ARF family G proteins and their regulators: roles in membrane transport, development and disease

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Abstract | Members of the ADP-ribosylation factor (ARF) family of guanine-nucleotide-binding (G) proteins, including the ARF-like (ARL) proteins and SAR1, regulate membrane traffic and organelle structure by recruiting cargo-sorting coat proteins, modulating membrane lipid composition, and interacting with regulators of other G proteins. New roles of ARF and ARL proteins are emerging, including novel functions at the Golgi complex and in cilia formation. Their function is under tight spatial control, which is mediated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) that catalyse GTP exchange and hydrolysis, respectively. Important advances are being gained in our understanding of the functional networks that are formed not only by the GEFs and GAPs themselves but also by the inactive forms of the ARF proteins.

The ADP-ribosylation factor (ARF) family of low molecular weight guanine-nucleotide-binding (G) proteins controls membrane traffic and organelle structure, and each member is regulated through a cycle of GTP binding and GTP hydrolysis, which activate and inactivate the G protein, respectively[1,2]. ARFs have several important functions, including the recruitment of coat proteins that promote sorting of cargo into vesicles, the recruitment and activation of enzymes, such as the phosphatidylinositol (PtdIns) kinases, that alter membrane lipid composition, and interaction with cytoskeletal factors (TABLE 1). There are six mammalian ARF proteins that can be divided into three classes based on sequence homology: Class I (ARF1, ARF2 and ARF3), Class II (ARF4 and ARF5) and Class III (ARF6) (FIG. 1). The Class II ARFs arose late in animal cell evolution, possibly in metazoans, but Class I ARFs are highly conserved and are present in all eukaryotes examined to date. Hence, in *Drosophila melanogaster* and *Caenorhabditis elegans*, each class has a single ARF orthologue, but yeast lacks the Class II ARFs. Plants have Class I ARFs, and potentially also Class III ARFs (BOX 1).

In addition, there are over 20 ARF-like (ARL) proteins, which seem to have broader roles than ARFs. Some ARL proteins, including ARL1, ARL2 and ARF-related protein 1 (ARFRP1), are ancient and have homologues in plants, yeast and metazoans, whereas others, such as ARL11, arose later in evolution and are present only in vertebrates[2]. SAR1, which is present in all eukaryotes examined to date, is also considered to be a member of the ARF family, owing to the presence of an amino-terminal amphipathic helix and the functional similarity of this protein to ARF1 in recruiting a coat complex during vesicle budding.

The study of ARF protein function was aided greatly by the early discovery of the regulators of ARF GTP binding and GTP hydrolysis. The ARF guanine nucleotide exchange factors (GEFs) contain a conserved SEC7 domain that catalyses GDP release from, and GTP binding to, their substrate ARFs. The GTPase -activating proteins (GAPs) catalyse the hydrolysis of GTP-bound ARFs and are critically important because ARFs have negligible intrinsic GTP-hydrolysis activity. ARF GAPs contain a conserved zinc-finger GAP catalytic domain. The conserved, signature catalytic domains in the GEFs and GAPs are what facilitated the identification of these ARF regulators in all organisms from yeast to man. However, the ARF substrate specificity for these GEFs and GAPs remains unclear, and whether they also work on ARL proteins has yet to be determined.

In the past, G protein activity was viewed as a linear signalling pathway, with the GDP-bound form being inactive and the GTP-bound form initiating effector functions until it returned to the inactive, GDP-bound state. The GEFs and GAPs were thought of as 'activators' and 'inactivators', respectively, that controlled this on–off switch (FIG. 1c). However, work on ARF and ARL proteins over the past decade has revealed that their

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Table 1 | **ARF and ARL effectors**

Effector	ARF	Location	ARF-interacting region
Coat complexes			
COPII	SAR1	ER exit sites	SEC23
COPI	ARF1,3	Golgi, ERGIC	γ-COP, β-COP
AP1–clathrin	ARF1,3	TGN, endosomes	γ-AP1, β-AP1
GGA1,2,3–clathrin	ARF1,3	TGN, endosomes	GAT domain
AP3	ARF1,3	Endosomes, TGN	–
AP4	ARF1,3	TGN	ε-AP4, μ-AP4 (also binds GDP-bound form)
BBSome	ARL6	PM	–
Lipid-modifying enzymes			
FAPP1,2	ARF1	Golgi	PH domain
CERT	ARF1	Golgi	PH domain
PtdIns4K	ARF1	Golgi	–
PtdIns4P5K	ARF1–6	PM (ARF6)	–
PLD	ARF1–6, ARL1	PM (ARF6)	–
Tethers			
GMAP210	ARF1	*cis*-Golgi	C-terminal GRAB domain
CC golgins*	ARL1	TGN	C-terminal GRIP domain
Exocyst	ARF6	PM	SEC10
GARP (VFT)	ARL1	TGN, endosome	–
G protein regulators			
ARHGAP21	ARF1,6	Golgi, PM	PH domain, C-terminal helix
Cytohesin (ARNO)	ARF6, ARL4	PM	PH domain
Scaffolding proteins			
JIP3,4	ARF6	Endosomes, intercellular bridge	LZII
FIP3,4	ARF5,6	Recycling endosomes, midbody	CC domain
Tubulin folding chaperone			
Cofactor D	ARL2	Cytosol	–
Cargo			
Rhodopsin	ARF4	TGN	VXPX targeting motif
Other			
NM23-H1	ARF6	PM, cell junctions	–
PDEδ	ARL2,3	Recruitment of prenylated proteins	β-sheet region
HRG4	ARL2,3	–	–
ARFAPTIN1,2	ARF1, ARL1	Golgi, TGN	BAR domain
SCOCO	ARL1	Golgi	CC
BART2	ARL2	Mitochondria, nucleus	α-helices 3, 4 and 5 of BART2

AP, adaptor protein; ARF, ADP-ribosylation factor; ARFAPTIN, ARF-interacting protein; ARHGAP21, Rho GTPase-activating protein 21; ARL, ARF-like; BAR, Bin–amphiphysin–Rvs; CC, coiled-coil; CERT, ceramide transfer; COP, coatomer protein; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; GGA, Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding protein; GMAP210, Golgi-associated microtubule-binding protein 210; GRAB, GRIP-related ARF-binding; JIP, JNK-interacting protein; LZII, Leu zipper domain II; PDEδ, phosphodiesterase-δ; PLD, phospholipase D; PH, pleckstrin homology; PM, plasma membrane; PtdIns4K, phosphatidylinositol 4-kinase; PtdIns4P5K, phosphatidylinositol-4-phosphate 5-kinase; TGN, *trans*-Golgi network. *Including golgin 245, golgin 97, GCC88, GCC185 (mammalian cells) and Imh1 (yeast).

signalling is more complex and that GEFs and GAPs can initiate their own physiological responses. We see evidence of ARF proteins acting in pairs or in series in the endoplasmic reticulum (ER)–Golgi system and at the plasma membrane. In this Review, we emphasize how ARF proteins function as a network in which the ARF regulators participate. These regulators also integrate ARF activities with other G protein signalling networks, as well as initiating their own distinct signalling pathways. We highlight new ARF and ARL activities, discuss

Guanine nucleotide exchange factors (GEFs). Proteins that promote the release of GDP from guanine-nucleotide-binding (G) proteins, which allows GTP to bind. These proteins often stabilize the nucleotide-free form and then are released upon GTP binding.

GTPase-activating proteins (GAPs). Proteins that promote GTP hydrolysis on GTP-bound guanine-nucleotide-binding (G) proteins. For ADP-ribosylation factor (ARF) proteins, GAPs are critical, as ARFs have negligible intrinsic GTPase activity. The catalytic regions of GAPs often include an Arg-finger motif that inserts into the GTP-binding pocket to stimulate hydrolysis of GTP.

