S., Cai, Y., Lazarova, D., Fu, C., Reinisch, K., Hay, J. C. and Ferro-Novick, S. (2007) TRAPPI tethers COPII vesicles by binding the coat subunit Sec23. Nature 445, 941–944.
92 Barlowe, C. (1997) Coupled ER to Golgi transport reconstituted with purified cytosolic proteins. J. Cell Biol. 139, 1097–1108.
93 Vanrheenen, S. M., Reilly, B. A., Chamberlain, S. J. and Waters, M. G. (2001) Dsl1p, an essential protein required for membrane traffic at the endoplasmic reticulum/Golgi interface in yeast. Traffic 2, 212–231.
94 Andag, U., Neumann, T. and Schmitt, H. D. (2001) The coatomer-interacting protein Dsl1p is required for Golgi-to-endoplasmic reticulum retrieval in yeast. J. Biol. Chem. 276, 39150–39160.
95 Kamena, F. and Spang, A. (2004) Tip20p prohibits back-fusion of COPII vesicles with the endoplasmic reticulum. Science 304, 286–289.
96 Fulton, A. B. (1982) How crowded is the cytoplasm? Cell 30, 345–347.
97 Guigas, G., Kalla, C. and Weiss, M. (2007) The degree of macromolecular crowding in the cytoplasm and nucleoplasm of mammalian cells is conserved. FEBS Lett. 581, 5094–5098.
98 Meurer, S., Pioch, S., Wagner, K., Muller-Esterl, W. and Gross, S. (2004) AGAP1, a novel binding partner of nitric oxide-sensitive guanylyl cyclase. J. Biol. Chem. 279, 49346–49354.
99 Ficarro, S. B., McCleland, M. L., Stukenberg, P. T., Burke, D. J., Ross, M. M., Shabanowitz, J., Hunt, D. F. and White, F. M. (2002) Phosphoproteome analysis by mass spectrometry and its application to Saccharomyces cerevisiae. Nat. Biotechnol. 20, 301–305.
100 Echarri, A., Muriel, O. and Del Pozo, M. A. (2007) Intracellular trafficking of raft/caveolae domains: insights from integrin signaling. Semin. Cell Dev. Biol. 18, 627–637.
101 Gaus, K., Le Lay, S., Balasubramanian, N. and Schwartz, M. A. (2006) Integrin-mediated adhesion regulates membrane order. J. Cell Biol. 174, 725–734.
102 del Pozo, M. A., Balasubramanian, N., Alderson, N. B., Kiosses, W. B., Grande-Garcia, A., Anderson, R. G. and Schwartz, M. A. (2005) Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. Nat. Cell Biol. 7, 901–908.
103 Kaksonen, M., Toret, C. P. and Drubin, D. G. (2006) Harnessing actin dynamics for clathrin-mediated endocytosis. Nat. Rev. Mol. Cell Biol. 7, 404–414.
104 Liu, J., Kaksonen, M., Drubin, D. G. and Oster, G. (2006) Endocytic vesicle scission by lipid phase boundary forces. Proc. Natl. Acad. Sci. USA 103, 10277–10282.