Chapter 8
Arf Proteins and Their Regulators: At the Interface Between Membrane Lipids and the Protein Trafficking Machinery

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Abstract The Arf small GTP-binding (G) proteins regulate membrane traffic and organelle structure in eukaryotic cells through a regulated cycle of GTP binding and hydrolysis. The first function identified for Arf proteins was recruitment of cytosolic coat complexes to membranes to mediate vesicle formation. However, subsequent studies have uncovered additional functions, including roles in plasma membrane signalling pathways, cytoskeleton regulation, lipid droplet function, and non-vesicular lipid transport. In contrast to other families of G proteins, there are only a few Arf proteins in each organism, yet they function specifically at many different cellular locations. Part of this specificity is achieved by formation of complexes with their guanine nucleotide-exchange factors (GEFs) and GTPase activating proteins (GAPs) that catalyse GTP binding and hydrolysis, respectively. Because these regulators outnumber their Arf substrates by at least 3-to-1, an important aspect of understanding Arf function is elucidating the mechanisms by which a single Arf protein is incorporated into different GEF, GAP, and effector complexes. New insights into these mechanisms have come from recent studies showing GEF–effector interactions, Arf activation cascades, and positive feedback loops. A unifying theme in the function of Arf proteins, carried out in conjunction with their regulators and effectors, is sensing and modulating the properties of the lipids that make up cellular membranes.

Keywords ADP ribosylation factor (Arf) • membrane • vesicular trafficking • lipid trafficking • membrane contact site (MCS) • lipid kinase • vesicle coat protein • guanine nucleotide-exchange factor (GEF) • GTPase activating protein (GAP)
8.1 Introduction

Arf proteins are low molecular weight GTP-binding (G) proteins that are regulated through a cycle of GTP binding and hydrolysis, in which binding of GTP activates and GTP hydrolysis inactivates the G protein (Donaldson and Jackson 2011; Gillingham and Munro 2007b) (Fig. 8.1). In their active GTP-bound form, Arf proteins are tightly associated with the membrane surface. Hence they bring their effectors, proteins that bind specifically to the GTP-bound form, into close contact with the lipid bilayer. These Arf effector proteins include coat complexes that deform membranes and promote cargo sorting, enzymes such as the phosphatidylinositol kinases that alter membrane lipid composition, and actin cytoskeletal components (Table 8.1). Arf1 is the founding member of the family, and was originally identified as a protein factor required for the ADP-ribosylation of the adenylate cyclase activator Gsα by cholera toxin (Schleifer et al. 1982). Although subsequent studies led to the discovery that the major cellular function of Arf1 is regulation of membrane trafficking, its name comes from this initial finding. The original discovery that Arf1 has an essential function in the secretory pathway at the level of the Golgi came from studies in yeast (Stearns et al. 1990a, b). Soon thereafter, the role of Arf1 in recruiting the COPI coat complex to membranes of the early secretory pathway to mediate COPI vesicle budding was demonstrated both in vitro and in cells. The reconstitution in vitro of COPI vesicle budding provided important mechanistic insights (Rothman and Wieland 1996), and the function of Arf1 in cells was greatly aided by use of the specific inhibitor of Arf1 activation, brefeldin A (Klausner et al. 1992).

Mammalian Arf proteins can be divided into three classes based on sequence homology: Class I (Arfs1–3), Class II (Arfs 4–5), and Class III (Arf6). Class I Arfs are highly conserved and are present in all eukaryotes, whereas the Class II Arfs arose during animal cell evolution, diverging from the Class I Arfs in the animal lineage after fungi separated, but before choanoflagellates did (Manolea et al. 2010; Schlacht et al. 2013). Consequently, in all invertebrates, such as D. melanogaster and C. elegans, there is one member of each of the three Arf classes. In certain lineages, some Arf classes have undergone expansion. For example, vertebrates have multiple members of Class I and II Arf proteins (Li et al. 2004). Although yeast lack Class II Arfs, they have two highly similar Class I Arf proteins and a single Class III member. Plants have numerous Class I Arfs (six in Arabidopsis) that are all more closely related to each other than to Class I Arfs of other eukaryotic supergroups, as well as highly diverged Arfs with some characteristics of mammalian Class III Arf6 (Gebbie et al. 2005). The Arf proteins are part of a larger family that also includes the Arf-like (Arl) proteins. There are more than 20 Arl proteins, with a wide range of functions including membrane trafficking, targeting of proteins to cilia, microtubule regulation, and lysosome function (Donaldson and Jackson 2011; Gillingham and Munro 2007b). The most divergent Arl protein is Sar1, an evolutionarily ancient small G protein that shares a highly related function...
to that of the Class I Arfs, the recruitment of a coat complex to membranes to mediate vesicle formation (Lee et al. 2004).

Studies of the evolution of membrane trafficking protein families, including Arf, coat, adaptor, Rab, and SNARE proteins, have revealed a high level of complexity and sophistication of the endomembrane system in the early ancestors of modern
Table 8.1 Arf effectors

| Effector | Arf | Location | Arf interacting region |
|----------|-----|----------|------------------------|
| **Coat complexes** | | | |
| COPI | Arf1 | Golgi, ERGIC | γ-COP β-COP |
| | Arfs 4, 5 | Golgi, ERGIC | ND |
| AP1/clathrin | Arfs 1, 3 | TGN, endosomes | γ-AP-1, β-AP-1 |
| GGA1, 2, 3/clathrin | Arfs 1, 3 | TGN, endosomes | GAT domain |
| AP3 | Arfs 1, 3 | Endosomes, TGN | Endosomes, TGN |
| AP4 | Arfs 1, 3 | TGN | ε-AP-4, μ-AP-4 (also binds GDP-bound form) |
| **Lipid-modifying enzymes** | | | |
| FAPP1, FAPP2 | Arf1 | trans-Golgi | PH domain |
| CERT | Arf1 | trans-Golgi | PH domain |
| OSBP | Arf1 | trans-Golgi | PH domain |
| PI 4 kinase | Arf1 | trans-Golgi | trans-Golgi |
| PI4P-5 kinase | Arfs1–6 | PM (Arf6) | PM (Arf6) |
| Phospholipase D | Arfs1–6 | PM (Arf6) | PM (Arf6) |
| **Tethers** | | | |
| GMAP-210 | Arf1 | cis-Golgi | C-terminal GRAB domain |
| Golgin160 | Arf1 | Golgi | N-terminus |
| Exocyst | Arf6 | PM | Sec10 |
| **G protein regulators** | | | |
| ARHGAP21 | Arf1, 6 | Golgi, PM | PH domain, C-terminal helix |
| Cytohesin/Arno | Arf6, Arf4 | PM | PH domain |
| **Scaffolding proteins** | | | |
| JIP3, JIP4 | Arf6 | Endosomes, intercellular bridge | Leucine zipper domain (LZII) |
| FIP3, FIP4 | Arfs 5, 6 | Recycling endosomes, midbody | CC |
| Nm23-H1 | Arf6 | PM, cell junctions | ND |
| Arfaptin1, arfaptin2 | Arf1 | Golgi, TGN | BAR domain |
| **Cargo** | | | |
| Rhodopsin | Arf4 | TGN | VxPx targeting motif |

AP adaptor protein, BAR Bin/Amphiphysin/Rvs, CC coiled-coil, COP coatamer protein, ER endoplasmic reticulum, ERGIC ER-Golgi intermediate compartment, GAT GGA (Golgi-localized, γ-adaptin homologous, ADP-ribosylation factor-binding protein) and TOM1 homologous, GRAB GRIP (golgin-97/RabBP2α/Imh1p/p230)-related Arf binding, PM plasma membrane, TGN trans-Golgi network; ND, not determined

eukaryotic cells (Dacks and Field 2007). Intriguingly, the last eukaryotic common ancestor (LECA) likely possessed only one Arf family member, in contrast to having already nearly 20 Rab proteins (Koumandou et al. 2013). However, multiple Arf GEFs and GAPs existed in this ancient eukaryotic ancestor, supporting the idea that a key feature of Arf function is a single Arf protein participating in multiple GEF and GAP regulatory complexes (Koumandou et al. 2013; Schlacht et al. 2013). This feature is conserved in modern organisms, for example, in humans, which have only 5 Arf proteins, yet at least 15 GEFs and 31 GAPs. Perhaps a clue as to the
nature of the primordial Arf protein comes from the protozoan parasite *Trypanosoma brucei*, which expresses a single Arf protein that has characteristics of both Class I and Class III mammalian Arfs. TbArf1 is a basic protein with a high pI similar to that of human Arf6, but contains the Golgi-targeting motif MxxE (Price et al. 2007), found in human Arf1 and Arf3 (Honda et al. 2005). TbArf1 localizes to the Golgi, and functional studies indicate that it has roles in both endocytosis and in Golgi–lysosome trafficking (Price et al. 2007).

The spatiotemporal control of Arf protein function is mediated by regulators of Arf GTP binding and GTP hydrolysis (Fig. 8.1d). The Arf guanine nucleotide-exchange factors (GEFs) catalyse GDP release from their substrate Arf, allowing GTP, which is more abundant in cells, to bind. This nucleotide-exchange activity is carried out by the Sec7 domain, a highly evolutionarily conserved domain first identified as a homology domain in the yeast Sec7p protein, and whose function was first identified in the yeast Gea1p protein (Peyroche et al. 1996). A Sec7 domain is present in all Arf GEFs identified to date. The Arf GTPase activating proteins (GAPs) catalyse the hydrolysis of GTP on their substrate Arf, a function carried out by a conserved GAP domain, characterized by the presence of a zinc finger. The Arf GAPs are essential because Arf proteins have negligible intrinsic GTP hydrolysis activity (Kahn and Gilman 1986). The primary sequence homology of the catalytic domains of the GEFs and GAPs facilitated their identification, but their Arf substrate specificity has not been fully elucidated. A recent study of the Arf GAPs has revealed that they have likely co-evolved with their Arf substrates (Schlacht et al. 2013). These results support the conclusion that the Arfs function in a tightly coordinated manner with their regulators.

