ARF GTPases and their GEFs and GAPs: concepts and challenges

Elizabeth Sztul[a,†], Pei-Wen Chen[b], James E. Casanova[c], Jacqueline Cherfils[d], Joel B. Dacks[e], David G. Lambright[f], Fang-Jen S. Lee[g], Paul A. Randazzo[h], Lorraine C. Santy[i], Annette Schürmann[j], Ilka Wilhelmi[j], Marielle E. Yohe[k], and Richard A. Kahn[l,†,*]

[a]Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL 35294; [b]Department of Biology, Williams College, Williamstown, MA 01267; [c]Department of Cell Biology, University of Virginia, Charlottesville, VA 22908; [d]Laboratoire de Biologie et Pharmacologie Appliquée, CNRS and Ecole Normale Supérieure Paris-Saclay, 94235 Cachan, France; [e]Division of Infectious Disease, Department of Medicine, University of Alberta, Edmonton, AB T6G 2H7, Canada; [f]Program in Molecular Medicine and Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Amherst, MA 01605; [g]Institute of Molecular Medicine, College of Medicine, National Taiwan University, Taipei 10002, Taiwan; [h]Laboratory of Cellular and Molecular Biology and [k]Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892; [i]Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802; [j]German Institute of Human Nutrition, 85764 Potsdam-Rehbrücke, Germany; [l]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322-3050

ABSTRACT Detailed structural, biochemical, cell biological, and genetic studies of any gene/protein are required to develop models of its actions in cells. Studying a protein family in the aggregate yields additional information, as one can include analyses of their coevolution, acquisition or loss of functionalities, structural pliability, and the emergence of shared or variations in molecular mechanisms. An even richer understanding of cell biology can be achieved through evaluating functionally linked protein families. In this review, we summarize current knowledge of *three* protein families: the ARF GTPases, the guanine nucleotide exchange factors (ARF GEFs) that activate them, and the GTPase-activating proteins (ARF GAPs) that have the ability to both propagate and terminate signaling. However, despite decades of scrutiny, our understanding of how these essential proteins function in cells remains fragmentary. We believe that the inherent complexity of ARF signaling and its regulation by GEFs and GAPs will require the concerted effort of many laboratories working together, ideally within a consortium to optimally pool information and resources. The collaborative study of these three functionally connected families (≥70 mammalian genes) will yield transformative insights into regulation of cell signaling.

Monitoring Editor
William Bement
University of Wisconsin

Received: Jan 2, 2019
Revised: Feb 26, 2019
Accepted: Mar 11, 2019

DOI:10.1091/mbc.E18-12-0820

[†]These authors contributed equally to this work.

*Address correspondence to: Richard A. Kahn (rkahn@emory.edu).

Abbreviations used: ARFRP1, activated ARF-related protein 1; ARL, ARF-like; BRAG, Brefeldin A–resistant ARF GEF (now IQSec); EFA6, exchange factor for ARF6; ER, endoplasmic reticulum; ERES, ER exit sites; FA, focal adhesion; GAP, GTPase-activating protein; GEEC, glycophosphatidyl-inositol–enriched endosomal compartment; GEF, guanine nucleotide exchange factor; LECA, last eukaryotic common ancestor; PIP, phosphoinositide; PM, plasma membrane; PSD, pleckstrin homology and Sec7 domain; TBCD, tubulin cochaperone cofactor D; TGN, *trans*-Golgi network.

© 2019 Sztul *et al*. This article is distributed by The American Society for Cell Biology under license from the author(s). Two months after publication it is available to the public under an Attribution–Noncommercial–Share Alike 3.0 Unported Creative Commons License (http://creativecommons.org/licenses/by-nc-sa/3.0).

"ASCB®," "The American Society for Cell Biology®," and "Molecular Biology of the Cell®" are registered trademarks of The American Society for Cell Biology.

INTRODUCTION

Members of the family of regulatory GTPases that include ARFs, ARF-like (ARLs), and SARs have emerged as key regulators of cellular signaling involved in almost all aspects of cell biology (Tables 1–3, Figure 1, and Supplemental Tables I–III) (D'Souza-Schorey and Chavrier, 2006; Donaldson and Jackson, 2011; Jackson and Bouvet, 2014). Their importance is underscored by findings showing that complete or conditional deletions or mutations result in embryonic lethality or organ-specific defects, with links to a variety of diseases (Table 4) (Seixas *et al*., 2013; Zhang *et al*., 2013). ARF family GTPases control key cellular processes, including bidirectional membrane trafficking (secretion and endocytosis), ciliogenesis, lipid metabolism, energy use, motility, division, apoptosis, and transcriptional regulation. Like all regulatory GTPases, ARF family GTPases operate

	GTPase	Localization	Function(s)	Interactors
1	Arf1	Cytosol, Golgi	Recruitment of coat complexes, activation of PLD, PI kinases	COP-I, GGAs, MINTs, Cholera toxin, Arfaptin, MKLP1, ARF GEFs, ARF GAPs
2	Arf3	Cytosol, Golgi	Recruitment of coat complexes, activation of PLD, PI kinases	COP-I, GGAs, MINTs, Cholera toxin, Arfaptin, MKLP1, ARF GEFs, ARF GAPs
3	Arf4	Cytosol, Golgi, endosomes		COP-I, GGAs, MINTs, Cholera toxin, Arfaptin, MKLP1, ARF GEFs, ARF GAPs
4	Arf5	Cytosol, Golgi, endosomes	Recruitment of coat complexes, activation of PLD, PI kinases	COP-I, GGAs, MINTs, Cholera toxin, Arfaptin, MKLP1, ARF GEFs, ARF GAPs
5	Arf6	PM, endosomes, RE, cortical actin	Cortical actin rearrangement, endocytosis, PLD activation	β-arrestin, POR1, PLD, Cytohesins, MKLP1, FilGAP
6	Arl1	Golgi, TGN	Endosome–Golgi secretory traffic, LD formation	Arfaptin, MKLP1, PDEd, HRG4, Golgins, GRIP-domain proteins
7	Arl2	Cytosol, mitochondria, centrosomes, basal bodies, cilia, RRs	Tubulin heterodimer assembly, mitochondrial fusion, Prenyl-protein traffic	TBCD/β-tubulin, TBCD, ELMOD1-3, BART/ARL2BP, PDEd, HRG4/UNC119
8	Arl3	Cytosol, centrosomes, cilia, mitotic spindle, midbody, Golgi	Cytokinesis, Prenyl- and Myr-protein traffic	PDE6δ, HRG4/UNC119, Golgins, ARL13B, BART/ARL2BP
9	Arl4a	Cytosol, nucleus, TGN, endosomes, PM	Endosome–Golgi traffic, actin remodeling, cell migration	ELMO, GCC185, Robo1, Cytohesin2
10	Arl4c	Cytosol, nucleus, PM	Cholesterol traffic, filopodia, cell migration, tumorigenesis	α-Tubulin, filamin-A, Cytohesin2
11	Arl4d	Cytosol, mitochondria, nucleus, PM, actin	Actin remodeling, neurite outgrowth	HP1, importin-α, Cytohesin2,
12	Arl5a	Nucleus	Endosome–Golgi traffic	HP1α, GARP, Ragulator
13	Arl5b	Nucleus	Endosome–Golgi traffic	HP1α, GARP, Ragulator
14	Arl5c			
15	Arl6	Cilia		BBSome, Sec61β
16	Arl8a	Lysosomes, phagolysosomes	Lysosomal traffic and fusion	SKIP-kinesin1b, HOPS complex
17	Arl8b	Lysosomes, phagolysosomes	Lysosomal traffic and fusion	SKIP-kinesin1b, HOPS complex
18	Arl9			
19	Arl10	Nuclei, mitochondria		
20	Arl11			p-ERK
21	Arl13a			
22	Arl13b	Cilia, EE, CDRs, centrosomes	Regulation of ciliary formation/maintenance, axoneme, Hh signaling, EEs	ARL3, INPP5E, PDE6δ, tubulin, FIP5, UBC9, MYH9
23	Arl14			
24	Arl15	Cytosol, Golgi	Genetic links to adiponectin levels and type 2 diabetes	ASAP2
25	Arl16	Cytosol, mitochondria, nucleus, cilia		RIG-I
26	Arfrp1	*trans*-Golgi	Recruitment of Arl1 and Golgin-97/245 to *trans*-Golgi	Sec7-1, Cytohesin
27	Sar1a	ER		
28	Sar1b	ER		
29	Trim23	Lysosomes, Golgi, autophagosomes	Ubiquitin ligase, viral infection, membrane trafficking	UBE2D2, TBK1, Cytohesin1

National Center for Biotechnology Information (NCBI) gene names are listed, along with cellular localization, identified functions, and protein interactors. Abbreviations used include CDR, circular dorsal ruffles; EE, early endosomes; PLD, phospholipase D; PM, plasma membrane; RE, recycling endosomes; RRs, rods and rings. Additional information is included in Supplemental Table I.

TABLE 1: Human ARF family GTPases.

	GEF	Localization	Function(s)	Interactors
1	Arfgef1/BIG1	TGN, sorting endosomes	Activation of Arf1/3, recruitment of AP1/AP3, myelination	Arl1, Arf1/3, ARF4/5
2	Arfgef2/BIG2	TGN, sorting endosomes	Activation of Arf1/3, recruitment of AP1/AP3	Arl1, Arf1/3, ARF4/5
3	Cyth1	PM	Cell adhesion/migration, integrin regulation	GRASP/tamalin, CNKSR1-3, CASP, Arl4A, Arl4D, Arf6
4	Cyth2/ARNO	PM, REs, Ruffles	Cell adhesion/migration, integrin regulation, actin remodeling, endosome traffic	GRASP/tamalin, CNKSR1-3, CASP, Arl4A, Arl4D, Arf6, paxillin, RLIP76, β-arrestin, pallidin
5	Cyth3/ARNO3/GRP1	PM, (Glut4-positive) endosomes	Glut4 exocytosis, cell migration	GRASP/tamalin, CNKSR1-3, CASP, Arl4A, Arl4D, Arf6
6	Cyth4			
7	GBF1	Golgi	Membrane traffic at *cis*-Golgi	p115, Rab1b, COG4, γ-COP, GGA1-3, ATLG, Gmh1
8	Iqsec1/BRAG2/GEP100	PM	Integrin endocytosis/cell adhesion regulation of AMPA receptor traffic	Calmodulin, MAP4K4, Arf5, Arf6
9	Iqsec2/BRAG1	PSDs	Regulation of AMPA receptor traffic	Calmodulin, PSD95, IRSp53, Arf6
10	Iqsec3	PSDs	Regulation of GABAergic synapse formation	Calmodulin, gephyrin, Arf6
11	Psd/EFA6	PM, tight junctions, axons, PSDs, endosomes	Tight junction formation, epithelial lumen formation	α-Actinin-1, 4, Arf6
12	Psd2/EFA6C			
13	Psd3/EFA6D			
14	Psd4/EFA6B	PM, epithelial tight junctions	Tight junction formation, epithelial lumen formation	α-Actinin-1, 4, Arf6
15	Fbox8			

NCBI gene names are listed, along with cellular localization, identified functions, and protein interactors. Abbreviations used include PM, plasma membrane; PSD, postsynaptic densities, RE, recycling endosomes; TGN, *trans*-Golgi network. Additional information is included in Supplemental Table II.

TABLE 2: Human ARF GEFs.

as "molecular switches" by interconverting between inactive (GDP-bound) and active (GTP-bound) conformations. Upon binding GTP, the activated GTPases alter their conformations, which increases their affinity for effectors and can alter their localization in cells, each of which contributes to the generation of a specific biological output. Activated (GTP-bound) ARF family GTPases propagate their effects through a specific redistribution of effectors (e.g., recruitment to a membrane), allosteric activation of effector enzymatic activity, conformational changes within the effector resulting in increased affinity for other cellular components (proteins, lipids, etc.), or a combination of such changes. As a consequence, the signal output of these GTPases is tightly controlled by the regulated binding of GTP and the half-life of the activated state. These are in turn controlled by the stimulation of the release of bound GDP (to allow GTP to bind spontaneously) by guanine nucleotide exchange factors (GEFs) and of their intrinsic GTPase activity by GTPase-activating proteins (GAPs) (Casanova, 2007; Inoue and Randazzo, 2007; Randazzo *et al.*, 2007; Bui *et al.*, 2009; Spang *et al.*, 2010; East and Kahn, 2011; Wright *et al.*, 2014; Vitali *et al.*, 2017). Thus, the triad of GEF–GTPase–GAP can be viewed as a minimal component in signaling pathways that alter a large fraction of cellular behaviors. Yet, despite their clear importance in cell biology and links to human pathologies, our understanding of the pathways involved and molecular mechanisms remain fragmentary. In this review, we briefly summarize the known roles of the ARF family GTPases, their GEFs and GAPs, their localization in cells, and their interactors. Rather than describing in detail any one of the many pathways in which they operate, we instead emphasize the extensive overlap in specificities and actions between family members, as this represents the largest challenge to achieving a deep understanding of their mechanisms of action. Because every pathway requires the minimum GEF–GTPase–GAP triad, we argue for a systematic approach to study each family and the three families together. We end our review by highlighting some key questions and challenges in ARF signaling, and hope that it inspires more collaborative efforts to address the large, complex, but vitally important area of ARF signaling.

ARF FAMILY GTPASES

Families of ARF GTPases and their cellular functions

Included within the ~30 members of the mammalian ARF family are the six "true ARFs" (humans lack ARF2, thus the discrepancy between this number and Table 1), the 21 ARF-like (ARL) proteins, two SARs, and Trim23 (Table 1; additional information included in Supplemental Table I) (Li *et al.*, 2004; Kahn *et al.*, 2006). The six mammalian ARFs are highly conserved, sharing > 65% sequence identity, and perform similar and/or overlapping functions. ARLs are more divergent, sharing typically 40–60% identity, and largely perform distinct cellular functions. The two mammalian SARs share ~90%

	GAP	Localization	Function(s)	Interactors
1	Arfgap1	Golgi	ER protein retrieval	γ-Adaptin (AP-1), KDEL receptor/ ERD2, p24
2	Arfgap2	Golgi		
3	Arfgap3	TGN, EEs	EE–LE transport of M6PR and EGFR	γ-COP (COPI), GGA1/2
4	Acap1/CENTB1	Rab11 REs	Integrin and TfnR recycling	β1-Integrin, TfnR, clathrin heavy chain
5	Acap2/CENTB2	PM, phagocytic cup, ARF6 endosomes	Neurite outgrowth, FcγR-mediated phagocytosis	Rab35
6	Acap3/CENTB5		Neurite outgrowth, neuronal migration	
7	Adap1/CENTA1	Membrane ruffles, mito-chondria, dendrites, synapse	*Salmonella* invasion, beta2-AR internalization, dendritic differentiation	Kif13b
8	Adap2/CENTA2			
9	Agap1	AP-3 endosomes	Endosome–lysosome transport	AP-3, Kif2A
10	Agap2/PIKE	FAs, Rab4/AP-1endosomes	Cell migration, neurite outgrowth, invasion, TfnR recycling	FAK, RACK1, Akt, Homer, AP-1
11	Agap3	Endosomes		
12	Agfg1/HRB, RIP	Clathrin/AP-2/EPS15 vesicles	TfnR endocytosis, HIV-1 replication	Rev
13	Agfg2			
14	Arap1	EEs, CDRs, podosomes	EGFR endocytosis, macropinocyto-sis, secretory lysosomes	CIN85, AP-3
15	Arap2	FAs, APPL EEs	FA turnover, SF formation, integrin endocytosis	RhoA, Arf6, APPL1
16	Arap3	Podosome-like adhesions	Cell migration, invasion, RhoGAP stimulation	Rap1, RhoGAP
17	Asap1	PM, FAs, podosomes/inva-dopodia, CDRs	Cell migration, invasion, SF forma-tion, integrin and EGFR recycling	FAK, Crk, CrkL, Src, cortactin, NM2A, PRKD2, CIN85, CDAP
18	Asap2	Cell periphery, phagocytic cup	Cell migration, FcγR-mediated phagocytosis	Selenoprotein K
19	Asap3	PM, CDRs	Cell migration, integrin recycling, invasion	Grb2
20	Git1	FAs, SNX27 endosomes, REs, EEs	Cell migration, invasion, EGFR traffic/degradation	PIX, Arf6, paxillin, MEK1, FAK, SNX6
21	Git2	PM, FAs	Cell migration, invasion, beta2-Adrenergic R down-regulation	Vav2, paxillin, GRKs
22	Smap1	PM	TfnR endocytosis	Clathrin heavy chain
23	Smap2	EE, TGN	EE–TGN transport	Clathrin heavy chain, CALM
24	ELMOD1	Golgi, nuclear speckles, LDs		
25	ELMOD2	ER, mitochondria, LDs, cen-trosomes, RRs	Mitochondrial fusion	ARL2, other ARF family GTPases
26	ELMOD3	PM, actin, lagging edge		
27	RP2	PM, microtubules, nucleus	Ciliary traffic	ARL3, UNC119, G protein β1

NCBI gene names are listed, along with cellular localization, identified functions, and protein interactors. Abbreviations used include CDR, circular dorsal ruffles; EE, early endosomes; EGFR, epidermal growth factor receptor; FA, focal adhesions; LD, lipid droplets; LE, late endosomes; PM, plasma membrane; RE, recycling endosomes; RRs, rods and rings; SF, stress fibers. Additional information is included in Supplemental Table III.

TABLE 3: Human ARF GAPs.

primary sequence identity (but < 30% to any other family member), and have a specialized role in traffic from the endoplasmic reticulum (ER) to the Golgi. The ARF family GTPases are distinct from the other families of small, regulatory GTPases (RAS, RHO, RAB) in having an N-terminal extension of ~14 amino acids and covalent modifications at or near this end. All six ARFs are N-myristoylated, while ARLs are

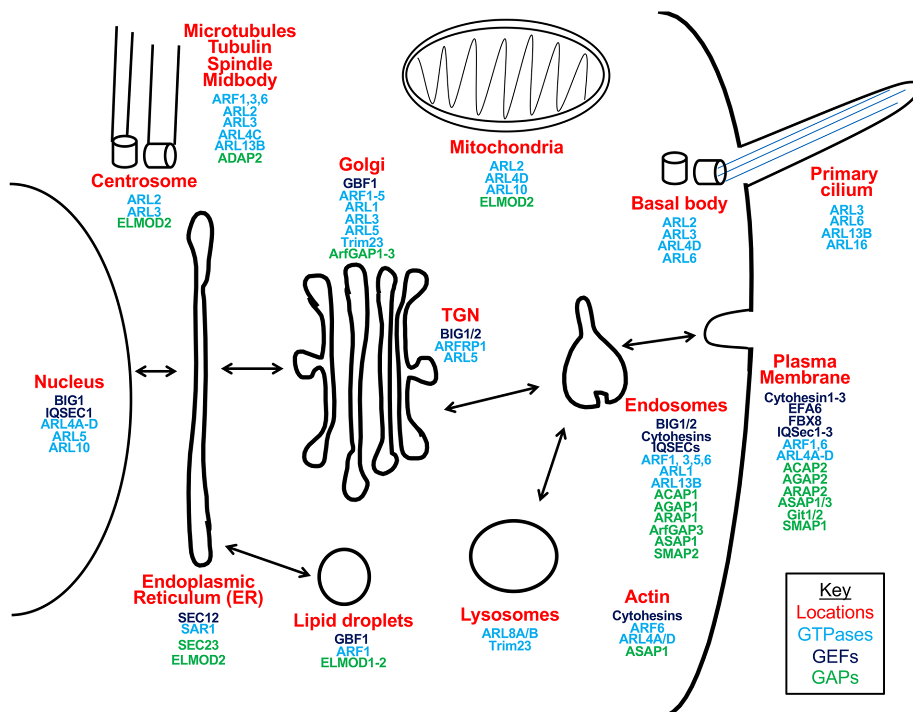

FIGURE 1: Subcellular localization of the ARF family GTPases, ARF GEFs, and ARF GAPs. A schematic cell with organelles (in red) showing the localization of the GTPases (in light blue), GEFs (in purple), and GAPs (in green). More detailed information for these localizations is provided in references cited in the text.

myristoylated (e.g., ARL1), palmitoylated (e.g., ARL13B), or acetylated (e.g., ARFRP1), with each modification critical for activity. In this section, we summarize briefly the actions of the different ARF GTPases. This should not be taken as an exhaustive description of their actions, and we apologize to the many researchers whose work is not included in the interest of space.

