Published in final edited form as:

Cell Mol Life Sci. 2014 September ; 71(18): 3419–3438. doi:10.1007/s00018-014-1602-7.

Regulating the large Sec7 ARF guanine nucleotide exchange factors: the when, where and how of activation

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Abstract

Eukaryotic cells require selective sorting and transport of cargo between intracellular compartments. This is accomplished at least in part by vesicles that bud from a donor compartment, sequestering a subset of resident protein “cargos” destined for transport to an acceptor compartment. A key step in vesicle formation and targeting is the recruitment of specific proteins that form a coat on the outside of the vesicle in a process requiring the activation of regulatory GTPases of the ARF family. Like all such GTPases, ARFs cycle between inactive, GDP-bound, and membrane-associated active, GTP-bound, conformations. And like most regulatory GTPases the activating step is slow and thought to be rate limiting in cells, requiring the use of ARF guanine nucleotide exchange factor (GEFs). ARF GEFs are characterized by the presence of a conserved, catalytic Sec7 domain, though they also contain motifs or additional domains that confer specificity to localization and regulation of activity. These domains have been used to define and classify five different sub-families of ARF GEFs. One of these, the BIG/GBF1 family, includes three proteins that are each key regulators of the secretory pathway. GEF activity initiates the coating of nascent vesicles via the localized generation of activated ARFs and thus these GEFs are the upstream regulators that define the site and timing of vesicle production. Paradoxically, while we have detailed molecular knowledge of how GEFs activate ARFs, we know very little about how GEFs are recruited and/or activated at the right time and place to initiate transport. This review summarizes the current knowledge of GEF regulation and explores the still uncertain mechanisms that position GEFs at “budding ready” membrane sites to generate highly localized activated ARFs.

Keywords

Membrane traffic; ARF GTPases; Guanine nucleotide exchange factors (GEFs); COP-I and clathrin coats; Adaptor proteins

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Introduction

Eukaryotic cells contain numerous membrane-enclosed compartments, each with a distinct biochemical function and composition. Proteins and lipids are continuously exchanged between these compartments, and this process is largely mediated through vesicular traffic. This traffic occurs via transport vesicles (typically 50–100 nm in diameter) that bud from the donor membrane, translocate through the cell and then fuse with a specific acceptor compartment. The integrity and specificity of this process is essential to the maintenance of distinct compartments/organelles in eukaryotes and the organism as a whole, as evidenced by the hundreds of human disorders that result from defects in membrane traffic [1, 2]. The formation of a vesicle is a complex process that involves the sequestration of cargo proteins and vesicle identifiers into a nascent bud in a process that includes the deformation of the planar membrane and eventually the scission of the mature bud from the donor membrane. At the core of these events is the recruitment of proteinaceous coats onto the cytoplasmic aspects of the nascent buds to facilitate both protein sequestration and membrane deformation to produce a vesicle. Directed transport of the mature carriers, docking and fusion at the appropriate acceptor membrane, and recycling of needed components to continue this process are also key steps in this constitutive process in all eukaryotic cells but are not discussed in any detail in this review.

One assumes (albeit this has not been experimentally tested) that vesicles are formed only when transport between compartments is necessary and when all the machinery required for transport is available. This implies that the cell has mechanisms for sensing the growing need for vesicle generation in each compartment, the availability of sufficient vesicle budding factors and other traffic regulators, and the means to convey that information to the relevant initiators of vesicle budding to initiate vesicle formation at the relevant site. Members of the ARF family of regulatory GTPases have been found to be key components in the initiation process and they in turn are thought to be activated in cells by ARF guanine nucleotide exchange factors (GEFs). Yet, our understanding of how ARF GEFs are “notified” to initiate coating in time and space is incomplete. This contrasts with the wealth of knowledge on the regulation of GEFs for other small GTPases such as Ras and the trimeric G proteins. Indeed, members of the large family of heptahelical G protein coupled receptors (GPCRs) are GEFs that activate G proteins via promotion of guanine nucleotide exchange on the alpha subunits and are the paradigm for all GEFs. GPCRs are ligand stimulated GEFs, allowing acute regulation of their activity. Their localization on the plasma membrane and specificity for both ligand activators and downstream signaling activation has made them ideal targets for drug development. For these reasons we believe ARF GEFs are also strong candidates for drug discovery, though efforts toward this end are currently hampered by our limited understanding of the activation process for ARF GEFs. As ARF GEFs operate most commonly on intracellular membranes, the development of drugs that

can gain access to them is more challenging, but the identification of several agents that specifically target intracellular ARF GEFs (e.g.: brefeldin A, Golgicide, LG186 [3-5]) is proof of concept and we hope propels additional attempts to drug this important family of cell regulators. Can we utilize paradigms developed from those earlier studies of other GTPases and apply them to ARFs and ARF GEFs to discover key regulatory aspects of their function? With numerous human diseases with links to defects in membrane traffic it should be obvious that ARF GEFs represent outstanding pharmacological targets. However, lacking biologically relevant “ligands” we will have to work in reverse, from the GTPase to the GEF to the initiator that regulates its function.

ARF GEFs are divided into five families, based on domain organization and conservation of primary amino acid sequence: GBF/BIG, cytohesins, EFA6, BRAGs, and F-box (reviewed in [6]). Each family contains multiple members with a total of 16 genes in humans. The GEFs involved in vesicular traffic belong to the GBF1/BIG family and all are sensitive to brefeldin A (BFA). Only this family of GEFs is present in all eukaryotes, in keeping with their fundamental roles in secretion and organellogenesis. The BFA-sensitive GEFs are large proteins (>200 kDa) and thus we use the terms large GEFs and GBF1/BIG family interchangeably herein.

In this review we will summarize the current knowledge of how the GBF1/BIG class of GEFs are regulated in the immediate and long-term to respond to cellular demands for vesicle traffic. We discuss issues regarding specificity for their ARF substrates, mechanisms by which the large GEFs are recruited to membranes, the paradoxical role of the active GTPases in GEF recruitment, and roles for lipids and cargo in recruitment of the GEFs. We then turn to the regulation of the GEF activity itself and the roles of intrinsic and extrinsic factors in that regulation. Throughout this review we pose key outstanding questions regarding GEF regulation that remain to be experimentally resolved or even addressed.

Specificity of ARF activation by GEFs

ARFs were originally discovered as auxiliary agents in cholera-toxin mediated ADP-ribosylation of the α -subunit of heterotrimeric G_s protein [7-14]. This activity of ARFs was found to be absent in the closely related (35–60 % identical) members of the ARF-like proteins (ARLs) family [15]. Along with SAR1, the ARFs and ARLs comprise the ARF family [16]. These proteins were present in the earliest eukaryotes and have been predicted to have arisen in prokaryotes [17]. ARFs have been shown to regulate membrane traffic both directly through recruitment of protein adaptors (the focus of this review) and perhaps less directly via regulation of lipid modifying enzymes that contribute to membrane deformation or signaling. Some of the other non-ARF members of the family (most notably ARL1, ARFRP1, and SAR1) also regulate aspects of integrity or morphology of organelles in the secretory pathway and regulate membrane traffic, though these roles and activities are not discussed in any detail herein.

Mammalian cells contain six ARFs, designated ARF1-6 (ARF2 is absent in humans). The six ARFs are grouped into three classes based on sequence homology at the genomic and protein level and phylogenetic analyses [18-20]. Class I contains ARF1, ARF2, and ARF3

that share ~96 % identity. Class II contains ARF4 and ARF5 that share ~90 % identity and ~80 % identity with other ARFs. ARF6 constitutes the sole member of Class III and is the least similar to the other ARFs, though still sharing >60 % identity with the others. The high degree of conservation of ARFs in evolution is evident by the 74 % primary sequence identity between human and *S. cerevisiae* ARFs as well as their functional conservation [21]. Class I and class II ARFs are evolutionarily conserved and at least one member of each class has been described in all eukaryotic organisms. Class I and II ARFs primarily localize to the ER Golgi intermediate compartment (ERGIC), the Golgi, the *trans*-Golgi network (TGN) and endosomes, and function in regulating membrane traffic. In contrast, the sole class III ARF, ARF6, localizes to the plasma membrane and regulates actin dynamics and endocytosis. The greater divergence in primary sequence of ARF6, together with its known functions, suggests a late evolutionary origin. Indeed, ARF6 appears to be unique to metazoans [22].