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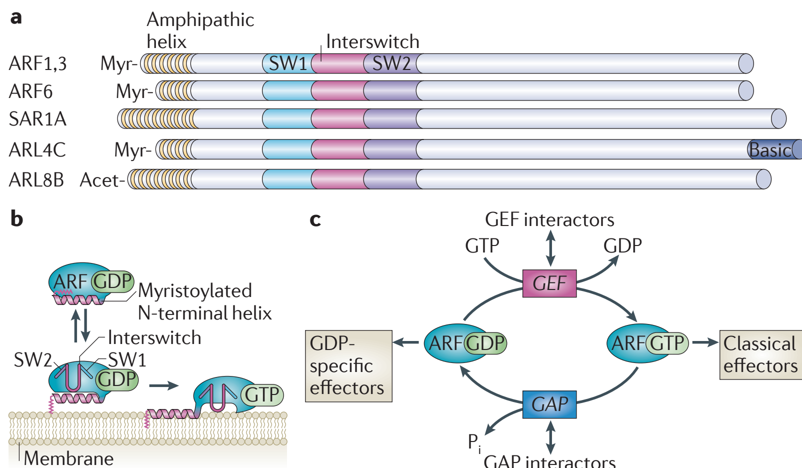

Figure 1 | **The domain structure and regulation of ARF and ARLs. a** | A schematic of representative ADP-ribosylation factor (ARF), SAR1 and ARF-like (ARL) proteins, indicating the conserved amino-terminal amphipathic helix and the protein-specific lipid modifications at the N terminus. These include myristoylation (Myr) and acetylation (Acet), both of which ensure tight membrane association. The effector regions of the guanine-nucleotide-binding (G) protein, switch 1 (SW1) and SW2, and the interswitch region between them, are depicted. These regions change conformation upon exchange of GDP for GTP and are involved in interactions with effectors. **b** | ARF•GDP reversibly associates with the membrane surface, and the myristoylated N-terminal helix ensures tight membrane association of ARF•GTP. The switch and interswitch regions are also shown, and these undergo a conformational change upon GTP binding to enter the hydrophobic pocket that the N-terminal amphipathic helix occupies in the GDP-bound form. **c** | ARF family G proteins undergo a cycle of GTP binding and hydrolysis, mediated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively. The GTP-bound form is thought to carry out G protein functions through interaction with 'classical effectors', including vesicle coat proteins and enzymes that can modify membrane lipid composition; however, increased attention has focused on networks of effectors that are targeted by proteins that interact with GEFs and GAPs themselves and unique effectors that associate specifically with the GDP-bound form of ARF proteins.

how GEFs and GAPs can act as scaffolds, both as effectors and in initiating signalling responses, and describe how they participate in development and disease. The reader is referred to two excellent prior reviews: one deals comprehensively with ARF1 and ARF6 function[1] and the other more broadly with ARF, ARL, GEF and GAP proteins[2].

Localization and activity of ARFs and ARLs

ARFs localize to membranes throughout the cell, including the plasma membrane and the membranes of the secretory, endosomal and lysosomal pathways. A distinguishing feature of ARF family G proteins is the presence of an N-terminal amphipathic helix that is critical for membrane binding (FIG. 1a,b). In addition, all ARF proteins are modified by myristoylation at the N terminus and this co-translational modification is required for membrane recruitment and biological activity. The myristoyl group and associated N-terminal amphipathic helix are inserted into the membrane upon GTP binding[3]. 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[4] (FIG. 1). This distinguishes them from other small G proteins of the Ras superfamily, including the Ras, Rho and Rab families, which have a long carboxy-terminal linker to which their lipid membrane anchor is attached[2]. ARF effectors are thus constrained to a position close to the membrane surface, in contrast to those of Rab and Rho, which can be located some distance from the membrane[2]. Some ARL proteins are myristoylated but most seem to lack this modification. In ARL8B, loss of hydrophobic residues in the amphipathic helix abolishes lysosomal localization[5]. Interestingly, ARFRP1 (Arl3 in *Saccharomyces cerevisiae*), ARL8A and ARL8B are acetylated rather than myristoylated at their N terminus[2]. In SAR1, the N-terminal amphipathic helix binds directly to membranes and induces membrane curvature[6].

Myristoylation
A lipid modification, occurring co- or post-translationally, in which a myristoyl moiety is attached to a Gly residue at the second position from the amino terminus, after cleavage of the N-terminal Met residue.

GDP dissociation inhibitor
(GDI). A protein that binds specifically to the GDP-bound form of a GTP-binding protein, preventing guanine nucleotide exchange.

Unlike for Rab and Rho G proteins, no GDP dissociation inhibitor (GDI) proteins have been identified for ARFs or ARLs. ARF1 and ARF3 appear to be released from membranes on GTP hydrolysis in cells. ARF6, however, remains bound to membranes in its GDP-bound conformation, and there is evidence that ARF4 and ARF5 remain bound to ER–Golgi intermediate compartment (ERGIC) membranes in their GDP-bound form[7,8]. This raises the possibility that ARF proteins that are bound to membranes in their GDP-bound form might interact with membrane-localized partners and mediate signalling. Evidence for this idea is emerging for ARF6 (see below), and suggests that distinct signalling pathways might be coordinated through the nucleotide state of these constitutively membrane-bound ARF family proteins. SAR1 and some of the ARLs, such as ARL1, ARL4 and ARL8, are cytosolic when GDP-bound, similarly to ARF1 (REFS 2,9), and it remains to be determined whether this is true for other ARL proteins.

In humans, there are 15 ARF GEFs, which are divided into six subfamilies, as well as the SAR1 GEF SEC12 (TABLE 2). No specific ARL GEFs have yet been identified, although the ARF1 GEF Syt1 (suppressor of Ypt3 1) in yeast apparently also has activity towards ARL1 (REF. 10). The 31 identified mammalian ARF GAPs fall into nine major subgroups based on their domain structure (TABLE 2). Two ARL GAPs have been identified (see below). GEFs and GAPs are recruited to very specific sites within cells to not only catalyse GTP exchange and hydrolysis, respectively, but also to assemble protein complexes at these sites independently of their catalytic activity (FIG. 1c). In this way, versatile signalling networks can be assembled that can respond dynamically to extracellular and intracellular signals.

Expanding the roles of ARFs and ARLs

Following activation on membranes, GTP-bound ARFs recruit coat proteins, lipid-modifying enzymes, tethers and other effector molecules that influence membrane trafficking and organelle structure[1,2] (TABLE 1). For example, ARF1 recruits the cytosolic coatomer complex I (COPI) to Golgi membranes, allowing sorting of cargo proteins into COPI-coated vesicles[11]. ARF proteins at the *trans*-Golgi network (TGN) also recruit heterotetrameric clathrin adaptor protein 1 (AP1), AP3 and AP4, as well as the three monomeric Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding

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Box 1 | **ARF function in plants and protists**

Plants have numerous ADP-ribosylation factors (ARFs) that are homologous to human ARF1 (REF. 125) and were originally thought to lack Class III ARF6-like proteins. However, in *Arabidopsis thaliana*, ARFB (also called ARFB1A) localizes to the plasma membrane and lacks the Golgi-targeting motif (MXXE) that is found in other ARF1 homologues in plants[126] and in mammals[17]. Nevertheless, only the GBF and BIG subfamilies of ARF guanine nucleotide exchange factors (GEFs) seem to be present in plants, and these function in both endocytic and Golgi trafficking pathways[127]. *A. thaliana* GNOM (also known as EMB30) is a homologue of mammalian GBF1 but acts at endosomes and the plasma membrane during the polar transport of the plant hormone auxin during development[127,128]. Another GBF-like protein in *A. thaliana*, GNOM-like 1 (GNL1), functions at the Golgi similarly to mammalian GBF1, but is also involved in endosomal trafficking[129]. BIG5 (also known as BEN1 and ATMIN7) was identified in a screen for *A. thaliana* mutants defective for internalization of the PIN auxin transporter from the plasma membrane. This ARF GEF is most closely related to BIG1 and BIG2 in mammalian cells, localizes predominantly to the *trans*-Golgi network (TGN) and early endosomes, and is involved in early endosomal trafficking[130]. Interestingly, BIG5 is targeted for degradation by a plant bacterial pathogen, *Pseudomonas syringae*, to protect the latter from host defence systems at the cell wall[131].

A. thaliana ARF GAPs include four members of a family of mammalian ACAP homologues that are known as VASCULAR NETWORK 3 (VAN3)-like after the first member to be characterized[125]. VAN3 (also known as SCARFACE and AGD3) regulates formation of plant vascular networks[132,133]. In addition to its roles on endosomes, VAN3 cooperates with GNOM during clathrin-mediated endocytosis of the PIN auxin transporter[128]. Another ARF GAP in *A. thaliana*, ARF GAP DOMAIN 5 (AGD5; also known as NEVERSHED), is a homologue of yeast ARF GAP effector 2 (Age2) and mammalian SMAP family ARF GAPs that localizes to the TGN[134]. AGD5 is required for floral organ cell separation[125] and regulates membrane trafficking though TGN–early endosomal compartments to trigger organ abscission[135].