The ARFs are best known for their roles in recruitment of coat proteins/complexes and initiation of vesicle formation in membrane trafficking, particularly at the Golgi. However, a brief glance at Figure 1, which depicts localizations for all ARF family members, as well as the known GEFs and GAPs, reveals far more complexity in locations and presumed functions. ARFs are found not only in all regions of the Golgi, but also at the plasma membrane (PM), endosomes, lipid droplets, and midbodies. ARFs often show overlapping distribution (e.g., ARF1, 4, and 5 localize to the *cis*-Golgi), and one ARF can localize to multiple sites (e.g., ARF1 can be recruited to the Golgi, lipid droplets, and the PM; however, it is likely that a large cytosolic pool remains). ARFs have both redundant and distinct functions, as seen in studies showing that small interfering RNA–mediated knockdown of any one ARF1/3/4/5 yielded no obvious phenotypes, while each double knockdown of distinct pairs of these GTPases resulted in specific phenotypes (Volpicelli-Daley *et al.*, 2005). ARFs affect cellular processes by recruiting effectors. The term "effector" is used herein to indicate a protein that binds preferentially to the activated (GTP-bound) form, resulting in a signal or output that changes some aspect of cell biology. There are more than 20 known ARF effectors and most of these are essential components in membrane traffic in all cells (Table 1). The best-characterized ARF effectors are adaptors for coat proteins or the coat proteins themselves, including both monomeric (e.g., GGAs or MINTs) (Boman *et al.*, 2000; Dell'Angelica *et al.*, 2000; Hill *et al.*, 2003) and oligomeric complexes (e.g., COPI, AP-1, AP-3, and AP-4) (Serafini *et al.*, 1991; Stamnes and Rothman, 1993; Traub *et al.*, 1993; West *et al.*, 1997; Ooi *et al.*, 1998; Hirst *et al.*, 1999; Drake *et al.*, 2000; Donaldson and Jackson, 2011). ARFs also recruit non-coat proteins to membranes (e.g., golgin-160, GCC88) (Derby *et al.*, 2004; Gilbert *et al.*, 2018) and can activate lipid-modifying enzymes (e.g., phospholipase D, PI(4)5-kinase) (Brown *et al.*, 1993; Cockcroft *et al.*, 1994; Honda *et al.*, 1999; Jones *et al.*, 2000) and/or lipid transporters (e.g., FAPP1) (Godi *et al.*, 2004). ARF1, ARF3, and ARF6 are implicated in cell division and/or cytokinesis, but the effectors involved remain to be identified (Altan-Bonnet *et al.*, 2003; Hanai *et al.*, 2016; Nakayama, 2016). ARF6 appears to act predominantly in the cell periphery, where it regulates both endosomal recycling and cortical actin dynamics, which are also linked to RAC GTPase signaling (Donaldson, 2003; Schweitzer *et al.*, 2011).

ARLs localize to and have functions in processes involving tubulin/microtubules at centrosomes, spindles, midbodies, basal bodies, and cilia (Figure 1). ARLs are much more divergent in action, with some ARLs functioning in parallel with those of the ARFs in membrane traffic, while others regulate completely different processes. For example, ARL1 is most closely related to ARFs in primary sequence, localizes to the *trans*-Golgi network (TGN), and acts in membrane trafficking through its ability to recruit proteins to that site (Yu and Lee, 2017). At the TGN, ARL1 effectors include BIG1 (an ARF GEF), Golgin-97, and Golgi-245. ARL1 also localizes to sorting endosomes, where it recruits BIG1 to activate ARF1 and ARF3 (D'Souza *et al.*, 2014). In yeast, GTP-bound ARL1 is located at the Golgi complex and facilitates the exit of vesicles from the TGN (Munro, 2005). In mammalian cells, ARL1 is involved in cell polarity (Lock *et al.*, 2005); innate immunity (Murray and Stow, 2014); and the secretion of insulin (Gehart *et al.*, 2012), chromogranin A (Cruz-Garcia *et al.*, 2013), and matrix metalloproteinases (Eiseler *et al.*, 2016). In marked contrast, ARL2 localizes to multiple cellular sites to perform surprisingly distinctive functions. ARL2 is found in 1) cytosol, as a 1:1:1 trimer with the tubulin cochaperone cofactor D (TBCD) and β-tubulin, and is required for αβ-tubulin biogenesis (Francis *et al.*, 2017a,b); 2) the mitochondrial intermembrane space, where it promotes mitochondrial fusion (Newman *et al.*, 2014, 2017); 3) at centrosomes (Zhou *et al.*, 2006); 4) in the nucleus; and 5) at rods and rings, which are implicated in guanine nucleotide metabolism (Schiavon *et al.*, 2018). Despite its involvement in such disparate activities, ARL2 is ubiquitously expressed in eukaryotes and has not duplicated into paralogues that might allow separation of functions (Francis *et al.*, 2016). One intriguing hypothesis born from these observations is that the use of a shared cell regulator at multiple locations might serve as a means of communication between those sites, a phenomenon termed "higher-order signaling" (Francis *et al.*, 2016).

ARL2 and ARL3 display both overlapping and distinct actions and interactions (Van Valkenburgh *et al.*, 2001; Zhou *et al.*, 2006). Both are linked to microtubules, localize to centrosomes, and share the ability to bind PDEδ in a GTP-dependent manner and promote

GTPase	Conventional knockout	Conditional knockout	Reference
ARF1	Embryonically lethal (E5.5)	—	Hayakawa *et al.* (2014)
ARF4		*Postnatal deletion (Arf4*^{flox}*/CagCreER)*: Reduced viability; reduced size of the pancreas; yellowish feces in lower intestine; hair turned from black to gray	Pearring *et al.* (2017)
		Photoreceptor cells (Arf4^{flox}*/iCre75)*: Normal rhodopsin localization; no retinal degeneration	Pearring *et al.* (2017)
		Kidney (Arf4^{flox}*/HoxB7Cre)*: No cystic disease	Pearring *et al.* (2017)
		Pancreas (Arf4^{flox}*/CagCreER):* Degeneration of exocrine pancreas; infiltration of adipocytes in exocrine pancreas; normal islet size and organization	Pearring *et al.* (2017)
ARF6	Embryonically lethal (midgestation); smaller liver with progressive apoptosis; defective hepatic cord formation	—	Suzuki *et al.* (2006)
ARF6		*Endothelial cells (Arf6*^{flox}*/Tie2-Cre)*: Reduced tumor angiogenesis via impaired HGF-induced endothelial β1-integrin recycling	Hongu *et al.* (2015)
		Neuronal cells (Arf6^{flox}*/Nestin-Cre)*: Smaller size of fimbria of hippocampus and corpus callosum; impaired oligodendrocyte myelination in the hippocampal fimbria and the corpus callosum during development, due to reduced secretion of fibroblast growth factor-2	Akiyama *et al.* (2014)
		Platelets (Arf6^{flox}*/PF4-Cre)*: Impaired αIIbβ3-integrin trafficking resulting in reduced fibrinogen uptake and storage	Huang *et al.* (2016)
		Podocyte specific (Arf6^{flox}*-Nphs2-Cre)*: Normal kidney development In model of acute podocyte effacement: protection from podocyte effacement In model for immune complex–mediated injury: aggravated proteinuria	Lin *et al.* (2017)
ARL3	Early death (3 wk of age); abnormal development of renal, hepatic, and pancreatic epithelial tubule structures; abnormal epithelial cell proliferation and cyst formation; photoreceptor degeneration (at P14)	—	Schrick *et al.* (2006)
		Retina specific (Arl3^{flox}*/Six3-Cre)*: Impaired ciliogenesis; no formation of connecting cilia and outer segments; degeneration of retina at 2 mo; inability of retina to respond to light rapidly	Hanke-Gogokhia *et al.* (2016)
		Rod photoreceptor specific (Arl3^{flox}*/iCre75)*: Photopic responses started to decline at the age of 1 mo; degeneration of rods and cones at 2 mo; decline of retinal thickness Trafficking deficiencies of lipidated phototransduction proteins, e.g., farnesylated rhodopsin kinase (GRK1)	Hanke-Gogokhia *et al.* (2016)

TABLE 4: Phenotypes of mice with mutations/deletions of ARF family GTPases.

Continues

GTPase	Conventional knockout	Conditional knockout	Reference
ARL4	Reduction of testis weight (30%) and sperm count (60%) without affecting fertility	—	Schurmann *et al.* (2002)
ARL6 (BBS3)	Development of BBS-associated phenotypes: retinal degeneration, male infertility, loss of sperm flagella, severe hydrocephalus, thinning of the cerebral cortex; reduced size of hippocampus and corpus striatum, reduced number and misshaping of ependymal cell cilia, increased body fat	—	Zhang *et al.* (2011)
ARL13B (a GEF of ARL3)	Hennin (hnn) mutation (ENU-induced mutation) corresponding to Arl3 null allele: Embryonically lethal; at ED 9.5, open neural tube in the head, caudal spinal cord, and randomized heart looping; at ED 14, abnormal eyes and axial polydactyly Nodal cilia half the normal length; abnormal structure of the axoneme Specific disruption of the Sonic hedgehog (Shh) signaling pathway		Caspary *et al.* (2007)
		Kidney specific (Arl13B^{flox}-Ksp-Cre): Defective cilia biogenesis and rapid kidney cyst formation due to an overproliferation followed by fibrosis; increased kidney-to-body weight ratio; mutant mice dead at around P60	Li *et al.* (2016)
		Retina specific (Arl13B^{flox}/Six3-Cre): Absence of outer segments of the retina (starting at P6); photoreceptor rhodopsin: failure to form mature transition zones and outer segments and rapid degeneration; normal docking of basal bodies of photoreceptors to cell membranes	Hanke-Gogokhia *et al.* (2017)
		Tamoxifen-inducible Cre/loxP recombination (Arl13B^{flox}-CAG-CreER) at 1 mo of age: Destabilization of axonemes and transition zones, leading to progressive photoreceptor degeneration; impairment of anterograde intraflagellar transport (IFT) due to marked reduction of IFT88 protein at basal bodies; impaired retinogenesis, including early postnatal proliferation of retinal progenitor cells, development of photoreceptor cilia, and morphogenesis of photoreceptor outer segment; mislocalization of rhodopsin, prenylated phosphodiesterase-6 (PDE6), and IFT88	Hanke-Gogokhia *et al.* (2017); Dilan *et al.* (2018)
		Tamoxifen-inducible Cre/loxP recombination (Arl13B^{flox}-CAGG-CreER) at postnatal day 4: Mutant mice smaller than the control littermates; ~two-thirds dead from cystic kidneys between P27 and P51 Normal cerebellar size and foliation	Bay *et al.* (2018)
ARFRP1	Embryonically lethal; apoptotic epiblast cells within ectoderm at ED 6.0 and 7.0 Mistargeting of E-cadherin to intracellular compartments		Mueller *et al.* (2002); Zahn *et al.* (2008)

TABLE 4: Phenotypes of mice with mutations/deletions of ARF family GTPases.

Continues

GTPase	Conventional knockout	Conditional knockout	Reference
		Intestine specific (Arfrp1^{flox}*/villin-Cre)*: Lower abundance of E-cadherin at the lateral membrane of the cell surface of crypts and villi (E-cadherin is associated with intracellular membranes); marked growth retardation due to impaired lipid uptake; impaired chylomicron lipidation and reduced release of ApoA-I	Zahn *et al.* (2008); Jaschke *et al.* (2012)
		Liver specific (Arfrp1^{flox}*/alb-Cre)*: Early growth retardation due to reduced secretion of hepatic insulin-like growth factor 1 (IGF1); decreased glucose transport and glycogen storage; intracellular retention of glucose transporter GLUT2 Impaired VLDL lipidation resulting in reduced plasma triglyceride levels in the fasted state	Hesse *et al.* (2012)
		Adipocyte specific (Arfrp1^{flox}*/ap2-Cre)*: Nearly abolished triglyceride storage in adipocytes, smaller lipid droplets, impaired lipid droplet fusion, and enhanced lipolysis Impaired sorting of the glucose transporter GLUT4 to intracellular storage compartment	Hommel *et al.* (2010); Hesse *et al.* (2010)
		Inducible adipocyte specific Tamoxifen-inducible Cre/loxP recombination Arfrp1^{flox}*/CreERT2)*: Impaired secretion of adiponectin and recycling of insulin receptor; decreased insulin signaling in adipose tissue and liver.	Rodiger *et al.* (2018)

Indicated are the ARF and ARL proteins deleted either as whole-body knockout (conventional knockout) or in a cell-type or tissue-specific manner, including inducible deletions (conditional knockout). E, embryonic day; ENU, *N*-ethyl-*N*-nitrosourea; HGF, hepatocyte growth factor; P, postnatal day; VLDL, very low density lipoprotein.

TABLE 4: Phenotypes of mice with mutations/deletions of ARF family GTPases. Continued

the release of isoprenylated cargoes (Van Valkenburgh *et al.*, 2001; Ismail *et al.*, 2011). While they both also bind HRG4, only ARL3 binding results in the release of N-myristoylated cargoes from this carrier (Ismail *et al.*, 2012). ARL2 is involved in tubulin heterodimer biogenesis, while ARL3 functions in cytokinesis (Zhou *et al.*, 2006). ARL3 also appears to act at the Golgi, though how such action may be integrated with its other roles remains unclear (Zhou *et al.*, 2006). ARL3 is essential in photoreceptor cells that employ an elaborate variation of cilia in outer segments (Panic *et al.*, 2003; Zhou *et al.*, 2006; Hanke-Gogokhia *et al.*, 2018) (Table 4). An unexpected finding is that ARL3 is activated by ARL13B (Gotthardt *et al.*, 2015; Ivanova *et al.*, 2017), which raises the possibility that other ARLs may also serve as ARL GEFs, particularly as so few ARL GEFs have been identified to date.

Primary cilia are a major site of action for four different ARLs: ARL3, 6, 13B, and 16 (Figure 1). Each of these GTPases plays a critical role(s) in ciliary biology, with details and mechanisms still under investigation. Their activity is particularly important during development (Zhang *et al.*, 2013), as mutations in these genes cause ciliopathies and developmental disorders in mammals (Chiang *et al.*, 2004; Caspary *et al.*, 2007; Wiens *et al.*, 2010; Liew *et al.*, 2014; Alkanderi *et al.*, 2018) (Table 4). Although best known for its role in cilia and in Hedgehog signaling, ARL13B also functions outside the cilium by interacting with a kinesin to facilitate axon guidance (Higginbotham *et al.*, 2012; Casalou *et al.*, 2014). ARL13B is also distinctive, as it is nearly twice the size of other GTPases in the ARF family, having a C-terminal domain as large as the GTPase domain. ARL6 also goes by the name BBS3, as its mutation is linked to Bardet-Biedl syndrome and defects in Wnt signaling (Chiang *et al.*, 2004; Wiens *et al.*, 2010; Zhang *et al.*, 2011). ARL6 acts in ciliary trafficking through recruitment of the BBSome complex (Jin and Nachury, 2009; Jin *et al.*, 2010; Ye *et al.*, 2018).

The theme of ARF GTPases sharing a similar function is evident in the recruitment of Golgins to the Golgi. As mentioned earlier, ARFs recruit Golgin-160 and GCC88 to the Golgi, while ARL1 recruits Golgin-97 and Golgin-245 (Derby *et al.*, 2004). This is just one of several examples in which ARLs and ARFs act in a common pathway. However, ARL4A, which performs a similar function by interacting with GCC185 at the TGN to modulate the integrity of the Golgi, also has a completely distinct function in endosome-to-Golgi transport (Lin *et al.*, 2011). ARL4A also plays a role in actin cytoskeleton rearrangement involving ELMO/DOCK180-induced RAC signaling (Patel *et al.*, 2011). ARL4C and ARL4D modulate actin remodeling and cell migration through their interacting partners, filamin-A and Cytohesin 2, respectively (Li *et al.*, 2007; Chiang *et al.*, 2017). Expression of ARL4C in normal epithelial cells promotes migration and proliferation, indicating a role in epithelial morphogenesis (Matsumoto *et al.*, 2014). Each ARL4 paralogue can recruit the ARF GEF Cytohesins to the PM (Hofmann *et al.*, 2007; Li *et al.*, 2007). ARL5 localizes to the Golgi and influences endosome–Golgi traffic through interactions with the GARP complex (Rosa-Ferreira *et al.*, 2015), while ARL8 also acts in vesicular trafficking, predominantly at lysosomes, where it can influence the motility of this organelle (Khatter *et al.*, 2015). With the exceptions of ARL2 and ARL3, which act in cytosol to affect the assembly and dynamics of microtubules and centrosomes, almost all ARFs and ARLs act at membranes. (Though nothing is currently known about the locations and functions of ARLs 9, 11, 14, or 15.)

Activated ARF-related protein 1 (ARFRP1) also localizes to the TGN and has been implicated in vesicular trafficking of vesicular stomatitis virus G protein (Shin *et al.*, 2005; Nishimoto-Morita *et al.*, 2009), glucose transporters (Hesse *et al.*, 2012), and other PM

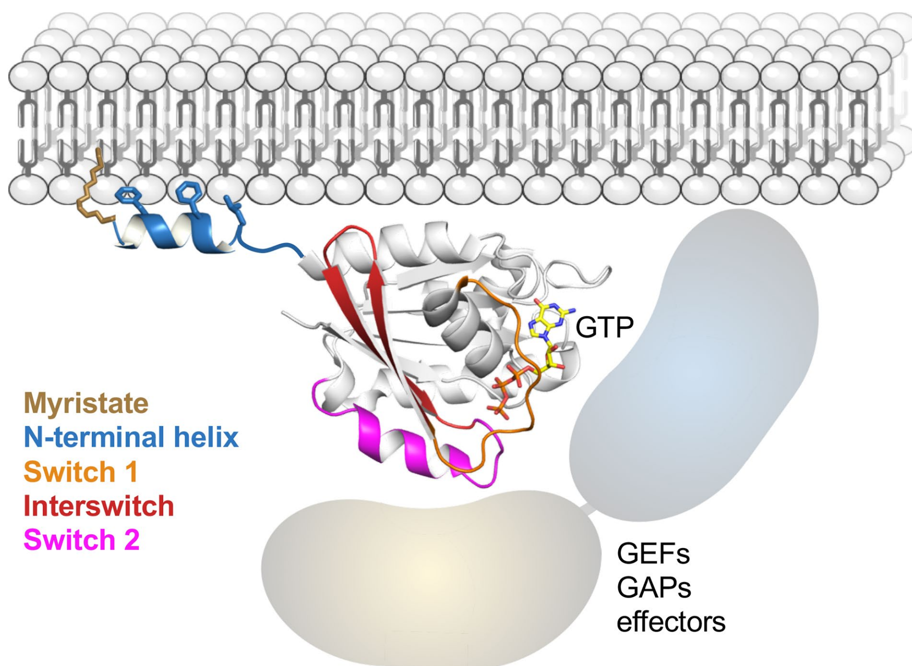

FIGURE 2: Structural determinants of ARF association with membranes and interactors. ARFs have four regions that change conformation between GDP- and GTP-bound forms: the canonical switch 1 (in orange) and switch 2 (in magenta) that directly sense the nature of the bound nucleotide; the myristoylated N-terminal helix (in blue), which is autoinhibitory in ARF-GDP and binds the membrane in ARF-GTP; and the interswitch (in red) that functions as a push button to ensure allosteric communication between the membrane- and the nucleotide-binding sites. GEFs, GAPs, and effectors generally bind to switch 1, switch 2, and/or the interswitch by one domain (in light yellow) and carry other domains that bind to the membrane (in light blue). The membrane bilayer is denoted in gray.

proteins. ARL1 and its effectors Golgin-97 and Golgin-245 are recruited to the TGN by ARFRP1 (Setty *et al.*, 2003; Zahn *et al.*, 2006). In addition to acting in membrane trafficking, ARFRP1 also plays roles in regulating metabolism, especially lipid and fat storage. Knockout of *Arfrp1* in different mouse tissues causes severe metabolic defects (Hommel *et al.*, 2010; Jaschke *et al.*, 2012; Rodiger *et al.*, 2018) (Table 4). Despite their similarities to ARFs and ARL1, neither GEFs nor GAPs for ARFRP1 have been identified, making descriptions of pathways challenging.