There are seven subfamilies of Arf GEFs in eukaryotic cells (Cox et al. 2004) (Table 8.2, Fig. 8.2). The GBF/Gea and BIG/Med7 GEFs are localized to the Golgi, and use Arf1 as a substrate (Donaldson and Jackson 2011). In animal cells and yeast, these GEFs act sequentially, with GBF/Gea proteins functioning at the early Golgi, and BIG/Med7 proteins at the trans-Golgi and trans-Golgi network (TGN) (Franzusoff et al. 1991; Peyroche et al. 2001; Zhao et al. 2002). The cytohesin/Arno, EFA6, and IQSEC/BRAG subfamilies function primarily in endosomal–PM trafficking pathways at the cell periphery, and primarily act on Arf6 (Casanova 2007; Cox et al. 2004; Gillingham and Munro 2007b). The EFA6 GEFs regulate endocytosis, endosomal membrane recycling, and actin cytoskeleton remodelling (Casanova 2007; Franco et al. 1999). Yel1p is the orthologue of EFA6 in budding yeast, *Saccharomyces cerevisiae*, exhibiting exchange activity in vitro on Arf3p (the yeast Class III member) (Gillingham and Munro 2007a), which is involved in endocytosis (Smaczynska-de Rooij et al. 2008). Yel1p has a homology domain in the C-terminus resembling that of EFA6 family members (Gillingham and Munro 2007a). Syt1p has a Sec7 domain that most closely resembles that of the IQSEC/BRAG family in mammalian cells, although it lacks an IQ motif (Cox et al. 2004), and functions with Arl3p and Arl1p at the trans-Golgi in yeast (Chen et al. 2010). The FBXO8 Arf GEFs contain an F-box in addition to the Sec7 domain, and are present in vertebrates, and at least one invertebrate (Gillingham and Munro 2007b), but are not present in yeast, worms, or flies. Little is known of its function, although
| Common protein name (human) | Human gene name | Human uniprot name, accession number | S. cerevisiae name, uniprot accession # | C. elegans | D. melanogaster |
|-----------------------------|-----------------|-------------------------------------|----------------------------------------|------------|-----------------|
| GBF1                        | GBF1            | GBF1, Q92538                        | Gea1p, P47102                          | C24H11.7 (gbf-1) | CG8487 (garz) |
| BIG1                        | ARFGEF1         | BIG1, Q9Y6D6                        | Sec7p, P11075                          | Y6B3A.1     | CG7578 (sec71)  |
| BIG2                        | ARFGEF2         | BIG2, Q9Y6D5                        | Yel1p, P34225                          | Y55D9A.1 (efa-6) | CG31158 (Efa6) |
| EFA6A                       | PSD1            | PSD1, A5PKW4                        |                                        |            |                 |
| EFA6B                       | PSD4            | PSD4, Q8NDX1                        |                                        |            |                 |
| EFA6C                       | PSD2            | PSD2, Q9BQ17                        |                                        |            |                 |
| EFA6D                       | PSD3            | PSD3, Q9NY10                        |                                        |            |                 |
| IQSEC1/BRAG2/GEP100         | IQSEC1          | IQEC1, Q6DN90                       | Syt1p, Q06836                          | M02B7.5     | CG32434 (siz, loner, schizo) |
| IQSEC2/BRAG1                | IQSEC2          | IQEC2, Q5U85                        |                                        |            |                 |
| IQSEC3/BRAG3/ synArfGEF     | IQSEC3          | IQEC3, Q9UPP2                       |                                        |            |                 |
| Cytoselin1                  | PSCD1           | CYH1, Q15438                       | none                                   | K06H7.4 (grp-1) | CG11628 (Grp1, step/steppke) |
| Cytoselin2/Armo             | PSCD2           | CYH2, Q99418                       |                                        |            |                 |
| Cytoselin3/Grp              | PSCD3           | CYH3, Q43739                       |                                        |            |                 |
| Cytoselin4                  | PSCD4           | CYH4, Q9UIA0                       |                                        |            |                 |
| FBXO8                       | FBXO8           | FBX8, Q9NRD0                       | –                                      | –          | –               |
recently a form of bilateral cleft lip has been associated with a deletion of a chromosomal region including the FBXO8 gene (Calcia et al. 2013). RalF is the founding member of a family of bacterial Arf GEFs, first identified in *Legionella pneumophila* and *Rickettsia prowazekii* (Amor et al. 2005; Cox et al. 2004). These GEFs do not have a prokaryotic origin, but rather were incorporated into bacterial genomes through horizontal transfer from their eukaryotic hosts (Nagai et al. 2002). The Sec7 domain of RalF is autoinhibited by the capping domain of the protein, which is relieved by interaction with a specific lipid environment (Alix et al. 2012; Folly-Klan et al. 2013).

There are 11 subfamilies of Arf GAPs, 10 of which are found in humans (Schlacht et al. 2013) (Table 8.3). A recent phylogenetic study has indicated that six Arf GAP families (ArfGAP1, ArfGAP2/3, SMAP, ACAP, AGFG, and the newly identified ArfGAPC2 family) are ancient, probably existing in the LECA prior to separation of the eukaryotic supergroups. On the other hand, ASAP, ARAP, and GIT families arose more recently in evolution, being found only in animals. Through the course of evolution, the Arf GAPs have undergone extensive domain shuffling, losing and gaining different domains in a complex pattern to give rise to the proteins existing today (Schlacht et al. 2013). The PH, BAR, RhoGAP, ankyrin repeat, and C2 domains of Arf GAP proteins are conserved across eukaryotes, and therefore were probably present in the primordial Arf GAPs, whereas other domains such as GOLD, CALM, and SH3 domains are restricted to specific lineages (Schlacht et al. 2013).

The first Arf GAP identified was ArfGAP1 (Cukierman et al. 1995), which has two ALPS motifs in its C-terminal region that mediate specific binding to the highly

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**Fig. 8.2** The domain structure of the major cellular families of Arf GEFs. The domains of the five major families of Arf GEFs are shown, approximately to scale. Representative members shown are from humans and budding yeast. The well-characterized Arf substrates for each family are listed, and the sensitivity (Sens) or resistance (Resist) of their exchange activity to brefeldin A is indicated. DCB dimerization and cyclophilin binding, HUS homology upstream of Sec7, HDS homology downstream of Sec7, CC coiled coil, PH pleckstrin homology, E6H EFA6 homology, IQ isoleucine–glutamine motif.

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| Common protein name (other name) | Human gene name (other Names) | Human uniprot name, accession number | S. cerevisiae, uniprot accession (other fungi) | C. elegans | D. melanogaster |
|----------------------------------|-----------------------------|--------------------------------------|-----------------------------------------------|------------|----------------|
| ArfGAP1 | ARFGAP1 | ARFG1, Q8N6T3 | Gcs1p, P35197 | K02B12.7 | CG4237 (Gap69C ) |
| ArfGAP2 | ARFGAP2 (ZNF289) | ARFG2, Q8N6H7 | Glo3p, P38682 | F07F6.4 | CG6838 |
| ArfGAP3 | ARFGAP3 | ARFG3, Q9NP61 | | | |
| ARFGAPC2 | | | | | |
| SMAP1 | SMAP1 | SMAP1, Q8IYB5 | | | |
| SMAP2 | SMAP2 (SMAP1L) | SMAP2, Q8WU79 | | W09D10.1 | CG8243 |
| AGFG1 (Hrb) | AGFG1 (HRB, RAB, RIP) | AGFG1, P52594 | (F4NRR3_BATDJ, B. dendrobatidis) | – | CG3365 (dromgo) |
| AGFG2 (HrBL) | AGFG2 (HRBL, RABR) | AGFG2, Q95081 | | | |
| ACAP1 (Centaurin β1) | ACAP1 (CENTB1, KIAA0050) | ACAP1, Q15027 | (I1C4D2_RHIO9, Rhizopus delemar) | Y17G7B.15 | CG6742 (cnt-1) (cenB1A) |
| ACAP2 (Centaurin β2) | ACAP2 (CENTB2, KIAA0041) | ACAP2, Q15057 | | | |
| ACAP3 (Centaurin β5) | ACAP3 (CENTB5, KIAA1716) | ACAP3, Q96P50 | | | |
| AGAP1 (Centaurin γ2) | AGAP1 (CENTG2, KIAA1099) | AGAP1, Q9UPQ3 | (I1CMQ2_RHIO9, Rhizopus delemar) | Y39A1A.15 | CG31811 (cnt-2) (cenG1A) |
| AGAP2 (Centaurin γ1) | AGAP2 (CENTG1, KIAA0167) | AGAP2, Q99490 | | | |
| AGAP3 (Centaurin γ3) | AGAP3 (CENTG3) | AGAP3, Q96P47 | | | |
| AGAP4-11 | AGAP4 (CTGLF1, MRIP2); AGAP5-10 (CTGLF2-7); AGAP11 (KIAA1975) | AGAP4, Q96P64; AGAP5, A6NIR3; AGAP6, Q5VW22; AGAP7, Q5VU15; AGAP8, Q5SRD3; AGAP9, Q5VTM2; AGA10, Q5T2P9; AGA11, Q8TF27; |
| AGAP5-10 | AGAP5 (CTGLF2-7) |
| AGAP11 | AGAP11 (KIAA1975) |

| ADAP1 | ADAP1 (CENTA1) | ADAP1, Q75689 | – | – | – |
| ADAP2 | ADAP2 (CENTA2) | ADAP2, Q9NP8 |
| GIT1 | GIT1 | GIT1, Q9Y2X7 | – |
| GIT2 | GIT2 (KIAA0148) | GIT2, Q14161 |

| ASAP1 | ASAP1 (DDEF1, KIAA1249) | ASAP1, Q9ULH1 | – | – |
| ASAP2 | ASAP2 (DDEF2, KIAA0400) | ASAP2, O43150 |
| ASAP3 | ASAP3 (DDEF1L1, UPLC1) | ASAP3, Q8TDY4 |

| ARAP1 | ARAP1 (CENTD2, KIAA0782) | ARAP1, Q96P48 | – |
| ARAP2 | ARAP2 (CENTD1, KIAA0850) | ARAP2, Q8WZ64 |
| ARAP3 | ARAP3 (CENTD3) | ARAP3, Q8WWN8 |

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curved membranes of COPI vesicles (Bigay et al. 2003, 2005; Mesmin et al. 2007). In an elegant self-organizing mechanism, COPI vesicles are generated through activation of Arf1 at the Golgi, which recruits COPI to deform the membrane into a highly curved vesicle, which then recruits ArfGAP1 through its ALPS motif to hydrolyse the GTP on Arf1, releasing the coat and allowing fusion of the vesicle with its target membrane (Bigay et al. 2003). ArfGAP2 and 3 also bind to COPI vesicles, but do so through direct interaction with the COPI coat, and hence can also bind to COPI-coated regions that are not highly curved (Kliouchnikov et al. 2009; Weimer et al. 2008). ArfGAP1, 2, and 3 and their yeast orthologues Ges1p and Glo3p function at the early Golgi (Spang et al. 2010), whereas most of the other Arf GAPs function at the cell periphery in TGN–endosomal–plasma membrane trafficking and actin cytoskeleton remodelling (Inoue and Randazzo 2007; Sabe et al. 2006). The following chapter in this volume will describe the latter class of Arf GAPs in depth.