Interestingly, the protozoan parasite *Trypanosoma brucei* expresses a single ARF protein that has characteristics of both ARF1 and ARF6. *T. brucei* ARF1 is a basic protein with a calculated isoelectric point (pI) value of 9.1, which is similar to that of human ARF6, but *T. brucei* ARF1 contains the Golgi-targeting motif MXXE[136] that is found in human ARF1 and ARF3 (REF. 17). Depletion of *T. brucei* ARF1 by small interfering RNA causes a major decrease in endocytosis and the formation of intracellular flagella, but the Golgi remains intact[136]. Trypanosomes also express an ARF-like 2 (ARL2) homologue, which is involved in microtubule biogenesis and cytokinesis[137], and an ARL1 homologue, which is important for Golgi structure and exocytosis of glycosyl phosphatidylinositol (GPI)-anchored proteins[138]. ARF and ARL proteins in trypanosomes are myristoylated, a modification that is required for their activity. Trypanosomes cause African sleeping sickness, a disease with no successful therapy. A selective inhibitor of trypanosomal *N*-myristoyl transferase has been shown to be effective in blocking trypanosome viability in a mouse model of this disease[139].

Cilium
A slender extension on the cell surface. A non-motile, primary cilium is present on nearly all epithelial cells in the body and serves as a sensory organ that is important for regulating cell differentiation and division.

proteins (GGAs), GGA1, GGA2 and GGA3[12]. These various coat proteins specifically bind cargo proteins and incorporate them into newly forming vesicles for sorting and transport to their correct destinations. ARFs can also recruit and activate enzymes that alter membrane lipid composition. Phospholipase D (PLD), which hydrolyses phosphatidylcholine to generate phosphatidic acid, is activated by all ARF proteins and also by ARL1 (REF. 13). All ARF proteins can both recruit and activate PtdIns-4-phosphate 5-kinase (PtdIns4P5K), an enzyme that phosphorylates PtdIns4P at the 5-position to generate PtdIns-4,5-bisphosphate (PtdIns(4,5)P_2)[1]. For example, ARF6 at the cell periphery directly affects the activity of PtdIns4P5K at the plasma membrane, and thus regulates PtdIns(4,5)P_2 levels there[1]. At the Golgi, ARF1 recruits and stimulates the activity of PtdIns 4-kinase (PtdIns4K), forming PtdIns4P, which is an important membrane lipid for Golgi function[14]. ARF1 also binds to PtdIns4P-specific pleckstrin homology (PH) domains contained in a family of oxysterol-binding proteins that are believed to function in lipid homeostasis at the Golgi[14].

New functions for Golgi-associated ARFs. The five ARF proteins in humans, ARF1, ARF3, ARF4, ARF5, and ARF6, are ubiquitously expressed. Studies to date have focused mainly on ARF1 at the Golgi and ARF6 at the plasma membrane, but ARF3, ARF4 and ARF5 are also present on Golgi membranes (FIG. 2a). Surprisingly, depletion experiments using RNA interference (RNAi) show that no single ARF, including ARF1, is required for Golgi function; instead, ARFs function in pairs at particular steps in Golgi transport[15]. For example, ARF1 and ARF4 act redundantly during transport in the early secretory pathway[15]. Consistent with this observation, ARF4 localizes to the ERGIC and *cis*-Golgi[8] and, together with ARF1 at the *cis*-Golgi, it organizes trafficking between these compartments[16].

ARF1 and ARF3 are identical except for seven amino acid differences in their N-terminal and C-terminal regions, and previously they were thought to function and localize identically. However, a Golgi-targeting sequence contained within the α3 helix of ARF1 and ARF3 targets a chimaera of ARF6 and ARF1 to the early Golgi[17]. Furthermore, ARF3 localizes specifically to the TGN (FIG. 2a), and this localization depends on four ARF3-specific amino acids contained in the N-terminal amphipathic helix, which are conserved among ARF3 homologues[18]. ARF3, but not ARF1, becomes cytosolic at 20°C, the temperature at which exit from the TGN is blocked[18]. Thus, ARF3 might have an additional crucial role during exit from the Golgi.

Several important functions for Class II ARFs at the TGN have now been defined (FIG. 2a). In an elegant series of studies, ARF4 was found to specifically recognize the VXPX cytosolic targeting motif in retinal rhodopsin to facilitate its transport into the rod outer segment, which is a specialized cilium[19] (FIG. 2b). This ciliary targeting complex includes, in addition to ARF4, Rab11, FIP3 (a shared ARF and Rab11 effector) and ASAP1 (ARF GAP containing SH3, ankyrin repeat and PH domains 1)[20]. Exactly how this complex facilitates the packaging of rhodopsin into post-Golgi carriers has yet to be determined but, interestingly, rhodopsin itself initiates complex formation by recruiting ARF4. The rhodopsin-binding site on ARF4 is in the α3 helix[19], the same region that in ARF1 binds the SNARE protein membrin (also known as GOSR2) to mediate targeting to the early Golgi[17]; thus, this region might generally allow ARF protein binding to membrane receptors. ARF4 and ARF5 can also directly bind to CAPS (calcium-dependent activator protein for secretion), which regulates exocytosis of dense core vesicles from nerve terminals[21]. It is the GDP-bound form of the ARF that binds to the PH domains of CAPS proteins, and knockdown of CAPS, ARF4 or ARF5 causes retention of chromagrannin (a marker for dense core vesicles) in the Golgi, suggesting that ARF4 and ARF5, together with CAPS, regulate the release of dense core vesicles from the Golgi (FIG. 2a). How these roles of ARF4 and ARF5 at the TGN in specialized cells can be reconciled

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Table 2 | **ARF family GEF and GAP proteins**

Name	Aliases and orthologues	Substrate	Location	Motifs and domains	Interactors
ARF GEFs					
GBF1	Gea1,2 (*Sc*), GARZ (*Dm*), GNL1 (*At*)	ARF1,3,5	ERGIC, Golgi	DCB	p115 tether, Rab1, γCOP, Drs2 (with Gea2)
BIG1	p200 ARF GEP, Sec7 (*Sc*)	ARF1,3	TGN, endosome, nucleus	DCB, AKAP	Myosin IXb, Exo70
BIG2	BIG5 (BEN1, AtMIN7) (*At*)	ARF1,3	TGN, endosome	DCB, AKAP	GABA receptor
Cytohesin 1	PSCD1, Steppke (*Dm*)	ARF1,6	PM, endosome	CC, PH, Polybasic	ARFRP1, CASP, ARL4, CNK1
Cytohesin 2	ARNO, PSCD2, Steppke (*Dm*)	ARF1,3,6	PM, endosome	CC, PH, Polybasic	CASP, GRASP (tamalin), IPCEF, A2AR, β-arrestin, V-ATPase, ARL4, ARF6, CNK1, ERBB receptor
Cytohesin 3	GRP1, ARNO3, PSCD3, Steppke (*Dm*)	ARF1,6	PM, endosome	CC, PH, Polybasic	CASP, GRASP (tamalin), THR, ARL4, ARF6, CNK1
Cytohesin 4	PSCD4	ARF1,5	–	CC, PH, Polybasic	–
EFA6A–D	PSD1–4, Yel1 (*Sc*), Syt1 (*Sc*), EFA6 (*Dm*)	ARF6	PM, endosome	PH, CC, pro	TWIK1 K^+ channel
BRAG1	IQSEC2, IQARFGEF, Loner (*Dm*), Schizo (*Dm*)	ARF6	PSD	PH, CC, IQ	IRSp53
BRAG2	GEP100, IQSEC1, Loner (*Dm*), Schizo (*Dm*)	ARF6	PM, endosome, nucleus	PH, CC, IQ	AMPA receptor
BRAG3	IQSEC3, SYNARFGEF, Loner (*Dm*), Schizo (*Dm*)	ARF6	PSD	PH, CC, IQ	PSD95, Homer, utrophin (dystrophin), S-SCAM
*ARF GAPs**					
ARFGAP1	Gcs1 (*Sc*)	ARF1–5	Golgi	ALPS	–
ARFGAP2,3	ZNF289, Glo3 (*Sc*)	ARF1–5	Golgi	Polybasic, Glo3 (ISS repeat)	COPI coat
ADAP1,2	Centaurin α1,β	–	–	Two PH	–
SMAP1,2	–	ARF1,6	–	Clathrin box, CALM	–
AGFG1,2	HRB1,2	–	–	FG repeats	–
GIT1,2	CAT1,2, p95APP1,2, GIT (*Dm*)	ARF6	PM	ANK, SHD, CC, PBS	PIX, PLCγ, MEK1, FAK, GPCR kinase
ASAP1–3	AMAP1,2, DEF1, PAG2,3, PAP, ASAP (*Dm*)	ARF1,5,6‡	PM, FA	BAR, PH, ANK, Pro, SH3	CIN85 (SH3KBP1), cortactin, CRK, SRC, FAK, PYK2
ACAP1–3	Centaurin β1,β2,β5	ARF6	PM, endosome	BAR, PH, ANK	β1 integrin, cellubrevin, transferrin R
AGAP1–11	Centaurin-γ	–	–	GLD, PH, ANK	–
ARAP1–3	Centaurin δ1,δ2,δ3	–	–	SAM, five PH, ANK, RhoGAP, RA	RhoA, CIN85, Rap1
ARL GAPs					
RP2	Cin2 (*Sc*), XRP2	ARL3	PM, periciliary ridge, cilial basal body	–	–
ELMOD2	–	ARL2,3	–	–	–