Two members of the ARF family are much larger than the 20- to 25-kDa norm, ARL13B with 428 residues and Trim23/ARD1 with 574 residues (Supplemental Table I). Homologues of the C-terminal domain of ARL13B are not found in other proteins, and we have little information on its function, save the presence of a VXPX ciliary localization motif. Trim23 is a multidomain protein having a RING finger, two B-boxes, and a coiled-coil motif (thus a tripartite motif [TRIM]) (Vichi *et al.*, 2005). These domains have polyubiquitination activity and act in the antiviral defense system and adipocyte differentiation (Arimoto *et al.*, 2010; Watanabe *et al.*, 2015; Sparrer *et al.*, 2017).

The functions and mechanisms of SARs are well characterized. SAR1 is activated by the Sec12 GEF, leading to its recruitment to the site of protein export from the ER, ERES (ER exit sites) (Figure 1). Activated SAR1 recruits the Sec23/24 complex and later the Sec13/31 complex to form the COPII coat necessary to generate COPII vesicles. The Sec13/31 complex promotes the GAP activity of Sec23 to "inactivate" SAR that serves to recycle all components. Sec12 and Sec23 are an atypical GEF and GAP pair as they lack a canonical GEF or GAP domain, and thus this regulatory system is of limited use in modeling mechanisms of the ARF/ARL GEFs and GAPs. Consequently, SARs are omitted from further discussion in this review.

Structural insight into the actions of ARF GTPases

While all ARF family members share the canonical G domain with nucleotide-sensitive switch 1 and 2 loops (Amor *et al.*, 1994), they display structural signatures that strikingly distinguish them from other small GTPase families (Figure 2) (Pasqualato *et al.*, 2002). The hallmark of members of the ARF family is an allosteric structural feature, which allows their nucleotide-binding site to communicate with regions located on the other side of the GTPase. It is based on an interswitch region (as it connects switch 1 and switch 2), that toggles like a push button between the inactive and the active conformations (Yu *et al.*, 2012). The ability of the interswitch to toggle is encoded in a conserved sequence signature at the beginning of the switch 2 (Pasqualato *et al.*, 2002). In toggling between these two positions, the interswitch simultaneously modifies the conformation of both the nucleotide-binding site and the other side of the protein. In ARF and related GTPases, the rearrangement of the interswitch is coupled to a variable N-terminal extension, which is autoinhibitory in the GDP-bound form and swings out to facilitate activation. In ARF GTPases, this region is a myristoylated amphipathic helix that interacts with membranes through the myristoyl group and the neighboring residues (Antonny *et al.*, 1997; Liu *et al.*, 2009, 2010). This is a prerequisite for their activation by GTP, thus coupling the activation of the GTPase to its interaction with the membrane bilayer (see also the *ARF GEFs* section).

Because of these major differences compared with classical RAS-like GTPases, caution is needed when using mutants and fusions to manipulate the activation state of ARF GTPases. The glutamine at the beginning of switch 2 is generally critical for GAP-stimulated GTP hydrolysis (Cherfils and Zeghouf, 2013), and this is also the case for ARF GTPases (e.g., Q71 in ARF1 or ARL1, Q70 in ARL2) (Zhang *et al.*, 1994; Van Valkenburgh *et al.*, 2001). However, another classical mutation, a P-loop serine/threonine to asparagine substitution, which gives rise to a dominant-negative version in many small GTPases by reducing their affinity for guanine nucleotide and titrating their GEFs, may not function the same in all ARF GTPases (Macia *et al.*, 2004). Alternatively, mutation of another threonine, located in switch 1, trapped ARF6 in a GDP-bound form, a mutation that could in principle also function in related ARF GTPases, many of which share the same structural feature. Another important aspect is that, given the regulatory role of the N-terminus and the need for lipid modifications, ARFs should be tagged only at the C-terminus, and even then only with caution (Jian *et al.*, 2010).

ARFs function by binding effectors, and structural studies of many ARF/ARL–effector complexes show that ARFs have similar conformations in all complexes and bind most effectors in the

same area centered on an invariant triad of aromatic residues in the switch/interswitch regions (Khan and Menetrey, 2013). In contrast, the *effectors* bind ARF-GTP through binding sites that are distinct in primary, secondary, ternary, and quaternary structures (Cherfils, 2014). Thus, the solved structures of known ARF–effector complexes do not inform on structural determinants that could be used to predict the binding of other effectors to ARF/ARLs. Interestingly, at least one ARF effector (i.e., coatamer) enhances the GTP hydrolytic activity of an ARF GAP, suggesting the formation of a ternary ARF-effector-GAP complex. Supporting evidence for formation of such a ternary complex is provided by a composite structural model for the ARF1/coatamer/ArfGAP1 complex (Yu *et al.*, 2012) and by the recent cryoelectron microscopy study of ARF1/COP1/ArfGAP complex reconstituted on a lipid vesicle (Dodonova *et al.*, 2017). A common feature of ARF–effector interactions is that they are predicted to position effectors in precise orientations in apposition to the membrane (DiNitto *et al.*, 2007; Liu *et al.*, 2009, 2010; Cherfils, 2014). This "solid phasing" will impart orientation constraints for effector interactions that are important to produce signals. As a consequence, biochemical assays used to determine affinities for and activities of effectors should incorporate membranes and are subject to changes in response to different lipid components of those membranes.

Regulating ARFs

ARFs are N-myristoylated (a cotranslational covalent modification that is not reversible), but this modification, while critical to activity, is unlikely to be regulatory. Other posttranslational modifications (e.g., phosphorylation, ubiquitination) occur on the GTPases, GEFs, and GAPs, but have been largely underexplored. The details of ARF activation and deactivation are discussed in the *ARF GEFs* and *ARF GAPs* sections.

ARF GEFS

Families of ARF GEFs and their cellular functions

ARFs require GEFs to accelerate nucleotide exchange. This is likely true of the ARLs as well, though relatively weak affinity for guanine nucleotides by ARL2, ARL13B, and perhaps others suggests the possibility of other means of regulating their activation process. As no GEFs for ARLs have been identified, except that of ARL13B for ARL3 (Gotthardt *et al.*, 2015), this section is limited to GEFs that act on mammalian ARFs. The human genome encodes 15 ARF GEFs divided into six families based on sequence relatedness, domain organization, and phylogenetic analyses: GBF, BIGs, Cytohesins, EFA6/Psd, BRAG/IQSec, and FBX (Table 2, Figure 3A, and Supplemental Table II). All ARF GEFs share a common catalytic Sec7 domain (Sec7d) and a mechanism of action, but display diversity in their actions and regulation in cells (Peyroche *et al.*, 1996; Cherfils and Chardin, 1999; Jackson and Casanova, 2000; Casanova, 2007). The locations of ARF GEFs in cells parallel those of ARF1-6 at the Golgi, endosomes, and the PM (Figure 1). In this section, we briefly describe the actions of human ARF GEFs.

GBF1 and BIG1/2 are key regulators of membrane traffic within the secretory and endosomal pathways (Wright *et al.*, 2014). GBF1 preferentially localizes to the Er-Golgi intermediate compartment (ERGIC) and the *cis*-Golgi, where it mediates ARF activation required for COPI vesicle formation. GBF1 likely activates ARF1 and ARF4, as only the simultaneous removal of these, but not any other pair of ARFs, inhibits COPI traffic (Zhao *et al.*, 2002, 2006; Volpicelli-Daley *et al.*, 2005; Szul *et al.*, 2007; Manolea *et al.*, 2008). GBF1 also acts

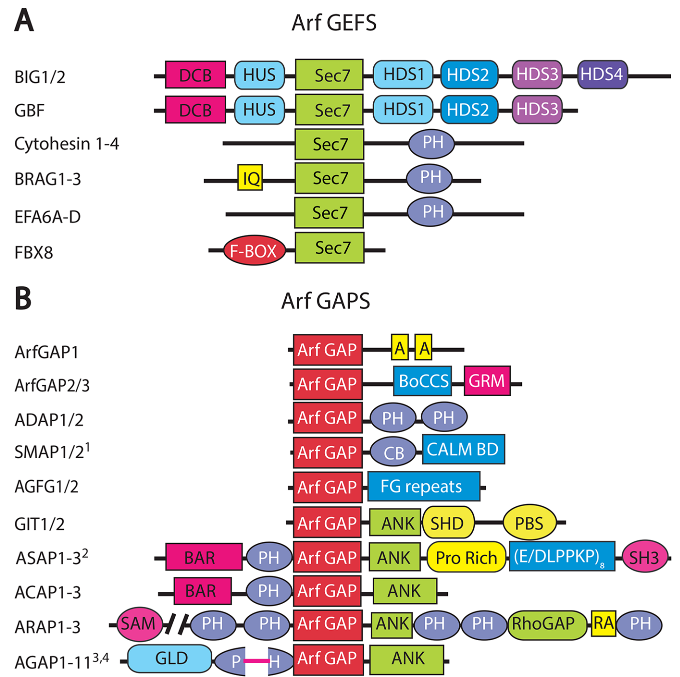

FIGURE 3: Domain organization of ARF GEFs and ARF GAPs. A schematic of the domains present in each subfamily of the ARF GEFs (A) and ARF GAPs (B). The defining ARF GEF/Sec7 domain and the ARF GAP domain are aligned. Protein lengths are not drawn to scale. Abbreviations (in alphabetical order): A, ARF GAP lipid-packing sensor (ALPS); ANK, ankyrin repeat; BAR, Bin/Amphiphysin/Rvs; BoCCS, binder of coatomer, cargo, and SNARE; CALM BD, calm binding domain; CB, clathrin box; DCB, dimerization and cyclophilin binding; E/DLPPKP$_8$, 8 repeats of this primary sequence (single letter code); F-BOX, cyclin F protein interaction motif; FG repeats, multiple copies of XXFG repeated; GLD, GTP binding protein–like domain; GRM, Glo3 regulatory motif; HDS(1-4), homology downstream of Sec7; HUS, homology upstream of Sec7; IQ, isoleucine/glutamine calmodulin-binding motif; PBS, Paxillin binding site; PH, pleckstrin homology; Pro-rich, proline rich; RA, Ras association; Rho GAP, Rho GTPase-activating protein; SAM, sterile α motif; SHD, Spa homology domain.

at the TGN to support the recruitment of BIG1 and BIG2 through activating ARF4 and ARF5 (Lowery *et al.*, 2013). GBF1 facilitates lipid droplet formation (Ellong *et al.*, 2011; Takashima *et al.*, 2011; Bouvet *et al.*, 2013), is detected at PM sites involved in active migration and chemotaxis (Mazaki *et al.*, 2012; Busby *et al.*, 2017), and in some cells facilitates traffic through the glycophosphatidyl-inositol–enriched endosomal compartments (GEECs) pathway (Gupta *et al.*, 2009), but the ARFs activated for these functions are unknown. BIG1 and BIG2 localize to the TGN and endosomes, where they mediate ARF activation required for endosome–PM recycling, TGN–PM recycling, TGN–late endosome transport, and in some cells TGN–granule transport (Shinotsuka *et al.*, 2002a; Zhao *et al.*, 2002). BIG1 and BIG2 facilitate the recruitment of the clathrin adaptors AP1 and AP3 through activation of ARF1 and ARF3 (Pacheco-Rodriguez *et al.*, 2002; Shinotsuka *et al.*, 2002a,b; Ishizaki *et al.*, 2008). BIG1 seems to play additional nontrafficking roles, as it is detected in the nucleus of serum-starved cells (Padilla *et al.*, 2004, 2008).

The Cytohesins localize to and regulate endosomal trafficking (Figure 1), including the stimulated recycling of the glucose transporter GLUT4, integrins, and other proteins (Caumont *et al.*, 2000; Oh and Santy, 2010, 2012; Li *et al.*, 2012; Salem *et al.*, 2015).

Cytohesins can be recruited to the PM in response to insulin, epidermal growth factor (EGF), or nerve growth factor (NGF) (Venkateswarlu *et al.*, 1998a,b), where they are required for signaling by these hormones (Li *et al.*, 2003; Fuss *et al.*, 2006; Hafner *et al.*, 2006; Lim *et al.*, 2010; Attar and Santy, 2013; Hahn *et al.*, 2013; Pan *et al.*, 2013; Reviriego-Mendoza and Santy, 2015). Cytohesins also stimulate Rac activation and actin polymerization at the cell periphery (Frank *et al.*, 1998; Santy and Casanova, 2001; Santy *et al.*, 2005; Li *et al.*, 2007; White *et al.*, 2010; Reviriego-Mendoza and Santy, 2015), resulting in increased cell migration (Santy and Casanova, 2001; Santy *et al.*, 2005; Torii *et al.*, 2010; Attar and Santy, 2013). Cytohesins perform these functions by activating ARF1 and/or 6. However, all ARF isoforms are efficiently activated by Cytohesins in vitro, raising the question of how specific ARF isoforms are selected in vivo.

The BRAGs (Brefeldin A–resistant ARF GEF, later renamed IQSecs) contain a calmodulin-binding IQ motif in the N-terminal third of each protein (Figure 3A). IQSec1 and 3 are highly enriched in the central nervous system, while IQSec2 is ubiquitous. IQSecs localize to endosomes and the PM in nonneuronal cells, and to postsynaptic densities in neurons (reviewed in D'Souza and Casanova, 2016) (Figure 1), In general, IQSecs appear to control the internalization of adhesive and/or signaling molecules. Examples include the adhesion proteins dumbfounded (Duf), roughest (rst), and N-cadherin in myoblasts (Bach *et al.*, 2010); the semaphorin, Sema3E, and its cognate receptor plexin D1 in endothelial cells (Sakurai *et al.*, 2011); synaptic AMPA receptors in neuronal pathfinding (Charych *et al.*, 2004; Scholz *et al.*, 2010; Elagabani *et al.*, 2016); and β1 integrins in metastasizing breast cancer cells (Moravec *et al.*, 2012). IQSecs perform these functions through the activation of ARF5 and/or ARF6. Like Cytohesins, IQSecs efficiently activate all ARFs in vitro (Peurois *et al.*, 2017), and the mechanisms that determine their selectivity in cells remain elusive. IQSec1 also acts in the nucleus (Dunphy *et al.*, 2007), raising the question of how its various functions are integrated and regulated.

The EFA6 (exchange factor for ARF6)/PSD (pleckstrin homology and Sec7 domain) proteins activate ARF6 and regulate actin cytoskeleton dynamics at the cell surface (Franco *et al.*, 1999; Macia *et al.*, 2001) (Figure 1). They appear to support distinct functions in specific cells and during development, as suggested by their varied tissue distribution (all EFA6 proteins except EFA6B are abundant in brain but are differentially distributed within different brain regions, with EFA6C showing the most selective localization to only Purkinje cells and the choroid plexus; Matsuya *et al.*, 2005) and changing expression levels during development (Sakagami *et al.*, 2006). EFA6 has been implicated in dendritic branching and spine formation (Inaba *et al.*, 2004) and might regulate endocytosis of neurotransmitter receptors (Decressac *et al.*, 2004). It also has been implicated in clathrin-mediated endocytosis through a regulatory interaction with endophilin (Boulakirba *et al.*, 2014).

FBX8 is the sole representative of the last GEF subfamily and is the least understood (Table 2). The role of the N-terminal F-box domain is unclear (Figure 3B). Paradoxically, though serving as a GEF for ARF6 at the cell surface, it also exhibits a suppressive effect on ARF6 activity, perhaps through a poorly described effect on mono-ubiquitination of ARF6 (Yano *et al.*, 2008). Interestingly, FBX8 shows no GEF activity in vitro, raising uncertainty as to whether it is a bona fide GEF.

Structural insight into the mechanisms of ARF activation by GEFs

All ARF GEFs use the same mechanism to promote nucleotide exchange through the highly conserved, catalytic ~200-residue Sec7 domain (Sec7d), so named based on homology to the domain in the *Saccharomyces cerevisiae* Sec7 protein (Peyroche *et al.*, 1996). To be activated by a Sec7d, ARF-GDP must be primed by membranes that displace the autoinhibitory N-terminal helix, thus allowing the GEF to promote the toggle of the interswitch and secure ARF-GDP on the membrane before GDP dissociation (Renault *et al.*, 2003). Next, the Sec7d inserts an invariant glutamate (also called the "glutamic finger"; Beraud-Dufour *et al.*, 1998; Renault *et al.*, 2003) into the active site, which competes with the phosphates of GDP to promote its dissociation and the formation of a nucleotide-free complex that can bind GTP (Goldberg, 1998). Charge-reversal mutation of the glutamic finger renders a Sec7d catalytically inactive (Beraud-Dufour *et al.*, 1998). Thus, in a manner that is unique to ARF GEFs, stimulation of GDP/GTP exchange has an absolute requirement for a membrane, which can be likened to a cofactor. Interestingly, the Sec7d of GBF and BIG is generally, although not always, the target of the fungal toxin Brefeldin A, which traps an abortive ARF–GDP–BFA–Sec7 complex (Peyroche *et al.*, 1999; Mossessova *et al.*, 2003; Renault *et al.*, 2003).

Regulating GEFs

ARF signaling is tightly regulated in cells, implying that the activating GEFs are catalytically active only at specific times and places. GEFs are regulated by multiple molecular mechanisms that impact their membrane association and/or catalytic activity. All inactive ARF GEFs are cytosolic, but activate ARF only on membranes, suggesting that membrane recruitment represents a regulatory step. Recruitment strategies differ among the GEFs, albeit some commonalities are emerging. GBF1 and BIG1/2 share a common domain architecture composed of domains located upstream and downstream of the Sec7d, coined HUS and HDS domains, respectively (Mouratou *et al.*, 2005). These proteins are recruited through an interaction of their N-terminal regions (up to the Sec7d) with a small GTPase: Rab1b for GBF1 (Alvarez *et al.*, 2003; Monetta *et al.*, 2007) and ARL1 (Christis and Munro, 2012; McDonold and Fromme, 2014) and ARF4/5 (Lowery *et al.*, 2013) for BIG1 and BIG2. Such a system is reminiscent of the "cascade" of Rab GTPases working at several stages of membrane trafficking (Jones *et al.*, 1999; Stalder and Antonny, 2013). The C-terminal regions of GBF1 and BIG1/2 are also important, as intact HDS domains are required for their membrane association (McDonold and Fromme, 2014; Chen *et al.*, 2017; Gustafson and Fromme, 2017; Meissner *et al.*, 2018; Pocognoni *et al.*, 2018). It is likely that multiple domains position these GEFs on the membrane, but how such interactions are ordered and whether or not they display cooperativity is unknown. The catalytic activity of GBF1, BIG1, and BIG2 appears to be regulated through allosteric mechanisms. The activity of the yeast Sec7p (orthologue of BIG1/2) is stimulated through conformational changes induced by binding of Ypt (yeast Rab orthologues) GTPases to its N- and C-terminal domains (McDonold and Fromme, 2014). In addition, binding of ARF-GTP also stimulates activity in a forward loop where the generated product further activates Sec7d. There are three allosteric binding sites on Sec7p, two for Ypts and one for activated ARF, leaving open the catalytic GEF site for binding ARF-GDP. Such a regulatory/stimulatory effect may ensure a concentrated burst of activated ARFs to locally recruit a plethora of effectors. However, mammalian BIG1/2 and GBF1 do not show an analogous regulatory mechanism. Instead, the catalytic activity of GBF1 may be stimulated by a HDS1-phosphoinositide (PIP) interaction (Meissner *et al.*, 2018), analogous to the PH domain regulating the catalytic activity of BRAG/IQSecs.

