Sensing Membrane Curvature

Transport vesicle formation and fusion are highly regulated processes that require the sequential assembly and disassembly of proteinacious membrane coats. Recent work suggests that one of the coat proteins senses the local changes in lipid packing that accompany membrane bending during vesiculation, and thus acts as a timer for coat disassembly once a complete vesicle is formed.

Membrane transport along the endocytic and secretory pathways relies on the formation and fusion of high-curvature lipid-bound vesicles and tubules. In the early secretory pathway, rounds of vesicle budding and fusion are linked to the GTPase cycle of the small G protein, ARF1 (ADP-ribosylation factor 1; Rothman, 1994). Upon a GDP to GTP exchange catalyzed by a guanine nucleotide exchange factor (GEF), $ARF1_{GTP}$ exposes an amphipathic α helix and becomes tightly associated with the Golgi membrane. Recruitment of ARF1 triggers the assembly of a protein coat complex termed coatomer or COPI. Coat assembly performs two major tasks: the concentration of cargo molecules and transport machinery (e.g., SNAREs) into a specialized region of the membrane, and the mechanical deformation of this region into a coated vesicle. Hydrolysis of ARF1-bound GTP catalyzed by a GTPase-activating protein (GAP) is thought to trigger vesicle uncoating, a prerequisite for vesicle fusion. Consequently, ARF-GEFs and ARF-GAPs are key regulators of vesicular transport. Whereas GEFs help define the region in the membrane where the cytosolic form of ARF and coat proteins are recruited, GAPs would act to ensure a timely release of the coat to allow fusion of the naked vesicle with its target membrane. Understanding of what regulates these regulators currently poses a major challenge in the membrane traffic field.

The application of cell-free assays has been instrumental for tracking down components of the vesicular transport machinery and for reconstructing the precise order of events required for generating fusion-competent vesicles. A striking observation was that COPI vesicles formed from purified Golgi membranes in the presence of nonhydrolyzable GTP analogs or a constitutively active ARF1 mutant are depleted of cargo compared to vesicles formed in the presence of GTP and wild-type ARF1 (Lanoix et al., 1999; Yang et al., 2002). This indicated that GTP hydrolysis on ARF1 plays a role in cargo uptake into COPI vesicles. Moreover, it was found that ARF-GAP1, the prototypic GAP for ARF1, binds directly to the sorting motif of certain cargo proteins (Lanoix et al., 2001) and that its level on reconstituted vesicles, which depends on GTP hydrolysis as well, is stoichiometric to COPI (Yang et al., 2002). From these and previous data, it emerged that ARF-GAP1 has two seemingly contradictory roles, namely one promoting vesicle formation as a functional component of the COPI coat and one predicted to antagonize vesicle formation by stimulating coat release. Clearly, these two activities must be separated in order to allow formation of a productive budding complex. One element of regulation by which this could be accomplished became apparent when synthetic peptides containing the sorting signals of specific cargo proteins were found to retard COPI-stimulated GTP hydrolysis by directly suppressing the catalytic activity of ARF-GAP1 (Goldberg, 2000; Lanoix et al., 2001). Based on these data, it was postulated that ARF-GAPs form part of a "proofreading" mechanism that ensures that only cargo-associated coated structures become stable and give rise to a coated vesicle. This idea is consistent with in vivo studies demonstrating that there is a rapid exchange of coat proteins between membrane-bound and cytosolic pools, even in the absence of vesicle formation (Presley et al., 2002).

However, if its interaction with cargo proteins prevents ARF-GAP1 from acting on ARF1 during formation of a coated vesicle, there must be an additional mechanism for switching ARF-GAP1 back on to ensure a timely release of the coat and prepare the vesicle for fusion. In an elegant contribution, Antonny and coworkers now present evidence that this may be provided by the ability of ARF-GAP1 to sense the local changes in lipid packing that accompany membrane vesiculation (Bigay et al., 2004). Previous work from the same group had shown that ARF-GAP1 binds preferentially to liposomes containing conical lipids *versus* cylindrical lipids (Antonny et al., 1997). Binding increased gradually as the size of the lipid polar heads decreased and as the cross-section of the acyl chains increased, indicating a key role for hydrophobic membrane interactions (Figures 1A and 1B). Using time-resolved assays for COPI dynamics on liposomes of decreasing size, the authors now show that the rate of ARF-GAP1-catalyzed GTP hydrolysis in ARF1 and the rate of COPI disassembly increase dramatically as the curvature of the lipid bilayer approaches that of a typical 50 nm transport vesicle (Bigay et al., 2004). The authors infer that the spacing between lipid head groups in the outer leaflet becomes large enough to accommodate ARF-GAP1 only when the bilayer is sufficiently bent (Figures 1D and 1E). This would allow hydrophobic residues of ARF-GAP1 to insert into the coat-facing membrane leaflet, hence facilitating interactions of the protein with membrane-bound $ARF1_{GTP}$. Consequently, as a polymerized COPI coat enhances membrane curvature, the number of $ARF1_{GTP}$ molecules that hold the coat should decrease. Antonny and coworkers speculate that, because the membrane curvature at the periphery of the coated area is negative, a ring of ARF1-GTP molecules should be protected from ARF-GAP1 and may keep the coat in a metastable state until fission has occurred and membrane curvature becomes entirely positive (Figures 1G and 1H). This model would explain previous observations that the rate of ARF1 release from Golgi membranes is faster than that of COPI (Presley et al., 2002), and that vesicles reconstituted in the presence of ARF-GAP1 and GTP have a low ARF1/COPI ratio (Yang et al., 2002).