A2AR, adenosine A2A receptor; ADAP, ARF GAP with dual PH domain-containing; AGFG, ARF GAP domain and FG repeats-containing; AKAP, A kinase-anchoring protein; ALPS, amphipathic lipid packing sensor; ANK, ankyrin repeat; ARF, ADP-ribosylation factor; ARL, ARF-like; ASAP, ARF GAP containing SH3, ankyrin repeat and PH domains; *At*, *Arabidopsis thaliana*; BAR, Bin–amphiphysin–Rvs; CALM, clathrin assembly lymphoid myeloid; CASP, cytohesin-associated scaffolding protein; CC, coiled-coil; Cin2, chromosome instability protein 2; CIN85, CBL-interacting protein 85; COPI, coatomer complex I; DCB, dimerization and cyclophilin-binding domain; *Dm*, *Drosophila melanogaster*; ERGIC, ER–Golgi intermediate compartment; FA, focal adhesion; FAK, focal adhesion kinase; FG, phenylalanine, glycine repeats; GABA, γ-aminobutyric acid; GAP, GTPase-activating protein; Gea, ARF guanine nucleotide exchange factor; GEF, guanine nucleotide exchange factor; GEP, guanine nucleotide exchange protein; GLD, GTP-binding protein like domain; GNL1, guanine nucleotide-binding protein-like 1; GPCR, G protein-coupled receptor; GRASP, GRP1-associated scaffold protein; IPCEF, interaction protein for cytohesin exchange factors; IQ, IQ motif; MEK, MAPK/ERK kinase; PAG, Paxillin-associated protein with ARF GAP activity; PBS, paxillin binding site; PH, pleckstrin homology; PLC, phospholipase C; PM, plasma membrane; Pro, proline-rich; PSD, post-synaptic density; PYK, proline-rich tyrosine kinase; RA, Ras association motif; RP2, retinitis pigmentosa 2; SAM, sterile motif; *Sc*, *Saccharomyces cerevisiae*; SH3, SRC homology 3; SHD, SRC homology domain; S-SCAM, synaptic scaffolding molecule; TGN, *trans*-Golgi network; THR, thyroid hormone receptor; TWIK1, tandem of P domains in a weak inward-rectifying K^+ channel 1; ZNF289, zinc-finger 289. *Consensus name used from REF. 140.
‡ASAPs work better on ARF1 and ARF5 than on ARF6.

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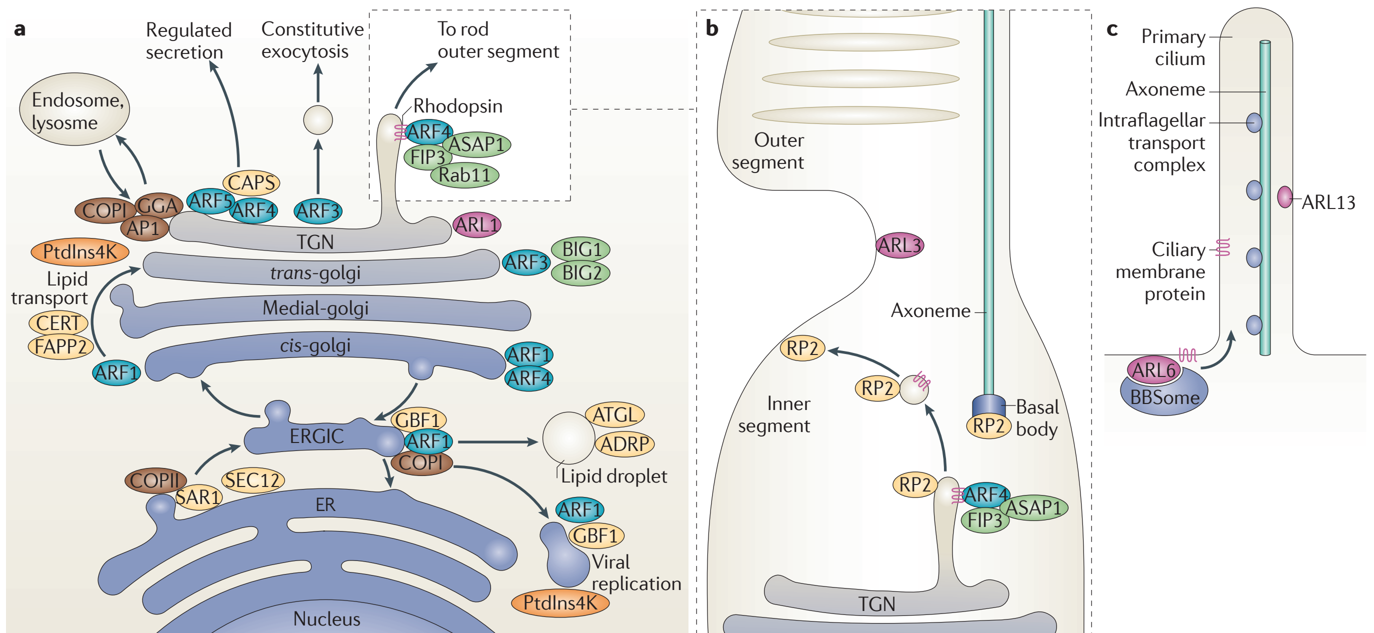

Figure 2 | **ARF and ARL functions in the secretory pathway and in specialized transport. a** | ADP-ribosylation factor (ARF) proteins have distinct localizations and functions in the endoplasmic reticulum (ER)–Golgi system. ARF1 and ARF4 localize to the early *cis*-Golgi and ARF3 specifically localizes to the *trans*-Golgi network (TGN). In addition to the recruitment of coat proteins (coatomer complex I (COPI), GGA (Golgi-localized, γ-ear-containing, ADP-ribosylation factor-binding protein) and adaptor protein 1 (AP1)) to the Golgi, ARF1 binds to ceramide transfer (CERT) and FAPP2 to mediate the transport of ceramide and glucosylceramide lipids from the *cis*-Golgi to the *trans*-Golgi. At the ER–Golgi intermediate compartment (ERGIC), ARF1 and its guanine nucleotide exchange factor (GEF) GBF1 act with COPII to regulate the formation of lipid droplets and for the replication of several viruses. CAPS (Calcium-dependent activator protein for secretion), which is involved in regulated secretion, is recruited to the TGN by ARF4 and ARF5. At the ER, SAR1, activated by SEC12, recruits COPII to allow vesicle transport to the Golgi. **b** | In retinal cells, ARF4 binds specifically to rhodopsin in the TGN membrane and, together with FIP3, ASAP (ARF GAP containing SH3, ankyrin repeat and PH domains) and Rab11, it facilitates the transport of rhodopsin in transport vesicles from the inner segment to the outer segment, which is a specialized cilium. ARF-like 3 (ARL3) has been found to be localized to the connecting cilium, and retinitis pigmentosa 2 (RP2; also known as XRP2), an ARL3 GAP, localizes to the TGN, the basal body and the membrane adjacent to the connecting cilium. **c** | In primary cilia, ARL6 recruits the BBSome coat complex that facilitates the transport of membrane proteins into the cilium. ARL13 is localized to the cilium and has been implicated in intraflagellar transport. ADRP, adipose differentiation-related protein (also known as adipophilin); ATGL, adipose triglyceride lipase; PtdIns4K, phosphatidylinositol 4-kinase.

with findings of ARF4 localization to, and ARF4 and ARF5 functioning at, the early Golgi in other cells[8,15,16] is not known.