Cytohesins, BRAG/IQSecs, and EFA6/PSDs contain a PH domain downstream of their Sec7 domain (Figure 3A) that facilitates membrane recruitment by interacting with PIPs and other anionic

phospholipids and, in some cases, the active forms of ARF/ARL GTPases. The binding properties and structural modalities, however, diverge between the families. The PH domains of Cytohesins play multiple roles, including specific recognition of PIP_2 and PIP_3 by the canonical lipid-binding site (DiNitto *et al.*, 2003; Cronin *et al.*, 2004), autoinhibition of the Sec7 active site (DiNitto *et al.*, 2007), and implementation of a positive-feedback loop by binding to ARF-GTP or ARL4-GTP (Cohen *et al.*, 2007; Hofmann *et al.*, 2007; Malaby *et al.*, 2013; Stalder and Antonny, 2013). An important determinant is the polybasic region located immediately downstream of the PH domain, which contributes both to autoinhibition and recruitment to the membrane. Other layers of regulation have been described. One of these is an autoinhibitory interaction mediated by the N-terminal coiled coil, a domain involved in cytohesin dimerization. Autoinhibition is relieved by AKT-dependent phosphorylation of a threonine residue in the PH domain, which allows the recruitment of Cytohesins to membranes (Li *et al.*, 2012; Hiester and Santy, 2013). Phosphorylation of protein kinase C (PKC) sites in the polybasic regions of Cyth1 and Cyth2/ARNO also stimulates their GEF activity, presumably by destabilizing the autoinhibited state (DiNitto *et al.*, 2007). Grp1 lacks these PKC sites but can be phosphorylated by AKT on a serine near the catalytic site in the Sec7d and a threonine in the $\beta1/\beta2$ loop in the PH domain, thereby influencing GEF activity and PIP affinity/specificity, respectively (Li *et al.*, 2012).

The PH domains of BRAG/IQSecs differ from those in Cytohesins in that they do not autoinhibit the Sec7 domain (Jian *et al.*, 2012; Aizel *et al.*, 2013) and they bind several anionic lipids instead of recognizing a single phosphoinositide with high specificity (Karandur *et al.*, 2017). PIP_2 binding increases their catalytic activity, likely by positioning the GEF in an optimal membrane-based orientation with respect to the ARF GTPase (Karandur *et al.*, 2017). In contrast to Cytohesins, ARF-GTP has no effect on BRAG/IQSec activity. In addition, BRAG/IQSecs are unique among the GEFs in their sensitivity to calcium due to the noncanonical IQ motif in the N-terminus (Figure 3A), which fits the consensus for binding to calcium-free calmodulin. BRAG1/IQSec2 binds to Ca^{2+}-free calmodulin in vitro, and addition of Ca^{2+} causes its dissociation from membranes (Aizel *et al.*, 2013; Roy *et al.*, 2016). Whether this dissociation is due to a calmodulin-based regulation, to competition of Ca^{2+} with phospholipids for binding to the PH domain, or both acting in synergy remains to be established but raises the question of possible cross-talk between Ca^{2+} and ARF signaling.

EFA6/PSD is also recruited to anionic membranes by its PH domain and a polybasic element in its C-terminus, but its activity is inhibited by ARF-GTP, indicating negative-feedback regulation (Padovani *et al.*, 2014). Its GEF activity is enhanced by direct interaction with endophilin in clathrin-mediated endocytosis (Boulakirba *et al.*, 2014).

ARF GAPS

Families of ARF GAPs and their cellular functions

ARF GAPs are defined by the presence of the conserved, catalytic GAP domain (Figure 3B), first identified in ArfGAP1, which binds to ARF-GTP to promote hydrolysis of GTP to GDP. The human genome encodes at least 28 proteins containing an ARF GAP domain or having GAP activity (Gillingham and Munro, 2007; Kahn *et al.*, 2008; Donaldson and Jackson, 2011) (Table 3; additional information included in Supplemental Table III). There are eight additional ARF GAP genes on chromosome 10, but it is not known whether these are expressed. ARF GAPs are divided into 10 subtypes based on sequence similarity and shared domain structure (Figure 3B) (Randazzo and Hirsch, 2004; Inoue and Randazzo, 2007; Spang *et al.*, 2010). Each GAP subtype, and even members within a particular subtype, display distinct localizations (Figure 1) and functions, and those can be either ARF dependent or ARF independent (Gillingham and Munro, 2007; Spang *et al.*, 2010; Donaldson and Jackson, 2011; Vitali *et al.*, 2017). An exception is the ELMOD family proteins that lack the ARF GAP domain, yet have in vitro activity against a wide range of ARF family GTPases, including both ARFs and several ARLs (Bowzard *et al.*, 2007; East *et al.*, 2012; Ivanova *et al.*, 2014). The three mammalian proteins, ELMOD1-3, share an ELMO domain and an apparent catalytic arginine (East *et al.*, 2012). Their cellular locations are shown in Figure 1, but are not discussed further.

With the well-established role of ARFs in membrane trafficking, most studies of ARF GAPs focused in this area, and specifically in coat/adaptor recruitment to membranes, predominantly at the Golgi and PM (Gillingham and Munro, 2007; Spang *et al.*, 2010; Donaldson and Jackson, 2011; Shiba and Randazzo, 2012; Vitali *et al.*, 2017). At least six subtypes of GAPs are involved in the recruitment of ARF-dependent adaptors, including the COPI coatomer, GGAs, and clathrin and its adaptor AP-3. Because GAPs can inactivate ARFs, the early models posited that GAPs function exclusively as terminators of ARF signaling (Weimer *et al.*, 2008). However, the role of GAPs is far more complex. Compelling evidence for function of GAPs in supporting ARF activities, rather than solely acting as signal terminators, initially came from a screen for high-copy suppressors of ARF insufficiency in yeast that showed that all ARF GAPs in that organism could compensate for the ARF deficiency (Zhang *et al.*, 1998). The idea of GAPs being involved in propagation of an ARF signal was further supported by the finding that a number of GAPs drive coat assembly and cargo selection during the formation of transport vesicles (Yang *et al.*, 2002; Lee *et al.*, 2005; Spang *et al.*, 2010; Bai *et al.*, 2011; Shiba *et al.*, 2011). These observations suggest that GAPs can serve as ARF effectors, or that ARF activity requires multiple rounds of inactivation/activation cycles that require GAPs, or both.

ARF GAPs also regulate the actin cytoskeleton and associated adhesive structures (Hoefen and Berk, 2006; Randazzo *et al.*, 2007; Ha *et al.*, 2008; Casalou *et al.*, 2016; Zhou *et al.*, 2016; Luo *et al.*, 2017; Vitali *et al.*, 2017); for example, at least seven GAPs (GIT1, GIT2, ASAP1-3, ARAP2, and AGAP2) associate with focal adhesions (FAs) and function therein (Figure 1; listed under PM in this figure to save space). GAP effects are mediated in part by regulating traffic of FA components to the nascent structures and through effects on RHO GTPase signaling, including acting as scaffolds for components in the Rho family GTPase pathways (Zhao *et al.*, 2000; Lamorte *et al.*, 2003; Yin *et al.*, 2005; Frank *et al.*, 2006) and directly binding to and altering the functions of actin, non-muscle myosin 2 (Chen *et al.*, 2016), and Kif2A (Luo *et al.*, 2016). Some effects on the cytoskeleton can be propagated by GAP mutants lacking catalytic activity but able to bind ARF-GTP (Randazzo *et al.*, 2000), again supporting the role of GAPs as effectors rather than simply signal terminators. Some GAPs (e.g., ARAPs) contain both ARF GAP and RHO GAP domains, with functions that can be attributed to either activity (Miura *et al.*, 2002; Krugmann *et al.*, 2002; Stacey *et al.*, 2004; Nishiya *et al.*, 2005; Yoon *et al.*, 2006, 2008; Gambardella *et al.*, 2011, 2012; Chen *et al.*, 2013, 2014; Luo *et al.*, 2018).

ARF GAPs also affect the activities of protein kinases (e.g., AGAP2 binds and activates AKT; Liu *et al.*, 2007; while ARAP2 reduces AKT phosphorylation, and thereby its activity, by an unknown mechanism; Luo *et al.*, 2018). Thus, a single ARF GAP can affect multiple signaling pathways, and multiple ARF GAPs may impinge

on a single pathway. Unfortunately, our knowledge of the many functions in signaling and integration of multiple signaling pathways to elicit distinct phenotypic responses is still fragmentary.

Structural insight into the mechanisms of ARF GAPs

Soon after the discovery of the first ARF GAP (Cukierman *et al.*, 1995), the role of the catalytic arginine (aka an "arginine finger"; Ahmadian *et al.*, 1997; Scheffzek *et al.*, 1998; Cherfils and Zeghouf, 2013) in the hydrolysis of the β-γ phosphate bond by the ARF was established (Randazzo *et al.*, 2000). The use of a highly conserved, catalytic arginine in GAP-stimulated GTP hydrolysis is also present in RHO GAPs (Barrett *et al.*, 1997; Amin *et al.*, 2016). Similar to many GAPs, the ARF GAP domain inserts the arginine finger into the nucleotide-binding site to stabilize the transition state of the reaction, and this requires the conserved glutamine in the switch 2 region (Ismail *et al.*, 2010; Cherfils and Zeghouf, 2013). Loss of GAP activity upon mutation of the arginine finger is fully consistent with its catalytic function. The arginine finger mechanism appears to be shared by both the ARF GAPs and ELMOD1-3, despite their disparate structures (East *et al.*, 2012). Calcium stimulates the in vitro GAP activity of ASAP but not of other GAPs, while also competing with its association to the membrane, again raising the intriguing issue of cross-talk between Ca^{2+} and ARF signaling (Ismail *et al.*, 2010).

Regulating ARF GAPs

Membranes play a central role in regulating ArfGAPs, by restricting both their activities to specific subcellular locations and allosteric control of their GAP activity. Recruitment to membranes and allosteric activation of GAPs is commonly conferred by their PH domains, which are N-terminal to the GAP domains; this is true for the ASAP, ACAP, ARAP, and AGAP subfamilies (Kam *et al.*, 2000; Nie *et al.*, 2002; Campa *et al.*, 2009; Jian *et al.*, 2015) (Figure 3B). Other domains also contribute to regulating GAP activity. The curvature-sensing BAR domain of ASAP1 positions an autoinhibitory motif to contact the PH and GAP domains, thus inhibiting GAP activity (Jian *et al.*, 2009), while a BAR domain binding partner, NM2A, stimulates ASAP1 activity, perhaps by relieving the autoinhibition (Chen *et al.*, 2016). In a landmark study of ArfGAP1, recognition of membrane curvature by an ALPS motif, a peptide that folds as a helix to bind curved membranes, was shown to stimulate its GAP activity (Bigay *et al.*, 2005). The catalytic activity of ArfGAP1 and ArfGAP2 also can be allosterically regulated by coatomer and cargo binding (Goldberg, 2000; Luo and Randazzo, 2008; Luo *et al.*, 2009). In another example, nonmuscle myosin 2A stimulates ASAP1 activity, perhaps by relieving autoinhibition (Chen *et al.*, 2016).

For several ARF GAPs that function in FAs, including GITs, ASAP1, and AGAP2, targeting is achieved by binding to specific FA components (Turner *et al.*, 2001; Randazzo *et al.*, 2007; Vitali *et al.*, 2017). ARF GAPs that regulate the Golgi and endocytic compartments also are targeted by binding to vesicle coat proteins, including SMAPs binding through clathrin boxes to clathrin heavy chain and ArfGAP1 binding the δ-COP component of COPI coatomer (Tanabe *et al.*, 2005; Natsume *et al.*, 2006; Weimer *et al.*, 2008; Spang *et al.*, 2010; Suckling *et al.*, 2015). These studies highlight the general principle, in which membrane and protein features that define the environmental conditions are coupled to the regulation of the GAP activity. They also show that specific mechanisms are remarkably diverse, likely to allow diverse ARF functions, and the need to unravel these mechanisms to allow a clear understanding of ARF GAP functions in cells.

EVOLUTIONARY PARALLELS BETWEEN ARF GTPASES AND THEIR GEFS AND GAPS

The complexity of human ARF GTPases and their GEFs and GAPs presents a major challenge in defining their functionalities. An evolutionary approach can help by categorizing the proteins based on their evolutionary history and presence or absence in different organisms with diverging cell biologies (Figure 4A). It can also help to connect the human complement to that of other model (and nonmodel) organisms by detailing how the diversity of the human proteins arose. Functional diversity can most easily be conceptualized as arising at three levels: 1) vertebrate-specific machinery that arose in the lineage giving rise to animals, 2) machinery present in the common ancestor of all eukaryotes, and 3) machinery present in the archaeal contributor to the origin of eukaryotes. The human complement of proteins in these three families includes a large number of subfamilies, shared among vertebrates, for example, humans, mice, rats, and fish. These are largely explained by the series of whole-genome duplications that took place at the dawn of vertebrates and gave rise to ARFs 1-5 (Manolea *et al.*, 2010), 2-3 paralogues in all ARF GAPs (except for ArfGAP1; Schlacht *et al.*, 2013), and 2-4 paralogues in almost all ARF GEFs (Figure 4B and Tables 2 and 3). The human complement also partly reflects expansion of the families in the lineage that gave rise to animals, whether at the ancestor of all animals (i.e., holozoan) or of animals plus fungi (i.e., opisthokont). Examples include the duplication that gave rise to class I versus class II ARFs in holozoans (Manolea *et al.*, 2010), the emergence of the GAP ASAP (Schlacht *et al.*, 2013), or that of the GEF EFA6, each of which arose in the opisthokonts (S. V. Pipaliya, A. Schlacht, C. M. Klinger, R. A. Kahn, and J. Dacks, personal communication). These are quite ancient (arising around a billion years ago; Eme *et al.*, 2014), but still reflect ARF and regulatory machinery that is restricted to a relatively limited subset of eukaryotes, later expanded in vertebrates. We share these proteins with basal animal lineages and fungi, meaning that molecular cell biological data from model organisms (e.g., *Drosophila melanogaster*, *Caenorhabditis elegans*, and *S. cerevisiae*) can meaningfully be applied to understand these proteins in human cells. However, there are no orthologues of these proteins in other eukaryotes, including plants, which likely reflects important functional differences.

To understand ARF signaling and regulatory biology common to all eukaryotic organisms, we need to look for proteins that arose before the common ancestor of all eukaryotic life (around 2 billion years ago; Eme *et al.*, 2014) and contributed common machinery in its descendent lineages. We know that this last eukaryotic common ancestor (LECA) was sophisticated, possessing a complement of membrane trafficking machinery that rivals that found in many eukaryotes today. LECA contained 16 ancient ARF GTPases, two of which were true ARFs (R. Petrželková and M. Eliáš, personal communication). It also had six ARF GAPs (SMAP, AGFG, ArfGAP1, ArfGAP2, ACAP, ArfGAP_C2; Schlacht *et al.*, 2013) (Figure 4A). This last protein is absent from human and yeast, but present in other eukaryotic lineages like plants and plant pathogens such as Phytophthora. The existence of ArfGAP_C2 highlights the fundamental eukaryotic cell biology left to be discovered, especially that not present in mammals. The LECA also possessed at least two ELMOD GAPs, which work on both ARFs and ARLs (East *et al.*, 2012) (Figure 4A). GBF1 and BIG were also present in the LECA (Bui *et al.*, 2009), as was Cytohesin (S. V. Pipaliya *et al.*, personal communication). Clearly, the complexity of ARF signaling had already been well developed at the dawn of eukaryotes. Recently, it has been possible to dig even deeper into the origins of ARFs with the discovery of

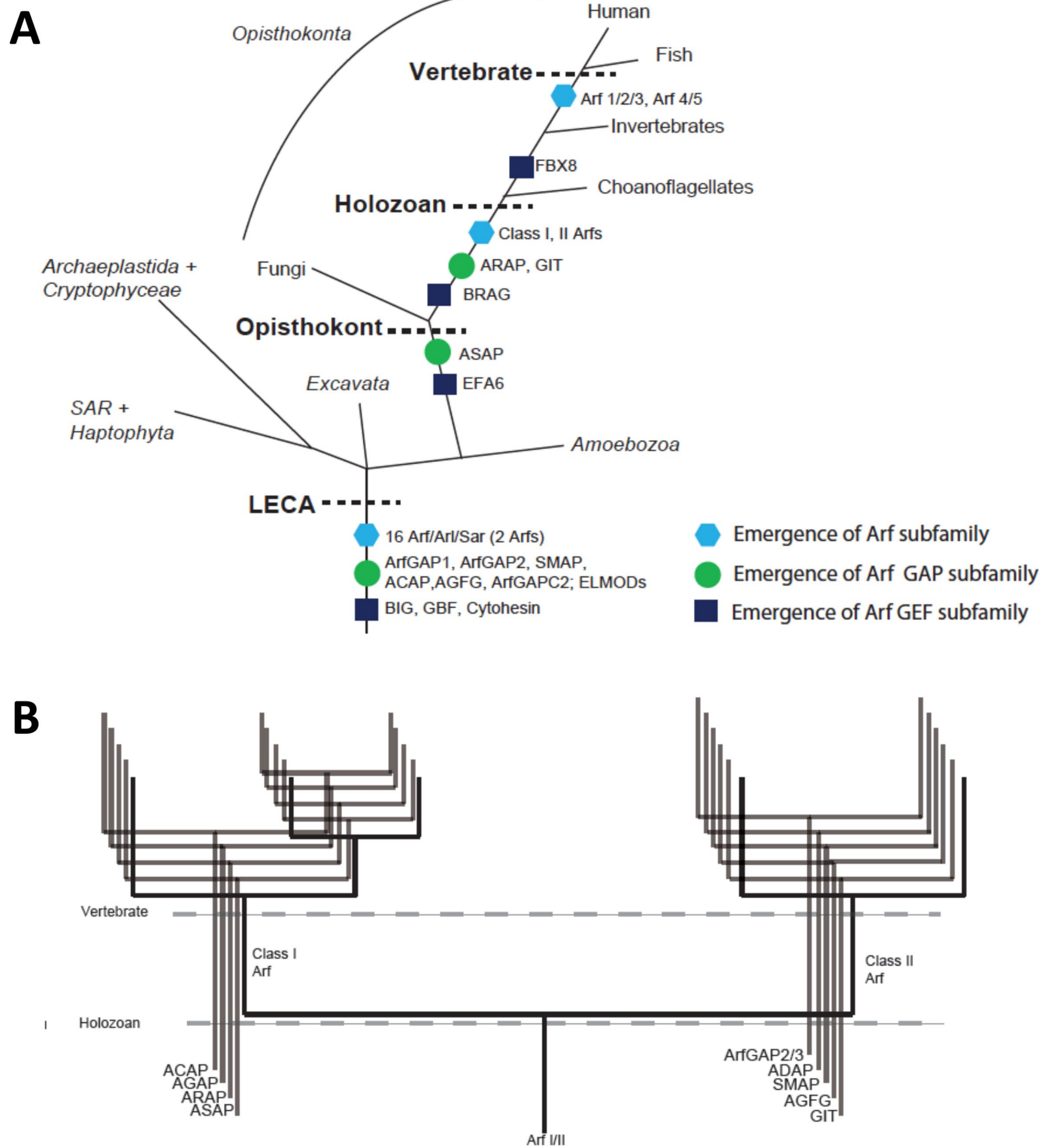

FIGURE 4: Evolution of the ARF family and its regulators. (A) The timing of the emergence of the relevant protein subfamilies is shown mapped on a simplified tree of eukaryotes. The polygons, circles, and squares denote the latest point by which the ARF GTPases, GAPs, and GEFs must have evolved, respectively, with the names of the subfamilies given to the right. The names of the eukaryotic supergroups are in italics, while the relevant "reconstructed ancestor" discussed in the text are in bold and noted by a dashed line. (B) Overlay of ARF1-5 evolution with that of the nine ARF GAP subfamilies that possess multiple paralogues. ARF evolution is depicted in black and Arf GAP in gray, with duplications at the base of Holozoa and Vertebrata. Relevant evolutionary transitions are illustrated by dashed lines.

the Asgard archaea, metagenomic assemblies of what appear to be the closest living descendants of the archaeal lineage that contributed to the birth of eukaryotes (Spang *et al.*, 2015; Zaremba-Niedzwiedzka *et al.*, 2017). Within these genomes can be found GTPases that are not ARFs, ARLs, or SARs, but are clearly close relatives of the GTPases from which the ARF family later arose (Spang *et al.*, 2015; Klinger *et al.*, 2016). Examples of some proteins with domains similar to possible ARF GAPs and GEFs were also found, in some cases fused to the GTPases themselves.