Despite the evidence supporting these generalizations about sites of localization and actions of the different ARFs caution is advised in assuming, for example, that if an ARF is involved at the cell surface it must be ARF6. One reason for this uncertainty is that ARFs can be very abundant proteins, e.g., representing between 0.1 and 1.0 % of total brain protein. Another is that the specificity with which ARF GEFs act on GTPase substrates may not always be conserved between their actions in cells and in *in vitro* assays. As highlighted below the *in vitro* ARF GEF assays typically depend upon use of the isolated Sec7d or require other accommodations that compromise the specificity that is so important in cells.

Like all RAS superfamily GTPases, ARFs cycle between a GDP-bound “inactive” conformation and a GTP-bound “active” conformation, though it is more accurate to simply state that the two conformations have different affinities for binding partners. The main differences in these conformational states are found in two regions termed switch I and II, found in all regulatory GTPases as well as an “inters-witch toggle” found only in the ARF family [23, 24]. ARFs are also unique in having a third nucleotide-sensitive region, an amphipathic alpha helix at the very N-terminus that includes and is stabilized by the co-translational and covalent addition of myristic acid on the N-terminal glycine [25, 26]. *N*-myristoylation is essential to ARF actions and confers a lipid/membrane dependence to the activation process. The translocation of ARF from cytosol to the membrane surface is coincident with activation, and is closely linked to the binding and activity of GEFs. ARF activation is mediated by nucleotide exchange, in which the off-rate of GDP is increased and GTP is then free to enter the empty site. The release of GDP is catalyzed by the highly conserved Sec7 domain (Sec7d) present in all ARF GEFs and originally identified in the yeast protein Sec7p [27]. The Sec7d alone is sufficient for catalysis of nucleotide exchange on ARFs. The mechanism of nucleotide exchange on ARFs has been described in exquisite structural detail in work from the Cherfils lab and others [24, 28-34].

Structural studies of the ~200 amino acid Sec7d from various species show ten α -helices, designated A–J, which condense in the tertiary structure to form two sub-domains. The first of these is a superhelix of the seven N-terminal alpha helices and the second forms a dense bundle immediately C-terminal to the superhelix. The crystal structures of the Sec7d from human BIG1 (PDB ID: 3LTL), BIG2 (PDB ID: 3L8N), and the yeast homologues of GBF1,

Gea1p (PDB ID: 1RE0) and Gea2p (PDB ID: 1KU1), have been solved and offer us detailed views of both the conserved features and differences.

ARF activation by the Sec7d has been characterized in great structural detail. The current model for the catalytic sequence begins with the binding of the two switch regions in ARF-GDP to a hydrophobic groove on the surface of the Sec7d at the junction of the two sub-domains. This binding triggers a conformational change in ARF that brings the nucleotide binding site into close proximity of a key, highly conserved glutamate residue in the Sec7d, often referred to as the “glutamic finger” (this residue is indicated in Fig. 1 as E794). This juxtaposes the glutamate with the β -phosphate of GDP. The mutual electrostatic repulsion of the glutamate and phosphate expels the GDP from the nucleotide binding site. GTP then enters the empty nucleotide binding site of ARF and in so doing lowers its affinity for the Sec7d, resulting in its dissociation.

The large GEFs show selectivity for their ARF substrates. Experimental evidence shows GBF1 acts preferentially with class I and class II ARFs (ARF1, ARF3, ARF4, and ARF5) [35], while BIG1 and BIG2 appear to preferentially activate class I ARFs (ARF1 and ARF3) [36,37]. None of the large GEFs appears to activate class III ARF6. The selectivity of the process is remarkable: despite the fact that ARF1 and ARF3 differ at only seven amino acids in their entire sequence [38], GBF1 interacts with ARF1 with much greater efficiency than ARF3 [35]. It is worth noting that there might be differences in substrates for GBF1 when assessed by different assays. In vitro assays using crude preparations of GBF1 and ARFs show selectivity to ARF1 and ARF5 [35]. However, in vivo data indicate that GBF1 preferentially binds ARF1 and ARF4 [39]. The detailed mechanism(s) governing the selectivity of GEFs in recognizing their ARF substrates is unknown. Members of the large GEF family also contain several highly conserved regions in addition to the catalytic Sec7d (Fig. 1). As discussed in more detail below there is growing and already convincing evidence that sequences outside of the Sec7d play important regulatory roles in ARF activation, much of which was lost in earlier studies that employed the isolated Sec7d. This situation, often dictated by the large size and lack of suitable preparations of full length ARF GEFs, has begun to change.

Specificity among the six mammalian ARFs for GEFs, GAPs and effectors, is a complicated and poorly understood issue. That these interactions all occur on biological membranes and that the lipid composition likely plays an important part further complicate attempts to resolve the issues. In addition, ARFs may operate at least at times in tandem, as evidenced by the more severe phenotypes observed with dual knockdowns by siRNAs than with any single ARF siRNA [40]. One possible scenario that may help us understand this observation would be if two different ARFs are capable of binding to the Sec7d's in an ARF GEF dimer. Finally, an issue that will need to be further analyzed is the mechanism(s) involved in recruiting ARFs to membranes. Because we know that activated ARFs have higher affinity for membranes than the GDP-bound ARFs it has been assumed that the actions of GEFs lead to the stable association of ARFs during the activation process. While we don't dispute a role for ARF GEFs in such recruitment, there is a growing appreciation for the role played by a smaller, nucleating pool of ARF or ARF family members that may participate in recruiting or activating the GEF. Binding sites for ARF family members on GEFs that are

outside of the Sec7d are likely contributors to recruitment of the activators. Whether distinct mechanisms are involved in recruiting these two pools of ARFs, or whether perhaps every membrane has on it small pools of loosely bound ARFs, and whether different ARFs play distinct roles are currently poorly understood. Another key question regarding GEF selectivity is whether the GEFs select specific ARFs for activation (assuming that there are multiple possible ARF substrates on the membrane) or whether the ARFs are pre-selected by some other mechanism and the GEFs merely activate whatever ARF is in their proximity. We believe that a better understanding of these questions and the nature of the signal(s) involved in ARF GEF activation, the “ligand equivalents” to the GPCRs, will provide important new information that is essential to an understanding of the cell biology of membrane traffic and its potential for chemotherapeutic interventions. We focus here on the roles of ligand equivalents as activators of the respective GEFs, as we presume that the nature of the GPCR ligands and the ARF GEF “ligands” differs in structure and localization. As we suggest below, ARF GEFs are likely impacted by a collection of low affinity molecular interactions that modulate activity in aggregate and may include protein and lipid components. In contrast, individual GPCRs are usually activated by high affinity ligand binding. While these differences are notable, it remains likely that both classes of GEF need highly localized activators that contribute to the specificity of the generated signal.

Localization of large GEFs to subcellular compartments

The mammalian large GEFs show distinct localization patterns: GBF1 localizes to ER exit sites (ERES), the ER-Golgi intermediate compartment (ERGIC), *cis*- and medial Golgi stacks [35, 41-45]. The presence of GBF1 in more than one compartment raises the interesting question of how GEFs recognize analogous and dissimilar membrane surfaces. It is possible that GBF1 recruitment to diverse membranes is mediated through one common molecular mechanism but we cannot yet exclude the involvement of different components at each site. BIG1 and BIG2 primarily localize to the TGN [42, 46, 47]. However, BIG2 is also found at recycling endosomes, late endosomes, neuronal axons, and both pre-synaptic and post-synaptic membranes [48-51]. Whether the same or different mechanisms regulate BIG1/2 membrane recruitment to different compartments is also unknown.

Intrinsic factors that regulate membrane recruitment of GEFs

GEFs activate ARFs only when associated with membranes and promote vesicle formation only from specific membrane sites. This implies that the association of GEFs with membranes is key in regulating the time and place of vesicle formation. Identifying the upstream signaling events regulating GEF recruitment is essential to understand the when and where of vesicle formation. It appears that multiple levels of interactions coordinate GEF recruitment to membranes. We first describe the intrinsic information within GEFs sufficient and required for membrane recruitment. Nothing in biology is simple and it appears that positioning GEFs on membranes is a complex, multi-components process that involves multiple GEF domains. This may reflect a coincidence detection mechanism or the sequential pairings of necessary components, as described below.

N-terminus

The information sufficient for membrane targeting of large GEFs, excepting the yeast Sec7p, appears to reside in their N-termini: amino acids 1–560 of GBF1, 1–559 of BIG1, 1–552 of BIG2 and 1–453 of the *Drosophila* BIG ortholog Sec71 alone target to membranes when expressed in cells [42, 52–54] (but see below regarding the requirement for HDS1 and HDS2 domains). In addition to being sufficient, the N-terminus also appears to be absolutely required for membrane recruitment: deletion of amino acids 1–294 of GBF1 results in loss of membrane association [55], and even deletion of a much smaller region (amino acids 1–37) leads to decreased membrane association of GBF1 [56].

