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COMMENTARY

Arfs and membrane lipids: sensing, generating and responding to membrane curvature

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Arf family GTP-binding proteins function in the regulation of membrane-trafficking events and in the maintenance of organelle structure. They act at membrane surfaces to modify lipid composition and to recruit coat proteins for the generation of transport vesicles. Arfs associate with membranes through insertion of an N-terminal myristoyl moiety in conjunction with an adjacent amphipathic α-helix, which embeds in the lipid bilayer when Arf1 is GTP-bound. In this issue of the *Biochemical Journal*, Lundmark et al. report that myristoylated Arfs in the presence of GTP bind to and cause tubulation of liposomes, and that GTP exchange on to Arfs is more efficient in the presence of liposomes of smaller diameter (increased curvature). These findings suggest that Arf protein activation and membrane interaction may initiate membrane curvature that will be enhanced further by coat proteins during vesicle formation.

Key words: amphipathic α-helix, Arf, membrane curvature, myristoylation.

The Ras superfamily of low-molecular-weight GTP-binding proteins regulate a wide range of cellular activities, including cell-cycle regulation, differentiation, cell–cell interactions, cell migration and intracellular vesicular membrane transport. These GTPases switch between GDP-bound and GTP-bound forms, corresponding to the inactive and active conformations respectively. In the active, GTP-bound form nearly all of these GTPases are bound to membrane surfaces, where engagement and activation of downstream effector proteins takes place. Ras, Rho and Rab family GTPases are modified post-translationally, with C-terminal lipid modification (geranylgeranylation or farnesylation), which together with C-terminal amino acid sequences determine sites of membrane interaction and specificity of function. Arf (ADP-ribosylation factor) family proteins, by contrast, are co-translationally modified at the N-terminus by myristoylation, which together with the N-terminal amphipathic helix embed the active Arf in the membrane. Recent structural studies have revealed that Arf proteins and their effectors are in intimate association with membranes, in contrast with Rab proteins, which appear to extend out from the membrane by their C-terminal extensions [1].

Arfs are highly conserved among eukaryotes from yeast to humans [1,2]. The Arfs are divided into three classes based on amino acid sequence, with class I comprising Arfs 1–3, class II comprising Arfs 4 and 5, and Class III containing only Arf6. Most metazoans have at least one representative from each class. In addition to the Arfs, there are other family members including Sar1 and Arf-related proteins or Arls. In cells, the Arf GTP-binding and hydrolysis cycle is regulated by GEFs (guanine-nucleotide-exchange factors) and GAPs (GTPase-activating proteins). There are 14 GEFs and 24 GAPs identified to date, and these regulatory proteins may contribute specificity to Arf function by activating (for GEFS) and inactivating (for GAPs) Arfs at specific locations [1,2]. Additionally, as many of these proteins contain multiple interaction domains and hence could provide a scaffold function, they themselves might recruit effectors and thus be part of Arf function.

Arf1 and Arf6 are the most studied and best characterized of the family members [1,2]. In cell-free biochemical assays both Arf1 and Arf6 can recruit coat proteins to membranes and activate PLD (phospholipase D) and PIP5K (phosphatidylinositol-4-phosphate 5-kinase). In cells, however, Arf1 is associated primarily with the Golgi complex, where it mediates the binding of the COPI (coatamer protein I) coat, the clathrin APs (adaptor proteins) 1, 3 and 4, and the Golgi-localized GGAs (Golgi-associated γ-adaptin ear homology domain Arf-interacting protein). These coat proteins act to sort membrane cargo proteins into vesicle carriers for trafficking in the ER (endoplasmic reticulum)/Golgi system (for COPI) and at the *trans*-Golgi network/endosomal–lysosomal system. Arf6, on the other hand, is localized to the plasma membrane and endosomal membranes, and does not appear to recruit coat proteins to membranes directly. Rather, it remodels membranes and regulates membrane traffic through its actions on PLD and PIP5K [1,2]. The resultant generation of PA (phosphatidic acid) and PIP_2 (phosphatidylinositol 4,5-bisphosphate) is important for recycling of endosomal membrane back to the PM (plasma membrane) and for the changes to the cortical actin structure at the PM induced by activation of Arf6. Despite this apparent separation of localization and function for Arf1 and 6, there is evidence that Arf1 can affect lipid composition and even actin assembly at the Golgi [2], and Arf6 might in the future be found to recruit coat proteins in the periphery. Overall, both Arf1 and Arf6 are involved in regulating trafficking between membrane compartments, and this presumably involves a role in vesicle formation.