Defining evolutionary patterns for the ARF, GEF, and GAP families individually is extremely informative, but comparisons among the three families can be even more powerful. Human GAPs show very similar duplication patterns to the ARFs. Class I ARFs gave rise to ARFs 1-3, while class II ARFs gave rise to ARF4 and 5 (Manolea *et al.*, 2010) (Figure 4B). The ARF GAPs show the same breakdown of two (ADAP, AGFG, GIT, SMAP) or three (ARFGAP, ACAP, AGAP, ARAP, ASAP) paralogues within these subfamilies (Schlacht *et al.*, 2013). This is a correlative observation, but raises testable models of coordinated activity of these proteins for which the substrate

specificity and biological functions are poorly and incompletely understood. Whether the expansions in the GEFs reflect a coevolutionary or functional relationship with the ARFs and GAPs is an open but exciting question. Interestingly, during the progressive expansion of the ARFs and their regulatory machinery from the LECA to humans, some protein families have resisted expansion. There is only a single GBF1 (but two orthologues, Gea1 and Gea2, in yeast) and a single ArfGAP1, both of which act in the early secretory pathway, suggesting a selective constraint on the plasticity of this pathway, as compared with the late secretory and endocytic systems. Similarly, there is a single ARL2 that acts at multiple sites in distinct pathways but has resisted segregating those functions via duplication with paralogues having distinct localization and actions (Sharer and Kahn, 1999; Bowzard *et al.*, 2005; Zhou *et al.*, 2006; Newman *et al.*, 2014). Evolutionary cell biology of cofunctioning families of proteins can reveal unexpected aspects of diverse organelle functions (for examples, see Dacks and Robinson, 2017) and provide crossover insights into possible mechanisms of action and regulatory networks for each family. While the ARF family is typically described as being one of four large families within the RAS superfamily, the evolutionary analysis (Klinger *et al.*, 2016), together with the unique and unifying structural mechanism employed by ARFs (Pasqualato *et al.*, 2002; see also earlier discussion), argue that ARF GTPases form their own superfamily.

ARF GTPASES/GEFS/GAPS IN CANCER AND OTHER DISEASES

As for most small GTPases, ARFs (and their GEFs and GAPs) have been associated with human diseases, and many more roles in pathologies continue to be discovered. A number of GTPases in the RAS superfamily have strong links to cancers, with RAS being the most commonly found mutated oncogene in human cancers and a RAS GAP, NF1, prominently altered in neurofibromatosis (Downward, 2003). We asked whether such links might be found within the human ARF families described herein. Interrogation of available next-generation sequencing data in the Cancer Genome Atlas (via http://cbioportal.com) reveals that ARF signaling is altered in cancer (albeit less commonly than is RAS or RHO signaling). Importantly, the mechanisms by which ARF GTPase/GEF/GAP signaling is genetically altered in cancers differs markedly from those seen in RAS and RHO families. RAS signaling is most commonly altered by missense mutation or amplification of the GTPases, deletion of RAS GAPs, and/or mutation of RAS effectors (Downward, 2003). Similarly, RHO signaling is most often altered by missense mutation or amplification of the GTPase, overexpression of GEFs, loss of GAPs, alterations in posttranslational modifications, and/or alternative splicing (Porter *et al.*, 2016). In contrast, missense mutations that render ARF GAPs inactive are largely not observed, and the most common genetic alterations observed are amplification events, particularly of the GTPases ARF4 and ARL14 and the ARF GAPs AGAP2 and ASAP1. ARF4 is commonly amplified in prostate cancer (20%) and is an important regulator of breast cancer cell migration (Jang *et al.*, 2012). ARL14 has yet to be studied in the lab, but its high amplification rate in squamous cell lung cancer (23%), esophageal cancer (13%), and ovarian cancer (11%) merits further investigation. AGAP2 is often coamplified with CDK4 and promotes cancer cell survival, migration, and invasion in glioblastoma models (Qi *et al.*, 2017). ASAP1 expression correlates with metastatic potential in uveal melanoma, colon cancer, prostate cancer, and laryngeal squamous cell carcinoma (Ehlers *et al.*, 2005; Muller *et al.*, 2010; Li *et al.*, 2014) and is associated with increased motility and invasiveness of uveal melanoma and breast cancer cells (Ehlers *et al.*, 2005; Onodera *et al.*, 2005). Furthermore, AGAP2 and ASAP1 amplification is associated with decreased overall and progression-free survival (Ehlers *et al.*, 2005). In addition, a number of reports implicate ARF4 (Jang *et al.*, 2012) and ARF6 (Hashimoto *et al.*, 2004; Hongu *et al.*, 2015; Li *et al.*, 2017) in cancer cell migration, invasion, and metastasis. However, the molecular mechanisms through which the changes in ARF GTPases and their GAPs elicit pathology remain to be defined. In addition to these amplification events, genomic deletions of at least one GTPase are observed. ARL11 (aka ADP-ribosylation factor-like tumor suppressor gene 1 [ARLTS1]), is commonly deleted in prostate cancer and sarcoma, and the expression of this gene in lung cancer is down-regulated due to promoter hypermethylation (Yendamuri *et al.*, 2008). Likewise, the ARF GEF BRAG2 has been implicated in breast cancer and uveal melanoma (Morishige *et al.*, 2008; Yoo *et al.*, 2016).

Mutations in ARF GEFs have been identified as causes of human neurological disease. For example, a large number of mutations in BRAG1/IQSec2 have been identified and implicated in nonsyndromic X-linked intellectual disability (Mignot *et al.*, 2018), a subset of which occur within either the IQ motif or the Sec7d. These mutations alter the trafficking of AMPA receptors in hippocampal neurons, suggesting a molecular basis for the deficits in learning and memory associated with this disease. Schwann cell–specific deletion of BIG1 prevents myelination (Miyamoto *et al.*, 2018), while mutations in BIG2 cause periventricular heterotopia with microcephaly (Sheen *et al.*, 2004; Lu *et al.*, 2006; Grzmil *et al.*, 2010). In both cases, we lack an understanding of the underlying mechanisms causing the GEF dysfunction.

TISSUE-SPECIFIC FUNCTIONS OF ARF GTPASES/GEFS/GAPS

There is growing evidence that at least some of these GTPases/GEFs/GAPs (especially those arising late in evolution) show differential tissue expression patterns and act in a tissue-specific manner or are expressed and function during specific stages of development. This is evident from studies in which specific GTPases have been mutated/deleted in mice (either total or tissue-specific knockout) and cause a variety of phenotypes (Table 4) (Mueller *et al.*, 2002; Schurmann *et al.*, 2002; Schrick *et al.*, 2006; Suzuki *et al.*, 2006; Caspary *et al.*, 2007; Zahn *et al.*, 2008; Hesse *et al.*, 2010, 2012; Hommel *et al.*, 2010; Zhang *et al.*, 2011; Jaschke *et al.*, 2012; Akiyama *et al.*, 2014; Hayakawa *et al.*, 2014; Hongu *et al.*, 2015; Hanke-Gogokhia *et al.*, 2016, 2017; Huang *et al.*, 2016; Li *et al.*, 2016; Lin *et al.*, 2017; Bay *et al.*, 2018; Dilan *et al.*, 2018; Pearring *et al.*, 2017; Rodiger *et al.*, 2018). As might be expected for ancient and highly conserved proteins, several GTPases are essential, and their deletion results in embryonic lethality. However, the use of tissue-specific deletions provides a wealth of new information and highlights the importance of these proteins in cells, tissues, and whole organisms, as exemplified by deletions of ARF6 in endothelial and neuronal cells as well as in platelets and podocytes and of the essential gene *ARFRP1* in liver, adipocytes, or intestine (Table 4). Tissue-specific expression of designer mutations in GTPases/GEFs/GAPs is another approach yielding novel insights (e.g., expression of the dominant active [Q70L]ARL2 in photoreceptor cells; Wright *et al.*, 2018). There are also large efforts underway to systematically knock out each mouse gene, and these will add both key reagents and important information on the biology of the three families of proteins discussed herein. We did not include such data, but they can be found at the following sites: National Institutes of Health (NIH) Knockout Mouse Project (www.komp.org), Mouse Genome

Informatics (www.informatics.jax.org), and International Mouse Phenotyping Consortium (www.mousephenotype.org).

In addition, many ARF GTPase/GEF/GAP genes give rise to multiple splice isoforms, yet we know little or nothing about how the expression of such isoforms is regulated in different tissues, whether the isoforms have distinct cellular localizations, perform distinct actions, or are regulated through different mechanisms. For example, Cytohesin 1 isoforms differing by the inclusion of a three-nucleotide glycine-coding microexon in the PH domain display differential affinity for $PI(4,5)P_2$ and $PI(3,4,5)P_3$ and localize either to the PM (triglycine isoform) or to the leading edge (diglycine isoform) (Ratcliffe *et al.*, 2018). Whether they perform different functions at those sites is unknown.

KEY QUESTIONS AND CHALLENGES

We reviewed key facets of current knowledge of ARF GTPases and their regulatory GEFs and GAPs. Here, we highlight what we consider the most glaring deficiencies that, if addressed experimentally, will advance our understanding of the underlying mechanisms and regulation of a broad array of essential cell processes.

1. *Functionalities of ARF family GTPases in cells:* We are largely ignorant of how many different functions a single GTPase can perform in a cell, which GTPases support which cellular functions, and the extent to which functional redundancy between different GTPases occurs. In some cases, these functions may be very similar (e.g., ARF1 regulating multiple steps of membrane trafficking), while in others they may be distinct (e.g., ARL2 acting from the intermembrane space to regulate mitochondrial fusion and from the cytosol to regulate $\alpha\beta$-tubulin assembly). When a single GTPase performs multiple functions at distinct intracellular sites, how is its distribution regulated, and how are the distinct functions coordinated to achieve integrated cellular homeostasis?
2. *What subset of ARFs, GEFs, and GAPs is used in a given cellular response?* It is well accepted that, if a regulatory GTPase is involved in a specific pathway, it will need an upstream GEF and a downstream GAP/effector to serve that regulatory role. In vitro studies using purified components reconstituted on membranes provide a powerful means to decipher complex regulatory properties at the molecular level, determine affinities and specificities, and generate testable hypotheses to interrogate these mechanisms in the cell. However, in vitro conditions are poor mimetics of those in a cell, and it is challenging to identify how such mechanisms are mobilized, altered, or combined by the cell to generate a specific response.
3. *ARF/GEF/GAP effectors/interactomes:* We are largely ignorant of the proteins that bind to each GTPase/GEF/GAP and how such interactions influence their activity and/or downstream events. Do the effectors/interactomes differ depending on location, and what defines the order, hierarchy, and cooperativity of such interactions? For example, do GEFs participate in the selection of effectors, that is, do GEFs both activate ARFs and bind ARF effectors/GAPs to promote the specificity of the downstream event, perhaps serving as a scaffold, as shown for GBF1 binding the γ-COP component of the coatomer (Deng *et al.*, 2009)? Our fragmentary knowledge of ARF family effectors and the downstream actions they perform is largely due to three technical difficulties. First, many GTPase-GAP/effector interactions occur within the constricted diffusion of effectors "solid phased" on the membrane surface and have relatively weak affinities in solution, making many common techniques of interactor identification (e.g., coimmunoprecipitation, affinity chromatography, or copurification) of limited utility. Second, ARFs often work in concert with phospholipids in so-called coincidence detection mechanisms, in which the interactions may require a particular lipid composition or membrane curvature. One example of this is the recruitment of the AP-1 clathrin adaptor complex to endosomal membranes, which requires its simultaneous binding to both ARF1 and the phosphoinositide PI(4)P (Ren *et al.*, 2013). Identification of new ARF effectors may therefore require affinity isolation approaches that incorporate lipids. Just such an approach recently identified a lipid-dependent interaction between ARF1 and the actin regulatory WAVE complex (Koronakis *et al.*, 2011). Third, most of these protein are cytosolic and may only transiently and incompletely associate with membranes to perform their key regulatory function(s), making it common for databases designed to catalogue localizations of proteins in cells or interactomes to miss important sites of action (e.g., compare our Figure 1 with data in the Human Protein Atlas: www.proteinatlas.org).
4. *Posttranslational modifications:* ARF GTPases/GEFs/GAPs are subject to posttranslational modifications that include phosphorylation and ubiquitination. These modification are transient and are likely to play important roles in localization, activation, selection of binding partners, and biological outputs. However, very few studies have analyzed the consequences of posttranslational modifications on protein function(s) or identified the responsible kinase or other modifiers. We also are ignorant of how the functional or metabolic status of a cell influences phosphorylation of specific proteins to evoke the appropriate functional response.
5. *Identification of ARL GEFs and GAPs:* This review focuses on the ARF GEFs and GAPs, largely because so little is known about the identity of ARL GEFs, GAPs, or effectors. Although ARLs comprise the largest group of the ARF family, we know the least about them and their regulators/interactors. We believe that the identification and characterization of each new GAP/GEF will provide important new insights into the regulation of essential cell processes. The finding that ELMODs, purified from mammalian tissues based on their GAP activity toward ARL2, also act on ARFs, showcases our ignorance of important and unexpected means of regulating ARFs as well as ARLs. Such studies increase the complexity and the challenges in sorting out specificities and pathways, but without such missing information, we risk fundamentally misinterpreting a lot of what we think we know about signaling by the ARF family.

SUMMARY

Surprisingly, despite decades of accumulated knowledge on ARF GTPases and their GEFs and GAPs, including an atomic understanding of the GDP/GTP exchange and GTP hydrolysis reactions, we remain ignorant of fundamental and key aspects of their action and regulation. Defining the answers posed here for even a single protein is a daunting task for any investigator. Yet, we argue that studying the entire ARF family of GTPases together and in concert with the families of their GEFs and GAPs will provide substantially more information and is critical to our understanding of 1) sources of specificity and functional redundancies, 2) complexities resulting from one protein acting at multiple sites, 3) enigmas of coordination between multiple GTPases to perform a single function, 4) the interconnections between ARF signaling and other cellular functions, and 5) how the actions of the GTPases/GEFs/GAPs are integrated with cellular physiology and/or contribute to pathology when gone awry. No one laboratory can hope to make more than a small dent in the black box before us. Thus, we hope that this review might serve as

an argument in support of more collaborative efforts to address this large, complex, but vitally important field of ARF signaling.

ACKNOWLEDGMENTS

We regret the many omissions of citations of outstanding work in the field that could not be cited or discussed in greater detail due to space limitations. This work was supported by grants from the NIH (R35GM122568 to R.A.K., R01DK093729 to L.C.S., R01GM127361 to J.E.C., R01GM122802 to E.S., R01GM056324 to D.G.L.), the National Science Foundation (MCB-1615607 to E.S.), the German Research Foundation DFG (SFB 958 to I.W. and A.S.), the National Health Research Institutes (NHRI) in Taiwan (NHRI-EX106-10601B1 to F.-J.S.L.), the NIH Intramural Program (project BC 007365 to P.A.R. and BC011805 to M.E.Y.), Alex's Lemonade Stand Foundation Young Investigator Award (M.E.Y.), the Institut National du Cancer (INCa, grant number 2014-160 to J.C.), and the Fondation pour la Recherche Médicale (FRM, grant number DEQ20150331694 to J.C.). J.B.D. is the Canada Research Chair (Tier II) in Evolutionary Cell Biology.

REFERENCES

Ahmadian MR, Stege P, Scheffzek K, Wittinghofer A (1997). Confirmation of the arginine-finger hypothesis for the GAP-stimulated GTP-hydrolysis reaction of Ras. Nat Struct Biol 4, 686–689.

Aizel K, Biou V, Navaza J, Duarte LV, Campanacci V, Cherfils J, Zeghouf M (2013). Integrated conformational and lipid-sensing regulation of endosomal ArfGEF BRAG2. PLoS Biol 11, e1001652.

Akiyama M, Hasegawa H, Hongu T, Frohman MA, Harada A, Sakagami H, Kanaho Y (2014). *Trans*-regulation of oligodendrocyte myelination by neurons through small GTPase Arf6-regulated secretion of fibroblast growth factor-2. Nat Commun 5, 4744.

Alkanderi S, Molinari E, Shaheen R, Elmaghloob Y, Stephen LA, Sammut V, Ramsbottom SA, Srivastava S, Cairns G, Edwards N, *et al.* (2018). ARL3 mutations cause Joubert syndrome by disrupting ciliary protein composition. Am J Hum Genet 103, 612–620.

Altan-Bonnet N, Phair RD, Polishchuk RS, Weigert R, Lippincott-Schwartz J (2003). A role for Arf1 in mitotic Golgi disassembly, chromosome segregation, and cytokinesis. Proc Natl Acad Sci USA 100, 13314–13319.

Alvarez C, Garcia-Mata R, Brandon E, Sztul E (2003). COPI recruitment is modulated by a Rab1b-dependent mechanism. Mol Biol Cell 14, 2116–2127.

Amin E, Jaiswal M, Derewenda U, Reis K, Nouri K, Koessmeier KT, Aspenstrom P, Somlyo AV, Dvorsky R, Ahmadian MR (2016). Deciphering the molecular and functional basis of RHOGAP family proteins: a systematic approach toward selective inactivation of RHO family proteins. J Biol Chem 291, 20353–20371.

Amor JC, Harrison DH, Kahn RA, Ringe D (1994). Structure of the human ADP-ribosylation factor 1 complexed with GDP. Nature 372, 704–708.

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.

Arimoto K, Funami K, Saeki Y, Tanaka K, Okawa K, Takeuchi O, Akira S, Murakami Y, Shimotohno K (2010). Polyubiquitin conjugation to NEMO by triparite motif protein 23 (TRIM23) is critical in antiviral defense. Proc Natl Acad Sci USA 107, 15856–15861.

Attar MA, Santy LC (2013). The scaffolding protein GRASP/Tamalin directly binds to Dock180 as well as to cytohesins facilitating GTPase crosstalk in epithelial cell migration. BMC Cell Biol 14, 9.

Bach AS, Enjalbert S, Comunale F, Bodin S, Vitale N, Charrasse S, Gauthier-Rouviere C (2010). ADP-ribosylation factor 6 regulates mammalian myoblast fusion through phospholipase D1 and phosphatidylinositol 4,5-bisphosphate signaling pathways. Mol Biol Cell 21, 2412–2424.

Bai M, Gad H, Turacchio G, Cocucci E, Yang JS, Li J, Beznoussenko GV, Nie ZZ, Luo RB, Fu LW, *et al.* (2011). ARFGAP1 promotes AP-2-dependent endocytosis. Nat Cell Biol 13, 559–U144.

Barrett T, Xiao B, Dodson EJ, Dodson G, Ludbrook SB, Nurmahomed K, Gamblin SJ, Musacchio A, Smerdon SJ, Eccleston JF (1997). The structure of the GTPase-activating domain from p50rhoGAP. Nature 385, 458–461.

Bay SN, Long AB, Caspary T (2018). Disruption of the ciliary GTPase Arl13b suppresses Sonic hedgehog overactivation and inhibits medulloblastoma formation. Proc Natl Acad Sci USA 115, 1570–1575.

Beraud-Dufour S, Robineau S, Chardin P, Paris S, Chabre M, Cherfils J, Antonny B (1998). A glutamic finger in the guanine nucleotide exchange factor ARNO displaces Mg^{2+} and the beta-phosphate to destabilize GDP on ARF1. EMBO J 17, 3651–3659.