The N-terminal regions of large GEFs contain two domains with extremely high sequence conservation across species and phyla: the DCB (dimerization and cyclophilin-binding) and the HUS (homology upstream of Sec7d) domain. The DCB domain was first described in the plant *A. thaliana* large GEF GNOM and shown to mediate DCB–DCB interactions and GNOM dimerization [57]. Similarly, DCB domains of mammalian GBF1 and BIG2 have been shown to interact with the analogous DCB domains by yeast two-hybrid analysis [58]. Based on the high sequence conservation between DCBs in orthologs in all species, it is likely that DCBs facilitate dimerization of all large GEFs. Despite the clear requirement for DCB in membrane association of at least GBF1, the role dimerization may play in GEF association with membranes has not been experimentally addressed.

The N-terminal HUS domain also may participate in membrane recruitment. The HUS domain, and specifically the most conserved region called the HUS box (marked in Fig. 1 as residues 538–545 (LYINYDCD in human GBF1), interacts with the DCB domain in yeast two-hybrid assays [58]. Mutations within the HUS domain (E646G, F481L, F477S, D485G, and F477S) in the yeast GBF1 ortholog Gea2p decrease its membrane association [59], suggesting that HUS might also regulate membrane dynamics of GEFs.

The N-termini of different GEFs have been shown to interact with small GTPases (ARL1, ARF4, ARF5 and RAB1B), coat components (γ -COPI and GGA), tethering proteins (Exo 70), motor proteins (non-muscle myosin 2) and kinases (PKA, AMY-1). Currently known interactors of large GEFs are listed in Table 1 and those involved in regulating GEF recruitment and/or activation are described below.

Sec7d

All large GEFs are cytosolic proteins that move between cytosol and membranes in a regulated fashion that is incompletely understood. Fluorescence recovery after photobleaching (FRAP) analyses of exogenously expressed GFP-tagged GBF1 showed that it associates with membranes in a dynamic manner and rapidly ($t_{1/2} \sim 18$ s) exchanges between cytosol and membranes [45, 60]. The Sec7d appears to influence the residence of the GEF on the membrane through its interactions with its substrate, ARF. Specifically, binding the ARF substrate stabilizes GBF1 on the membrane, as shown by increased residence time on membranes of the catalytically inactive E794K mutant that binds the ARF-GDP substrate but doesn't stimulate GDP expulsion. Similarly, expression of the GDP-restricted T31N mutant of ARF1 increases GBF1 residence on membranes, as does BFA

treatment, each of which are thought to increase affinity of the GEF for the GTPase. The cycling dynamics of other large GEFs and any changes that may accompany the interaction of their Sec7d with substrates have not been examined so the generality of these observations remains to be determined.

The Sec7d also can modulate membrane association independently of substrate binding. This is based upon the observation that a mutation of a single residue (G579R) in the Sec7d of GNOM causes membrane dissociation without altering the catalytic activity of this GEF [61]. Whether this mechanism is general to all large ARF GEFs or specific to one or a small subset of GEFs is unknown.

C-terminus

The C-terminal regions of GEFs also may influence membrane association of at least some GEFs. This region contains 3 (GBF1) or 4 (BIG1 and BIG2) HDS (homology downstream of Sec7d) domains (see Fig. 1). Deletion of portions of the HDS4 domain (amino acids 1836–1883) in the yeast Sec7p (a BIG1/2 ortholog) results in decreased association with the Golgi [62]. Larger deletions up to the HDS1 domain also decrease association and deletion of the entire C-terminus, including the HDS1 domain, results in near complete loss of Sec7p from membranes. Furthermore, deletion or mutation of only the HDS1 domain also greatly diminishes membrane association of Sec7p. The effects of deletions of HDS1 may be restricted to Sec7p as they are not observed in the yeast Gea1p (a GBF1 ortholog) [63]. However, a C-terminal truncation (amino acids 982–1983) of *Drosophila* Garz (a GBF1 ortholog) decreases targeting to the Golgi [64], as does deletion of the C-terminus (removal of amino acids 1060–1859) in GBF1 (E.S., unpublished data). The extreme C-terminus (amino acids 1762–1859) of mammalian GBF1 is dispensable for membrane recruitment [44]. Although the N-terminal region of GBF1 up to the Sec7d appears sufficient for Golgi association in studies from the Melancon group [42, 52] and in our unpublished results, a recent study from the Jackson group reported that deletion of either HDS1 or HDS2 within the context of the full-length protein prevented GBF1 association with the Golgi [65]. They identified HDS1 as a lipid-binding domain, but showed that HDS1 alone did not target to the Golgi. Thus, the mechanism through which HDS1 and HDS2 participate in GBF1 recruitment remains to be determined.

Though the mechanism(s) through which the C-terminal regions of large GEFs facilitate membrane recruitment are unknown, the C-termini of different GEFs have been shown to interact with motor proteins (myosin IXb and KIF21A), tethering proteins (p115) and putative scaffolding proteins (KANK).

In addition to Golgi recruitment, GFP-tagged GBF1 has been detected decorating the surface of lipid droplets, suggesting that GBF1 may target to monolayer, as well as bilayer membranes [65, 66]. The association with lipid droplets appears mediated by HDS1-2 because GFP-tagged HDS1 and HDS2 of GBF1 target to lipid droplets (but not to Golgi) in cells when used either individually or in tandem [66, 67]. The association of HDS1 with lipid droplets was shown to be mediated by an amphipathic α -helix at the C-terminus of the domain [66]. However, another study did not detect association of endogenous GBF1

(detected by antibody staining) with lipid droplets [68]. Thus, whether GBF1 is targeted to diverse intracellular lipid surfaces remains controversial.

Together, the deletion and mutagenesis results show that large GEF recruitment involves the participation of multiple domains and likely in a synergistic or interactive fashion. The differences in requirements for membrane association between large GEFs from various species are somewhat puzzling given that yeast, plant and metazoan GBF1/BIGs have been demonstrated on multiple occasions to be remarkably similar with respect to interacting partners and functions [62, 69, 70]. These differences may be the result of subtle differences in the lipid microenvironments of different membranes between different species. Also, within the model of coincidence detection, there may be different complements of interacting partners in different species to accommodate GEF recruitment. For example, yeast have fewer GTPases than mammalian cells and the abundance of different GTPases could permit recruitment of the N-terminus in the absence of the C-terminus.

Regulation of GEF membrane recruitment by small GTPases of the ARF, ARL and RAB families

The ability of the N-terminal regions of large GEFs to target to appropriate membranes suggests that at least some of the specificity of recruitment is mediated by interactions between the N-termini and specific membrane proteins. The N-termini of large ARF GEFs have been shown to bind directly to ARF, ARL and RAB GTPases (Table 1). ARFs are not particularly “fussy” and associate with multiple compartments, making them less than perfect to act as compartment-specific zip codes. Due to the high degree of primary sequence conservation (e.g., ARF1-3 share ~97 % identity) antibodies capable of distinguishing between the ARFs typically work only in immunoblots. And there are well-documented concerns in using tagged ARF family members to define their localization in cells [71]. Thus, we lack detailed knowledge of the endogenous localizations of ARFs. One exception to this is ARF3, which is found predominantly at the TGN [72]. In contrast, ARL and Rab proteins show precise selectivity in their subcellular localization, which intuitively makes them good candidates for providing spatial information for GEF targeting. Given the overlap of ARFs, ARLs, and Rabs in cellular compartments, it is tempting to propose a “GTPase code” that identifies individual compartments and perhaps even microdomains of those compartments that are distinct in function. These GTPases together with their GEFs and GAPs would then define a continuum of regulators that in turn define the progression of the various trafficking pathways.

ARF

The N-terminus of BIG2 (amino acids 1–250) binds ARF4 and ARF5 in a GTP-dependent manner and activated ARF4 and ARF5 facilitate membrane recruitment of BIG1 and BIG2 to membranes in cells [54]. The activation cascade for ARF4 and ARF5 has been explored and it appears that GBF1 is the upstream GEF that mediates ARF4/5 activation and subsequent BIG1/2 recruitment. This is consistent with the findings that depletion of GBF1 causes dissociation of AP-1 and GGA3 (coat proteins whose recruitment to the Golgi are regulated by BIG1/2-dependent activation of ARFs at that site) from membranes [73], and

that inactivation of GBF1 during Coxsackie virus infection causes dispersal of BIGs throughout the cell [74-76]. Furthermore, point mutations (E646G, F481L, F477S, D485G, and F477S) in the HUS domain of the yeast GBF1 ortholog Gea2p cause a loss of TGN structures and reduction in the association of the yeast BIG ortholog Sec7 with membranes [59]. Thus, it appears that one GEF is utilized to regulate membrane recruitment of other GEFs. Of course this only raises more questions of the chicken and egg variety since we now must understand how GBF1, which also targets to ERES, ERGIC and the Golgi, is also selectively recruited to the TGN membrane sites optimized for vesicle budding.