8.2 Localization and Functions of Arf Proteins

A major distinguishing feature of the Arf proteins is the presence of a myristoylated amino-terminal amphipathic helix that is necessary for membrane binding (Fig. 8.1a, b, c). Myristoylation is a cotranslational modification required for the essential functions of Arf1 in vivo (Kahn et al. 1995). In cells, myristoylation is required for correct localization of Arf proteins, including Golgi localization of Arf1 and PM localization of Arf6 (Donaldson and Jackson 2011). In vitro, the myristoylated group of the GDP-bound form of Arf1 is available for membrane insertion, permitting a weak association of the inactive form of Arf1 with membranes (Franco et al. 1995). Upon GTP binding, the N-terminal amphipathic helix of Arf1 is released and inserts into the membrane, resulting in tight membrane association (Antonny et al. 1997) (Fig. 8.1c). Structural studies revealed the details of this change in conformation, showing that the interswitch region changes position to occlude the hydrophobic pocket that harbours the amphipathic N-terminal helix in the GDP-bound form of Arf1 (Goldberg 1998). NMR studies of N-myristoylated Arf1-GTP further confirmed this mechanism (Liu et al. 2010). Thus, in addition to changes in the effector binding regions upon exchange of GDP for GTP, Arf proteins undergo a second change in conformation that brings them into very close contact with the membrane (Fig. 8.1c) (Antonny et al. 1997; Chavrier and Menetrey 2010). This property distinguishes them from other small G proteins of the Ras superfamily, including the Ras, Rho, and Rab Families, which have a long C-terminal linker to which their lipid membrane anchor is attached (Gillingham and Munro 2007b). Arf effectors are thus constrained to a position close to the membrane, in contrast to those of Rab and Rho, which can be located at a distance from the membrane surface (Gillingham and Munro 2007b; Khan and Menetrey 2013).
Another distinction between Arf proteins and those of the Rab and Rho families is that no guanosine diphosphate dissociation inhibitors (GDI) have been identified. All Arfs are tightly membrane bound in their active GTP-bound conformation because of nucleotide regulation of the position of the N-terminal amphipathic helix, and simply hydrolysing the GTP on Arf proteins is sufficient to render them soluble in vitro. Indeed, Arf1 and Arf3 appear to be released from membranes into the cytosol upon GTP hydrolysis in cells. However, Arf6 remains bound to membranes in cells in its GDP-bound conformation. There is also evidence that Arf4-GDP and Arf5-GDP remain membrane bound in cells, in the latter case, to the ER–Golgi intermediate compartment (ERGIC) (Chun et al. 2008; Duijsings et al. 2009). This membrane association is likely due to interaction of the GDP-bound forms with membrane-associated proteins. For Arf6, members of the Kalirin family of Rho GEFs have been shown to bind specifically to the GDP-bound form through their spectrin-like repeat domain (Koo et al. 2007). Arf6-GDP recruits Kalirin to the membrane where it subsequently activates Rac and RhoG to regulate actin dynamics (Koo et al. 2007). Arf6-GDP binds several TBC (Tre-2/Bub/Cdc16) domain-containing proteins, which have Rab GAP activity (Haas et al. 2007), including TBC1D24, a protein mutated in familial infantile myoclonic epilepsy (Falace et al. 2010), and the TRE17 oncogene (Martinu et al. 2004). Hence interactions with the GDP-bound form of a G protein could provide a mechanism for a single Arf protein to trigger alternative signalling pathways depending on the nucleotide bound, which could have important implications in human disease (Donaldson and Jackson 2011).

Following activation on membranes, GTP-bound Arfs recruit coat proteins, lipid-modifying enzymes, tethers, and other effector molecules that modulate the properties of membranes and mediate vesicle trafficking (Table 8.1). The first function of the Arf proteins to be identified was their ability to recruit cytosolic coat proteins to membranes. In the early secretory pathway, Arf1 recruits coatomer complex I (COPI), which sorts cargo proteins into COPI-coated vesicles as it curves the membrane to form the vesicle (Beck et al. 2009). Arf1 at the trans-Golgi network (TGN) also recruits the heterotetrameric clathrin adaptor proteins (AP), AP-1, AP-3, and AP-4 and the three monomeric Golgi-localized γ-ear-containing, ADP-ribosylation factor-binding proteins (GGAs 1–3) (Bonifacino and Lippincott-Schwartz 2003). These various coat proteins specifically bind to cargo proteins and incorporate them into forming vesicles for sorting and transport to their correct destination.

Arf proteins can also recruit and activate enzymes that alter membrane lipid composition. The first of these enzymes to be identified was phospholipase D (PLD), which hydrolyses phosphatidylcholine to generate phosphatidic acid (Brown et al. 1993; Cockcroft et al. 1994). PLD is activated by all Arf proteins and also by Arl1 (Hong et al. 1998). PLD activation by Arf6 is involved in a number of processes at the cell periphery, including regulated endocytosis and cell migration (D’Souza-Schorey and Chavrier 2006).

Another major function of Arf proteins is regulation of phosphoinositide levels in cells. All Arf proteins can both recruit to membranes and stimulate the activity of
phosphatidylinositol 4-phosphate, 5-kinase (PIP5K), an enzyme that phosphorylates inositol 4-phosphate (PI4P) at the 5-position to generate phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) (Honda et al. 1999). In cells, it is primarily Arf6 that colocalizes with PIP5K at the PM to generate PI(4,5)P2, which in turn stimulates PM ruffling (Honda et al. 1999). At the Golgi, Arf1 recruits and stimulates the activity of phosphatidylinositol 4-kinase, forming PI4P, an important membrane lipid for Golgi function (De Matteis and Godi 2004a).

8.3 New Insights into Coat Recruitment by Arf Proteins

As described above, Arf1 can recruit the COPI coat to early Golgi membranes, as well as the heterotetrameric clathrin adaptor complexes (AP-1, AP-3, AP-4) and the three monomeric adaptors (GGA1, GGA2, and GGA3) to trans-Golgi, TGN, and endosomal membranes. How a single Arf protein can recruit multiple coats to different membrane sites in cells is still not fully understood, but one important contribution to specificity comes from the Arf GEFs. In both mammalian and yeast cells, GBF1 and Gea1/2, respectively, interact directly with COPI (Deng et al. 2009), and knockdown of GBF1 inhibits COPI recruitment to membranes in mammalian cells (Deng et al. 2009; Ishizaki et al. 2008; Manolea et al. 2008; Szul et al. 2007). In contrast, knockdown of BIG1 and BIG2 Arf1 GEFs inhibits AP-1 and GGA recruitment to the trans-Golgi (Ishizaki et al. 2008; Manolea et al. 2008).

In yeast, a single class I Arf is responsible for recruiting COPI, AP-1, and GGA coats to membranes in cells, and hence mechanisms such as Arf GEF-mediated specificity are required. In mammalian cells, Arfs 3–5 could contribute an additional layer of specificity. It has been shown recently that a specific subset of Arf family members (Arf1, Arf4, and Arf5, but not Arf3 or Arf6) are incorporated into COPI vesicles reconstituted using cytosol (Popoff et al. 2011). A full understanding of the mechanisms determining the specificity of coat recruitment by Arf family members and their regulators is an important open question in the field.

Important insights into the recruitment of coats by Arf1 have come from recent structural studies (Ren et al. 2013; Yu et al. 2012). The structure of Arf1-GTP bound to a subcomplex of the COPI coat revealed a binding site on the γ subunit, which would position the entire COPI complex on the membrane surface in a conformation very similar to the membrane-bound AP-2/clathrin complex (Yu et al. 2012). COPI, AP-2/clathrin, and AP-1/clathrin complexes share a remarkable level of structural similarity, although AP-2 is recruited to membranes by plasma membrane PI(4,5)P2 rather than an activated Arf protein (Jackson et al. 2010). All of these coats have two large subunits that are symmetrically located within the complex, which in the case of COPI are the β and γ subunits. Biochemical studies confirmed a second Arf1-GTP binding site on the β subunit of COPI (Yu et al. 2012). The structure of Arf1 in complex with the entire central trunk region of the AP-1 adaptor complex revealed only one of the two Arf1-GTP binding sites on AP-1, on the β1 subunit (Ren et al. 2013). This Arf1–AP-1 structure...
is almost identical to the active, cargo-bound AP-2 complex (Jackson et al. 2010), and reveals a second interaction, involving the back side of Arf, opposite the switch regions, to a second site on the AP-1 γ subunit. Biochemical and cell biological analyses showed that recruitment of the AP-1 adaptor to membranes requires two binding sites for Arf1-GTP (one on each of the symmetrical β1 and γ subunits), in a manner analogous to those found for COPI. However, the conformation of the active Arf1-GTP–AP-1 complex docked to a membrane bilayer is not compatible with binding of Arf1 molecules at both recruitment sites (Ren et al. 2013). Hence the proposed model based on these results is recruitment of a closed conformation of Arf1 by two Arf1 molecules, a change in conformation to the open cargo-bound form of the adaptor with one molecule of cargo engaged, and then a large conformational change mediated by binding of the trunk of the γ subunit to the back side of Arf1-GTP, concomitantly releasing the γ recruitment site (Ren et al. 2013). Given the structural similarities, this model will likely apply to other Arf1-GTP–coat complexes, including COPI.

8.4 New Functions of Golgi-Localized Arfs

The majority of initial studies on the Arf proteins focused mainly on Arf1 at the Golgi and Arf6 at the cell periphery (D’Souza-Schorey and Chavrier 2006; Donaldson and Jackson 2011). This exclusivity was warranted, as Arf1 is the most highly expressed of the Arf proteins in cells, and is essential for viability both in mammalian cells (Reiling et al. 2013) and in budding yeast, S. cerevisiae (Stearns et al. 1990a). Arf6 has functions at the cell periphery in cell adhesion and motility that are involved in numerous human pathologies such as cancer, and is the only member of the class III Arfs in mammalian cells. Arf3, Arf4, and Arf5, similarly to Arf1, all localize to internal membranes, including the Golgi. As described above, phylogenetic studies support the conclusion that these other Class I and II Arfs arose late in animal cell evolution, so may be involved in more specialized functions, or in increasing the spatial or temporal resolution of Arf1-mediated processes (Manolea et al. 2010). Interestingly, partial depletion experiments show that under conditions where single knockdowns have little if any phenotypic effects, siRNA depletion of pairs of Arf1–Arf5 has effects on specific membrane trafficking steps (Volpicelli-Daley et al. 2005). For example, the double knockdown of Arf1 and Arf4 affects transport in the early secretory pathway, inhibiting COPI coat recruitment, but has little effect on several other trafficking pathways (Volpicelli-Daley et al. 2005). Consistent with this observation, Arf4 localizes to the ERGIC and cis-Golgi (Chun et al. 2008), and together with Arf1 at the cis-Golgi, it organizes trafficking between these compartments (Ben-Tekaya et al. 2010).

Arf1 and Arf3 differ only at seven amino acid positions in their amino- and carboxy-terminal regions and previously they were thought to function and localize identically in cells. However, a Golgi-targeting sequence contained within the α-3
helix of Arf1 and Arf3 targets a chimaera between Arf3 and Arf1 to the early Golgi (Honda et al. 2005). Furthermore, Arf3 localizes specifically to the trans-Golgi and TGN and this localization depends on four Arf3-specific amino acids contained in the N-terminal amphipathic helix, which are conserved among Arf3 homologues (Manolea et al. 2010) (Fig. 8.1b). Arf3, but not Arf1, becomes cytosolic at 20 °C, the temperature at which exit from the TGN is blocked (Manolea et al. 2010). These results support the conclusion that Arf3 has a critical role during exit from the Golgi.

Recently, Class II Arfs have been shown to participate in novel functions at the TGN. D. Deretic and colleagues have found that Arf4 specifically recognizes the VxPx cytosolic targeting motif in retinal rhodopsin to facilitate its transport into the rod outer segment, a specialized cilium (Deretic et al. 2005). This ciliary targeting complex includes Rab11, FIP3 (a dual Arf and Rab11 effector), and ASAP1, an Arf GAP (Mazelova et al. 2009), in addition to Arf4. The mechanism by which this complex facilitates the packaging of rhodopsin into post-Golgi carriers has not yet been determined, but it is known that rhodopsin itself initiates complex formation by recruiting Arf4. The rhodopsin-binding site of Arf4 has been mapped to the α-3 helix (Deretic et al. 2005), which corresponds to the region of Arf1 that binds the SNARE protein membrin to mediate targeting to the early Golgi (Honda et al. 2005). Hence, the α-3 helix might generally allow Arf protein binding to membrane receptors. Arf4 and Arf5 can also directly bind to the calcium-dependent activator protein for secretion (CAPS), which regulates exocytosis of dense core vesicles from nerve terminals (Sadakata et al. 2010). Arf1 and Arf4 together have been shown to play a role in endosome–TGN trafficking; the double knockdown causes tubulation of the recycling endosome and an inhibition of TGN38 and mannose-6-phosphate receptor trafficking from there back to the TGN (Nakai et al. 2013). How these roles of Arf4 and 5 at the TGN in cells can be reconciled with findings of Arf4 localization to, and Arf4 and 5 functioning at, the early Golgi (Ben-Tekaya et al. 2010; Chun et al. 2008; Popoff et al. 2011) is not known.