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.

Boman AL, Zhang C, Zhu X, Kahn RA (2000). A family of ADP-ribosylation factor effectors that can alter membrane transport through the *trans*-Golgi. Mol Biol Cell 11, 1241–1255.

Boulakirba S, Macia E, Partisani M, Lacas-Gervais S, Brau F, Luton F, Franco M (2014). Arf6 exchange factor EFA6 and endophilin directly interact at the plasma membrane to control clathrin-mediated endocytosis. Proc Natl Acad Sci USA 111, 9473–9478.

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.

Bowzard JB, Cheng D, Peng J, Kahn RA (2007). ELMOD2 is an Arl2 GTPase-activating protein that also acts on Arfs. J Biol Chem 282, 17568–17580.

Bowzard JB, Sharer JD, Kahn RA (2005). Assays used in the analysis of Arl2 and its binding partners. Methods Enzymol 404, 453–467.

Brown HA, Gutowski S, Moomaw CR, Slaughter C, Sternweis PC (1993). ADP-ribosylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity [see comments]. Cell 75, 1137–1144.

Bui QT, Golinelli-Cohen MP, Jackson CL (2009). Large Arf1 guanine nucleotide exchange factors: evolution, domain structure, and roles in membrane trafficking and human disease. Mol Genet Genomics 282, 329–350.

Busby T, Meissner JM, Styers ML, Bhatt J, Kaushik A, Hjelmeland AB, Sztul E (2017). The Arf activator GBF1 localizes to plasma membrane sites involved in cell adhesion and motility. Cell Logist 7, e1308900.

Campa F, Yoon HY, Ha VL, Szentpetery Z, Balla T, Randazzo PA (2009). A PH Domain in the Arf GTPase-activating protein (GAP) ARAP1 binds phosphatidylinositiol 3,4,5-trisphosphate and regulates Arf GAP activity independently of recruitment to the plasma membranes. J Biol Chem 284, 28069–28083.

Casalou C, Faustino A, Barral DC (2016). Arf proteins in cancer cell migration. Small GTPases 7, 270–282.

Casalou C, Seixas C, Portelinha A, Pintado P, Barros M, Ramalho JS, Lopes SS, Barral DC (2014). Arl13b and the non-muscle myosin heavy chain IIA are required for circular dorsal ruffle formation and cell migration. J Cell Sci 127, 2709–2722.

Casanova JE (2007). Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. Traffic 8, 1476–1485.

Caspary T, Larkins CE, Anderson KV (2007). The graded response to Sonic Hedgehog depends on cilia architecture. Dev Cell 12, 767–778.

Caumont AS, Vitale N, Gensse M, Galas MC, Casanova JE, Bader MF (2000). Identification of a plasma membrane-associated guanine nucleotide exchange factor for ARF6 in chromaffin cells. Possible role in the regulated exocytotic pathway. J Biol Chem 275, 15637–15644.

Charych EI, Yu W, Miralles CP, Serwanski DR, Li X, Rubio M, De Blas AL (2004). The brefeldin A-inhibited GDP/GTP exchange factor 2, a protein involved in vesicular trafficking, interacts with the beta subunits of the GABA receptors. J Neurochem 90, 173–189.

Chen J, Wu X, Yao L, Yan L, Zhang L, Qiu J, Liu X, Jia S, Meng A (2017). Impairment of cargo transportation caused by gbf1 mutation disrupts vascular integrity and causes hemorrhage in zebrafish embryos. J Biol Chem 292, 2315–2327.

Chen PW, Jian X, Heissler SM, Le K, Luo R, Jenkins LM, Nagy A, Moss J, Sellers JR, Randazzo PA (2016). The Arf GTPase-activating protein, ASAP1, binds nonmuscle myosin 2A to control remodeling of the actomyosin network. J Biol Chem, 291, 7517–7526.

Chen PW, Jian XY, Yoon HY, Randazzo PA (2013). ARAP2 signals through Arf6 and Rac1 to control focal adhesion morphology. J Biol Chem 288, 5849–5860.

Chen P-W, Luo R, Jian X, Randazzo PA (2014). The Arf6 GTPase-activating proteins ARAP2 and ACAP1 define distinct endosomal compartments that regulate integrin $\alpha5\beta1$ traffic. J Biol Chem 289, 30237–30248.

Cherfils J (2014). Arf GTPases and their effectors: assembling multivalent membrane-binding platforms. Curr Opin Struct Biol 29, 67–76.

Cherfils J, Chardin P (1999). GEFs: structural basis for their activation of small GTP-binding proteins. Trends Biochem Sci 24, 306–311.

Cherfils J, Zeghouf M (2013). Regulation of small GTPases by GEFs, GAPs, and GDIs. Physiol Rev 93, 269–309.

Chiang AP, Nishimura D, Searby C, Elbedour K, Carmi R, Ferguson AL, Secrist J, Braun T, Casavant T, Stone EM, *et al*. (2004). Comparative genomic analysis identifies an ADP-ribosylation factor-like gene as the cause of Bardet-Biedl syndrome (BBS3). Am J Hum Genet 75, 475–484.

Chiang TS, Wu HF, Lee FS (2017). ADP-ribosylation factor-like 4C binding to filamin-A modulates filopodium formation and cell migration. Mol Biol Cell 28, 3013–3028.

Christis C, Munro S (2012). The small G protein Arl1 directs the *trans*-Golgi-specific targeting of the Arf1 exchange factors BIG1 and BIG2. J Cell Biol 196, 327–335.

Cockcroft S, Thomas GM, Fensome A, Geny B, Cunningham E, Gout I, Hiles I, Totty NF, Truong O, Hsuan JJ (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.

Cronin TC, DiNitto JP, Czech MP, Lambright DG (2004). Structural determinants of phosphoinositide selectivity in splice variants of Grp1 family PH domains. EMBO J 23, 3711–3720.

Cruz-Garcia D, Ortega-Bellido M, Scarpa M, Villeneuve J, Jovic M, Porzner M, Balla T, Seufferlein T, Malhotra V (2013). Recruitment of arfaptins to the *trans*-Golgi network by PI(4)P and their involvement in cargo export. EMBO J 32, 1717–1729.

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.

Dacks JB, Robinson MS (2017). Outerwear through the ages: evolutionary cell biology of vesicle coats. Curr Opin Cell Biol 47, 108–116.

Decressac S, Franco M, Bendahhou S, Warth R, Knauer S, Barhanin J, Lazdunski M, Lesage F (2004). ARF6-dependent interaction of the TWIK1 K^+ channel with EFA6, a GDP/GTP exchange factor for ARF6. EMBO Rep 5, 1171–1175.

Dell'Angelica EC, Puertollano R, Mullins C, Aguilar RC, Vargas JD, Hartnell LM, Bonifacino JS (2000). GGAs: a family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex. J Cell Biol 149, 81–94.

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.

Derby MC, van Vliet C, Brown D, Luke MR, Lu L, Hong W, Stow JL, Gleeson PA (2004). Mammalian GRIP domain proteins differ in their membrane binding properties and are recruited to distinct domains of the TGN. J Cell Sci 117(Pt 24), 5865–5874.

Dilan TL, Moye AR, Salido EM, Saravanan T, Saravanan K, Goldberg AFX, Ramamurthy V (2018). ARL13B, a Joubert syndrome-associated protein, is critical for retinogenesis and elaboration of mouse photoreceptor outer segments. J Neurosci 39, 1347–1364.

DiNitto JP, Cronin TC, Lambright DG (2003). Membrane recognition and targeting by lipid-binding domains. Sci STKE 2003, re16.

DiNitto JP, Delprato A, Gabe Lee MT, Cronin TC, Huang S, Guilherme A, Czech MP, Lambright DG (2007). Structural basis and mechanism of autoregulation in 3-phosphoinositide-dependent Grp1 family Arf GTPase exchange factors. Mol Cell 28, 569–583.

Dodonova SO, Aderhold P, Kopp J, Ganeva I, Rohling S, Hagen WJH, Sinning I, Wieland F, Briggs JAG (2017). 9A structure of the COPI coat reveals that the Arf1 GTPase occupies two contrasting molecular environments. Elife 6, eLife.26691.

Donaldson JG (2003). Multiple roles for Arf6: sorting, structuring, and signaling at the plasma membrane. J Biol Chem 278, 41573–41576.

Donaldson JG, Jackson CL (2011). ARF family G proteins and their regulators: roles in membrane transport, development and disease. Nat Rev Mol Cell Biol 12, 362–375.

Downward J (2003). Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 3, 11–22.

Drake MT, Zhu Y, Kornfeld S (2000). The assembly of AP-3 adaptor complex-containing clathrin-coated vesicles on synthetic liposomes. Mol Biol Cell 11, 3723–3736.

D'Souza RS, Casanova JE (2016). The BRAG/IQSec family of Arf GEFs. Small GTPases 7, 257–264.

D'Souza RS, Semus R, Billings EA, Meyer CB, Conger K, Casanova JE (2014). Rab4 orchestrates a small GTPase cascade for recruitment of adaptor proteins to early endosomes. Curr Biol 24, 1187–1198.

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

Dunphy JL, Ye K, Casanova JE (2007). Nuclear functions of the Arf guanine nucleotide exchange factor BRAG2. Traffic 8, 661–672.

East MP, Bowzard JB, Dacks JB, Kahn RA (2012). ELMO domains, evolutionary and functional characterization of a novel GTPase-activating protein (GAP) domain for Arf protein family GTPases. J Biol Chem 287, 39538–39553.

East MP, Kahn RA (2011). Models for the functions of Arf GAPs. Semin Cell Dev Biol 22, 3–9.

Ehlers JP, Worley L, Onken MD, Harbour JW (2005). DDEF1 is located in an amplified region of chromosome 8q and is overexpressed in uveal melanoma. Clin Cancer Res 11, 3609–3613.

Eiseler T, Wille C, Koehler C, Illing A, Seufferlein T (2016). Protein Kinase D2 assembles a multiprotein complex at the *trans*-Golgi network to regulate matrix metalloproteinase secretion. J Biol Chem 291, 462–477.

Elagabani MN, Brisevac D, Kintscher M, Pohle J, Kohr G, Schmitz D, Kornau HC (2016). Subunit-selective N-methyl-D-aspartate (NMDA) receptor signaling through Brefeldin A-resistant Arf guanine nucleotide exchange factors BRAG1 and BRAG2 during synapse maturation. J Biol Chem 291, 9105–9118.

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.

Eme L, Sharpe SC, Brown MW, Roger AJ (2014). On the age of eukaryotes: evaluating evidence from fossils and molecular clocks. Cold Spring Harb Perspect Biol 6, a016139.

Francis JW, Goswami D, Novick SJ, Pascal BD, Weikum ER, Ortlund EA, Griffin PR, Kahn RA (2017a). Nucleotide binding to ARL2 in the TBCDARL2β-tubulin complex drives conformational changes in β-tubulin. J Mol Biol 429, 3696–3716.

Francis JW, Newman LE, Cunningham LA, Kahn RA (2017b). A trimer consisting of the tubulin-specific chaperone D (TBCD), regulatory GTPase ARL2, and β-Tubulin is required for maintaining the microtubule network. J Biol Chem 292, 4336–4349.

Francis JW, Turn RE, Newman LE, Schiavon C, Kahn RA (2016). Higher order signaling: ARL2 as regulator of both mitochondrial fusion and microtubule dynamics allows integration of 2 essential cell functions. Small GTPases 7, 188–196.

Franco M, Peters PJ, Boretto J, van Donselaar E, Neri A, D'Souza-Schorey C, Chavrier P (1999). EFA6, a sec7 domain-containing exchange factor for ARF6, coordinates membrane recycling and actin cytoskeleton organization. EMBO J 18, 1480–1491.

Frank SR, Adelstein MR, Hansen SH (2006). GIT2 represses Crk- and Rac1-regulated cell spreading and Cdc42-mediated focal adhesion turnover. EMBO J 25, 1848–1859.

Frank SR, Hatfield JC, Casanova JE (1998). Remodeling of the actin cytoskeleton is coordinately regulated by protein kinase C and the ADP-ribosylation factor nucleotide exchange factor ARNO. Mol Biol Cell 9, 3133–3146.

Fuss B, Becker T, Zinke I, Hoch M (2006). The cytohesin Steppke is essential for insulin signalling in *Drosophila*. Nature 444, 945–948.

Gambardella L, Anderson KE, Jakus Z, Voigt S, Hawkins PT, Stephens L, Mocsai A, Vermeren S (2012). ARAP3 functions downstream of PI3K to regulate neutrophils. Eur J Clin Invest 42, 25–25.

Gambardella L, Anderson KE, Nussbaum C, Segonds-Pichon A, Margarido T, Norton L, Ludwig T, Sperandio M, Hawkins PT, Stephens L, *et al*. (2011). The GTPase-activating protein ARAP3 regulates chemotaxis and adhesion-dependent processes in neutrophils. Blood 118, 1087–1098.

Gehart H, Goginashvili A, Beck R, Morvan J, Erbs E, Formentini I, De Matteis MA, Schwab Y, Wieland FT, Ricci R (2012). The BAR domain protein Arfaptin-1 controls secretory granule biogenesis at the *trans*-Golgi network. Dev Cell 23, 756–768.

Gilbert CE, Sztul E, Machamer CE (2018). Commonly used trafficking blocks disrupt ARF1 activation and the localization and function of specific Golgi proteins. Mol Biol Cell 29, 937–947.

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

Godi A, Di Campli A, Konstantakopoulos A, Di Tullio G, Alessi DR, Kular GS, Daniele T, Marra P, Lucocq JM, De Matteis MA (2004). FAPPs control Golgi-to-cell-surface membrane traffic by binding to ARF and PtdIns(4)P. Nat Cell Biol 6, 393–404.

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

Goldberg J (2000). Decoding of sorting signals by coatomer through a GTPase switch in the COPI coat complex. Cell 100, 671–679.

Gotthardt K, Lokaj M, Koerner C, Falk N, Giessl A, Wittinghofer A (2015). A G-protein activation cascade from Arl13B to Arl3 and implications for ciliary targeting of lipidated proteins. Elife 4, eLife.11859.

Grzmil P, Enkhbaatar Z, Gundsambuu B, Oidovsambuu O, Yalcin S, Wolf S, Engel W, Neesen J (2010). Early embryonic lethality in gene trap mice with disruption of the *Arfgef2* gene. Int J Dev Biol 54, 1259–1266.
Gupta GD, Swetha MG, Kumari S, Lakshminarayan R, Dey G, Mayor S (2009). Analysis of endocytic pathways in *Drosophila* cells reveals a conserved role for GBF1 in internalization via GEECs. PLoS One 4, e6768.
Gustafson MA, Fromme JC (2017). Regulation of Arf activation occurs via distinct mechanisms at early and late Golgi compartments. Mol Biol Cell 28, 3660–3671.
Ha VL, Luo RB, Nie ZZ, Randazzo PA (2008). Contribution of AZAP-type Arf GAPs to cancer cell migration and invasion. Adv Canc Res, 101, 1–28.
Hafner M, Schmitz A, Grune I, Srivatsan SG, Paul B, Kolanus W, Quast T, Kremmer E, Bauer I, Famulok M (2006). Inhibition of cytohesins by SecinH3 leads to hepatic insulin resistance. Nature 444, 941–944.
Hahn I, Fuss B, Peters A, Werner T, Sieberg A, Gosejacob D, Hoch M (2013). The *Drosophila* Arf GEF Steppke controls MAPK activation in EGFR signaling. J Cell Sci 126, 2470–2479.
Hanai A, Ohgi M, Yagi C, Ueda T, Shin H-W, Nakayama K (2016). Class I Arfs (Arf1 and Arf3) and Arf6 are localized to the Flemming body and play important roles in cytokinesis. J Biochem 159, 201–208.
Hanke-Gogokhia C, Frederick JM, Zhang H, Baehr W (2018). Binary function of ARL3-GTP revealed by gene knockouts. Adv Exp Med Biol 1074, 317–325.
Hanke-Gogokhia C, Wu Z, Gerstner CD, Frederick JM, Zhang H, Baehr W (2016). Arf-like protein 3 (ARL3) regulates protein trafficking and ciliogenesis in mouse photoreceptors. J Biol Chem 291, 7142–7155.
Hanke-Gogokhia C, Wu Z, Sharif A, Yazigi H, Frederick JM, Baehr W (2017). The guanine nucleotide exchange factor, Arf-like protein 13b, is essential for assembly of the mouse photoreceptor transition zone and outer segment. J Biol Chem 292, 21442–21456.
Hashimoto S, Onodera Y, Hashimoto A, Tanaka M, Hamaguchi M, Yamada A, Sabe H (2004). Requirement for Arf6 in breast cancer invasive activities. Proc Natl Acad Sci USA 101, 6647–6652.
Hayakawa N, Ogoh H, Sumiyoshi M, Matsui Y, Nishikawa S, Miyamoto K, Maede Y, Kiyonari H, Suzuki M, Watanabe T (2014). The ADP-ribosylation factor 1 gene is indispensable for mouse embryonic development after implantation. Biochem Biophys Res Commun 453, 748–753.
Hesse D, Hommel A, Jaschke A, Moser M, Bernhardt U, Zahn C, Kluge R, Wittschen P, Gruber AD, Al-Hasani H, *et al.* (2010). Altered GLUT4 trafficking in adipocytes in the absence of the GTPase Arfrp1. Biochem Biophys Res Commun 394, 896–903.
Hesse D, Jaschke A, Kanzleiter T, Witte N, Augustin R, Hommel A, Puschel GP, Petzke KJ, Joost HG, Schupp M, *et al.* (2012). GTPase ARFRP1 is essential for normal hepatic glycogen storage and insulin-like growth factor 1 secretion. Mol Cell Biol 32, 4363–4374.
Hiester KG, Santy LC (2013). The cytohesin coiled-coil domain interacts with threonine 276 to control membrane association. PLoS One 8, e82084.
Higginbotham H, Eom TY, Mariani LE, Bachleda A, Hirt J, Gukassyan V, Cusack CL, Lai C, Caspary T, Anton ES (2012). Arl13b in primary cilia regulates the migration and placement of interneurons in the developing cerebral cortex. Dev Cell 23, 925–938.
Hill K, Li Y, Bennett M, McKay M, Zhu X, Shern J, Torre E, Lah JJ, Levey AI, Kahn RA (2003). Munc18 interacting proteins: ADP-ribosylation factor-dependent coat proteins that regulate the traffic of beta-Alzheimer's precursor protein. J Biol Chem 278, 36032–36040.
Hirst J, Bright NA, Rous B, Robinson MS (1999). Characterization of a fourth adaptor-related protein complex. Mol Biol Cell 10, 2787–2802.
Hoefen RJ, Berk BC (2006). The multifunctional GIT family of proteins. J Cell Sci 119, 1469–1475.
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.
Hommel A, Hesse D, Volker W, Jaschke A, Moser M, Engel T, Bluher M, Zahn C, Chadt A, Ruschke K, *et al.* (2010). The ARF-like GTPase ARFRP1 is essential for lipid droplet growth and is involved in the regulation of lipolysis. Mol Cell Biol 30, 1231–1242.
Honda A, Nogami M, Yokozeki T, Yamazaki M, Nakamura H, Watanabe H, Kawamoto K, Nakayama K, Morris AJ, Frohman MA, *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.
Hongu T, Funakoshi Y, Fukuhara S, Suzuki T, Sakimoto S, Takakura N, Ema M, Takahashi S, Itoh S, Kato M, *et al.* (2015). Arf6 regulates tumour angiogenesis and growth through HGF-induced endothelial beta1 integrin recycling. Nat Commun 6, 7925.
Huang Y, Joshi S, Xiang B, Kanaho Y, Li Z, Bouchard BA, Moncman CL, Whiteheart SW (2016). Arf6 controls platelet spreading and clot retraction via integrin $\alpha_{IIb}\beta_3$ trafficking. Blood 127, 1459–1467.
Inaba Y, Tian QB, Okano A, Zhang JP, Sakagami H, Miyazawa S, Li W, Komiyama A, Inokuchi K, Kondo H, *et al.* (2004). Brain-specific potential guanine nucleotide exchange factor for Arf, synArfGEF (Po), is localized to postsynaptic density. J Neurochem 89, 1347–1357.
Inoue H, Randazzo PA (2007). Arf GAPs and their interacting proteins. Traffic 8, 1465–1475.
Ishizaki R, Shin HW, Mitsuhashi 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.
Ismail SA, Chen YX, Miertzschke M, Vetter IR, Koerner C, Wittinghofer A (2012). Structural basis for Arl3-specific release of myristoylated ciliary cargo from UNC119. EMBO J 31, 4085–4094.
Ismail SA, Chen YX, Rusinova A, Chandra A, Bierbaum M, Gremer L, Triola G, Waldmann H, Bastiaens PI, Wittinghofer A (2011). Arl2-GTP and Arl3-GTP regulate a GDI-like transport system for farnesylated cargo. Nat Chem Biol 7, 942–949.
Ismail SA, Vetter IR, Sot B, Wittinghofer A (2010). The structure of an Arf-Arf-GAP complex reveals a Ca^{2+} regulatory mechanism. Cell 141, 812–821.
Ivanova AA, Caspary T, Seyfried NT, Duong DM, West AB, Liu Z, Kahn RA (2017). Biochemical characterization of purified mammalian ARL13B protein indicates that it is an atypical GTPase and ARL3 guanine nucleotide exchange factor (GEF). J Biol Chem 292, 11091–11108.
Ivanova AA, East MP, Yi SL, Kahn RA (2014). Characterization of recombinant ELMOD (cell engulfment and motility domain) proteins as GTPase-activating proteins (GAPs) for ARF family GTPases. J Biol Chem 289, 11111–11121.
Jackson CL, Bouvet S (2014). Arfs at a glance. J Cell Sci 127, 4103–4109.
Jackson CL, Casanova JE (2000). Turning on ARF: the Sec7 family of guanine-nucleotide-exchange factors. Trends Cell Biol 10, 60–67.
Jang SY, Jang SW, Ko J (2012). Regulation of ADP-ribosylation factor 4 expression by small leucine zipper protein and involvement in breast cancer cell migration. Cancer Lett 314, 185–197.
Jaschke A, Chung B, Hesse D, Kluge R, Zahn C, Moser M, Petzke KJ, Brigelius-Flohe R, Puchkov D, Koepsell H, *et al.* (2012). The GTPase ARFRP1 controls the lipidation of chylomicrons in the Golgi of the intestinal epithelium. Hum Mol Genet 21, 3128–3142.
Jian X, Cavenagh M, Gruschus JM, Randazzo PA, Kahn RA (2010). Modifications to the C-terminus of Arf1 alter cell functions and protein interactions. Traffic 11, 732–742.
Jian X, Gruschus JM, Sztul E, Randazzo PA (2012). The pleckstrin homology (PH) domain of the Arf exchange factor Brag2 is an allosteric binding site. J Biol Chem 287, 24273–24283.
Jian X, Tang WK, Zhai P, Roy NS, Luo R, Gruschus JM, Yohe ME, Chen PW, Li Y, Byrd RA, *et al.* (2015). Molecular basis for cooperative binding of anionic phospholipids to the PH domain of the Arf GAP ASAP1. Structure, doi:10.1016/j.str.2015.08.008.
Jian XY, Brown P, Schuck P, Gruschus JM, Balbo A, Hinshaw JE, Randazzo PA (2009). Autoinhibition of Arf GTPase-activating protein activity by the BAR domain in ASAP1. J Biol Chem 284, 1652–1663.
Jin H, Nachury MV (2009). The BBSome. Curr Biol 19, R472–R473.
Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, Bazan JF, Nachury MV (2010). The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. Cell 141, 1208–1219.
Jones DH, Morris JB, Morgan CP, Kondo H, Irvine RF, Cockcroft S (2000). Type I phosphatidylinositol 4-phosphate 5-kinase directly interacts with ADP-ribosylation factor 1 and is responsible for phosphatidylinositol 4,5-bisphosphate synthesis in the Golgi compartment. J Biol Chem 275, 13962–13966.
Jones S, Jedd G, Kahn RA, Franzusoff A, Bartolini F, Segev N (1999). Genetic interactions in yeast between Ypt GTPases and Arf guanine nucleotide exchangers. Genetics 152, 1543–1556.
Kahn RA, Bruford E, Inoue H, Logsdon JM, Nie ZZ, Premont RT, Randazzo PA, Satake M, Theibert AB, Zapp ML, *et al.* (2008). Consensus nomenclature for the human ArfGAP domain-containing proteins. J Cell Biol 182, 1039–1044.
Kahn RA, Cherfils J, Elias M, Lovering RC, Munro S, Schurmann A (2006). Nomenclature for the human Arf family of GTP-binding proteins: ARF, ARL, and SAR proteins. J Cell Biol 172, 645–650.
Kam JL, Miura K, Jackson TR, Gruschus J, Roller P, Stauffer S, Clark J, Aneja R, Randazzo PA (2000). Phosphoinositide-dependent activation of the ADP-ribosylation factor GTPase-activating protein ASAP1. Evidence for