In the yeast *S. cerevisiae*, ARF1 regulates Sec7p recruitment (and activation) via the HDS1 domain through a feed forward mechanism [63]. This appears to occur through a cooperative mechanism in which a small amount of active ARF leads to increased recruitment of a Sec7p, which in turn quickly generates a considerably larger pool of activated ARF. In addition, Sec7p is activated by its own product ARF1, resulting in a positive feedback loop that generates a localized burst of activated ARF on membranes. The integrated membrane recruitment and activation has been best documented for the small ARF GEFs ARNO/Cytohesin 2 and Grp1 [77-80]. ARNO recruitment to membranes is stimulated by the binding of its PH domain to activated ARF6 or ARL4 (within the context of phosphoinositide-containing membranes) [77, 78]. The GTPase binding also relieves PH-mediated auto-inhibition and results in active cytohesin [79-81]. Other non-ARF GEFs also have been shown to be regulated by positive feedback loops including the Ras GEF Sos, and the RhoA GEFs of the Lbc family [82-84]. In contrast, some ARF GEFs, like the bacterial RalF protein [85, 86], are thought to be activated as a result of relief of auto-inhibition. In this model, access of the substrate to the Sec7d is blocked until the ARF GEF docks on a specific membrane surface, causing domain reorganization and opening access to the substrate binding site. It is currently unknown whether HDS1 domains in GEFs other than Sec7p also regulate GEF activity.

ARL1

The ARF-like protein 1 (ARL1) binds the N-terminus (amino acids 1–453) of *Drosophila* Sec71 (BIG1/2 ortholog) and overexpression of ARL1 increases recruitment of Sec71 to membranes in *Drosophila* cells [53]. ARL1 appears to be required for GEF recruitment because depletion of ARL1 from mammalian cells causes dissociation of BIG1 and BIG2 from membranes [53]. Depletion of ARL1 has no effect on membrane recruitment of GBF1, indicating that different GEFs are recruited by distinct small GTPases. The requirement for activated ARL1 raises the obvious question of what initiates membrane recruitment of the recruiting ARL1.

In *S. cerevisiae*, ARL1 is recruited by ARL3 (the ortholog of mammalian ARFRP1) and is activated (at least partially) by the ARL1 GEF Syt1 [87]. Overexpression of Syt1p increases ARL1p-GTP production in vivo, and the Sec7d of Syt1 promotes nucleotide exchange on ARL1p in vitro. In contrast, deletion of SYT1 results in the majority of ARL1p being distributed diffusely throughout the cytosol. However, ARL1p appears to be regulated by multiple GEFs as *sytl* did not show defects in transport, unlike *arl1* or *arl3* mutants. This now begs the question of how Syt1 (and the other ARL1 GEFs) is recruited to

membranes to activate ARL1. In addition, levels of activated ARL1 are also regulated by the GAP activity of Gcs1p, a protein whose membrane association and activity are regulated by the golgin Imh1p [88]. Imh1p is an ARL1 effector that interacts via its GRIP domain with activated ARL1 to modulate the GTP hydrolysis of ARL1p. Thus a balance of Syt1p, Gcs1 and Imh1p activities defines the levels of active ARL1 on the membrane, and impacts GEF recruitment. The upstream proteins that regulate membrane positioning of Syt1, Gcs1 and Imh1 and perhaps other factors that impact on ARL1 activation and membrane recruitment must be identified and integrated into a complete model for BIG1/2 recruitment.

These findings raise a number of interesting questions regarding BIG1/2 membrane recruitment. Because the N-terminus of BIG1/2 binds ARL1, ARF4 and ARF5, do these GTPases bind cooperatively or do they compete for the same binding domain within BIG1/2? Do ARL1, ARF4 and ARF5 position BIG1/2 at different membrane sites? Does the presence of the ARL1 change the positioning of the GEF within the context of ARF4 or ARF5 and vice versa? Or perhaps the GTPases compete for BIG1/2 and depending on the abundance of ARL1, ARF4 and ARF5, the GEF will be recruited to a different site? These questions must be experimentally addressed to provide clues to the mechanisms that dictate the membrane recruitment of the large GEFs.

RAB

While ARFs and ARL1 regulate BIG1/2 membrane recruitment, GBF1 recruitment is influenced by a RAB (although the possible involvement of ARLs or ARFs in GBF1 recruitment has not been experimentally excluded). The N-terminus of GBF1 (amino acids 1–380) interacts directly with RAB1B in a GTP-dependent manner and RAB1B regulates GBF1 membrane association: expression of the GTP-restricted RAB1B mutant (RAB1B-Q67L) increases GBF1 association, while depletion of RAB1B by siRNA decreases GBF1 membrane association [55] (Fig. 2). The loss of the RAB1-binding region (amino acids 1–294) of GBF1 results in loss of membrane association [55], but this is unlikely to result solely from lack of RAB1B binding since depletion of RAB1 or expression of the dominant inactive RAB1 doesn't inhibit GBF1 association with membranes. Furthermore, expression of the RAB1B-binding N-terminal domain of GBF1 alone is insufficient to recruit GBF1 to membranes, consistent with a multi-component recruitment mechanism [55]. Genetic interactions between the yeast RAB1 ortholog YPT1 and Gea1/2 (GBF1 ortholog) have been detected, but whether YPT1 binds directly to the yeast GEF is unknown [89]. Whether other YPT/RABs may also interact with other GEFs (Sec7/BIG1/2) is under investigation.

The mechanisms through which small GTPases facilitate GEF recruitment to membranes remain to be defined. Possible models include coincidence detection in which all GTPases (and possibly other components) bind simultaneously to initiate recruitment of different GEFs onto a particular site on the membrane. Alternatively, GTPases may bind separately and compete with each other to position the GEF at different sites or at different times. Additional models exist and all must be experimentally tested.

Role of lipids and cargo in GEF recruitment

Lipids

Membranes of each compartment/organelle have distinct lipid compositions that may be characterized by enrichment for one or more phosphatidylinositides (PIs). In addition, localized activities of various lipid-metabolizing enzymes such as PI-kinases generate foci of specific and transient lipid composition that often serve a signaling function. Activities of PI kinases are temporally and spatially regulated through numerous signaling pathways and are optimally positioned for providing at least some level of selectivity for GEF recruitment. In agreement with this notion, specific lipid modifications have been implicated in GEF membrane association: inhibition or depletion of the lipid kinase PI4KIII α causes GBF1 dissociation from membranes [90]. This enzyme catalyzes the phosphorylation of PI to PI(4)P. The function of PI(4)P in GBF1 recruitment is independent of the RAB1 requirement since PtdIns(4)P is not required for membrane association of RAB1, suggesting that PtdIns(4)P and RAB1 act independently, perhaps via a coincidence detection mechanism. In addition, regions of the C-terminus of GBF1 have been shown to directly bind to PI(3,4,5)P₃ and PI(3,5)P₂, and this binding region is required for GBF1 recruitment to the leading edge in a motile leukocyte [91].

Cargo proteins

Key questions in the field, alluded to repeatedly above, include: what provides the initial signal for GEF recruitment and how does this signal determine which GEFs are recruited to what compartments and at what time? Recent studies suggest that cargo (the transmembrane proteins destined for packaging into vesicles) may confer specificity to the GEF recruitment/activation process. Expression of specific cargo proteins [furin, mannose-6-phosphate receptor (M6PR), and the amyloid precursor protein (APP)] and their arrest either at the Golgi, the TGN or endosomal membranes by experimental blocks resulted in an increased recruitment of ARF-dependent adaptors (AP-1, GGAs, and Mint3) in a cargo-dependent manner [92, 93]. Because adaptor recruitment is dependent on ARF activation, this model system reflects an indirect means of assessing the recruitment/activation of GEFs by the cargo. And because each of these cargos displayed strict specificity in the ARF-dependent adaptor recruited at each site we require a model that involves more than a general activation of a GEF. Importantly, cargos do not promote adaptor recruitment to all compartments in which they reside indicating that additional factors regulate the cargo's ability to promote ARF activation and adaptor recruitment. Whether the cargo proteins directly bind one or more GEFs was not explored in this study but remains the simplest model to explain the specificity observed in cells.