A recent study has found a specific role for Arf4 in mediating brefeldin A-induced apoptosis, a pathway used by human pathogens such as Chlamydia trachomatis and Shigella flexneri (Reiling et al. 2013). In response to brefeldin A and other Golgi-disrupting agents, cells upregulate expression of Arf4 at the transcriptional level, through proteolytic activation of the basic leucine zipper transcription factor CREB3 at the Golgi (Reiling et al. 2013). Interestingly, a previous study found that knockdown of GBF1 caused an upregulation of the unfolded protein response and apoptosis (Citterio et al. 2008), possibly through this newly defined CREB3/Arf4 pathway. These results suggest that Golgi stress might trigger a response through Arf4 aimed at re-establishing Golgi function, and if this fails, mediate a switch to induction of apoptosis (Reiling et al. 2013).
8.5 Arf1 in Lipid Trafficking

A novel function for Arf1 in lipid droplet (LD) metabolism has been uncovered recently (Beller et al. 2008; Guo et al. 2008; Soni et al. 2009). Lipid droplets are well known for their function in storage of energy in the form of triglycerides (Ducharme and Bickel 2008; Londos et al. 2005). More recently, their dynamic structure and integration with membrane trafficking pathways have revealed that they are in fact bona fide organelles (Walther and Farese 2012). LDs are distinct from other organelles in having a neutral lipid core surrounded by a phospholipid monolayer (Tauchi-Sato et al. 2002), rather than a bilayer encompassing an aqueous interior. In addition to triglycerides, which are the major energy storage molecules of eukaryotic cells, the neutral lipid core of LDs also contains esterified cholesterol. These two classes of neutral lipids serve as storage precursors of the two major lipid components of cellular membranes. Arf1, along with its GEF GBF1 and effector COPI, associates with LDs (Fig. 8.3) and is required for recruitment of a subset of lipid droplet associated proteins to the LD surface (Soni et al. 2009). In mammalian cells, these LD components include a triglyceride lipase (ATGL) and a perilipin family member (PLIN2) (Soni et al. 2009), and for ATGL at least, this function is conserved in Drosophila (Beller et al. 2008). GBF1 itself is recruited to LDs via HDS1, the domain just downstream of the catalytic Sec7 domain, which binds both liposomes and artificial droplets directly in vitro (Bouvet et al. 2013). This domain and the downstream HDS2 domain are both required for localization of GBF1 to the Golgi. However, when expressed alone as GFP fusions in cells, HDS1 and HDS2 are targeted only to LDs, not to the Golgi (Ellong et al. 2011). The Sec7 domain regulates HDS1 association with membranes, acting as an inhibitor of localization, and the N-terminal DCB and HUS domains are required in addition to target GBF1 to the Golgi (Bouvet et al. 2013).

B. Antonny and colleagues have proposed a novel mechanism of protein recruitment to the unique surface of LDs. In a recent study, they demonstrated that the density of phospholipids in the monolayer surrounding the neutral lipid core of an artificial LD could be decreased, leading as expected to an increase in surface tension (Thiam et al. 2013). Hence in contrast to a bilayer, which would tear with even a small decrease in phospholipid density in one leaflet, the phospholipids of the LD surface can be spread apart, albeit with the unfavourable effect of exposing the hydrophobic core to the aqueous environment of the cytosol. This increase in surface tension could favour the recruitment of cytosolic proteins to the LD, which would compensate for the lower density of phospholipids by helping to cover the LD surface. B. Antonny and F. Pincet further hypothesized that GBF1, Arf1, and COPI might function to recruit proteins to LDs by decreasing phospholipid density, as a result of their removal by budding of small droplets from the surface of a large LD. Indeed, Arf1-GTP and COPI were sufficient to cause budding of 60–100 nm diameter droplets from an artificial droplet surface in vitro (Thiam et al. 2013). In support of their hypothesis, these authors then found that the binding of α-synuclein, known to associate with LDs in cells (Cole et al. 2002), was promoted...
when the phospholipid density of artificial droplets decreased (Thiam et al. 2013). This result supports the hypothesis that LDs can become more “reactive” when they experience a decrease in phospholipid density on their surface, because of the unfavourable increase in surface tension. Together, these findings provide an elegant explanation for the function of GBF1, Arf1, and COPI in recruitment of proteins to the LD surface.

In addition to its function in lipid metabolism of LDs, Arf1 has an important function in lipid trafficking at the Golgi that is distinct from its role in vesicle formation. A number of lipid transfer proteins are recruited by Arf1 to membranes at sites of close contact between the ER and other organelles, known as membrane contact sites (MCS) (Levine and Rabouille 2005; Stefan et al. 2013). FAPP2, CERT, and OSBP transfer glucosylceramide, ceramide, and sterol, respectively, and all three possess a PH domain that requires both Arf1 and PI4P in order to bind to trans-Golgi membranes (De Matteis and Godi 2004b) (Table 8.1). The first clue that these proteins might bridge two different organelles came from studies...
indicating that in addition to a late Golgi-specific PH domain, they also carry a motif mediating binding to the ER-localized protein VAP (Lev 2010; Levine and Loewen 2006). A recent study showed that these two localization regions of OSBP are sufficient to mediate contact between the ER and Golgi, and moreover, that OSBP activity regulates this association (Mesmin et al. 2013). FAPP2-mediated delivery of glucosylceramide to the trans-Golgi has been shown to be dependent on Arf1 and also to be required for vesicular trafficking from the Golgi to the PM (D’Angelo et al. 2007). Hence Arf1, by recruiting proteins that mediate transfer of sphingolipid precursors and sterol, plays an essential role in establishing the characteristic lipid environment of the trans-Golgi, PM, and endosomal system of the cell, an important feature of cellular organization (Bigay and Antonny 2012). A key question regarding these transfer proteins is how they transport lipids such as sterols up their concentration gradient. For OSBP, B. Mesmin and colleagues showed that sterol is exchanged for PI4P at the trans-Golgi, where it is returned to the ER and the phosphate hydrolysed by the ER-resident PI4P phosphatase Sac1p. The energy from hydrolysis of phosphate on PI4P drives the transport of sterol up its concentration gradient (Mesmin et al. 2013).

### 8.6 New Insights into Arf6 Function

Arf6 is localized to the plasma membrane and regulates both the cortical actin cytoskeleton and endosomal membrane recycling. At the plasma membrane, Arf6 modulates membrane lipid composition through activation of PI4P 5-kinase (PIP5K) and PLD, resulting in production of PI(4,5)P2 and PA. These phospholipids can influence the sorting of membrane proteins within the PM, and are important for the formation of clathrin-coated pits during endocytosis. They are also required for the recruitment and activation of Rho family G proteins such as Rac to induce actin polymerization. Arf6 is associated with endosomal membranes derived from clathrin-independent forms of endocytosis, where it functions to recycle membrane components back to the plasma membrane (Grant and Donaldson 2009). Recycling through endosomal compartments by Arf6 is required for the polarized delivery of Cdc42, Rac, and the Par6 complex to the leading edge of migrating cells (Osmani et al. 2010), and for recycling of proteins involved in cell adhesion (Balasubramanian et al. 2007). Arf6 is also associated with clathrin-coated vesicles, where it mediates the rapid recycling of transferrin receptor back to the plasma membrane through interaction with the microtubule motor adaptor protein JIP4 after clathrin uncoating (Montagnac et al. 2011). Arf6 can interact with adaptor protein 2 (AP-2) (Paleotti et al. 2005) and with AP-2 and clathrin during G-protein coupled receptor cell signalling (Poupart et al. 2007). Hence Arf6 has a general function in mediating recycling of components back to the PM after endocytic internalization via different routes.

Studies of the Arf6 homologues in model organisms have illustrated the evolutionarily conserved nature of Arf6 function. Arf3p, the yeast Arf6 homologue,
affects polarization events such as bud site selection in *S. cerevisiae* (Huang et al. 2003), and the switch in cellular growth from monopolar extension to bipolar extension in fission yeast (Fujita 2008). Budding yeast Arf3p contributes to PM PI (4,5)P2 levels like its mammalian homologue (Smaczynska-de Rooij et al. 2008), and is involved in uncoating of clathrin-coated endocytic vesicles along with its specific GAP Gts1p (Toret et al. 2008). In the filamentous fungus *Aspergillus nidulans*, the Class III ArfB localizes to both the plasma membrane and endomembranes, and regulates endocytosis and polarity establishment during hyphal growth (Lee et al. 2008). Arf6 in Drosophila is also involved in endocytic recycling, which is required for cytokinesis in spermatocytes (Dyer et al. 2007). In mammalian cells, Arf6 is involved in cytokinesis through interaction with JIP4 (Montagnac et al. 2009). The crystal structure of Arf6 in complex with JIP4 shows that residues adjacent to the switch regions are structural determinants for the specific binding of JIP4 to Arf6 (Isabet et al. 2009).

In mammals, Arf6 is not required for early embryonic development, as demonstrated by the fact that homozygous knockout mice can develop to mid-gestation or even to birth, although they die shortly thereafter (Suzuki et al. 2006). Hence, the critical physiological roles of Arf6 in cell adhesion and cell migration appear to be more important for functions that become crucial late in development of the organism, such as wound healing and metastasis, rather than functions necessary for early development. Detailed descriptions of the functions of Arf6 in these processes have been shown in a large number of studies, and the reader is referred to recent excellent reviews on the subject (D'Souza-Schorey and Chavrier 2006; Schweitzer et al. 2011).