the pleckstrin homology domain functioning as an allosteric site. J Biol Chem 275, 9653–9663.

Karandur D, Nawrotek A, Kuriyan J, Cherfils J (2017). Multiple interactions between an Arf/GEF complex and charged lipids determine activation kinetics on the membrane. Proc Natl Acad Sci USA 114, 11416–11421.

Khan AR, Menetrey J (2013). Structural biology of Arf and Rab GTPases' effector recruitment and specificity. Structure 21, 1284–1297.

Khatter D, Sindhwani A, Sharma M (2015). Arf-like GTPase Arl8: moving from the periphery to the center of lysosomal biology. Cell Logist 5, e1086501.

Klinger CM, Spang A, Dacks JB, Ettema TJ (2016). Tracing the archaeal origins of eukaryotic membrane-trafficking system building blocks. Mol Biol Evol 33, 1528–1541.

Koronakis V, Hume PJ, Humphreys D, Liu T, Horning O, Jensen ON, McGhie EJ (2011). WAVE regulatory complex activation by cooperating GTPases Arf and Rac1. Proc Natl Acad Sci USA 108, 14449–14454.

Krugmann S, Anderson KE, Ridley SH, Risso N, McGregor A, Coadwell J, Davidson K, Eguinoa A, Ellson CD, Lipp P, *et al*. (2002). Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices. Mol Cell 9, 95–108.

Lamorte L, Rodrigues S, Sangwan V, Turner CE, Park M (2003). Crk associates with a multimolecular Paxillin/GIT2/beta-PIX complex and promotes Rac-dependent relocalization of Paxillin to focal contacts. Mol Biol Cell 14, 2818–2831.

Lee SY, Yang JS, Hong WJ, Premont RT, Hsu VW (2005). ARFGAP1 plays a central role in coupling COPI cargo sorting with vesicle formation. J Cell Biol 168, 281–290.

Li CC, Chiang TC, 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.

Li HS, Shome K, Rojas R, Rizzo MA, Vasudevan C, Fluharty E, Santy LC, Casanova JE, Romero G (2003). The guanine nucleotide exchange factor ARNO mediates the activation of ARF and phospholipase D by insulin. BMC Cell Biol 4, 13.

Li J, Malaby AW, Famulok M, Sabe H, Lambright DG, Hsu VW (2012). Grp1 plays a key role in linking insulin signaling to glut4 recycling. Dev Cell 22, 1286–1298.

Li M, Tian L, Yao H, Lu J, Ge J, Guo Y, Liu M, Xiao H (2014). ASAP1 mediates the invasive phenotype of human laryngeal squamous cell carcinoma to affect survival prognosis. Oncol Rep 31, 2676–2682.

Li R, Peng C, Zhang X, Wu Y, Pan S, Xiao Y (2017). Roles of Arf6 in cancer cell invasion, metastasis and proliferation. Life Sci 182, 80–84.

Li Y, Kelly WG, Logsdon JM Jr, Schurko AM, Harfe BD, Hill-Harfe KL, Kahn RA (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 Y, Tian X, Ma M, Jerman S, Kong S, Somlo S, Sun Z (2016). Deletion of ADP ribosylation factor-like GTPase 13B leads to kidney cysts. J Am Soc Nephrol 27, 3628–3638.

Liew GM, Ye F, Nager AR, Murphy JP, Lee JS, Aguiar M, Breslow DK, Gygi SP, Nachury MV (2014). The intraflagellar transport protein IFT27 promotes BBSome exit from cilia through the GTPase ARL6/BBS3. Dev Cell 31, 265–278.

Lim J, Zhou M, Veenstra TD, Morrison DK (2010). The CNK1 scaffold binds cytohesins and promotes insulin pathway signaling. Genes Dev 24, 1496–1506.

Lin JS, Jeon JS, Fan Q, Wong HN, Palmer MB, Holzman LB (2017). ARF6 mediates nephrin tyrosine phosphorylation-induced podocyte cellular dynamics. PLoS One 12, e0184575.

Lin YC, Chiang TC, Liu YT, Tsai YT, Jang LT, Lee FJ (2011). ARL4A acts with GCC185 to modulate Golgi complex organization. J Cell Sci 124, 4014–4026.

Liu X, Hu Y, Hao C, Rempel SA, Ye K (2007). PIKE-A is a proto-oncogene promoting cell growth, transformation and invasion. Oncogene 26, 4918–4927.

Liu Y, Kahn RA, Prestegard JH (2009). Structure and membrane interaction of myristoylated ARF1. Structure 17, 79–87.

Liu Y, Kahn RA, Prestegard JH (2010). Dynamic structure of membrane-anchored Arf*GTP. Nat Struct Mol Biol 17, 876–881.

Lock JG, Hammond LA, Houghton F, Gleeson PA, Stow JL (2005). E-cadherin transport from the *trans*-Golgi network in tubulovesicular carriers is selectively regulated by golgin-97. Traffic 6, 1142–1156.

Lowery J, Szul T, Styers M, Holloway Z, Oorschot V, Klumperman J, Sztul E (2013). The Sec7 guanine nucleotide exchange factor GBF1 regulates membrane recruitment of BIG1 and BIG2 to the *trans*-Golgi network (TGN). J Biol Chem 288, 11532–11545.

Lu J, Tiao G, Folkerth R, Hecht J, Walsh C, Sheen V (2006). Overlapping expression of ARFGEF2 and Filamin A in the neuroependymal lining of the lateral ventricles: insights into the cause of periventricular heterotopia. J Comp Neurol 494, 476–484.

Luo R, Chen PW, Kuo JC, Jenkins L, Jian X, Waterman CM, Randazzo PA (2018). ARAP2 inhibits Akt independently of its effects on focal adhesions. Biol Cell 110, 257–270.

Luo R, Chen PW, Wagenbach M, Jian X, Jenkins L, Wordeman L, Randazzo PA (2016). Direct functional interaction of the kinesin-13 family member kinesin-like protein 2A (Kif2A) and Arf GAP with GTP-binding protein-like, ankyrin repeats and PH domains 1 (AGAP1). J Biol Chem 291, 25761.

Luo R, Ha VL, Hayashi R, Randazzo PA (2009). Arf GAP2 is positively regulated by coatomer and cargo. Cell Signal 21, 1169–1179.

Luo R, Reed CE, Sload JA, Wordeman L, Randazzo PA, Chen PW (2017). Arf GAPs and molecular motors. Small GTPases 21, 1–14.

Luo RB, Randazzo PA (2008). Kinetic analysis of Arf GAP1 indicates a regulatory role for coatomer. J Biol Chem 283, 21965–21977.

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.

Macia E, Luton F, Partisani M, Cherfils J, Chardin P, Franco M (2004). The GDP-bound form of Arf6 is located at the plasma membrane. J Cell Sci 117, 2389–2398.

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, Chun J, Chen DW, Clarke I, Summerfeldt N, Dacks JB, Melancon P (2010). Arf3 is activated uniquely at the *trans*-Golgi network by brefeldin A-inhibited guanine nucleotide exchange factors. Mol Biol Cell 21, 1836–1849.

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.

Matsumoto S, Fujii S, Sato A, Ibuka S, Kagawa Y, Ishii M, Kikuchi A (2014). A combination of Wnt and growth factor signaling induces Arl4c expression to form epithelial tubular structures. EMBO J 33, 702–718.

Matsuya S, Sakagami H, Tohgo A, Owada Y, Shin HW, Takeshima H, Nakayama K, Kokubun S, Kondo H (2005). Cellular and subcellular localization of EFA6C, a third member of the EFA6 family, in adult mouse Purkinje cells. J Neurochem 93, 674–685.

Mazaki Y, Nishimura Y, Sabe H (2012). GBF1 bears a novel phosphatidylinositol-phosphate binding module, BP3K, to link PI3Kgamma activity with Arf1 activation involved in GPCR-mediated neutrophil chemotaxis and superoxide production. Mol Biol Cell 23, 2457–2467.

McDonold CM, Fromme JC (2014). Four GTPases differentially regulate the Sec7 Arf-GEF to direct traffic at the *trans*-Golgi network. Dev Cell 30, 759–767.

Meissner JM, Bhatt JM, Lee E, Styers ML, Ivanova AA, Kahn RA, Sztul E (2018). The ARF guanine nucleotide exchange factor GBF1 is targeted to Golgi membranes through a PIP-binding domain. J Cell Sci 131, doi:10.1242/jcs.210245.

Mignot C, McMahon AC, Bar C, Campeau PM, Davidson C, Buratti J, Nava C, Jacquemont ML, Tallot M, Milh M, *et al*. (2018). IQSEC2-related encephalopathy in males and females: a comparative study including 37 novel patients. Genet Med, doi:10.1038/s41436-018-0268-1.

Miura K, Jacques KM, Stauffer S, Kubosaki A, Zhu KJ, Hirsch DS, Resau J, Zheng Y, Randazzo PA (2002). ARAP1: a point of convergence for Arf and Rho signaling. Mol Cell 9, 109–119.

Miyamoto Y, Torii T, Tago K, Tanoue A, Takashima S, Yamauchi J (2018). BIG1/Arfgef1 and Arf1 regulate the initiation of myelination by Schwann cells in mice. Sci Adv 4, eaar4471.

Monetta P, Slavin I, Romero N, Alvarez C (2007). Rab1b interacts with GBF1, modulates both ARF1 dynamics and COPI association. Mol Biol Cell 18, 2400–2410.

Moravec R, Conger KK, D'Souza R, Allison AB, Casanova JE (2012). BRAG2/GEP100/IQSec1 interacts with clathrin and regulates $\alpha5\beta1$ integrin endocytosis through activation of ADP ribosylation factor 5 (Arf5). J Biol Chem 287, 31138–31147.

Morishige M, Hashimoto S, Ogawa E, Toda Y, Kotani H, Hirose M, Wei S, Hashimoto A, Yamada A, Yano H, *et al*. (2008). GEP100 links epidermal growth factor receptor signalling to Arf6 activation to induce breast cancer invasion. Nat Cell Biol 10, 85–92.