Numerous biochemical studies over the years have examined the role of the N-terminal α-helix and myristoyl group in the interaction of Arfs with membranes. The amphipathic helix has positively charged lysine residues on one face and hydrophobic residues on the other face, and thus can insert itself into the membrane parallel to the lipid bilayer. The structure of GDP-bound Arf1 revealed that the N-terminal helix folds back on the Arf structure hiding its hydrophobic face [3]. Furthermore, it has been known for some time that recombinant myristoylated Arf1 *in vitro* does not exchange nucleotides readily unless lipids are added in the form of liposomes or detergent/lipid micelles. The presence of the lipid allows the myristoylated amphipathic helix to

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embed in the membrane and opens up the Arf structure to facilitate nucleotide exchange under physiological conditions (i.e. 1 mM Mg^{2+}) in the absence of a GEF [4,5]. Indeed these observations led some investigators to study N-terminally truncated Arf proteins (Δ17 for Arf1 and Δ13 for Arf6) that could readily be loaded with GTP and interact with effector proteins in a soluble form. However, interactions of truncated Arfs may not reflect the full extent and behaviour of Arfs in cells, which takes place on membrane surfaces.

In this issue of the *Biochemical Journal*, Lundmark et al. [6] re-examine the interactions of myristoylated Arfs with membrane lipids. Using liposomes created from total brain lipids, they show that myristoylated Arf1 binds to liposomes and this is increased slightly in the presence of GTP. When they looked at the ability of liposomes to catalyse exchange of nucleotide on myristoylated Arfs, they found an increase in nucleotide exchange with liposomes of increasing curvature, i.e. smaller diameter. Optimal nucleotide exchange occurred with 50 nm liposomes, the approximate diameter of a typical vesicle. Furthermore, both Arf1 and 6, when presented with large (800 nm) diameter liposomes in the presence of GTP, would induce 50 nm tubules to form from these liposomes. Presumably, this geometrical transformation could in turn lead to increased activation of Arfs on the curved tubules. Charge-reversal mutations in the lysine residues in the helix abolished the ability of Arf6 to exchange nucleotide in the presence of liposomes and to form these tubules. The authors suggest that Arf protein activation could be influenced by membrane curvature and that Arf–GTP could itself promote initial curvature during coated vesicle formation.

This finding is reminiscent of studies reported for Sar1, an Arf family member that recruits the COPII coat on to vesicles forming from the ER. The N-terminal helix of Sar1, although lacking myristoylation, can nonetheless induce the formation of tubules from liposomes and this is inhibited when mutations are made in the hydrophobic face of the α-helix [7], which interestingly has acidic and not basic residues on its hydrophilic side. This mutated Sar1 is less efficient in supporting ER-to-Golgi traffic than wild-type Sar1, although it is still capable of recruiting COPII components, suggesting that Sar1-induced curvature is vital to functional vesicle formation [7]. The finding that Sar1 can promote membrane tubulation in cells is supported by the earlier findings of Aridor et al. [8], who reported massive tubulation from the ER in permeabilized cells treated with activated Sar1.

These activities of Arf proteins and Sar1 in generating membrane curvature are informative, but we also need to assess the functioning of these proteins in the presence of coat proteins, cargo proteins and the GEFs and GAPs, which make contributions of their own. Some of the ArfGEFs and GAPs are targeted to membranes through pleckstrin homology domains that recognize specific phosphoinositides; these interactions may also influence catalytic activity in some cases [1,2]. ArfGAP1, which is the GAP for most Golgi-associated Arfs, contains within its Golgi-targeting domain an ArfGAP lipid-packing sensor motif capable of sensing membrane curvature, and hence activating GAP activity on regions of high membrane curvature (i.e. vesicles) [9]. Some GAP proteins also contain BAR domains, which by themselves are capable of forming membrane tubules [10]. Further studies will be needed to understand how these components work together in cell-free systems and in intact cells.

Finally, the other aspect that warrants attention is the possible contribution that Arf stimulation of PIP5K and PLD could play in membrane traffic. The generation of PIP_2 or PA at defined membrane sites could influence membrane curvature and membrane fission during vesicle formation, and affect fusion of the vesicle with the target membrane. These membrane modelling functions of Arfs are likely to be as important as coat protein recruitment. The challenge for future studies is to discover how all these activities of Arfs come together to orchestrate membrane traffic at the Golgi complex and PM.

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