8.7 Arf GEFs: Cascades and Positive Feedback Loops

Of the seven subfamilies of Arf GEFs in eukaryotic cells, probably the most intensively studied are the cytohesin/Arno proteins. This subfamily was the first one to be identified in mammalian cells (Chardin et al. 1996), and in addition to a wide range of crucial physiological functions, its members have proved to be highly amenable to biochemical and structural characterization. The cytohesin/Arno GEFs function in plasma membrane–endosomal membrane trafficking routes, in cytoskeleton regulation, as well as in signal transduction pathways important for cell proliferation, immune response, and growth control (Casanova 2007; Kolanus 2007). Members of this GEF family can catalyse exchange on both Arf1 and Arf6 in vitro and in cells, although in vitro they are more efficient GEFs for Arf1 (Casanova 2007; Macia et al. 2001). Cytohesin activation is spatially regulated through relief of autoinhibition, positive feedback loops, and activation cascades. At the plasma membrane, the PH domains of cytohesin family members interact with PM-specific phosphoinositides and with the GTP-bound forms of Arf6 (Cohen et al. 2007) and Arl4 (Hofmann et al. 2007; Li et al. 2007), leading to cytohesin recruitment and further activation of Arf6 or Arf1 at the plasma membrane.
A crystal structure of the autoinhibited Sec7 domain in tandem with the PH domain of cytohesin3/Grp1 revealed that the C-terminal helix that follows the PH domain and the linker between the Sec7 and PH domains block the catalytic site (DiNitto et al. 2007). Interaction of the PH domain with Arf6-GTP and phosphoinositides, either PI(4,5)P$_2$ or PI(3,4,5)P$_3$, as well as the interaction of the polybasic C-terminus of cytohesin/Arno with acidic phospholipids, all contribute to relieving this autoinhibition (DiNitto et al. 2007). Reconstitution of the cytohesin/Arno exchange assay on liposomes, in the presence of both activating Arf6-GTP and substrate Arf1, revealed that mutations in the PH domain of cytohesin/Arno that abolished interaction with Arf6-GTP were completely inactive (Stalder et al. 2011). Hence interaction of the PH domain with an activating Arf protein is an absolute requirement for relief of cytohesin/Arno autoinhibition on membranes. A recent structure of the Sec7 and PH domains of Arno in complex with Arf6-GTP, when compared to the autoinhibited structure, reveals a large conformational change upon Arf6-GTP binding. Binding of Arf6-GTP creates grooves at the Arf6–PH domain interface into which the autoinhibitory elements bind, thus uncovering the binding site for the substrate Arf (Malaby et al. 2013). Together these studies demonstrate how precise spatial regulation of cytohesin/Arno activation is achieved: a specific phosphoinositide (PIP$_2$ and/or PIP$_3$), additional acidic phospholipids, and an active Arf localized to the plasma membrane must all coincide to relieve autoinhibition, thus restricting the membrane domain at which these GEFs can become active.

The activation of cytohesins by a GTP-bound Arf family member raises the question of whether they can engage in a positive feedback loop whereby the product of the reaction can stimulate exchange. Indeed, such a positive feedback loop has been demonstrated (Stalder et al. 2011). Cytohesin/Arno is one of the most efficient GEFs in vitro on its myristoylated Arf1 substrate ($k_{cat}/K_m \approx 10^6 \text{M}^{-1} \text{s}^{-1}$), and this is due in part to the stimulation of exchange by interaction of Arf1-GTP with the PH domain (Stalder et al. 2011). Given the high level of efficiency of this GEF, it would be reasonable to expect a tight regulation of its activity. As described above, one such mechanism is found in the absolute requirement for an activating Arf protein to relieve autoinhibition (Stalder et al. 2011). This study also showed that other Arf effectors are able to compete with the cytohesin/Arno PH domain for available Arf-GTP, suggesting that the activating Arf protein (Arl4-GTP or Arf6-GTP) must be present in excess of other effectors to reach a level sufficient to stimulate this GEF (Stalder et al. 2011). Hence, in order to activate cytohesin/Arno, a burst of Arl4- or Arf6-GTP must be produced to overcome autoinhibition, but once in its active conformation, this GEF has a high capacity to stimulate exchange on its Arf substrates due to the effect of positive feedback. Interestingly, stimulation of exchange activity by the product of the reaction has been demonstrated for the trans-Golgi localized Sec7p Arf1 GEF (Richardson et al. 2012), as well as for the Ras GEF Sos (Boykevisch et al. 2006). Hence, this type of positive feedback regulation may be a general property of small G proteins.

In the case of Sec7p, the HDS1 domain downstream of the catalytic Sec7 domain binds to membranes and to Arf1-GTP both in vitro and in cells, and like the PH
domain in the cytohesin proteins, is responsible for mediating relief of autoinhibition and the positive feedback effect (Richardson et al. 2012). For Sec7, the HDS1–Arf1-GTP interaction is required to maintain Golgi localization of Sec7p (Richardson et al. 2012). Interestingly, reduction of Arf1 levels in yeast cells is particularly detrimental to trans-Golgi function (Gall et al. 2000), a result that could potentially be explained by the need for Sec7 to have a high level of Arf1 for its autocatalytic activity. One consequence of this mechanism could be to maintain directionality in trafficking through the Golgi. As described above, the GBF/Gea family of GEFs act early in the secretory pathway, at the cis-Golgi, and Sec7/BIG GEFs act later at the trans-Golgi. Interestingly, the HDS1 domain of GBF1, also immediately downstream of the Sec7 domain, is a direct lipid binding domain that does not require Arf1-GTP for membrane binding like Sec7p does (Bouvet et al. 2013). These results taken together suggest that one mechanism to drive trafficking forward through the Golgi is initial recruitment of GBF1/Gea GEFs to produce Arf1-GTP, which then can recruit the later-acting Sec7/BIG GEFs to Golgi membranes.

Whether Arf6, Arf1, or both are the primary substrates for the cytohesin GEFs has been a long-standing controversy and is still an open question. However, Arf6-GTP is more efficient at relieving autoinhibition of cytohesins than Arf1-GTP, both in vitro and in cells (Cohen et al. 2007; DiNitto et al. 2007). This fact, combined with the requirement for acidic phospholipids in cytohesin membrane binding, would restrict activation of cytohesins to PM or endosomal membranes. These results illustrate the complexity of Arno activation and shed light on this long-standing debate over the physiological substrate of cytohesins in cells. The fact that Arf6-GTP can activate cytohesin/Arno, that the capacity to activate this GEF is highly dependent on relative levels of cytohesin/Arno and effectors, and that both Arf6 and Arf1 positive feedback loops exist, all need to be taken into consideration in evaluating in vivo results. Arf1 is required for specific processes at the PM such as recruitment of proteins to focal adhesions and in phagocytosis, and Arf1-GTP localizes to these sites (Beemiller et al. 2006; Furman et al. 2002; Kruljac-Letunic et al. 2003; Norman et al. 1998). In the forming phagocytic cup, Arf6-GTP is recruited early, followed by Arf1-GTP, at a stage that requires rapid insertion of new membrane. These results support the idea that the Arf6–cytohesin–Arf1 cascade may play an important role in processes that require a high level of Arf protein. Arf6 is less abundant than Arf1 in cells, and since both Arf1 and Arf6 can recruit effectors such as PI4P 5 kinase and PLD, processes requiring an acute activation of such effectors may rely on the more abundant Arf1 to provide an adequate supply. Another process in which this mechanism may operate is in the insulin signalling pathway, where both Arf1 and Arf6 were shown to contribute to activation of PI4P 5 kinase and PLD by cytohesin-2/Arno (Lim et al. 2010).
8.8 Arf Proteins and Their Regulators in Human Disease

The implication of Arf proteins and their GEFs and GAPs in human pathologies is a rapidly expanding area. Arfs and their regulators have been linked to neurodevelopmental disorders, neurodegenerative diseases, cancers, and both viral and bacterial infections. Here, I will describe examples of their roles in human disorders, and the reader is referred to other sources for a more comprehensive description of specific topics in this growing field (Dani et al. 2013; Lovrecic et al. 2010; Poincloux et al. 2009; Sabe et al. 2006, 2009; Seixas et al. 2013; Stafa et al. 2012; Tan and Evin 2012).

Mutations in the BIG2 Arf1 GEF have been linked to autosomal recessive periventricular heterotopia (ARPH), a disorder of neuronal migration that leads to severe malformation of the cerebral cortex (microcephaly) and developmental delay (Sheen et al. 2004). Two mutations in BIG2 have been identified in ARPH patients, one of which is a frame shift mutation that results in truncation of the majority of the protein (Sheen et al. 2004). The disease symptoms are a result of the failure of a specific class of neurons to migrate from their point of origin in the lateral ventricular proliferative zone to the cerebral cortex (Ferland et al. 2009; Sheen et al. 2004). This defect arises from a defect in vesicular trafficking that alters the adhesion properties of these neurons (Ferland et al. 2009). In addition to BIG2, mutations in the gene encoding filamin A also cause ARPH. A recent study has found a mechanistic connection between these two proteins in a mouse model of the disease (Zhang et al. 2013).

The IQSEC/BRAG Arf GEFs are highly expressed in the postsynaptic density of the central nervous system (Casanova 2007), and play important roles in signalling during synaptic transmission (Myers et al. 2012). This family of Arf GEFs use Arf6 as a substrate, but can also act on Arf5 (Moravec et al. 2012). BRAG1/IQSEC2 is mutated in X-linked nonsyndromic intellectual disability, a form of mental retardation. Three point mutations isolated from patients map to the Sec7 domain and result in proteins that cannot activate Arf6 normally (Shoubridge et al. 2010a, b). BRAG2 has been linked to alterations in synaptic content during long-term depression (LTD). Signalling through AMPA-type glutamate receptors facilitates LTD, and downregulation of activated AMPA receptors is normally regulated by AMPA receptor-mediated recruitment of BRAG2, which in turn activates Arf6 and endocytosis (Scholz et al. 2010).

The GEFs and GAPs for Arf1 and Arf6 at the cell periphery have roles which are becoming more clearly defined in the progression of numerous cancers (D’Souza-Schorey and Chavrier 2006; Muller et al. 2010; Peng et al. 2013; Sabe et al. 2006, 2009; Sangar et al. 2014; Yoo et al. 2012). Cytohesin/Arno GEFs affect signalling through epidermal growth factor (EGF)/ErbB, promoting conformational changes that increase trans-phosphorylation of EGFRs upon ligand-induced dimerization (Bill et al. 2010). Importantly, these GEFs are specifically inhibited by SecinH3, which holds promise as anti-cancer drug, since treatment of an EGF receptor-
dependent lung cancer cell line with SecinH3 resulted in reduced proliferation (Bill et al. 2010).

Arf proteins and their regulators are hijacked by numerous bacterial and viral pathogens (Dautry-Varsat et al. 2005; Goody and Itzen 2013; Hsu et al. 2010; Humphreys et al. 2013; Matto et al. 2011). The Arf GEF GBF1 is required for the replication of numerous viruses, including enteroviruses, hepatitis C virus, and coronaviruses (Belov et al. 2008; Goueslain et al. 2010; Lanke et al. 2009; Winchester et al. 2008). These viruses remodel ER and early secretory pathway membranes to form replication complexes, for which they subvert the function of GBF1. Interestingly, a major requirement for replication of several of these viruses is gaining control of host lipid trafficking and metabolism pathways (Alvisi et al. 2011; Hsu et al. 2010; Ilnytska et al. 2013).

8.9 Conclusions and Perspectives

The activity of Arf proteins is regulated in a spatiotemporal manner by their GEFs and GAPs, highlighting the importance of precise localization of these regulators. The mechanisms of this spatiotemporal control are now beginning to emerge. Coincidence detection mechanisms involving binding to specific lipids and protein partners play an important role, and for the cytohesins, a fairly complete description of how relief of autoinhibition is coupled to precise spatial cues has been obtained. Arf activation cascades have been demonstrated for the cytohesins, but may play an important role in the activation of other families of GEFs as well. Such cascades, similarly to those demonstrated for Rab G proteins, could be involved in transforming one membrane domain into another during highly dynamic membrane trafficking maturation events. These transformations involve coordinate changes in both the lipid and protein composition of each membrane domain, a specific function of the Arf family members, which recruit both lipid-modifying enzymes and protein effectors such as coats and tethers. The signature feature of Arf family proteins, their N-terminal membrane-binding amphipathic helix, ensures that they are closely associated with the lipid bilayer in their GTP-bound form. Future studies on how Arf family proteins function will therefore continue to require in vitro reconstitution on model membranes.