Recent discoveries show that ARF1 regulates lipid transfer proteins within the Golgi and promotes the formation of lipid droplets at the ERGIC (FIG. 2a). At the Golgi, ARF1 recruits the lipid transfer proteins ceramide transfer (CERT) and FAPP2 (REF. 14) through interaction with their PH domains, which can also bind PtdIns4P. CERT mediates the non-vesicular transport of ceramide from the ER to the Golgi and FAPP2 mediates the transfer of glucosylceramide from the cytosolic side of the early Golgi to the *trans*-Golgi[22]. Exactly how the directionality of this transfer occurs, and the role that ARF1 has, is not yet clear. The finding that ARF1 associates with GBF1 and COPI during lipid droplet formation was unexpected. These proteins were identified in an RNAi screen of lipid droplet formation in *D. melanogaster*[23] and also appeared in proteomic analyses of lipid droplets along with other trafficking proteins, which led to the idea that lipid droplets interface with multiple membrane trafficking pathways[24]. In particular, the delivery of two proteins, adipose triglyceride lipase (ATGL) and adipose differentiation-related protein (ADRP; also known as adipophilin), to the surface of lipid droplets requires ARF1, GBF1 and COPI, and possibly the COPII machinery, in mammalian cells[25]; similar results were obtained in *D. melanogaster* S2 cells[26]. Another ARF family member, ARFRP1, is highly expressed in adipocytes, and mice that lack ARFRP1 in adipose tissue show severe defects in lipid storage and enhanced lipolysis[27]. Finally, in some cell types ARF1 at the plasma membrane affects endocytosis of proteins anchored to the membrane by a glycosyl PtdIns (GPI) linkage[28]. This may also require the ARF GEF GBF1 (REF. 29) and could be related to the other lipid-regulating functions of ARF1.

New understanding of ARF6 function. A great deal of work on ARF6 function has been summarized in a previous review[1], so here we focus on more recent advances. In mammals, ARF6 is not required for early embryonic development, but ARF6-knockout mice die at

Lipid droplets
Lipid storage organelles that are surrounded by a phospholipid monolayer.

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Figure 3 | **The localization and function of ARF and ARL proteins in endosomal–lysosomal trafficking.** At the plasma membrane, ADP-ribosylation factor 6 (ARF6) activates phosphatidylinositol-4-phosphate 5-kinase (PtdIns4P5K) to generate PtdIns-4,5-bisphosphate (PtdIns(4,5)P_2) and, together with ARF-like 4 (ARL4), recruits cytohesin (also known as ARNO) guanine nucleotide exchange factors (GEFs) that can lead to further activation of ARF6 or ARF1. Cytohesins associate with the IPCEF (interactor protein for cytohesin exchange factors)–DOCK180 complex, which activates Rac, but another Rac GEF, Kalirin, can be recruited to membranes by ARF6•GDP. ARF6 at the plasma membrane can regulate the membrane lipid composition, alterations in cortical actin to drive protrusions (for example, during cell migration), and endocytosis of ligand-activated guanine-nucleotide-binding (G) protein-coupled receptors (GPCR) via clathrin-dependent endocytosis. ARF6 and the microtubule motor adaptor protein JNK-interacting protein 4 (JIP4) promote rapid recycling of endosomal membrane back to the cell surface, and ARF6, together with the exocyst complex, also affects slow recycling from sorting endosomes. ARF1 has been implicated in clathrin-independent endocytosis of glycosyl PtdIns (GPI)-anchored proteins in some cells. ARF6 and the ARF6 GEFs cytohesin and BRAG2 have been implicated in both assembly and disassembly of adherens junctions. Two ARF GTPase-activating proteins (GAPs), ASAP1 (ARF GAP containing SH3, ankyrin repeat and PH domains 1) and GIT1, localize to focal adhesions that mediate adhesion to the extracellular matrix (ECM), and GIT1 interacts with PIX, a GEF for CDC42. ARL8 is required for fusion of multivesicular late endosomes with lysosomes and is involved in transport along microtubules. HGF, hepatocyte growth factor.

mid-gestation or shortly after birth and exhibit impaired liver development[30]. This phenotype suggests that the critical physiological roles of ARF6 take place after birth and is consistent with reported effects of ARF6 on cell adhesion, cell migration, wound healing and metastasis.

ARF6 is present at the plasma membrane and influences both the cortical actin cytoskeleton and endosomal membrane recycling[1] (FIG. 3). At the plasma membrane, ARF6 changes the membrane lipid composition through activation of PtdIns4P5K and PLD, resulting in the generation of PtdIns(4,5)P_2 and phosphatidic acid. These lipids are important for sorting proteins within the membrane, for the formation of clathrin-coated pits during endocytosis, and for the recruitment and activation of Rho family G proteins, such as Rac, to alter actin polymerization. There is some evidence that ARF6 can interact with AP2 (REF. 31) and clathrin during G protein-coupled receptor (GPCR) cell signalling[32]. A recent study has found that ARF6 enters cells in clathrin-coated vesicles to facilitate the rapid recycling of the transferrin receptor back to the plasma membrane through interaction with the microtubule motor adaptor protein JNK-interacting protein 4 (JIP4) after clathrin uncoating[33]. In some cells, ARF6 associates with endosomal membranes derived from clathrin-independent forms of endocytosis and mediates recycling of this membrane back to the plasma membrane[34]. Recycling endosomes return membrane proteins that are important for cell adhesion and migration back to the plasma membrane[34,35]. ARF6 regulation of such endosomal membrane trafficking is required for the polarized delivery of CDC42, Rac and the PAR6 complex to the leading edge of migrating cells[36], which can alter adhesion to the extracellular matrix through focal adhesions and actin-based protrusions. Hence, regardless of the mode of endocytosis, ARF6 is important for membrane recycling.

The crucial functions of ARF6 in membrane lipid modification, establishment of cell polarity and promotion of endocytic recycling are conserved in yeast and *D. melanogaster*[1]. Arf3, the yeast ARF6 homologue, contributes to PtdIns(4,5)P_2 levels at the plasma membrane[37] and also affects polarization events, such as bud site selection[38] in *S. cerevisiae* and new end take-off growth in *Schizosaccharomyces pombe*[39]. ArfB, the ARF6 homologue in the filamentous fungi *Aspergillus nidulans*, localizes to both the plasma membrane and endomembranes, and regulates endocytosis and polarity establishment during hyphal growth[40]. In *D. melanogaster*,

New end take-off
A switch in cellular growth of fission yeast, from monopolar extension to bipolar extension.

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deletion of the ARF6 homologue blocks the rapid endocytic recycling required for cytokinesis in spermatocytes, resulting in male sterility, but no other phenotypes were reported[41]. Interestingly, in mammalian cells ARF6 interacts with JIP4 to control a motor switch mechanism regulating endosomal trafficking in cytokinesis[42]. 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 (REF. 43).

ARF6 has been implicated in both the assembly and disassembly of adherens junctions in polarized epithelial cells[1] (FIG. 3). During adherens junction formation, PAR3 recruits a scaffolding protein, FRMD4A, that binds to cytohesin GEFs, which leads to activation of ARF6 (REF. 44). Treatment of fully polarized epithelial cells with hepatocyte growth factor leads to activation of ARF6, most likely through the ARF GEF BRAG2 (REF. 45), and activation of Rac, which causes disassembly of adherens junctions by stimulating endocytosis of epithelial cadherin (E-cadherin)[1]. Hence, depending on the signalling complex assembled, either formation or disassembly of adherens junctions can be achieved through activation of ARF6. There is also some evidence that the ARF6 GEF EFA6 affects tight-junction assembly[46]. ARF6 activation has also been reported at the onset of tubulogenesis (a developmental progression from polarized epithelia to tubular structures), and perturbation of the ARF6 GTP–GDP cycle inhibits tubule formation[47].

Importance of turning off ARF6. ARF proteins carry out their actions through a regulated cycle of GTP binding and hydrolysis. This allows ARFs to engage and disengage with their effectors with spatial and temporal specificity, and in some cases may allow ARF•GDP to bind other classes of effector. ARF6•GDP binds several TBC (Tre2–Bub2–Cdc16) domain-containing proteins, which often have Rab GAP activity[48]. ARF6•GDP binds both TBC1 domain family member 24 (TBC1D24; a protein mutated in familial infantile myoclonic epilepsy[49]) and the TRE17 oncogene[50]. TRE17 binding to ARF6 increases its activation[50]; although TRE17 does not itself have GEF activity towards ARF6, it may facilitate interaction of ARF6 with another GEF. ARF6•GDP also binds to the Kalirin family of Rho GEFs, through their spectrin-like repeat domain[51], and recruits Kalirin to the membrane, where it subsequently activates Rac and RhoG to regulate actin dynamics[51] (FIG. 3). Hence, ARF6•GDP and ARF6•GTP both interact with regulatory proteins of other small G proteins, allowing alternative signalling pathways to be activated depending on which nucleotide is bound (FIG. 1c). This raises the intriguing possibility that other GDP-bound ARF or ARL proteins might also bind unique effector proteins.