Cargo proteins can and do directly bind (and perhaps activate) GEFs, as shown for BIG2 interaction with the β 3IL subunit of the GABA_A receptor (amino acids 328–345 of the receptor interact with the C-terminal amino acids 1682–1791 of BIG2) [49, 94]. BIG2 and GABA_AR β 3IL partially colocalize in hippocampal neurons, and overexpression of BIG2 facilitates GABA_AR β 3 export from the ER [49]. In support for cargo-GEF coupling, microinjection of anti-BIG2 antibodies blocks GABA receptor activity at the cell surface [95]. Similarly, BIG1 interacts with the ATP-binding cassette transporter A-1 (ABCA1) [49,

94]. BIG1 depletion reduced surface ABCA1 on HepG2 cells, while BIG1 overexpression increased surface ABCA1. Thus, a cargo can interact with a GEF, and the presence of the GEF subsequently affects cargo traffic and function through the recruitment of ARFs, and consequently ARF-dependent adaptors, but also likely through recruitment or actions on other proteins.

That cargo may regulate its own traffic within select compartments by facilitating GEF action is further suggested by the findings that depletion of GBF1 causes different cargos to arrest in different compartments: while the adhesion protein integrin- $\alpha 5$ and the viral glycoprotein VSV-G are both retained in the ER, another adhesion factor E-selectin ligand 1 (ESL-1) arrests in *cis*-Golgi elements [39, 96]. Furthermore, GBF1 is not required for traffic of all cargos, and the adhesion proteins laminin and fibronectin are both efficiently trafficked and secreted in cells depleted of GBF1.

Another possible mechanism for cargo-mediated GEF recruitment could be through interactions between coats and GEFs. Cargoes directly interact with coat components and coats have been shown to interact with GEFs [97-99]. Thus, a cargo-coat complex could generate a temporally and spatially defined GEF recruitment site. In support of this model, the γ -COP subunit of the COPI coat binds the N-terminal (amino acids 434–521) region of yeast Gea1p and (amino acids 1–894) of its mammalian ortholog GBF1 [100]. The binding is enhanced by disruption of the DCB-HUS interaction in GBF1, suggesting that it may be modulated by conformational changes in the N-terminus of GBF1.

The N-terminal portion of GBF1 also interacts with Golgi-localized γ -ear-containing ARF-binding proteins (GGAs), adaptor molecules for clathrin recruitment that are important in traffic from the TGN to endosomes [73]. GGA1, GGA2, and GGA3 have been shown to bind GBF1 by co-immunoprecipitation and yeast two-hybrid analyses. The interaction is mediated by the VHS domain in the case of GGA1.

Cargo-GEF and coat-GEF interactions seem optimal for defining sites and times of GEF engagement as it seems wise to couple cargo directly to the traffic machinery so as to engage GEFs only when there is something to transport. Cargo/coat-GEF interactions also are well suited for amplification cascades in which cargo proteins transiently enrich coats on regions of the membrane that would subsequently recruit GEFs which will increase a restricted and local concentration of ARF to recruit more coats and then more GEF to further stimulate the coating process in a forward stimulatory loop. This would provide a rapid and highly localized burst of vesicle biogenesis within a cargo-defined area. Importantly, in this model cargo may be viewed as the upstream signal (ligand equivalent in GPCRs) that regulates the time and site of vesicle formation through activation of the GEF activity.

The coupling of cargo with GEFs may well be part of an even larger complex or assembly of components in a GTPase pathway that provides specificity as well as spatial and temporal control to the process. Such assemblies likely include GAPs and other effectors in addition to the initiator (cargo), GEFs and GTPases. For example, recent findings show that cargo sorting/packaging machinery exemplified by ARF-GAPs might also affect GEF function: the *Drosophila* ortholog of GBF1, Garz, has been shown to interact genetically with the ARF1

ortholog ARF79F and with the ARF1-GAP ortholog Gap69C [64]. Whether this interaction is conserved in other species and whether the ARF GAP influences the positioning and/or activity of the GEF is unknown.

Regulating GEF activity

In addition to recruitment to the membrane, another level of GEF regulation that is poorly understood is the extent and mechanisms of activation of the GEF activity itself. In this section we describe and discuss what is currently known about the regulation of the catalytic process that speeds the release of GDP and activation of the ARFs.

Activation by release of auto-inhibition

S. cerevisiae Sec7p localizes to the TGN and is regulated by both positive feedback and release of auto-inhibition [63,101]. The positive feedback arises through the stable recruitment of Sec7p to membranes via its HDS1 domain mediated by activated ARF1, as discussed above. In addition, the catalytic activity of Sec7p appears to be auto-inhibited, as revealed by assaying recombinant fragments of Sec7p in in vitro ARF GEF assays. The auto-inhibition is mediated by HDS1 and is relieved by binding to activated ARF within the context of lipid membranes. Whether all large ARF GEFs are auto-inhibited in vivo is currently unknown, but it seems prudent for a cell to keep its GEFs catalytically inactive until correctly positioned and engaged in a productive functional cycle. Such a mechanism is certainly not inconsistent with other families of GEFs. Cytohesins and the bacterial GEF RalF are known to be auto-inhibited in a similar manner. However, it is worth noting that auto-inhibition and positive feedback may not be linked for all GEFs. For example, in RalF auto-inhibition occurs but is not linked to a positive feedback loop [86, 102, 103].

Potential activation by Gmh1p

The transmembrane protein Gmh1p interacts with the yeast orthologs of GBF1, Gea1/2p (Table 1 and Fig. 2) [69]. Gmh1p is highly conserved across species and localizes to the *cis*-Golgi in yeast and mammalian cells. Gmh1p doesn't appear to regulate Gea1/2p association with membranes since Gea1/2 are still membrane associated in cells lacking Gmh1p. However, over-expression of Gmh1p in cells with mutations in Gea1p rescues cell viability and retrograde traffic defects, suggesting a functional relationship (perhaps through stimulating Gea1p activity) between the two proteins. This interaction is conserved in the *C. elegans* ortholog of Gmh1p, UNC-50, and defects in UNC-50 that prevent its interaction with the worm GBF1 ortholog cause defects in nicotinic receptor traffic [70]. While clearly important, the exact role Gmh1 plays in GEF function remains unknown.

Network regulation of GEF function: integrating GEFs with other traffic regulators

It makes intuitive sense that GEFs would be recruited to membrane sites and activated in conjunction with other traffic regulators to ensure that vesicles are formed only when all of the traffic machinery is available to facilitate subsequent steps of membrane deformation, vesicle maturation, scission, transport, tethering and fusion. In such a model, other traffic

regulators may also provide spatiotemporal information for the GEFs. In support, numerous traffic regulators have been shown to interact with GBF1, BIG1 and BIG2 or their orthologs (Table 1). Here we describe a number of proteins found to bind one or more of the large GEFs and speculate as to their relevance with regard to function, as data to posit more specific models are lacking.

Lipid flippases

The yeast GBF1 homolog Gea2p binds the yeast phospholipid flippase Drs2p [104, 105]. Phospholipid flippases mediate the transfer of phosphatidylserine (PS) between the luminal and the cytoplasmic leaflet of membrane bilayers, thus inducing curvature [106]. Such membrane remodeling is necessary for membrane deformation that precedes vesicle scission. The flippase-GEF interaction is mediated through the cytosolic C-terminal tail of Drs2p (amino acids 1230–1355) and a domain within the C-terminus of Sec7d of Gea2p (Fig. 2). Disruption of the interaction between Gea2p and Drs2p by the V698G mutation in Gea2p produces major traffic defects in yeast, suggesting that the interaction is functionally important [104]. In support of this conclusion, in vitro assays showed that Gea2p has a stimulatory effect on the flippase activity of Drs2p [105,107]. Whether the flippase may also influence the activity of the GEF is currently unknown. However, these results suggest a direct coupling between the machinery facilitating cargo selection and coating (GEFs) and machinery inducing membrane deformation (flippases), perhaps to coordinate their activities in the formation of a nascent vesicle.