Among the small G proteins, the Arf family is perhaps the most enigmatic. Why so few? What is the unifying principle in the broad range of functions defined for each one? The small number of Arf proteins in a given eukaryotic cell is an ancient property of the Arf proteins, since phylogenetic analyses indicate that the last common ancestor of the eukaryotes had only one Arf protein, despite a significant level of complexity, and numerous distinct Arf regulators and effectors (COPI, trans-Golgi clathrin-adaptor proteins, coiled-coil tether, and phospholipase D). One obvious consequence of having a single protein with different functions is that the various processes can be integrated in a simple manner. For example, the single pool of Arf1 must be distributed among all of the different GEF, GAP, and effector
complexes in the cell, which could provide a type of global regulation of all of the functions that these different complexes carry out. In particular, Arf1 is involved in the recruitment of coat complexes and membrane tethers at the Golgi that mediate vesicular trafficking, and also in lipid droplet metabolism and in recruitment of at least three lipid transfer proteins that mediate non-vesicular lipid trafficking in cells. Hence a fundamental role of the Arf proteins may be in coordinating both vesicular and non-vesicular lipid trafficking pathways in the cell.

Acknowledgements Work in the author’s laboratory is funded by grants from the Agence Nationale de la Recherche (ANR) and the Fondation pour la Recherche Médicale (FRM) and by the CNRS, France.

References

Alix E, Chesnel L, Bowzard BJ, Tucker AM, Delprato A et al (2012) The capping domain in RalF regulates effector functions. PLoS Pathog 8:e1003012

Alvisi G, Madan V, Bartenschlager R (2011) Hepatitis C virus and host cell lipids: an intimate connection. RNA Biol 8:258–269

Amor JC, Swails J, Zhu X, Roy CR, Nagai H et al (2005) The structure of RalF, an ADP-ribosylation factor guanine nucleotide exchange factor from Legionella pneumophila, reveals the presence of a cap over the active site. J Biol Chem 280:1392–1400

Antonny B, Beraud-Dufour S, Chardin P, Chabre M (1997) N-terminal hydrophobic residues of the G-protein ADP-ribosylation factor-1 insert into membrane phospholipids upon GDP to GTP exchange. Biochemistry 36:4675–4684

Balasubramanian N, Scott DW, Castle JD, Casanova JE, Schwartz MA (2005) The structure of RalF, an ADP-ribosylation factor guanine nucleotide exchange factor from Legionella pneumophila, reveals the presence of a cap over the active site. J Biol Chem 280:1392–1400

Beek R, Rawet M, Wieland FT, Cassel D (2009) The COPI system: molecular mechanisms and function. FEBS Lett 583:2701–2709

Beemiller P, Hoppe AD, Swanson JA (2006) A phosphatidylinositol-3-kinase-dependent signal transition regulates ARF1 and ARF6 during Fcgamma receptor-mediated phagocytosis. PLoS Biol 4:e162

Beller M, Sztalryd C, Southall N, Bell M, Jackie H et al (2008) COPI complex is a regulator of lipid homeostasis. PLoS Biol 6:e292

Belov GA, Feng Q, Nikovics K, Jackson CL, Ehrenfeld E (2008) A critical role of a cellular membrane traffic protein in poliovirus RNA replication. PLoS Pathog 4:e1000216

Ben-Tekaya H, Kahn RA, Hauri HP (2010) ADP ribosylation factors 1 and 4 and group VIA phospholipase A(2) regulate morphology and intraorganellar traffic in the endoplasmic reticulum-Golgi intermediate compartment. Mol Biol Cell 21:4130–4140

Bigay J, Antonny B (2012) Curvature, lipid packing, and electrostatics of membrane organelles: defining cellular territories in determining specificity. Dev Cell 23:886–895

Bigay J, Gounon P, Robineau S, Antonny B (2003) Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature. Nature 426:563–566

Bigay J, Casella JF, Drin G, Mesmin B, Antonny B (2005) ArfGAP1 responds to membrane curvature through the folding of a lipid packing sensor motif. EMBO J 24:2244–2253

Bill A, Schmitz A, Albertoni B, Song JN, Heukamp LC et al (2010) Cytohesins are cytoplasmic ErbB receptor activators. Cell 143:201–211

Bonifacino JS, Lippincott-Schwartz J (2003) Coat proteins: shaping membrane transport. Nat Rev 4:409–414
Bouvet S, Golinelli-Cohen MP, Contremoulins V, Jackson CL (2013) Targeting of the Arf-GEF GBF1 to lipid droplets and Golgi membranes. J Cell Sci 126:4794–4805
Boykevisch S, Zhao C, Sondermann H, Philippidou P, Hagegoua S et al (2006) Regulation of ras signaling dynamics by Sos-mediated positive feedback. Curr Biol 16:2173–2179
Brown HA, Gutowski S, Moomaw CR, Slaughter C, Sternweis PC (1993) ADP-ribosylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. Cell 75:1137–1144
Calcia A, Gat G, Di Gregorio E, Talarico F, Naretto VG et al (2013) Bilaterally cleft lip and bilateral thumb polydactyly with triphalangeal component in a patient with two de novo deletions of HSA 4q32 and 4q34 involving PDGFC, GRIA2, and FBXO8 genes. Am J Med Genet A 161:2656–2662
Casanova JE (2007) Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. Traffic 8:1476–1485
Chardin P, Paris S, Antony B, Robineau S, Beraud-Dufour S et al (1996) A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. Nature 384:481–484
Chavrier P, Menetrey J (2010) Toward a structural understanding of arf family:effector specificity. Structure 18:1552–1558
Chen KY, Tsai PC, Hsu JW, Hsu HC, Fang CY et al (2010) Syt1p promotes activation of Arl1p at the late Golgi to recruit Imh1p. J Cell Sci 123:3478–3489
Chun J, Shapovalova Z, Dejagaard SY, Presley JF, Melancon P (2008) Characterization of class I and II ADP-ribosylation factors (Arfs) in live cells: GDP-bound class II Arfs associate with the ER-Golgi intermediate compartment independently of GBF1. Mol Biol Cell 19:3488–3500
Citterio C, Vichi A, Pacheco-Rodriguez G, Aponte AM, Moss J, Vaughan M (2008) Unfolded protein response and cell death after depletion of brefeldin A-inhibited guanine nucleotide-exchange protein GBF1. Proc Natl Acad Sci USA 105:2877–2882
Cockcroft S, Thomas GM, Fensome A, Geny B, Cunningham E et al (1994) Phospholipase D: a downstream effector of ARF in granulocytes. Science 263:523–526
Cohen LA, Honda A, Varnai P, Brown FD, Balla T, Donaldson JG (2007) Active Arf6 recruits ARNO/cytohesin GEFs to the PM by binding their PH domains. Mol Biol Cell 18:2244–2253
Cole NB, Murphy DD, Grider T, Rueter S, Brasaemle D, Nussbaum RL (2002) Lipid droplet binding and oligomerization properties of the Parkinson’s disease protein alpha-synuclein. J Biol Chem 277:6344–6352
Cox R, Mason-Gamer RJ, Jackson CL, Segev N (2004) Phylogenetic analysis of Sec7-domain-containing Arf nucleotide exchangers. Mol Biol Cell 15:1487–1505
Cukierman E, Huber I, Rotman M, Cassel D (1995) The ARF1 GTPase-activating protein: zinc finger motif and Golgi complex localization. Science 270:1999–2002
D’Angelo G, Polishchuk E, Di Tullio G, Santoro M, Di Campli A et al (2007) Glycosphingolipid synthesis requires FAPP2 transfer of glucosylceramide. Nature 449:62–67
Dacks JB, Field MC (2007) Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode. J Cell Sci 120:2977–2985
Dani N, Barbosa AJ, Del Rio A, Di Girolamo M (2013) ADP-ribosylated proteins as old and new drug targets for anticancer therapy: the example of ARF6. Curr Pharm Des 19:624–633
Dautry-Varsat A, Subtil A, Hackstadt T (2005) Recent insights into the mechanisms of Chlamydia entry. Cell Microbiol 7:1714–1722
De Matteis MA, Godi A (2004a) PI-loting membrane traffic. Nat Cell Biol 6:487–492
De Matteis MA, Godi A (2004b) Protein-lipid interactions in membrane trafficking at the Golgi complex. Biochim Biophys Acta 1666:264–274
Deng Y, Golinelli-Cohen MP, Smirnova E, Jackson CL (2009) A COPI coat subunit interacts directly with an early-Golgi localized Arf exchange factor. EMBO Rep 10:58–64
Deretic D, Williams AH, Ransom N, Morel V, Hargrave PA, Arendt A (2005) Rhodopsin C terminus, the site of mutations causing retinal disease, regulates trafficking by binding to ADP-ribosylation factor 4 (ARF4). Proc Natl Acad Sci USA 102:3301–3306
DiNitto JP, Delprato A, Gabe Lee MT, Cronin TC, Huang S et al (2007) Structural basis and mechanism of autoregulation in 3-phosphoinositide-dependent Grp1 family Arf GTPase exchange factors. Mol Cell 28:569–583
Donaldson JG, Jackson CL (2011) ARF family G proteins and their regulators: roles in membrane transport, development and disease. Nat Rev 12:362–375

D’Souza-Schorey C, Chavrier P (2006) ARF proteins: roles in membrane traffic and beyond. Nat Rev 7:347–358

Ducharme NA, Bickel PE (2008) Lipid droplets in lipogenesis and lipolysis. Endocrinology 149:942–949

Duijsings D, Lanke KH, van Dooren SH, van Dommelen MM, Wetzels R et al (2009) Differential membrane association properties and regulation of class I and class II Arfs. Traffic 10:316–323

Dyer N, Rebollo E, Dominguez P, Elkhatib N, Chavrier P et al (2007) Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. Development 134:4437–4447

Ellong EN, Soni KG, Bui QT, Sougrat R, Golinelli-Cohen MP, Jackson CL (2011) Interaction between the triglyceride lipase ATGL and the Arf1 activator GBF1. PLoS One 6:e21889

Falace A, Filippello F, La Padula V, Vanni N, Madia F et al (2010) TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. Am J Hum Genet 87:365–370

Ferland RJ, Batiz LF, Neal J, Lian G, Bundock E et al (2009) Disruption of neural progenitors along the ventricular and subventricular zones in periventricular heterotopia. Hum Mol Genet 18:497–516

Folly-Klan M, Alix E, Stalder D, Ray P, Duarte LV et al (2013) A novel membrane sensor controls the localization and ArfGEF activity of bacterial RalF. PLoS Pathog 9:e1003747

Franco M, Chardin P, Chabre M, Paris S (1995) Myristoylation of ADP-ribosylation factor 1 facilitates nucleotide exchange at physiological Mg2+ levels. J Biol Chem 270:1337–1341

Franco M, Peters PJ, Boretto J, van Donselaar E, Neri A et al (1999) EFA6, a sec7 domain-containing exchange factor for ARF6, coordinates membrane recycling and actin cytoskeleton organization. EMBO J 18:1480–1491

Franzusoff A, Redding K, Crosby J, Fuller RS, Schekman R (1991) Localization of components involved in protein transport and processing through the yeast Golgi apparatus. J Cell Biol 112:27–37

Fujita A (2008) ADP-ribosylation factor arf6p may function as a molecular switch of new end take off in fission yeast. Biochem Biophys Res Commun 366:193–198