The model proposed by Antonny and colleagues is an appealing one, highlighting yet another example of the dynamic interplay between proteins and lipids in the regulation of membrane traffic. Simultaneously, it raises some important issues that need to be addressed in future research. First, the model predicts that ARF-GAP1 membrane interactions should be sensitive to regulation by inverted cone-shaped lipids and membrane-active proteins capable of filling voids (Figures 1C and 1F); this prediction seems worth testing. Second, it is currently

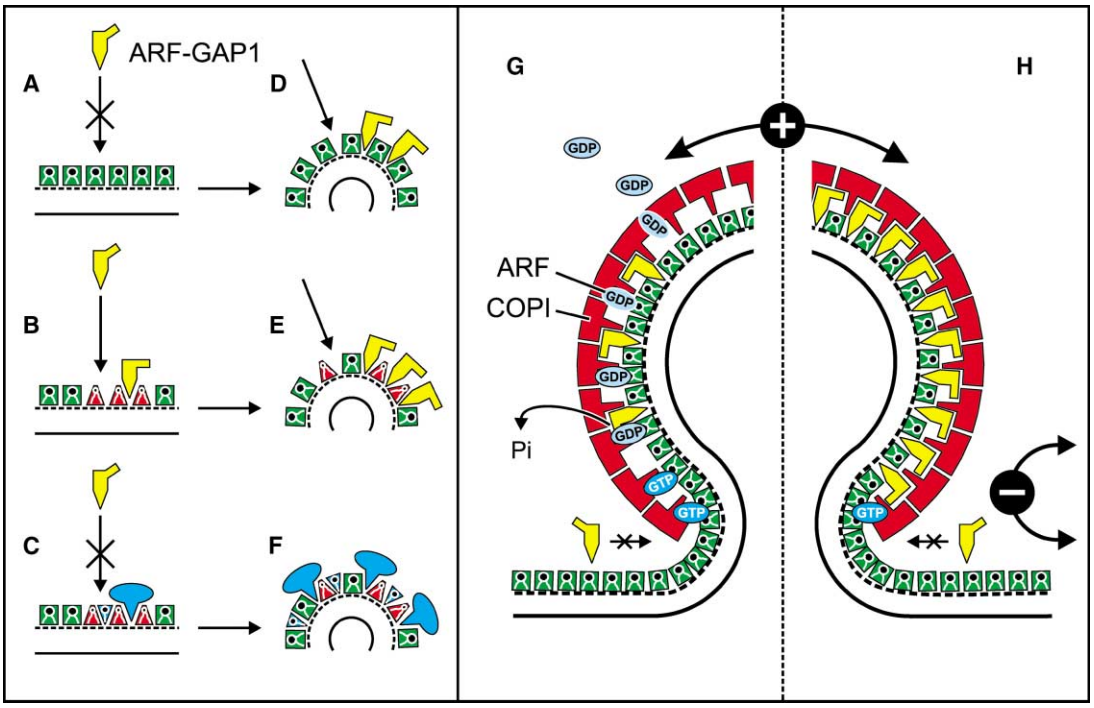

Figure 1. ARF-GAP1: A Sensor of Membrane Curvature?

Membrane binding of ARF-GAP1 is sensitive to local lipid head group packing. ARF-GAP1 binds poorly to flat membranes composed of phospholipids with an overall cylindrical shape (A), but efficiently to flat membranes containing cone-shaped lipids, creating voids for ARF-GAP1 insertion ([B]; Antonny et al., 1997). Voids are also created when membranes are forced into convex (positive) membrane curvature (D), in particular if cone-shaped lipids are present (E). ARF-GAP1 membrane interaction should be sensitive to regulation by inverted cone-shaped (lyso) lipids and competing membrane-active proteins capable of filling voids (C). Lysolipids and membrane-active proteins may also directly induce membrane curvature (F). Membrane curvature is positive in the outer membrane leaflet of the COPI-coated bud accelerating ARF-GAP1 membrane interaction, but slightly negative at the coat aperture in the membrane neck (arrows in [G] and [H]; Kozlovsky and Kozlov, 2003). As a result, ARF-GAP1 will preferentially catalyze GTP hydrolysis on ARF1 in the bud, leaving a ring of $ARF1_{GTP}$ at the coat aperture (G). After fission, GTP hydrolysis on ARF1 at the coat aperture may initiate uncoating. Alternatively, ARF-GAP1 may play a crucial role in membrane bending as a structural component of the membrane coat (H). According to this model, ARF-GAP1 would be an inducer, rather than a sensor, of membrane curvature.

unclear at what level ARF1 is present in COPI-coated vesicles in vivo, and, thus, the precise role of ARF1 and ARF-GAP1 in vesicle uncoating remains to be clarified. Third, Antonny and coworkers studied the interaction of ARF-GAP1 with highly curved liposomes, assuming that membrane curvature in vivo is generated by an external force, namely polymerization of COPI on the membrane surface. An alternative possibility, consistent with the aforementioned data, is that ARF-GAP1 is not recruited after membrane bending, but that membrane insertion of ARF-GAP directly contributes to membrane bending, similar to the role proposed for epsin in clathrin-coated vesicle formation (Ford et al., 2002; Figure 1H). Finally, a differential binding of ARF-GAP1 and the resulting gradient of $ARF1_{GTP}$ over the membrane bud may help define the polarity of the coated bud and coordinate the final step in vesicle formation, fission of the membrane neck. This fission event probably involves additional proteins, and these proteins may be recruited to the membrane or be activated in a manner dependent on ARF1 and ARF-GAP1.

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Selected Reading

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