Motor proteins

BIG1 interacts with the plus-end directed microtubule motor KIF21A: the C-terminus of BIG1 (amino acids 886–1849) binds the C-terminus of KIF21A (amino acids 999–1661) [108] (Table 1). KIF21A and BIG1 partially colocalize in cells and depletion of either protein alters the localization of the other [108]. Both BIG1 and KIF21A also bind KANK1, the product of the *Kank* gene, which encodes an ankyrin repeat-containing protein from kidneys, originally identified in the region showing a loss of heterozygosity in renal cell carcinoma [109, 110]. KANK1 is a regulator of actin remodeling dynamics and motility, but has no apparent enzymatic function, suggesting that it may work as an adaptor or a scaffold protein. Depletion of BIG1 or KANK1 phenocopy each other in wound healing assays in which cells lose the ability to polarize and reorganize their cytoskeleton to form a leading edge [109]. It is possible that BIG1 and KANK1 work together to facilitate directional traffic of adhesion proteins, but the mechanism of KANK1 involvement in GEF function is unclear.

BIG1 also interacts with the actin based motor myosin IXb: the C-terminus of BIG1 (amino acids 1305–1849) binds the C-terminus of myosin IXb (1532–1855) [111]. This interaction appears to have no effect on the catalytic activity of BIG1, but BIG1 binding inhibits the RhoGAP activity of myosin IXb and prevents Rho from binding to myosin IXb. The binding of myosin IXb and KIF21A appears to occur within the same C-terminal domain of BIG1, raising the possibility that these motors compete for the GEF, perhaps resulting in the generation of distinct classes of transport vesicles.

BIG1 and BIG2 have been shown to co-precipitate with non-muscle myosin IIA and depletion of BIG1 or BIG2 enhanced phosphorylation of myosin regulatory light chain (T18/S19) and F-actin content, which impaired cell migration in Transwell assays [112]. Thus, BIG1/2 appear to regulate myosin IIA function and processes that influence traffic of adhesion molecules. Whether the converse is true, i.e., whether the interaction with myosin influences GEF function, is unknown.

Collectively, these findings show that GEFs may influence the activity of actin-based motors with which they interact (e. g., myosin IXb and non-muscle myosin IIA), but that interactions with motor proteins don't appear to regulate GEF recruitment or activity. However, the direct interactions between GEFs and motor proteins may be required for directional traffic, perhaps to ensure that vesicle production is coupled to motor-driven vectorial transport.

Tethering factors

GBF1 and its yeast orthologs Gea1/2 interact with multiple tethers operational at the ER-Golgi interface, including p115, COG and TRAPP II (Table 1; Fig. 2). The C-terminal proline-rich region (amino acids 1762–1859) of mammalian GBF1 binds directly to the N-terminal globular head (amino acids 1–766) of p115 [44]. The p115 tether is required for ER-Golgi traffic and cells depleted of p115 arrest cargo at the ER [113–115]. Disruption of GBF1-p115 interaction does not affect GBF1 targeting to membranes. However, overexpression of the p115-binding proline-rich domain of GBF1 in cells causes disruption of the Golgi, suggesting that the p115-GBF1 interaction might be functionally relevant [44].

The N-terminus of Gea2p (amino acids 1–550) interacts with the Cog4 subunit (amino acids 331–860) of the COG complex [116] (Fig. 2). COG mediates vesicle tethering within the Golgi and lack of COG function results in untethered COPI vesicles accumulating near Golgi cisterna [117–121]. The functional relevance of the GEF-COG interaction has not been determined.

Gea2 also interacts with the TRAPP II complex; specifically, the C-terminal 801 amino acids (759–1460) of Gea2p bind the Trs65 subunit (amino acids 303–561) of the TRAPP II complex [122] (Fig. 2). TRAPP II is essential for Golgi homeostasis [123–125]. In addition to being a tether, TRAPP II is also a GEF for Ypt1p, the yeast ortholog of human RAB1B [123]. Gea2p and TRAPP II partially colocalize in cells, but their interaction does not have an effect on the exchange activity of either GEF. However, their functions are linked since recruitment of TRAPP II to membranes appears to be ARF-dependent and ARF activation is mediated (at least in part) by Gea2p. Similarly, TRAPP II GEF activity on Ypt1p promotes Gea2p recruitment to membranes (described previously). This inter-twined relationship may facilitate vesicle formation where Gea2p activates ARF which promotes COPI recruitment and also TRAPP II recruitment. TRAPP II, which interacts with COPI [126], could then stabilize COPI on vesicles in synergy with Gea2p. TRAPP II-mediated activation of Ypt1 would further stimulate Gea2p recruitment in a forward feedback loop. In addition, genetic interactions between ARF1 and another component of TRAPP II, TRS130, in yeast have been described [127].

Although experimental evidence is available only for different GEFs within the GBF1/Gea subfamily, it is likely that GBF1/Gea1/2 interact with all three tethers (p115, COG and TRAPPII). As diagrammed in Fig. 2, p115, COG and TRAPPII bind to distinct regions within GBF1/Gea1/2, raising the possibility that all these interactions might be simultaneous. Of course, it is also possible that GEF pairing with a specific tether occurs at different sites. This is supported by the slightly different distribution of the three tethers at the ER-Golgi interface (p115 is most *cis*- while COG is more medial and TRAPPII appears more distal), making it more likely that GEFs interacts with different tethers at distinct membrane sites [114, 128-132].

BIG2 also has been shown to interact with a tether: the N-terminus of BIG2 (amino acids 1–643) binds the full-length splice variant of the Exo70 subunit of the exocyst tethering complex [133] (Table 1). BIG2 and Exo70 colocalize at the microtubule organizing center (MTOC) and treatment with BFA disrupts the perinuclear localization of Exo70 suggesting that exocyst recruitment is dependent on GEF-mediated ARF activation. The role of the exocyst in BIG2 function is unknown. The interactions between GEFs (required for vesicle formation) and tethers (required for vesicle tethering prior to fusion) are especially interesting because they suggest that the mechanisms for vesicular fusion could be imprinted on a vesicle as it is forming. Thus, GEFs may impart or contribute to future targeting information on the forming vesicle.

Multi-nodal coincidence detection for positioning and activating GEFs at membranes?

The evidence reviewed above suggests that the association of GEFs with membranes is regulated through multiple domains and through multiple cellular components. This strengthens the currently favored model of coincidence detection where the functionally important association of GEFs occurs on membrane sites primed for vesicle budding through numerous distinct pathways involving small GTPases, lipids, cargo and cytosolic factors. The ever-increasing number of proteins shown to interact with GEFs complicates the analysis and makes it difficult to propose simplistic models of GEF recruitment and activation. Even for the known interactors described in Table 1 and Fig. 2, we lack a comprehensive knowledge of their functional relevance and still need to understand the interplay between all the binding partners in regulating GEF activation. Identifying all the components that together constitute a coincidence detection network for each GEF within the context of each cargo, ARF and coat represents a major challenge in the field.

Effects of cell physiology on GEF function

The cellular demand for secretory traffic changes in response to a number of environmental or internal cues so it is reasonable to suggest that as key regulators, the large ARF GEFs may be regulated in response to such cues. This may occur by cell-wide up-regulation of all GEFs to amplify the entire secretory/endocytic pathway, or through selective changes that regulate only one or a few specific GEFs. Our knowledge of how GEFs respond to physiological and developmental stimuli is minimal. Some of the stimuli might be intrinsic such as progression through the cell cycle and nutrient availability, and some could be

triggered through signaling pathways in response to external ligands. In addition, cells in certain “professional secretory” tissues, such as liver, may possess the ability to more acutely regulate the expression of GEFs to meet changing demands for secretory traffic.

Cell division

Entry of cells into mitosis is paralleled by inhibition in secretory protein traffic and the disassembly of the Golgi [134-136]. These changes occur at least in part through the actions of protein kinases [137], particularly the cyclin-dependent kinases (CDKs). A number of regulators and components of membrane traffic are phosphoproteins and consequences of such modifications include changes in protein–protein interactions and in association with membranes, with consequent loss of activity during mitosis [138-145]. For example, human GBF1 has been found to be phosphorylated at 40 different residues in at least one proteomics study and at least 28 of these sites have been confirmed in at least two additional independent studies (www.Phosphosite.org). The vast majority of these sites have not been studied for functional consequences. However, GBF1 is phosphorylated by cyclin-dependent kinase 1 (CDK1), causing its dissociation from Golgi membranes in mitosis (Fig. 3a) [146]. The CDK1-mediated dissociation of GBF1 from membranes may be at least partially responsible for the inhibition in protein secretion and Golgi disassembly observed in dividing cells.

The key role of mitotic kinases in modulating GEF function suggests that other kinases may regulate GEF activation in interphase cells in response to environmental factors. In support, recent studies (described below) show that GEF’s recruitment to membranes and/or activities are regulated in response to environmental conditions.