Furman C, Short SM, Subramanian RR, Zetter BR, Roberts TM (2002) DEF-1/ASAP1 is a GTPase-activating protein (GAP) for ARF1 that enhances cell motility through a GAP-dependent mechanism. J Biol Chem 277:7962–7969

Gall WE, Higginbotham MA, Chen C, Ingram MF, Cyr DM, Graham TR (2000) The auxilin-like phosphoprotein Swa2p is required for clathrin function in yeast. Curr Biol 10:1349–1358

Gebbie LK, Burn JE, Hocart CH, Williamson RE (2005) Genes encoding ADP-ribosylation factors in Arabidopsis thaliana L. Heyn.; genome analysis and antisense suppression. J Exp Bot 56:1079–1091

Gillingham AK, Munro S (2007a) Identification of a guanine nucleotide exchange factor for Arf3, the yeast orthologue of mammalian Arf6. PLoS One 2:e842

Gillingham AK, Munro S (2007b) The small G proteins of the Arf family and their regulators. Annu Rev Cell Dev Biol 23:579–611

Goldberg J (1998) Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. Cell 95:237–248

Goody RS, Itzen A (2013) Modulation of small GTPases by legionella. Curr Top Microbiol Immunol 376:117–133

Goueslain L, Alsaleh K, Horellou P, Roingeard P, Descamps V et al (2010) Identification of GBF1 as a cellular factor required for hepatitis C virus RNA replication. J Virol 84:773–787

Grant BD, Donaldson JG (2009) Pathways and mechanisms of endocytic recycling. Nat Rev 10:597–608

Guo Y, Walther TC, Rao M, Stuurman N, Goshima G et al (2008) Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. Nature 453:657–661

Haas AK, Yoshimura S, Stephens DJ, Preisinger C, Fuchs E, Barr FA (2007) Analysis of GTPase-activating proteins: Rab1 and Rab43 are key Rabes required to maintain a functional Golgi complex in human cells. J Cell Sci 120:2997–3010
Hofmann I, Thompson A, Sanderson CM, Munro S (2007) The Arl4 family of small G proteins can recruit the cytohesin Arf6 exchange factors to the plasma membrane. Curr Biol 17:711–716
Honda A, Nogami M, Yokozeki T, Yamazaki M, Nakamura H et al (1999) Phosphatidylinositol 4-phosphate 5-kinase alpha is a downstream effector of the small G protein ARF6 in membrane ruffle formation. Cell 99:521–532
Honda A, Al-Awar OS, Hay JC, Donaldson JG (2005) Targeting of Arf-1 to the early Golgi by membrin, an ER-Golgi SNARE. J Cell Biol 168:1039–1051
Hong JX, Lee FJ, Patton WA, Lin CY, Moss J, Vaughan M (1998) Phospholipid- and GTP-dependent activation of cholera toxin and phospholipase D by human ADP-ribosylation factor-like protein 1 (HARL1). J Biol Chem 273:15872–15876
Hsu NY, Ilnytska O, Belov G, Santiana M, Chen YH et al (2010) Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. Cell 141:799–811
Huang CF, Liu YW, Tung L, Lin CH, Lee FJ (2003) Role for Arf3p in development of polarity, but not endocytosis, in Saccharomyces cerevisiae. Mol Biol Cell 14:3834–3847
Humfrey D, Davidson AC, Hume PJ, Makin LE, Koronakis V (2013) Arf6 coordinates actin assembly through the WAVE complex, a mechanism usurped by Salmonella to invade host cells. Proc Natl Acad Sci USA 110:16880–16885
Ilnytska O, Santiana M, Hsu NY, Du WL, Chen YH et al (2013) Enteroviruses harness the cellular endocytic machinery to remodel the host cell cholesterol landscape for effective viral replication. Cell Host Microbe 14:281–293
Inoue H, Randazzo PA (2007) Arf GAPs and their interacting proteins. Traffic 8:1465–1475
Isabet T, Montagnac G, Regazzoni K, Raynal B, El Khadali F et al (2009) The structural basis of Arf effector specificity: the crystal structure of ARF6 in a complex with JIP4. EMBO J 28:2835–2845
Ishizaki R, Shin HW, Mitsushashi H, Nakayama K (2008) Redundant roles of BIG2 and BIG1, guanine-nucleotide exchange factors for ADP-ribosylation factors in membrane traffic between the trans-Golgi network and endosomes. Mol Biol Cell 19:2650–2660
Jackson LP, Kelly BT, McCoy AJ, Gaffry T, James LC et al (2010) A large-scale conformational change couples membrane recruitment to cargo binding in the AP2 clathrin adaptor complex. Cell 141:1220–1229
Kahn RA, Gilman AG (1986) The protein cofactor necessary for ADP-ribosylation of Gs by cholera toxin is itself a GTP binding protein. J Biol Chem 261:7906–7911
Kahn RA, Clark J, Rulka C, Stearns T, Zhang CJ et al (1995) Mutational analysis of Saccharomyces cerevisiae ARF1. J Biol Chem 270:143–150
Khan AR, Menetrey J (2013) Structural biology of Arf and Rab GTPases’ effector recruitment and specificity. Structure 21:1284–1297
Klausner RD, Donaldson JG, Lippincott-Schwartz J (1992) Brefeldin A: insights into the control of membrane traffic and organelle structure. J Cell Biol 116:1071–1080
Kliouchnikov L, Bigay J, Mesmin B, Parnis A, Rawet M et al (2009) Discrete determinants in ArfGAP2/3 conferring Golgi localization and regulation by the COPI coat. Mol Biol Cell 20:859–869
Kolanus W (2007) Guanine nucleotide exchange factors of the cytohesin family and their roles in signal transduction. Immunol Rev 218:102–113
Koo TH, Eipper BA, Donaldson JG (2007) Arf6 recruits the Rac GEF Kalirin to the plasma membrane recruiting Rac activation. BMC Cell Biol 8:29
Koumandou VL, Wickstead B, Ginger ML, van der Giezen M, Dacks JB, Field MC (2013) Molecular paleontology and complexity in the last eukaryotic common ancestor. Crit Rev Biochem Mol Biol 48:373–396
Krujic-Letunic A, Moellemen J, Kallin A, Wieland F, Blaukat A (2003) The tyrosine kinase Pyk2 regulates Arf1 activity by phosphorylation and inhibition of the Arf-GTPase-activating protein ASAP1. J Biol Chem 278:29560–29570
Lanke KH, van der Schaar HM, Belov GA, Feng Q, Duijsings D et al (2009) GBF1, a guanine nucleotide exchange factor for Arf, is crucial for coxsackievirus B3 RNA replication. J Virol 83:11940–11949
Lee MC, Miller EA, Goldberg J, Orci L, Schekman R (2004) Bi-directional protein transport between the ER and Golgi. Annu Rev Cell Dev Biol 20:87–123
Lee SC, Schmidtke SN, Dangott LJ, Shaw BD (2008) Aspergillus nidulans ArfB plays a role in endocytosis and polarized growth. Eukaryot Cell 7:1278–1288
Lev S (2010) Non-vesicular lipid transport by lipid-transfer proteins and beyond. Nat Rev 11:739–750
Levine T, Loewen C (2006) Inter-organelle membrane contact sites: through a glass, darkly. Curr Opin Cell Biol 18:371–378
Levine T, Rabouille C (2005) Endoplasmic reticulum: one continuous network compartmentalized by extrinsic cues. Curr Opin Cell Biol 17:362–368
Li Y, Kelly WG, Logsdon JM Jr, Schurko AM, Harfe BD et al (2004) Functional genomic analysis of the ADP-ribosylation factor family of GTPases: phylogeny among diverse eukaryotes and function in C. elegans. FASEB J 18:1834–1850
Li CC, Chiang WC, Wu TS, Pacheco-Rodriguez G, Moss J, Lee FJ (2007) ARL4D recruits cytohesin-2/ARNO to modulate actin remodeling. Mol Biol Cell 18:4420–4437
Lim J, Zhou M, Veenstra TD, Morrison DK (2010) The CNK1 scaffold binds cytohesins and promotes insulin pathway signaling. Genes Dev 24:1496–1506
Liu Y, Kahn RA, Prestegard JH (2010) Dynamic structure of membrane-anchored Arf*GTP. Nat Struct Mol Biol 17:876–881
Londos C, Zaal V, Ciechanover A, Karsenty G, Kandil S et al (2010) ADP-ribosylation factor guanine nucleotide-exchange factor 2 (ARFGEF2): a new potential biomarker in Huntington’s disease. J Int Med Res 38:1653–1662
Macia E, Chabre M, Franco M (2001) Specificities for the small G proteins ARF1 and ARF6 of the guanine nucleotide exchange factors ARNO and EFA6. J Biol Chem 276:24925–24930
Malaby AW, van den Berg B, Lambright DG (2013) Structural basis for membrane recruitment and allosteric activation of cytohesin family Arf GTPase exchange factors. Proc Natl Acad Sci USA 110:14213–14218
Manolea F, Claude A, Chun J, Rosas J, Melancon P (2008) Distinct functions for Arf guanine nucleotide exchange factors at the Golgi complex: GBF1 and BIGs are required for assembly and maintenance of the Golgi stack and trans-Golgi network, respectively. Mol Biol Cell 19:523–535
Manolea F, Chun J, Chen DW, Clarke I, Summerfeldt N et al (2010) Arf3 is activated uniquely at the trans-Golgi network by brefeldin A-inhibited guanine nucleotide exchange factors. Mol Biol Cell 21:1836–1849
Martinu L, Masuda-Robens JM, Robertson SE, Santy LC, Casanova JE, Chou MM (2004) The TBC (Tre-2/Bub2/Cdc16) domain protein TRE17 regulates plasma membrane-endoosomal trafficking through activation of Arf6. Mol Cell Biol 24:9752–9762
Mato M, Sklan EH, David N, Melamed-Book N, Casanova JE et al (2011) Role for ADP ribosylation factor 1 in the regulation of hepatitis C virus replication. J Virol 85:946–956
Mazelova J, Astuto-Gribble L, Inoue H, Tam BM, Schonteich E et al (2009) Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. EMBO J 28:183–192
Mesmin B, Drin G, Levi S, Rawet M, Cassel D et al (2007) Two lipid-packing sensor motifs contribute to the sensitivity of ArfGAP1 to membrane curvature. Biochemistry 46:1779–1790
Mesmin B, Bigay J, Moser von Fiselnk J, Lacs-Gervais S, Drin G, Antonny B (2013) A four-step cycle driven by Pl(4)P hydrolysis directs sterol/Pl(4)P exchange by the ER-Golgi tether OSBP. Cell 155:830–843
Montagnac G, Sibarita JB, Loubrey S, Daviet L, Romao M et al (2009) ARF6 Interacts with JIP4 to control a motor switch mechanism regulating endosome traffic in cytokinesis. Curr Biol 19:184–195

Montagnac G, de Forges H, Smythe E, Guedry C, Romao M et al (2011) Decoupling of activation and effector binding underlies ARF6 priming of fast endocytic recycling. Curr Biol 21:574–579

Moravec R, Conger KK, D’Souza R, Allison AB, Casanova JE (2012) BRAG2/GEF100/IQSec1 interacts with clathrin and regulates alpha5beta1 integrin endocytosis through activation of ADP ribosylation factor 5 (Arf5). J Biol Chem 287:31138–31147