Mossessova E, Corpina RA, Goldberg J (2003). Crystal structure of ARF1*Sec7 complexed with Brefeldin A and its implications for the guanine nucleotide exchange mechanism. Mol Cell 12, 1403–1411.
Mouratou B, Biou V, Joubert A, Cohen J, Shields DJ, Geldner N, Jurgens G, Melancon P, Cherfils J (2005). The domain architecture of large guanine nucleotide exchange factors for the small GTP-binding protein Arf. BMC Genomics 6, 20.
Mueller AG, Moser M, Kluge R, Leder S, Blum M, Buttner R, Joost HG, Schurmann A (2002). Embryonic lethality caused by apoptosis during gastrulation in mice lacking the gene of the ADP-ribosylation factor-related protein 1. Mol Cell Biol 22, 1488–1494.
Muller T, Stein U, Poletti A, Garzia L, Rothley M, Plaumann D, Thiele W, Bauer M, Galasso A, Schlag P, *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.
Munro S (2005). The Arf-like GTPase Arl1 and its role in membrane traffic. Biochem Soc Trans 33(Pt 4), 601–605.
Murray RZ, Stow JL (2014). Cytokine secretion in macrophages: SNAREs, Rabs, and membrane trafficking. Front Immunol 5, 538.
Nakayama K (2016). Regulation of cytokinesis by membrane trafficking involving small GTPases and the ESCRT machinery. Crit Rev Biochem Mol Biol 51, 1–6.
Natsume W, Tanabe K, Kon S, Yoshida N, Watanabe T, Torii T, Satake M (2006). SMAP2, a novel ARF GTPase-activating protein, interacts with clathrin and clathrin assembly protein and functions on the AP-1-positive early endosome/*trans*-Golgi network. Mol Biol Cell 17, 2592–2603.
Newman LE, Schiavon CR, Turn RE, Kahn RA (2017). The ARL2 GTPase regulates mitochondrial fusion from the intermembrane space. Cell Logist 7, e1340104.
Newman LE, Zhou CJ, Mudigonda S, Mattheyses AL, Paradies E, Marobbio CM, Kahn RA (2014). The ARL2 GTPase is required for mitochondrial morphology, motility, and maintenance of ATP levels. PLoS One 9, e99270.
Nie ZZ, Stanley KT, Stauffer S, Jacques KM, Hirsch DS, Takei J, Randazzo PA (2002). AGAP1, an endosome-associated, phosphoinositide-dependent ADP-ribosylation factor GTPase-activating protein that affects actin cytoskeleton. J Biol Chem 277, 48965–48975.
Nishimoto-Morita K, Shin HW, Mitsuhashi H, Kitamura M, Zhang Q, Johannes L, Nakayama K (2009). Differential effects of depletion of ARL1 and ARFRP1 on membrane trafficking between the *trans*-Golgi network and endosomes. J Biol Chem 284, 10583–10592.
Nishiya N, Kiosses WB, Han JW, Ginsberg MH (2005). An alpha(4) integrin-paxillin-Arf-GAP complex restricts Rac activation to the leading edge of migrating cells. Nat Cell Biol 7, U343–U347.
Oh SJ, Santy LC (2010). Differential effects of cytohesins 2 and 3 on beta1 integrin recycling. J Biol Chem 285, 14610–14616.
Oh SJ, Santy LC (2012). Phosphoinositide specificity determines which cytohesins regulate beta1 integrin recycling. J Cell Sci 125(Pt 13), 3195–3201.
Onodera Y, Hashimoto S, Hashimoto A, Morishige M, Mazaki Y, Yamada A, Ogawa E, Adachi M, Sakurai T, Manabe T, *et al.* (2005). Expression of AMAP1, an ArfGAP, provides novel targets to inhibit breast cancer invasive activities. EMBO J 24, 963–973.
Ooi CE, Dell'Angelica EC, Bonifacino JS (1998). ADP-Ribosylation factor 1 (ARF1) regulates recruitment of the AP-3 adaptor complex to membranes. J Cell Biol 142(2), 391–402.
Pacheco-Rodriguez G, Moss J, Vaughan M (2002). BIG1 and BIG2: brefeldin A-inhibited guanine nucleotide-exchange proteins for ADP-ribosylation factors. Methods Enzymol 345, 397–404.
Padilla PI, Pacheco-Rodriguez G, Moss J, Vaughan M (2004). Nuclear localization and molecular partners of BIG1, a brefeldin A-inhibited guanine nucleotide-exchange protein for ADP-ribosylation factors. Proc Natl Acad Sci USA 101, 2752–2757.
Padilla PI, Uhart M, Pacheco-Rodriguez G, Peculis BA, Moss J, Vaughan M (2008). Association of guanine nucleotide-exchange protein BIG1 in HepG2 cell nuclei with nucleolin, U3 snoRNA, and fibrillarin. Proc Natl Acad Sci USA 105, 3357–3361.
Padovani D, Folly-Klan M, Labarde A, Boulakirba S, Campanacci V, Franco M, Zeghouf M, Cherfils J (2014). EFA6 controls Arf1 and Arf6 activation through a negative feedback loop. Proc Natl Acad Sci USA 111, 12378–12383.
Pan T, Sun J, Zhou J, Fu Z, Hu Y, Zheng S, Zhang S (2013). Function and mode of action of cytohesins in the epidermal growth factor pathway in colorectal cancer cells. Oncol Lett 5, 521–526.
Panic B, Whyte JR, Munro S (2003). The ARF-like GTPases Arl1p and Arl3p act in a pathway that interacts with vesicle-tethering factors at the Golgi apparatus. Curr Biol 13, 405–410.
Pasqualato S, Renault L, Cherfils J (2002). Arf, Arl, Arp and Sar proteins: a family of GTP-binding proteins with a structural device for "front-back" communication. EMBO Rep 3, 1035–1041.
Patel M, Chiang T-C, Tran V, Lee F-JS, Côté J-F (2011). The Arf family GTPase Arl4A complexes with ELMO proteins to promote actin cytoskeleton remodeling and reveals a versatile Ras-binding domain in the ELMO proteins family. J Biol Chem 286, 38969–38979.
Pearring JN, San Agustin JT, Lobanova ES, Gabriel CJ, Lieu EC, Monis WJ, Stuck MW, Strittmatter L, Jaber SM, Arshavsky VY, *et al.* (2017). Loss of Arf4 causes severe degeneration of the exocrine pancreas but not cystic kidney disease or retinal degeneration. PLoS Genet 13, e1006740.
Peurois F, Veyron S, Ferrandez Y, Ladid I, Benabdi S, Zeghouf M, Peyroche G, Cherfils J (2017). Characterization of the activation of small GTPases by their GEFs on membranes using artificial membrane tethering. Biochem J 474, 1259–1272.
Peyroche A, Antonny B, Robineau S, Acker J, Cherfils J, Jackson CL (1999). Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain. Mol Cell 3, 275–285.
Peyroche A, Paris S, Jackson CL (1996). Nucleotide exchange on ARF mediated by yeast Gea1 protein. Nature 384, 479–481.
Pocognoni CA, Viktorova EG, Wright J, Meissner JM, Sager G, Lee E, Belov GA, Sztul E (2018). Highly conserved motifs within the large Sec7 ARF guanine nucleotide exchange factor GBF1 target it to the Golgi and are critical for GBF1 activity. Am J Physiol Cell Physiol 314, C675–C689.
Porter AP, Papaioannou A, Malliri A (2016). Deregulation of Rho GTPases in cancer. Small GTPases 7, 123–138.
Qi Q, Kang SS, Zhang S, Pham C, Fu H, Brat DJ, Ye K (2017). Co-amplification of phosphoinositide 3-kinase enhancer A and cyclin-dependent kinase 4 triggers glioblastoma progression. Oncogene 36, 4562–4572.
Randazzo PA, Andrade J, Miura K, Brown MT, Long YQ, Stauffer S, Roller P, Cooper JA (2000). The Arf GTPase-activating protein ASAP1 regulates the actin cytoskeleton. Proc Natl Acad Sci USA 97, 4011–4016.
Randazzo PA, Hirsch DS (2004). Arf GAPs: multifunctional proteins that regulate membrane traffic and actin remodelling. Cell Signal 16, 401–413.
Randazzo PA, Inoue H, Bharti S (2007). Arf GAPs as regulators of the actin cytoskeleton. Biol Cell 99, 583–600.
Ratcliffe CDH, Siddiqui N, Coelho PP, Laterreur N, Cookey TN, Sonenberg N, Park M (2018). HGF-induced migration depends on the PI(3,4,5)P3-binding microexon-spliced variant of the Arf6 exchange factor cytohesin-1. J Cell Biol, doi:10.1083/jcb.201804106.
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.
Renault L, Guibert B, Cherfils J (2003). Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. Nature 426, 525–530.
Reviriego-Mendoza MM, Santy LC (2015). The cytohesin guanosine exchange factors (GEFs) are required to promote HGF-mediated renal recovery after acute kidney injury (AKI) in mice. Physiol Rep 3, e12442.
Rodiger M, Werno MW, Wilhelmi I, Baumeier C, Hesse D, Wettschureck N, Offermanns S, Song K, Krauss M, Schurmann A (2018). Adiponectin release and insulin receptor targeting share *trans*-Golgi-dependent endosomal trafficking routes. Mol Metab 8, 167–179.
Rosa-Ferreira C, Christis C, Torres IL, Munro S (2015). The small G protein Arl5 contributes to endosome-to-Golgi traffic by aiding the recruitment of the GARP complex to the Golgi. Biol Open 4, 474–481.
Roy NS, Yohe ME, Randazzo PA, Gruschus JM (2016). Allosteric properties of PH domains in Arf regulatory proteins. Cell Logist 6, e1181700.
Sakagami H, Suzuki H, Kamata A, Owada Y, Fukunaga K, Mayanagi H, Kondo H (2006). Distinct spatiotemporal expression of EFA6D, a guanine nucleotide exchange factor for ARF6, among the EFA6 family in mouse brain. Brain Res 1093, 1–11.
Sakurai A, Jian X, Lee CJ, Manavski Y, Chavakis E, Donaldson J, Randazzo PA, Gutkind JS (2011). Phosphatidylinositol-4-phosphate 5-kinase and GEP100/Brag2 protein mediate antiangiogenic signaling by semaphorin 3E-plexin-D1 through Arf6 protein. J Biol Chem 286, 34335–34345.
Salem JC, Reviriego-Mendoza MM, Santy LC (2015). ARF-GEF cytohesin-2/ARNO regulates R-Ras and α5-integrin recycling through an EHD1-positive compartment. Mol Biol Cell 26, 4265–4279.
Santy LC, Casanova JE (2001). Activation of ARF6 by ARNO stimulates epithelial cell migration through downstream activation of both Rac1 and phospholipase D. J Cell Biol 154, 599–610.

Santy LC, Ravichandran KS, Casanova JE (2005). The DOCK180/Elmo complex couples ARNO-mediated Arf6 activation to the downstream activation of Rac1. Curr Biol 15, 1749–1754.

Scheffzek K, Ahmadian MR, Wittinghofer A (1998). GTPase-activating proteins: helping hands to complement an active site. Trends Biochem Sci 23, 257–262.

Schiavon CR, Griffin ME, Pirozzi M, Parashuraman R, Zhou W, Jinnah HA, Reines D, Kahn RA (2018). Compositional complexity of rods and rings. Mol Biol Cell 29, 2303–2316.

Schlacht A, Mowbrey 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.

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.

Schrick JJ, Vogel P, Abuin A, Hampton B, Rice DS (2006). ADP-ribosylation factor-like 3 is involved in kidney and photoreceptor development. Am J Pathol 168, 1288–1298.

Schurmann A, Koling S, Jacobs S, Saftig P, Krauss S, Wennemuth G, Kluge R, Joost HG (2002). Reduced sperm count and normal fertility in male mice with targeted disruption of the ADP-ribosylation factor-like 4 (Arl4) gene. Mol Cell Biol 22, 2761–2768.

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.

Serafini T, Orci L, Amherdt M, Brunner M, Kahn RA, Rothman JE (1991). ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for a GTP-binding protein. Cell 67, 239–253.

Setty SR, Shin ME, Yoshino A, Marks MS, Burd CG (2003). Golgi recruitment of GRIP domain proteins by Arf-like GTPase 1 is regulated by Arf-like GTPase 3. Curr Biol 13, 401–404.

Sharer JD, Kahn RA (1999). The ARF-like 2 (ARL2)-binding protein, BART. Purification, cloning, and initial characterization. J Biol Chem 274, 27553–27561.

Sheen VL, Ganesh VS, Topcu M, Sebire G, Bodell A, Hill RS, Grant PE, Shugart YY, Imitola J, Khoury SJ, *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.

Shiba Y, Luo R, Hinshaw JE, Szul T, Hayashi R, Sztul E, Nagashima K, Baxa U, Randazzo PA (2011). ArfGAP1 promotes COPI vesicle formation by facilitating coatomer polymerization. Cell Logist 1, 139–154.

Shiba Y, Randazzo PA (2012). ArfGAP1 function in COPI mediated membrane traffic: currently debated models and comparison to other coat-binding ArfGAPs. Histol Histopathol 27, 1143–1153.

Shin HW, Kobayashi H, Kitamura M, Waguri S, Suganuma T, Uchiyama Y, Nakayama K (2005). Roles of ARFRP1 (ADP-ribosylation factor-related protein 1) in post-Golgi membrane trafficking. J Cell Sci 118(Pt 17), 4039–4048.

Shinotsuka C, Waguri S, Wakasugi M, Uchiyama Y, Nakayama K (2002a). Dominant-negative mutant of BIG2, an ARF-guanine nucleotide exchange factor, specifically affects membrane trafficking from the *trans*-Golgi network through inhibiting membrane association of AP-1 and GGA coat proteins. Biochem Biophys Res Commun 294, 254–260.

Shinotsuka C, Yoshida Y, Kawamoto K, Takatsu H, Nakayama K (2002b). Overexpression of an ADP-ribosylation factor-guanine nucleotide exchange factor, BIG2, uncouples brefeldin A-induced adaptor protein-1 coat dissociation and membrane tubulation. J Biol Chem 277, 9468–9473.

Spang A, Saw JH, Jorgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C, Guy L, Ettema TJ (2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521, 173–179.

Spang A, Shiba Y, Randazzo PA (2010). Arf GAPs: gatekeepers of vesicle generation. FEBS Lett 584, 2646–2651.

Sparrer KMJ, Gableske S, Zurenski MA, Parker ZM, Full F, Baumgart GJ, Kato J, Pacheco-Rodriguez G, Liang C, Pornillos O, *et al.* (2017). TRIM23 mediates virus-induced autophagy via activation of TBK1. Nat Microbiol 2, 1543–1557.

Stacey TTI, Nie ZZ, Stewart A, Najdovska M, Hall NE, He H, Randazzo PA, Lock P (2004). ARAP3 is transiently tyrosine phosphorylated in cells attaching to fibronectin and inhibits cell spreading in a RhoGAP-dependent manner. J Cell Sci 117, 6071–6084.

Stalder D, Antonny B (2013). Arf GTPase regulation through cascade mechanisms and positive feedback loops. FEBS Lett 587, 2028–2035.

Stamnes MA, Rothman JE (1993). The binding of AP-1 clathrin adaptor particles to Golgi membranes requires ADP-ribosylation factor, a small GTP-binding protein. Cell 73, 999–1005.

Suckling RJ, Poon PP, Travis SM, Majoul IV, Hughson FM, Evans PR, Duden R, Owen DJ (2015). Structural basis for the binding of tryptophan-based motifs by delta-COP. Proc Natl Acad Sci USA 112, 14242–14247.

Suzuki T, Kanai Y, Hara T, Sasaki J, Sasaki T, Kohara M, Maehama T, Taya C, Shitara H, Yonekawa H, *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, Lowe M, Sztul E (2007). Dissecting the role of the ARF guanine nucleotide exchange factor GBF1 in Golgi biogenesis and protein trafficking. J Cell Sci 120(Pt 22), 3929–3940.

Takashima K, Saitoh A, Hirose S, Nakai W, Kondo Y, Takasu Y, Kakeya H, Shin HW, Nakayama K (2011). GBF1-Arf-COPI-ArfGAP-mediated Golgi-to-ER transport involved in regulation of lipid homeostasis. Cell Struct Funct 36, 223–235.

Tanabe K, Torii T, Natsume W, Braesch-Andersen S, Watanabe T, Satake M (2005). A novel GTPase-activating protein for ARF6 directly interacts with clathrin and regulates clathrin-dependent endocytosis. Mol Biol Cell 16, 1617–1628.

Torii T, Miyamoto Y, Sanbe A, Nishimura K, Yamauchi J, Tanoue A (2010). Cytohesin-2/ARNO, through its interaction with focal adhesion adaptor protein paxillin, regulates preadipocyte migration via the downstream activation of Arf6. J Biol Chem 285, 24270–24281.

Traub LM, Ostrom JA, Kornfeld S (1993). Biochemical dissection of AP-1 recruitment onto Golgi membranes. J Cell Biol 123, 561–573.

Turner CE, West KA, Brown MC (2001). Paxillin-ARF GAP signaling and the cytoskeleton. Curr Opin Cell Biol 13, 593–599.

Van Valkenburgh H, Shern JF, Sharer JD, Zhu X, Kahn RA (2001). ADP-ribosylation factors (ARFs) and ARF-like 1 (ARL1) have both specific and shared effectors: characterizing ARL1-binding proteins. J Biol Chem 276, 22826–22837.

Venkateswarlu K, Gunn-Moore F, Oatey PB, Tavare JM, Cullen PJ (1998a). Nerve growth factor- and epidermal growth factor-stimulated translocation of the ADP-ribosylation factor-exchange factor GRP1 to the plasma membrane of PC12 cells requires activation of phosphatidylinositol 3-kinase and the GRP1 pleckstrin homology domain. Biochem J 335(Pt 1), 139–146.

Venkateswarlu K, Oatey PB, Tavare JM, Cullen PJ (1998b). Insulin-dependent translocation of ARNO to the plasma membrane of adipocytes requires phosphatidylinositol 3-kinase. Curr Biol 8, 463–466.

Vichi A, Moss J, Vaughan M (2005). ADP-ribosylation factor domain protein 1 (ARD1), a multifunctional protein with ubiquitin E3 ligase, GAP, and ARF domains. Methods Enzymol 404, 195–206.

Vitali T, Girald-Berlingeri S, Randazzo PA, Chen PW (2017). Arf GAPs: a family of proteins with disparate functions that converge on a common structure, the integrin adhesion complex. Small GTPases, doi:10.1080/21541248.2017.1299271.

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.

Watanabe M, Takahashi H, Saeki Y, Ozaki T, Itoh S, Suzuki M, Mizushima W, Tanaka K, Hatakeyama S (2015). The E3 ubiquitin ligase TRIM23 regulates adipocyte differentiation via stabilization of the adipogenic activator PPARgamma. Elife 4, e05615.

Weimer C, Beck R, Eckert P, Reckmann I, Moelleken J, Brugger B, Wieland F (2008). Differential roles of ArfGAP1, ArfGAP2, and ArfGAP3 in COPI trafficking. J Cell Biol 183, 725–735.

West MA, Bright NA, Robinson MS (1997). The role of ADP-ribosylation factor and phospholipase D in adaptor recruitment. J Cell Biol 138, 1239–1254.

White DT, McShea KM, Attar MA, Santy LC (2010). GRASP and IPCEF promote ARF-to-Rac signaling and cell migration by coordinating the association of ARNO/cytohesin 2 with Dock180. Mol Biol Cell 21, 562–571.

Wiens CJ, Tong Y, Esmail MA, Oh E, Gerdes JM, Wang J, Tempel W, Rattner JB, Katsanis N, Park HW, *et al.* (2010). Bardet-Biedl syndrome-associated small GTPase ARL6 (BBS3) functions at or near the ciliary gate and modulates Wnt signaling. J Biol Chem 285, 16218–16230.

Wright J, Kahn RA, Sztul E (2014). Regulating the large Sec7 ARF guanine nucleotide exchange factors: the when, where and how of activation. Cell Mol Life Sci 71, 3419–3438.

Wright ZC, Loskutov Y, Murphy D, Stoilov P, Pugacheva E, Goldberg AFX, Ramamurthy V (2018). ADP-ribosylation factor-like 2 (ARL2) regulates cilia stability and development of outer segments in rod photoreceptor neurons. Sci Rep 8, 16967.

Yang JS, Lee SY, Gao MG, Bourgoin S, Randazzo PA, Premont RT, Hsu VW (2002). ARFGAP1 promotes the formation of COPI vesicles, suggesting function as a component of the coat. J Cell Biol 159, 69–78.
Yano H, Kobayashi I, Onodera Y, Luton F, Franco M, Mazaki Y, Hashimoto S, Iwai K, Ronai Z, Sabe H (2008). Fbx8 makes Arf6 refractory to function via ubiquitination. Mol Biol Cell 19, 822–832.
Ye F, Nager AR, Nachury MV (2018). BBSome trains remove activated GPCRs from cilia by enabling passage through the transition zone. J Cell Biol 217, 1847–1868.
Yendamuri S, Trapasso F, Calin GA (2008). ARLTS1—a novel tumor suppressor gene. Cancer Lett 264, 11–20.
Yin GY, Zheng QL, Yan C, Berk BC (2005). GIT1 is a scaffold for ERK1/2 activation in focal adhesions. J Biol Chem 280, 27705–27712.
Yoo JH, Shi DS, Grossmann AH, Sorensen LK, Tong Z, Mleynek TM, Rogers A, Zhu W, Richards JR, Winter JM, *et al.* (2016). ARF6 is an actionable node that orchestrates oncogenic GNAQ signaling in uveal melanoma. Cancer Cell 29, 889–904.
Yoon HY, Lee JS, Randazzo PA (2008). ARAP1 regulates endocytosis of EGFR. Traffic 9, 2236–2252.
Yoon HY, Miura K, Cuthbert EJ, Davis KK, Ahvazi B, Casanova JE, Randazzo PA (2006). ARAP2 effects on the actin cytoskeleton are dependent on Arf6-specific GTPase-activating-protein activity and binding to RhoA-GTP. J Cell Sci 119, 4650–4666.
Yu C-J, Lee F-JS (2017). Multiple activities of Arl1 GTPase in the *trans*-Golgi network. J Cell Sci 130, 1691–1699.
Yu X, Breitman M, Goldberg J (2012). A structure-based mechanism for Arf1-dependent recruitment of coatomer to membranes. Cell 148, 530–542.
Zahn C, Hommel A, Lu L, Hong W, Walther DJ, Florian S, Joost HG, Schurmann A (2006). Knockout of Arfrp1 leads to disruption of ARF-like1 (ARL1) targeting to the *trans*-Golgi in mouse embryos and HeLa cells. Mol Membr Biol 23, 475–485.
Zahn C, Jaschke A, Weiske J, Hommel A, Hesse D, Augustin R, Lu L, Hong W, Florian S, Scheepers A, *et al.* (2008). ADP-ribosylation factor-like GTPase ARFRP1 is required for *trans*-Golgi to plasma membrane trafficking of E-cadherin. J Biol Chem 283, 27179–27188.
Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Backstrom D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, *et al.* (2017). Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature 541, 353–358.
Zhang CJ, Cavenagh MM, Kahn RA (1998). A family of Arf effectors defined as suppressors of the loss of Arf function in the yeast *Saccharomyces-cerevisiae*. J Biol Chem 273, 19792–19796.
Zhang CJ, Rosenwald AG, Willingham MC, Skuntz S, Clark J, Kahn RA (1994). Expression of a dominant allele of human ARF1 inhibits membrane traffic in vivo. J Cell Biol 124, 289–300.
Zhang Q, Hu J, Ling K (2013). Molecular views of Arf-like small GTPases in cilia and ciliopathies. Exp Cell Res 319, 2316–2322.
Zhang Q, Nishimura D, Seo S, Vogel T, Morgan DA, Searby C, Bugge K, Stone EM, Rahmouni K, Sheffield VC (2011). Bardet-Biedl syndrome 3 (Bbs3) knockout mouse model reveals common BBS-associated phenotypes and Bbs3 unique phenotypes. Proc Natl Acad Sci USA 108, 20678–20683.
Zhao X, Claude A, Chun J, Shields DJ, Presley JF, Melancon P (2006). GBF1, a *cis*-Golgi and VTCs-localized ARF-GEF, is implicated in ER-to-Golgi protein traffic. J Cell Sci 119(Pt 18), 3743–3753.
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.
Zhao ZS, Manser E, Loo TH, Lim L (2000). Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. Mol Cell Biol 20, 6354–6363.
Zhou C, Cunningham L, Marcus AI, Li Y, Kahn RA (2006). Arl2 and Arl3 regulate different microtubule-dependent processes. Mol Biol Cell 17, 2476–2487.
Zhou W, Li XB, Premont RT (2016). Expanding functions of GIT Arf GTPase-activating proteins, PIX Rho guanine nucleotide exchange factors and GIT-PIX complexes. J Cell Sci 129, 1963–1974.