Turning off ARF6 is important for its biological function. In some cells, expression of the constitutively active mutant of ARF6, Q67L, leads to the accumulation of early endosomes containing plasma membrane proteins that enter cells independently of clathrin; failure to inactivate ARF6 blocks further trafficking of this membrane towards recycling or to other destinations[52]. Immediately upon platelet activation, ARF6•GTP levels fall, and this inactivation precedes, and is required for, the subsequent activation of Rac[53]. ARF6 is important for the disassembly of adherens junctions[1] and, more recently, active ARF6 was shown to disrupt the formation of epithelial cysts[54]. The Slit2–ROBO4 signalling pathway is important for maintaining barrier function in the vascular network, and ROBO4 interacts with paxillin to recruit ARF GAP proteins, such as GIT1, to inactivate ARF6 (REF. 55); this ARF6 inactivation suppresses protrusive activity of the endothelial cells and neovascularization. GIT2 and ARF6 inactivation are also important for maintaining the podosome, an actin-rich sealing zone in osteoclasts[56]. Finally, non-canonical ubiquitylation of ARF6, catalysed by FBX8 (an F-box and SEC7 domain-containing protein) seems to be another, unusual, way to turn off ARF6 (REF. 57). FBX8 is diminished or lacking in several cancer cell lines, which is consistent with roles for ARF6 in cancer cell metastasis[58].

Insights into ARL function. Similarly to ARF1, ARL1 and ARL2 arose early in evolution and share common effectors in plants, yeast and mammals. ARL1 recruits GRIP-domain golgins to the TGN[2]. It also mediates TGN-localization of ARF-interacting proteins (ARFAPTINs), which contain Bin–amphiphysin–Rvs (BAR) domains that induce the formation of tubules and vesicles at the TGN[59]. Whereas ARL1 functions in vesicle trafficking similarly to ARFs, ARL2 has a highly conserved function in regulating microtubule-based processes[2]. ARL3 is closely related to ARL2, but is found only in cells with cilia, where it regulates microtubule-based processes at the cilial basal body[2,60] (FIG. 2b). ELMOD2 has been reported to be a GAP for ARL2, but also has activity against ARF1 and ARF6, which is surprising given that it has no homology to ARF GAPs[61]; the physiological relevance of this activity remains to be determined. Retinitis pigmentosa 2 (RP2; also known as XRP2) acts as a GAP for ARL3 during intraflagellar transport and ciliogenesis.

ARL3, ARL6 and ARL13 affect intraflagellar transport and ciliogenesis (FIG. 2b,c). Cilia are vital for cell signalling and differentiation, and their impaired formation is responsible for many genetic disorders[62]. Bardet–Biedl syndrome is a complex genetic disease that can be caused by mutation in any one of 14 genes associated with ciliogenesis. Transport of membrane proteins into the cilium is driven by a complex of proteins, called the BBSome. BBSome subunits have ‘coat-like’ attributes and similar structural folds to those found in COPI and adaptor protein complexes, suggesting that the BBSome can sort specific cargo for transport (FIG. 2c). ARL6 is a BBS subunit (BBS3) and is required in its GTP-bound form to recruit the BBSome onto the plasma membrane to drive cargo sorting into cilia[63]. Structural and biochemical analyses have shown that one of the mutations in ARL6 that causes Bardet–Biedl syndrome, T31R, leads to a non-functional ARL6 that cannot bind GTP[64]. This supports the idea that ARL6 recruits the BBSome complex to membranes for formation of BBSome-coated vesicles. ARL13 is mutated in patients with Joubert syndrome,

Adherens junctions
Cellular adhesions that connect epithelial cells to form a polarized epithelium. Made up of homotypic cadherin interactions and associated intracellular proteins.

ROBO4
(Roundabout homologue 4). Acts as a receptor for Slit2 protein and regulates vascular integrity.

Podosome
An adhesive, ring-like, actin-rich structure that is formed on the ventral surface of cells.

BBSome
A complex of proteins that facilitates membrane traffic into the cilium. Mutant forms of several BBS components have been identified as causative agents for various ciliopathies.

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which is a rare, complex cerebral disorder that is characterized by developmental delays and cognitive disability. It is also involved in intraflagellar transport (FIG. 2c) and, in *C. elegans*, ARL-13 associates with the doublet segment of the cilium and its loss results in shortened cilia[65,66].

Retinitis pigmentosa is a retinal degeneration disease, and mutations in the *RP2* gene are responsible for a large fraction of the most severe X-linked form. RP2 was identified as a GAP for ARL3, and mutations associated with retinitis pigmentosa compromise ARL3 GAP activity[67]. ARL3 localizes to the photoreceptor segment connecting to the cilium (FIG. 2b), and *ARL3*$^{-/-}$ mice have abnormal kidney and photoreceptor development, indicating the importance of this protein in primary cilia[68]. RP2 localizes to the basal body and centriole at the base of the photoreceptor cilium, but also to the adjacent Golgi and apical plasma membrane[69]. Furthermore, RP2 promotes vesicle trafficking from the Golgi to the base of the cilium in mammalian cells[69], presumably acting together with ARF4, ASAP1 and FIP3. Intriguingly, *D. melanogaster* ARL3 (also called Dead end) regulates actin polymerization and vesicular trafficking to the plasma membrane, which are important for tracheal morphogenesis[70]. Hence, ARL3 appears to link microtubule-based processes and vesicular trafficking during development.

ARL8 might also coordinate microtubule and vesicular trafficking. ARL8 localizes to late endosomes and lysosomes (FIG. 3) in both humans and worms, and mediates transport of endocytic proteins between these two compartments[71]. ARL8 also facilitates the axonal transport of presynaptic cargo proteins in vesicles, preventing their premature aggregation[9]. Exactly how these two functions of ARL8 are related is not clear but they might both involve transport along microtubules[2].

ARF GEFs in physiology and disease

A great deal of progress has been made in identifying ARF GEFs, and an unexpectedly broad range of roles has been revealed for these regulators, including both the coordination of membrane trafficking with lipid homeostasis and signalling at the plasma membrane (TABLE 2). Because GEFs ensure the precise temporal and spatial activation of ARFs, their own localization mechanisms are crucial for understanding their cellular roles. These mechanisms are turning out to be quite complex, even for the simplest of the ARF GEFs, the members of the cytohesin (also known as ARNO) family. Membrane trafficking is crucial to numerous developmental and physiological processes, and the specific functions of different ARF GEFs in these pathways and their links to disease are now being revealed.

Mechanisms of ARF GEF recruitment. There is particular interest in understanding how ARF GEFs are recruited to membranes to regulate ARF activation. BIG1 and BIG2 localize to the TGN and endosomes, where they have both distinct and overlapping functions[72,73]. By contrast, GBF1 localizes predominantly to the *cis*-Golgi[74] (FIG. 2a), where it controls transport of membrane proteins through the secretory pathway[75]. The activity of phosphodiesterase 3A is important for recruitment of BIG1 and BIG2 to the *trans*-Golgi[76]. However, Rab1 (REF. 77) and PtdIns4P generated by PtdIns4KIIIα[78] are involved in recruitment of GBF1 to membranes. Other close connections between Golgi ARFs and PtdIns4P have emerged recently. In yeast there is an interesting synergy observed between the ARF1 GEF Gea2 and PtdIns4P produced by Pik1 (the yeast homologue of PtdIns4KIIIβ). Both are simultaneously required to activate the aminophospholipid translocase (flippase) Drs2 at the TGN during formation of AP1–clathrin vesicles[79].

PtdIns4Ks are essential for viral replication, and notably produce the PtdIns4P-enriched membrane environment that recruits the enteroviral RNA polymerases[80]. GBF1 is required for the replication of numerous viruses, including enteroviruses, hepatitis C virus and coronaviruses[81–84]. In enteroviral systems, GBF1 and PtdIns4KIIIβ are recruited coordinately to membranes by the viral 3A protein to promote formation of functional viral replication complexes[80] near ER exit sites (FIG. 2a).

Yel1 is an EFA6-like GEF for the ARF6 orthologue Arf3 in yeast, and localizes to the plasma membrane of the emerging bud[85]. Similarly to its mammalian orthologues, the PH domain of Yel1 is required for membrane targeting but, interestingly, multiple regions of the protein are important for precise spatial localization of this GEF[85].