Growth conditions

GBF1 appears to be inhibited under conditions of cellular stress. GBF1 is phosphorylated on threonine 1337 by the nutrient responsive AMP-activated protein kinase (AMPK) in cells treated with 2-deoxyglucose (2-DG), which blocks glucose utilization and increases intracellular AMP levels [147]. The 2-DG treatment causes fragmentation of the Golgi that can be reversed by expressing a GBF1 mutant in which threonine 1337 is replaced by alanine and thus can’t be phosphorylated. This suggests that the Golgi fragmentation phenotype caused by 2-DG is mediated by an AMPK-induced defect in GBF1. Importantly, GBF1 remains associated with the fragmented Golgi in cells treated with 2-DG, indicating that the AMPK-induced defect is not in GBF1 membrane recruitment, but may involve the catalytic activity of GBF1.

The cellular levels of cAMP fluctuate in response to nutrient availability and influence the activity of protein kinase A (PKA). BIG1 and BIG2 are substrates of PKA and are both phosphorylated in response to elevated cellular cAMP levels [148]. The phosphorylation affects the membrane association of BIG1 but not BIG2: BIG1 is released from the Golgi and translocated to the nucleus, while BIG2 remains associated with the TGN. Mutating the putative PKA phosphorylation site in BIG1 (serine at position 883 replaced with alanine) prevents the relocation of BIG1 into the nucleus, whereas replacement with aspartic acid (which mimics phosphorylated serine) causes nuclear accumulation of BIG1 even without

increasing cellular cAMP level [149]. In contrast to its differential effects on localization, PKA-mediated phosphorylation inhibits the catalytic activity of both BIG1 and BIG2 [148]. Incubation of BIG1 or BIG2 immunoprecipitated from cells with PKA catalytic subunits and ATP in vitro results in their phosphorylation and significantly decreases their GEF activity. BIG1 is phosphorylated by PKA on multiple sites including serine 883 located at the very C-terminus of the catalytic Sec7d. This region of the Sec7d is required for binding the ARF substrate since substitution of the KIAM motif within BIG2 with alanines inhibits ARF binding [150]. Thus, the PKA-mediated phosphorylation of BIG1 may directly interfere with substrate binding. BIG2 lacks serine 883, but is nevertheless inhibited by PKA-mediated phosphorylation; possibly another site within the Sec7d (or elsewhere) is phosphorylated in BIG2 to inhibit its activity.

These findings suggest a fine-tuned balance of phosphorylation (and dephosphorylation) reactions that allow for modulation in the levels of membrane-associated GEFs or in direct regulation of the GEF catalytic activity. It appears that both the Sec7d and those outside can be the targets of kinase-mediated regulation. These findings also illuminate the close link between physiological status of the cell and ARF activation, and the need to elucidate the details of the signaling pathways that bridge the cellular homeostasis with GEF phosphorylation and perhaps other as yet undetected modifications. It is likely that additional signaling pathways modulated by nutrient levels and other physiological parameters regulate cellular activities of GEFs.

Availability of binding partners and other traffic regulators

Steady-state levels of BIG2 protein are influenced by the presence of its binding partner, filamin A (an actin-binding protein that is essential for motility of neurons [151, 152]). The amount of BIG2 in cells was significantly increased in neuronal cells acutely depleted of filamin A by siRNA and in filamin A-null neural progenitor cells [153]. Whether this regulation is at the transcriptional or post-transcriptional level is unknown. These findings indicate that any changes in filamin A homeostasis will influence the levels (and perhaps overall activity) of BIG2 and suggest the possibility that other partners may share this ability to regulate the levels of GBF1/BIG family proteins.

Not only direct binders can affect steady state levels of GEFs. Depletion of the soluble *N*-ethylmaleimide-sensitive factor attachment protein α (α SNAP) by siRNA in cultured epithelial cells has been shown to stimulate autophagy, decrease mTOR signaling and lead to Golgi fragmentation [154]. Loss of α SNAP also results in the down-regulation of GBF1, BIG1 and BIG2 proteins. The mechanism through which α SNAP depletion decreases GEF levels is unknown and thus could be transcriptional or post-transcription.

RAB1B is one of the key regulators of traffic at the ER-Golgi interface [155-158]. As discussed previously, RAB1B directly binds GBF1 and promotes GBF1 recruitment to membranes. However, RAB1B also has transcriptional effects: overexpression of RAB1 in HeLa cells coordinately induced enlargement of the Golgi and up-regulated mRNA levels of GBF1 and numerous other traffic regulators such as KDELR3 and GM130 [159]. The signaling pathway responsible for this induction requires the activity of the p38 mitogen-activated protein kinase and the cAMP-responsive element-binding protein consensus

binding site in promoter regions of the target genes. Whether RAB1B also modulates expression of BIG1/2 was not monitored in this study. RAB1B expression has been shown to increase in a secretory thyroid cell line (FRTL5) in response to thyroid-stimulating hormone (TSH). It is likely that such hormonal stimulation induces RAB1B-mediated increase in numerous factors required to elicit a specific TSH response. These results stress a close relationship between components of the traffic machinery, and imply that cellular surveillance monitors levels of traffic proteins and coordinately regulates their levels.

Developmental regulation

The *Drosophila* GBF1 ortholog Gartenzwerg (Garz) is expressed ubiquitously during development. However, Garz mRNA is enriched in organs exhibiting tubular structure or glandular function such as salivary glands, trachea, and the hindgut, consistent with high demand for protein traffic in these tissues [160]. Whether this enrichment is due to increased transcription or mRNA stability is unknown. The developmental profile of Garz expression in the developing larva is available on FlyBase at <http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=0&text=garz> and a single snapshot is shown in Fig. 3b.

In *Drosophila*, the transcription of a variety of genes acting within the secretory pathway is mediated by the CrebA transcription factor [161]. This system appears conserved in mammalian cells and expression of the CrebA ortholog Creb3L1 in HeLa cells induced expression of numerous genes related to secretion [162]. GBF1, BIG1 and BIG2 were not analyzed in that study.

Alternative splicing may also contribute to GEF regulation. Splice variants of GBF1 have been identified in CHO cells, with three positions displaying small in-frame deletions and insertions [43]. Comparison of variants with larger deletions defined regions of 75 (exons 5–7) and 412 (exons 31–39) amino acids that differentially affected GBF1 function (were required for cell killing but were dispensable for promoting BFA resistance). This suggests that different splice variants (with different characteristics) might be produced in cells in response to different cellular conditions. The distribution of different splice forms of GBF1 during development or within an adult organism is unknown, as are the factors that may influence alternative splicing of the GBF1 gene.

Differential splicing mechanisms also are likely to regulate BIG1 and BIG2 function. In mice, BIG2 has been shown to possess a splice variant lacking exon 7 [163]. While both splice variants are present in all tissues, the relative abundance of these species is altered, suggesting different functions in different tissues.

These findings suggest transcriptional and post-transcriptional inputs into the regulation of steady state levels of functionally distinct isoforms of GEFs. The signals and pathways that regulate the production of distinct GEF isoforms in different tissues at different times remain to be elucidated.

Outstanding questions and future perspectives

Large ARF GEFs are highly conserved traffic regulators of vesicular traffic in all eukaryotic cells. The molecular details of how these and other GEFs act as catalysts of ARF activation has been defined at the atomic level. Similarly, the localization and cellular functions of large GEFs have been extensively studied and we have a number of emerging stories on the identification of specific binding partners and how those interactions may regulate GEF location or function. In contrast, the upstream regulators of GEFs are poorly characterized and we are still far from understanding the mechanisms that ensure that GEFs are selectively positioned and activated only at the sites programmed for vesicle budding.

Major questions regarding both the signals that initiate GEF activation and the pathways that link the initial signal with the GEF remain unanswered. It is very likely that characterization of the non-catalytic domains of GEFs and their interactors will be essential to uncover upstream and downstream regulators that may control GEF specificity. Future research will aim to identify all the factors that regulate large GEF functions, and elucidate how incoming signals are integrated and transduced to impart selectivity to vesicular traffic. We also must identify the environmental and developmental conditions that regulate the transcription and translation of functionally distinct GEF isoforms in different tissues. Finally, we need to explore the long-term regulatory mechanisms that respond to hormonal and physiological cues that alter GEF function and impact secretory capacity of cells in response to developmental cues. Only with an understanding of these issues and linkage to the roles of these large GEFs in providing specificity to ARF activation and signaling will we be positioned to explore opportunities for modulating these essential processes in designed fashions.