Muller T, Stein U, Poletti A, Garzia L, Rothley M et al (2010) ASAP1 promotes tumor cell motility and invasiveness, stimulates metastasis formation in vivo, and correlates with poor survival in colorectal cancer patients. Oncogene 29:2393–2403

Myers KR, Wang G, Sheng Y, Conger KK, Casanova JE, Zhu JJ (2012) Arf6-GEF BRAG1 regulates JNK-mediated synaptical removal of GluA1-containing AMPA receptors: a new mechanism for nonsyndromic X-linked mental disorder. J Neurosci 32:11716–11726

Nagai H, Kagan JC, Zhu X, Kahn RA, Roy CR (2002) A bacterial guanine nucleotide exchange factor activates ARF on Legionella phagosomes. Science 295:679–682

Nakai W, Kondo Y, Saitoh A, Naito T, Nakayama K, Shin HW (2013) ARF1 and ARF4 regulate recycling endosomal morphology and retrograde transport from endosomes to the Golgi apparatus. Mol Biol Cell 24:2570–2581

Norman JC, Jones D, Barry ST, Holt MR, Cockcroft S, Critchley DR (1998) ARF1 mediates paxillin recruitment to focal adhesions and potentiates Rho-stimulated stress fiber formation in intact and permeabilized Swiss 3T3 fibroblasts. J Cell Biol 143:1981–1995

Osmani N, Peglion F, Chavrier P, Etienne-Manneville S (2010) Cdc42 localization and cell polarity depend on membrane traffic. J Cell Biol 191:1261–1269

Paleotti O, Macia E, Luton F, Klein S, Partisani M et al (2005) The small G-protein Arf6GTP recruits the AP-2 adaptor complex to membranes. J Biol Chem 280:21661–21666

Peng H, Dara L, Li TW, Zheng Y, Yang H et al (2013) MAT2B-GIT1 interplay activates MEK1/ERK 1 and 2 to induce growth in human liver and colon cancer. Hepatology 57:2299–2313

Peyroche A, Paris S, Jackson CL (1996) Nucleotide exchange on ARF mediated by yeast Gea1 protein. Nature 384:479–481

Peyroche A, Courbeyrette R, Rambourg A, Jackson CL (2001) The ARF exchange factors Gea1p and Gea2p regulate Golgi structure and function in yeast. J Cell Sci 114:2241–2253

Poincloux R, Lizarraga F, Chavrier P (2009) Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia. J Cell Sci 122:3015–3024

Popoff V, Langer JD, Reckmann I, Hellwig A, Kahn RA et al (2011) Several ADP-ribosylation factor (Arf) isoforms support COP1 vesicle formation. J Biol Chem 286:35634–35642

Poupart ME, Fessart D, Cotton M, Laporte SA, Claing A (2007) ARF6 regulates angiotensin II type 1 receptor endocytosis by controlling the recruitment of AP-2 and clathrin. Cell Signal 19:2370–2378

Price HP, Stark M, Smith DF (2007) Trypanosoma brucei ARF1 plays a central role in endocytosis and golgi-lysosome trafficking. Mol Biol Cell 18:864–873

Reiling JH, Olive AJ, Sanyal S, Carette JE, Brummelkamp TR et al (2013) A CREB3–ARF4 signalling pathway mediates the response to Golgi stress and susceptibility to pathogens. Nat Cell Biol 15:1473–1485

Ren X, Farias GG, Canagarajah BJ, Bonifacino JS, Hurley JH (2013) Structural basis for recruitment and activation of the AP-1 clathrin adaptor complex by Arf1. Cell 152:755–767

Richardson BC, McDonold CM, Fromme JC (2012) The Sec7 Arf-GEF is recruited to the trans-Golgi network by positive feedback. Dev Cell 22:799–810

Rothman JE, Wieland FT (1996) Protein sorting by transport vesicles. Science 272:227–234

Sabe H, Onodera Y, Mazaki Y, Hashimoto S (2006) ArfGAP family proteins in cell adhesion, migration and tumor invasion. Curr Opin Cell Biol 18:558–564
Sabe H, Hashimoto S, Morishige M, Ogawa E, Hashimoto A et al (2009) The EGFR-GEP100-Arf6-AMAP1 signaling pathway specific to breast cancer invasion and metastasis. Traffic 10:982–993
Sadakata T, Shinoda Y, Sekine Y, Saruta C, Itakura M et al (2010) Interaction of calcium-dependent activator protein for secretion 1 (CAPS1) with the class II ADP-ribosylation factor small GTPases is required for dense-core vesicle trafficking in the trans-Golgi network. J Biol Chem 285:38710–38719
Sangar F, Schreurs AS, Umana-Diaz C, Claperon A, Desbois-Mouthon C et al (2014) Involvement of small ArfGAP1 (SMAP1), a novel Arf6-specific GTPase-activating protein, in microsatellite instability oncogenesis. Oncogene 33:2758–2767
Schlacht A, Mowbrek K, Elias M, Kahn RA, Dacks JB (2013) Ancient complexity, opisthokont plasticity, and discovery of the 11th subfamily of Arf GAP proteins. Traffic 14:636–649
Schleifer LS, Kahn RA, Hanski E, Northup JK, Sternweis PC, Gilman AG (1982) Requirements for cholera toxin-dependent Arf-ribosylation of the regulatory component of adenylate cyclase. J Biol Chem 257:20–23
Scholz R, Berberich S, Rathgeber L, Kolleker A, Kohr G, Kornau HC (2010) AMPA receptor signaling through BRAG2 and Arf6 critical for long-term synaptic depression. Neuron 66:768–780
Schweitzer JK, Sedgwick AE, D’Souza-Schorey C (2011) ARF6-mediated endocytic recycling impacts cell movement, cell division and lipid homeostasis. Semin Cell Dev Biol 22:39–47
Seixas E, Barros M, Seabra MC, Barral DC (2013) Rab and Arf proteins in genetic diseases. Traffic 14:871–885
Sheen VL, Ganesh VS, Topcu M, Sebire G, Bodell A et al (2004) Mutations in ARFGEF2 implicate vesicle trafficking in neural progenitor proliferation and migration in the human cerebral cortex. Nat Genet 36:69–76
Shoubridge C, Tarpey PS, Abidi F, Ramsden SL, Rujirabanjerd S et al (2010a) Mutations in the guanine nucleotide exchange factor gene IQSEC2 cause nonsyndromic intellectual disability. Nat Genet 42:486–488
Shoubridge C, Walikonis RS, Geicz J, Harvey RJ (2010b) Subtle functional defects in the Arf-specific guanine nucleotide exchange factor IQSEC2 cause non-syndromic X-linked intellectual disability. Small GTPases 1:98–103
Smaczynska-de Rooij II, Costa R, Ayscough KR (2008) Yeast Arf3p modulates plasma membrane PtdIns(4,5)P2 levels to facilitate endocytosis. Traffic 9:559–573
Soni KG, Mardones GA, Sougrat R, Smirnova E, Jackson CL, Bonifacino JS (2009) Coatamer-dependent protein delivery to lipid droplets. J Cell Sci 122:1834–1841
Spang A, Shiba Y, Randazzo PA (2010) Arf GAPS: gatekeepers of vesicle generation. FEBS Lett 584:2646–2651
Stafa K, Trancikova A, Webber PJ, Glauser L, West AB, Moore DJ (2012) GTPase activity and neuronal toxicity of Parkinson’s disease-associated LRRK2 is regulated by ArfGAP1. PLoS Genet 8:e1002526
Stalder D, Barelli H, Gautier R, Macia E, Jackson CL, Antonny B (2011) Kinetic studies of the Arf activator Arno on model membranes in the presence of Arf effectors suggest control by a positive feedback loop. J Biol Chem 286:3873–3883
Stein W, Kahn RA, Botstein D, Hoyt MA (1990a) ADP ribosylation factor is an essential protein in Saccharomyces cerevisiae and is encoded by two genes. Mol Cell Biol 10:6690–6699
Stein W, Willingham MC, Botstein D, Kahn RA (1990b) ADP-ribosylation factor is functionally and physically associated with the Golgi complex. Proc Natl Acad Sci USA 87:1238–1242
Stefan CJ, Manford AG, Emr SD (2013) ER-PM connections: sites of information transfer and inter-organelle communication. Curr Opin Cell Biol 25:434–442
Suzuki T, Kanai Y, Hara T, Sasaki J, Sasaki T et al (2006) Crucial role of the small GTPase ARF6 in hepatic cord formation during liver development. Mol Cell Biol 26:6149–6156
Szul T, Grabski R, Lyons S, Morohashi Y, Shestopal S et al (2007) Dissecting the role of the ARF guanine nucleotide exchange factor GBF1 in Golgi biogenesis and protein trafficking. J Cell Sci 120:3929–3940
Tan J, Evin G (2012) Beta-site APP-cleaving enzyme 1 trafficking and Alzheimer’s disease pathogenesis. J Neurochem 120:869–880
Tauchi-Sato K, Ozeki S, Houjou T, Taguchi R, Fujimoto T (2002) The surface of lipid droplets is a phospholipid monolayer with a unique Fatty Acid composition. J Biol Chem 277:44507–44512
Thiam AR, Antonny B, Wang J, Delacotte J, Wilfling F et al (2013) COPI buds 60-nm lipid droplets from reconstituted water-phospholipid-triacylglyceride interfaces, suggesting a tension clamp function. Proc Natl Acad Sci USA 110:13244–13249
Toret CP, Lee L, Sekiya-Kawasaki M, Drubin DG (2008) Multiple pathways regulate endocytic coat disassembly in Saccharomyces cerevisiae for optimal downstream trafficking. Traffic 9:848–859
Volpicelli-Daley LA, Li Y, Zhang CJ, Kahn RA (2005) Isoform-selective effects of the depletion of ADP-ribosylation factors 1-5 on membrane traffic. Mol Biol Cell 16:4495–4508
Walther TC, Farese RV Jr (2012) Lipid droplets and cellular lipid metabolism. Annu Rev Biochem 81:687–714
Weimer C, Beck R, Eckert P, Reckmann I, Moelleken J et al (2008) Differential roles of ArfGAP1, ArfGAP2, and ArfGAP3 in COPI trafficking. J Cell Biol 183:725–735
Winchester B, Bali D, Bodamer OA, Caillaud C, Christensen E et al (2008) Methods for a prompt and reliable laboratory diagnosis of Pompe disease: report from an international consensus meeting. Mol Genet Metab 93:275–281
Yoo SM, Antonyak MA, Cerione RA (2012) The adaptor protein and Arf GTPase-activating protein Cat-1/Git-1 is required for cellular transformation. J Biol Chem 287:31462–31470
Yu X, Breitman M, Goldberg J (2012) A structure-based mechanism for Arf1-dependent recruitment of coatamer to membranes. Cell 148:530–542
Zhang J, Neal J, Lian G, Hu J, Lu J, Sheen V (2013) Filamin A regulates neuronal migration through brefeldin A-inhibited guanine exchange factor 2-dependent Arf1 activation. J Neurosci 33:15735–15746
Zhao X, Lasell TK, Melancon P (2002) Localization of large ADP-ribosylation factor-guanine nucleotide exchange factors to different Golgi compartments: evidence for distinct functions in protein traffic. Mol Biol Cell 13:119–133