BRAG2, an ARF6 GEF, also has a PH domain that is critical for membrane targeting and in breast cancer cells is specifically recruited to the EGF receptor upon EGF stimulation, through direct interaction of its PH domain with the EGF receptor[86]. This interaction requires phosphorylation on specific Tyr residues and thus the recruitment of BRAG2 couples receptor activation to ARF6 activation[86]. BRAG2 is overexpressed in many breast cancer cell lines and depletion of BRAG2 by small interfering RNA blocks cell invasion *in vitro* and in animal tumour models[86]. These observations add to others that have implicated ARF6 and its activation in a number of models of cancer cell invasion and metastasis[1,58].

Autoinhibition of cytohesin GEFs. At the cell periphery, the cytohesin GEFs function in plasma membrane–endosomal membrane trafficking pathways, and in signal transduction pathways that are important for cell proliferation, immune response and growth control[87,88]. 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 (REF. 87). Recent insights have been gained into how cytohesin activation is spatially regulated, and how its autoinhibition is relieved (FIG. 4). In addition to phosphoinositide binding at the membrane, the PH domains of cytohesin family members interact with the GTP-bound forms of ARF6 (REF. 89) and ARL4 (REFS 90,91), leading to cytohesin recruitment and further activation of ARF6 or ARF1 at the membrane. A crystal structure of the SEC7 domain in tandem with the PH domain of cytohesin 3 (also known as GRP1) revealed that it adopts an autoinhibited conformation. The C-terminal helix that

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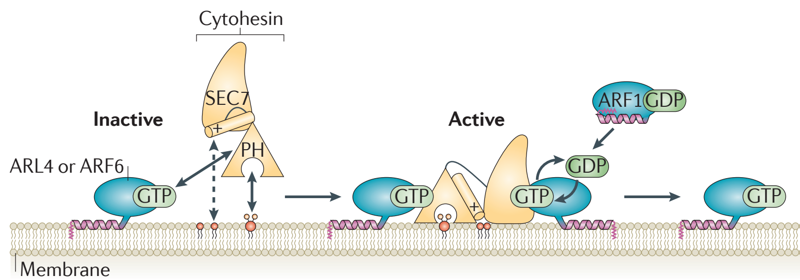

Figure 4 | **The recruitment of an ARF GEF to the membrane is coupled to relief of autoinhibition.** An active GTP-bound ADP-ribosylation factor (ARF) family member (either ARF-like 4 (ARL4) or ARF6), phosphoinositides (phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) or PtdIns-3,4,5-trisphosphate (PtdIns(3,4,5)P_3)), and additional acidic phospholipids such as phosphatidylserine, are all required for membrane recruitment of the cytohesin (also known as ARNO) guanine nucleotide exchange factor (GEF), to convert it from its cytosolic inactive form to its fully active membrane-bound form. Before recruitment, the SEC7 catalytic GEF domain, the pleckstrin homology (PH) domain and the carboxy-terminal α-helix of cytohesin are in an autoinhibited conformation (left), with the C-terminal α-helix (charged residues within this are shown as '+') and linker situated between the catalytic SEC7 domain and the PH domain, which blocks the ARF-binding site. Upon binding of the PH domain to the GTP-bound GEF at the membrane, the catalytic site is released from autoinhibition (right). This can in turn drive further activation of ARF proteins, such as ARF1, at the membrane, and may form the basis of an ARF protein activation cascade. Figure is modified, with permission, from REF. 93 © (2011) American Society for Biochemistry and Molecular Biology.

follows the PH domain and the linker between the SEC7 and PH domains block the catalytic site[92]. Interaction of the PH domain with ARF6•GTP and phosphoinositides (either PtdIns(4,5)P_2 or PtdIns-3,4,5-trisphosphate (PtdIns(3,4,5)P_3)), as well as the interaction of the polybasic C terminus of cytohesin with acidic phospholipids, all contribute to relieving this autoinhibition[92] (FIG. 4). Reconstitution of the cytohesin-exchange assay on liposomes, in the presence of both activating ARF6•GTP and substrate ARF1, revealed that mutations in the PH domain of cytohesin that abolished interaction with ARF6•GTP were completely inactive[93]. Together, these studies demonstrate how precise spatial regulation of cytohesin activation is achieved. A specific phosphoinositide (PtdIns(4,5)P_2 and/or PtdIns(3,4,5)P_3), additional acidic phospholipids and an active ARF localized in the plasma membrane must all coincide to relieve autoinhibition, thus restricting the membrane domain at which these GEFs can become active.

We do not know whether ARF6, ARF1 or both are the primary substrates for the cytohesins. However, ARF6•GTP is more efficient in relieving autoinhibition of cytohesins than ARF1•GTP, both *in vitro* and in cells[89,92]. 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 substrate itself can stimulate further exchange. Indeed, such a loop has been demonstrated *in vitro* for ARF1 (REF. 93).

Phagocytosis
A cellular endocytic process for engulfing large particles, such as bacteria, and bringing them inside the cell.

GEF-mediated cascades. There is also evidence that cytohesins might mediate a cascade of activation from ARF6 to ARF1. Cells expressing constitutively active ARF6Q67L have increased levels of ARF1•GTP[89]. ARF1 affects several processes at the plasma membrane, including recruitment of proteins to focal adhesions and during phagocytosis. In the forming phagocytic cup, ARF6•GTP is recruited earlier than ARF1•GTP, at a stage that requires rapid insertion of new membrane[94]. Hence, the ARF6–cytohesin–ARF1 cascade might ensure a high level of activated ARF protein here. ARF6 is less abundant than ARF1 in cells, and as both ARF1 and ARF6 can recruit effectors such as PtdIns4P5K and PLD, processes requiring acute activation of such effectors may rely on the more abundant ARF1 to provide an adequate supply. In support of this idea, both ARF1 and ARF6, through cytohesins, contribute to activation of PtdIns4P5K and PLD in the insulin signalling pathway[95]. In addition to ARF6–cytohesin–ARF1 or possible ARL4–cytohesin–ARF6 cascades, there is a conserved ARL cascade, in which yeast ARL3•GTP recruits ARL1 to TGN membranes[2]. In this case, it is not known whether an ARL GEF is involved. Hence, ARF family cascades could be common and could explain the Golgi ARFs that act in pairs.

ARF GEFs in scaffolding complexes. Use of the specific cytohesin inhibitor SecinH3 has revealed roles for this family of GEFs in the insulin and ERBB receptor Tyr kinase signalling pathways[96–98]. Cytohesins are positive activators of insulin signalling in both *D. melanogaster* and mammalian cells, and they are important for cell growth and for insulin sensitivity in human liver cells[97,98]. They regulate insulin signalling by binding CNK1, a scaffolding molecule that is important for Ras, phosphoinositide 3-kinase (PI3K) and AKT signalling[95]. CNK1 recruits cytohesins in an insulin-dependent manner to the plasma membrane, where they generate a PtdIns(4,5)P_2-enriched microdomain that is essential for PI3K–AKT activation. Other scaffolding proteins interact with the coiled-coil domain of cytohesin; these proteins include Golgi reassembly-stacking protein (GRASP) and IPCEF (interactor protein for cytohesin exchange factors), which mediate the interaction of DOCK180 with cytohesin[99]. Interestingly, assembly of this scaffolding complex promotes Rac activation and cell migration, indicating that these scaffolds assemble a signalling complex that determines a specific downstream output upon ARF activation[99]. Cytohesins also affect integrin signalling in the immune system, and cytohesin 1 can activate β2 integrins in dendritic cells[100], possibly through a scaffolding role of cytohesins.

GEFs in neuronal development and disease. Levels of ARF6 and the EFA6 and cytohesin family GEFs markedly increase in the mammalian brain after birth, suggesting important roles in postnatal nervous system development[101]. Experiments in isolated hippocampal neurons indicate that ARF6, EFA6 and the cytohesins might affect neurite and dendritic spine development[102,103].

In humans, mutations in the ARF1 GEF BIG2 are linked to autosomal recessive periventricular heterotopia (ARPH), a disease in which the cerebral cortex is severely underdeveloped owing to failure of neurons in the lateral ventricular proliferative zone to migrate to the cortex[104]. This impaired migration arises from a

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defect in vesicular trafficking that alters the adhesive properties of these neurons[105]. Disease alleles include an early frameshift mutation that deletes most of the BIG2 protein[104].

Members of the BRAG (or IQSEC) family of ARF GEFs are extremely abundant in neuronal postsynaptic densities, and can serve as GEFs for ARF6 (REF. 87). BRAG1 (also known as IQSEC2) and BRAG2 are vital for neuronal development. BRAG1 is mutated in X-linked nonsyndromic intellectual disability (also referred to as mental retardation). Three point mutations isolated from patients map to the SEC7 domain and result in proteins that cannot activate ARF6 normally[106]. BRAG2 has been linked to alterations in synaptic content during long-term depression (LTD). Signalling through AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-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[107]. Thus, BRAG GEFs and ARF6 are vital for neuronal development and learning.

ARF-independent GEF functions. Cytohesin GEFs may affect signalling through epidermal growth factor (EGF) or ERBB receptor Tyr kinase receptors independently of their GEF activity. EGF receptors (EGFRs) undergo ligand-induced dimerization and subsequent trans-phosphorylation, mediated by conformational changes in their cytoplasmic portion. Cytohesins bind directly to these cytoplasmic domains and promote conformational changes that increase phosphorylation[96]. Furthermore, treatment of an EGF receptor-dependent lung cancer cell line with the cytohesin inhibitor SecinH3 reduced proliferation[96]. Surprisingly, this function of the cytohesins does not require their GEF activity. Similarly, in *C. elegans,* the GEF EFA-6 regulates microtubule dynamics at the cell cortex independently of its substrate ARF6 (REF. 108). Furthermore, essential functions of GBF1 in poliovirus replication are independent of ARF1 activation[109]. The extent to which other ARF GEFs may have broader roles beyond ARF activation warrants further investigation. There are also suggestions that some multidomain ARF GAP proteins have functions that are independent of their GAP activity.

ARF GAPs as scaffolding effectors

All ARF GAPs contain the conserved zinc-finger ARF GAP catalytic domain in addition to other domains responsible for membrane recruitment, regulation of GAP activity and other scaffolding functions (TABLE 2). ARFGAP1, the first ARF GAP to be cloned[110], is Golgi-localized and, together with ARFGAP2 and ARFGAP3, mediates most ARF-bound GTP hydrolysis at the Golgi. The complex, multidomain structure of the other ARF GAP families has stimulated much research. Here, we highlight a few examples of how these multidomain ARF GAPs, by recognizing the GTP-bound form of their substrate ARF, act as downstream effectors in addition to signal terminators. Information about other ARF GAPs can be found in an excellent review article[111].

The ASAP proteins are the prototypical multidomain GAPs that interact with many signalling molecules, including SRC and focal adhesion kinase[111] (TABLE 2). ASAP1 resides in focal adhesions but, in response to SRC activation, it facilitates formation of podosomes[112], which are discrete actin-based structures that are formed at the cell substratum to degrade matrix. The crystal structure of ARF6 in complex with the catalytic domain of ASAP3 revealed that a catalytic Arg-finger of ASAP3 is responsible for GTP hydrolysis[113], similarly to many other GAPs, a finding that is consistent with an earlier structure of the GAP domain of ASAP2 (REF. 114). There is also some evidence that calcium might bind to the complex and regulate GAP activity[113], although this needs to be confirmed with full-length ASAP3 and ARF6 and in cells. The ASAPs all have N-terminal BAR domains that can induce membrane curvature and tubule formation in transfected cells and in cell-free systems. The BAR domain in ASAP1 negatively regulates its GAP activity towards ARF1 (REF. 115), and binding of the Rab11 effector FIP3 to the BAR domain of ASAP1 stimulates its GAP activity[116]. As mentioned earlier, ASAP1 also promotes ciliary targeting together with ARF4 and FIP3 (REF. 20) (FIG. 2b). ASAP1 is upregulated in breast, pancreatic and colorectal cancer[58]. CBL-interacting protein 85 (CIN85; also known as SH3KBP1) binds to ASAP1, recruiting the E3 ubiquitin ligase CBL, to trigger the monoubiquitylation of ASAP1; this modification is important for invasion of breast cancer cells[117] but the role for ubiquitylation of ASAP in cell invasion is not known. One caveat to observations made when ASAP is expressed in cells is that a study designed to systematically look at ARF GAP function and ARF specificity failed to detect an effect of ASAP1 expression on either ARF1•GTP or ARF6•GTP levels in cells[118]. This raises the possibility that the GAP activity of ASAP1 might not always be critical for some of ASAP1's specific functions.

The ARF GAP GIT1, originally identified as a GPCR kinase-interacting protein, can coordinate signalling by acting as a scaffold. GIT1 and its substrate ARF6 affect ligand-stimulated endocytosis of several GPCRs through either clathrin-dependent or clathrin-independent endocytic pathways[119]. Among the proteins interacting with GIT1 are the CDC42 and Rac GEF PIX, focal adhesion kinase and paxillin. GIT1, similarly to ASAP1, is sometimes observed in focal adhesions and its influence on the activation of CDC42 and Rac suggests that ARF inactivation and Rac activation are coordinated (FIG. 3). *D. melanogaster* GIT is required for muscle morphogenesis[120] and the GIT1-knockout mouse is defective in fear learning[121] and dendritic spine formation[122]. Rac3 interacts with GIT1, disrupting GIT1 binding to paxillin; this in turn stimulates GIT1 GAP activity, presumably towards ARF6 (REF. 123), and inhibits cell spreading and neuritogenesis. In endothelial cells, ROBO4 interacts with paxillin, which recruits GIT1 to inactivate ARF6, and this leads to vascular stability, blocking cellular protrusions and neovascular leak[55]. Thus, these examples provide insights into how modular ARF GAPs promote spatially and temporally restricted assembly of signalling complexes, and allow a precise physiological output in response to a signal.

Long-term depression
(LTD). A reduction in the efficacy or strength of neuronal synapses that is linked to learning and memory formation.

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Intracellular pathogens can use a fascinating GAP-blocking mechanism to rewire the host cell's signalling network for their own purposes. Enterohaemorrhagic *Escherichia coli* produce the EspG protein, which binds to GTP-bound ARF1 and ARF6, blocking their access to GAPs and disrupting the function of both early Golgi and recycling endosomes[124]. Moreover, EspG simultaneously binds to p21-activated kinase (PAK), an effector of a distinct G protein family member, CDC42, and promotes PAK localization at Golgi membranes rather than at the plasma membrane. This raises the possibility that EspG assembles its own signalling complex on intracellular membranes to subvert membrane trafficking and polarity processes in host cells.

Conclusions & perspectives

ARF activity is regulated in a spatiotemporal manner by the GEFs and GAPs, underlining the importance of precise localization of these regulators. In the case of cytohesins, such specificity can be achieved through a coincidence-detection mechanism, requiring both an activating ARF or ARL protein and a specific lipid composition. This example also reveals the existence of ARF family activation cascades and how relief of autoinhibition can be coupled to precise spatial cues. It will be interesting to see how widespread these mechanisms are among ARF family members. ARF cascades, similarly to those demonstrated for Rab G proteins, could transform one membrane domain into another during highly dynamic membrane trafficking. These transformations involve coordinated changes in the lipid and protein composition of each membrane domain, a specialty of many 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 require *in vitro* reconstitution on model membranes. There appears to be a particularly important link between ARF1 function and PtdIns4P, a lipid that has a central role in the function of the Golgi, which parallels the coordination of membrane trafficking and PtdIns(4,5)P_2 signalling by ARF6 at the plasma membrane.

The GAPs and GEFs for the ARF family proteins are multidomain proteins that can assemble signalling complexes and so place the ARFs and ARLs into larger networks. These networks include cytoskeleton regulators, and it appears that some ARL proteins (ARL2, for example) have evolved exclusively to regulate the cytoskeleton. The role of ARF6 in networks linking membrane trafficking to the actin cytoskeleton also involves interaction of ARF6 with GEFs and GAPs of the Rac and Rho small G proteins, actin cytoskeleton regulators. Another emerging concept is that some ARF family members remain membrane-bound in their GDP-bound form so that they can interact with signalling complexes and promote alternative signalling pathways. Ultimately, these ARF family signalling networks will need to be studied through systems level analysis.

So far, no GEFs and only two GAPs that are specific for an ARL have been identified. Several ARL proteins affect ciliogenesis and, in some cases, ciliopathies; other ARLs function in neurons and have been associated with neurodegenerative disorders. Hence, increased understanding of ARLs and their regulators should inform both fundamental questions in cell biology and disease mechanisms.

Finally, the use of model organisms to complement studies in mammalian cells has already provided valuable insights into the physiological roles of ARF family proteins. This approach holds great promise for uncovering the unknown functions of most ARLs, as well as defining the full range of activities of all ARF and ARL proteins.

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The authors declare no competing financial interests.

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CORRIGENDUM

ARF family G proteins and their regulators: roles in membrane transport, development and disease

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The authors would like to note that Catherine L. Jackson's address was incomplete as it appeared in the original version of this article. This has been corrected in the online version.