Acknowledgments

We thank Dr. Martin Lowe for providing unpublished images and Dr. Julie Brill for information regarding FlyBase. We apologize to all whose work we didn't cover due to our ignorance, inadvertent oversight or space constraints.

References

1. Aridor M, Hannan LA. Traffic jams II: an update of diseases of intracellular transport. *Traffic*. 2002; 3(11):781–790. [PubMed: 12383344]
2. Aridor M. Visiting the ER: the endoplasmic reticulum as a target for therapeutics in traffic related diseases. *Adv Drug Deliv Rev*. 2007; 59(8):759–781. [PubMed: 17681635]
3. Pan H, Yu J, et al. A novel small molecule regulator of guanine nucleotide exchange activity of the ADP-ribosylation factor and Golgi membrane trafficking. *J Biol Chem*. 2008; 283(45):31087–31096. [PubMed: 18799457]
4. Saenz JB, Sun WJ, et al. Golgicide A reveals essential roles for GBF1 in Golgi assembly and function. *Nat Chem Biol*. 2009; 5(3):157–165. [PubMed: 19182783]
5. Boal F, Guetzoian L, et al. LG186: an inhibitor of GBF1 function that causes Golgi disassembly in human and canine cells. *Traffic*. 2010; 11(12):1537–1551. [PubMed: 20854417]
6. Jackson CL, Casanova JE. Turning on ARF: the Sec7 family of guanine-nucleotide-exchange factors. *Trends Cell Biol*. 2000; 10(2):60–67. [PubMed: 10652516]
7. Moss J, Vaughan M. Cholera toxin activation of solubilized adenylate cyclase: requirement for GTP and protein activator for demonstration of enzymatic activity. *Proc Natl Acad Sci USA*. 1977; 74(10):4396–4400. [PubMed: 200916]

8. Enomoto K, Gill DM. Cholera toxin activation of adenylate cyclase. Roles of nucleoside triphosphates and a macromolecular factor in the ADP ribosylation of the GTP-dependent regulatory component. *J Biol Chem.* 1980; 255(4):1252–1258. [PubMed: 6766444]
9. Kahn RA, Gilman AG. Purification of a protein cofactor required for ADP-ribosylation of the stimulatory regulatory component of adenylate cyclase by cholera toxin. *J Biol Chem.* 1984; 259(10):6228–6234. [PubMed: 6327671]
10. Kahn RA, Gilman AG. The protein cofactor necessary for ADP-ribosylation of Gs by cholera toxin is itself a GTP binding protein. *J Biol Chem.* 1986; 261(17):7906–7911. [PubMed: 3086320]
11. Tsai SC, Noda M, et al. Stimulation of cholera enzymatic activities by GTP and two soluble proteins purified from bovine brain. *J Biol Chem.* 1988; 263(4):1768–1772. [PubMed: 3123477]
12. Noda M, Tsai SC, et al. Activation of immobilized, biotinylated cholera AI protein by a 19-kilodalton guanine nucleotide-binding protein. *Biochemistry.* 1989; 28(19):7936–7940. [PubMed: 2514798]
13. Noda M, Tsai SC, et al. Mechanism of cholera toxin activation by a guanine nucleotide-dependent 19 kDa protein. *Biochim Biophys Acta.* 1990; 1034(2):195–199. [PubMed: 2112955]
14. Vaughan M, Moss J. Activation of toxin ADP-ribosyl-transferases by the family of ADP-ribosylation factors. *Adv Exp Med Biol.* 1997; 419:315–320. [PubMed: 9193671]
15. Clark J, Moore L, et al. Selective amplification of additional members of the ADP-ribosylation factor (ARF) family: cloning of additional human and *Drosophila* ARF-like genes. *Proc Natl Acad Sci USA.* 1993; 90(19):8952–8956. [PubMed: 8415637]
16. Kahn RA, Bruford E, et al. Consensus nomenclature for the human ArfGAP domain-containing proteins. *J Cell Biol.* 2008; 182(6):1039–1044. [PubMed: 18809720]
17. Dong JH, Wen JF, et al. Homologs of eukaryotic Ras superfamily proteins in prokaryotes and their novel phylogenetic correlation with their eukaryotic analogs. *Gene.* 2007; 396(1):116–124. [PubMed: 17449198]
18. Lee CM, Haun RS, et al. Characterization of the human gene encoding ADP-ribosylation factor 1, a guanine nucleotide-binding activator of cholera toxin. *J Biol Chem.* 1992; 267(13):9028–9034. [PubMed: 1577740]
19. Lee FJ, Stevens LA, et al. Characterization of class II and class III ADP-ribosylation factor genes and proteins in *Drosophila melanogaster*. *J Biol Chem.* 1994; 269(34):21555–21560. [PubMed: 8063793]
20. Lee FJ, Stevens LA, et al. Characterization of a glucose-repressible ADP-ribosylation factor 3 (ARF3) from *Saccharomyces cerevisiae*. *J Biol Chem.* 1994; 269(33):20931–20937. [PubMed: 8063710]
21. Kahn RA, Kern FG, et al. Human ADP-ribosylation factors. A functionally conserved family of GTP-binding proteins. *J Biol Chem.* 1991; 266(4):2606–2614. [PubMed: 1899243]
22. Bui QT, Golinelli-Cohen MP, et al. Large Arf1 guanine nucleotide exchange factors: evolution, domain structure, and roles in membrane trafficking and human disease. *Mol Genet Genomics.* 2009; 282(4):329–350. [PubMed: 19669794]
23. Pasqualato S, Renault L, et al. Arf, Arl, Arp and Sar proteins: a family of GTP-binding proteins with a structural device for ‘front-back’ communication. *EMBO Rep.* 2002; 3(11):1035–1041. [PubMed: 12429613]
24. Renault L, Guibert B, et al. Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. *Nature.* 2003; 426(6966):525–530. [PubMed: 14654833]
25. Kahn RA, Goddard C, et al. Chemical and immunological characterization of the 21-kDa ADP-ribosylation factor of adenylate cyclase. *J Biol Chem.* 1988; 263(17):8282–8287. [PubMed: 3131341]
26. Kahn RA, Randazzo P, et al. The amino terminus of ADP-ribosylation factor (ARF) is a critical determinant of ARF activities and is a potent and specific inhibitor of protein transport. *J Biol Chem.* 1992; 267(18):13039–13046. [PubMed: 1618801]
27. Achstetter T, Franzusoff A, et al. SEC7 encodes an unusual, high molecular weight protein required for membrane traffic from the yeast Golgi apparatus. *J Biol Chem.* 1988; 263(24):11711–11717. [PubMed: 3042778]

28. Beraud-Dufour S, Robineau S, et al. A glutamic finger in the guanine nucleotide exchange factor ARNO displaces Mg^{2+} and the beta-phosphate to destabilize GDP on ARF1. *EMBO J.* 1998; 17(13):3651–3659. [PubMed: 9649435]
29. Cherfils J, Menetrey J, et al. Structure of the Sec7 domain of the Arf exchange factor ARNO. *Nature.* 1998; 392(6671):101–105. [PubMed: 9510256]
30. Goldberg J. Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell.* 1998; 95(2):237–248. [PubMed: 9790530]
31. Mossessova E, Gulbis JM, et al. Structure of the guanine nucleotide exchange factor Sec7 domain of human arno and analysis of the interaction with ARF GTPase. *Cell.* 1998; 92(3):415–423. [PubMed: 9476900]
32. Peyroche A, Antonny B, et al. Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain. *Mol Cell.* 1999; 3(3):275–285. [PubMed: 10198630]
33. Mossessova E, Corpina RA, et al. Crystal structure of ARF1*Sec7 complexed with Brefeldin A and its implications for the guanine nucleotide exchange mechanism. *Mol Cell.* 2003; 12(6):1403–1411. [PubMed: 14690595]
34. Pasqualato S, Senic-Matuglia F, et al. The structural GDP/GTP cycle of Rab11 reveals a novel interface involved in the dynamics of recycling endosomes. *J Biol Chem.* 2004; 279(12):11480–11488. [PubMed: 14699104]
35. Claude A, Zhao BP, et al. GBF1: a novel Golgi-associated BFA-resistant guanine nucleotide exchange factor that displays specificity for ADP-ribosylation factor 5. *J Cell Biol.* 1999; 146(1):71–84. [PubMed: 10402461]
36. Togawa A, Morinaga N, et al. Purification and cloning of a brefeldin A-inhibited guanine nucleotide-exchange protein for ADP-ribosylation factors. *J Biol Chem.* 1999; 274(18):12308–12315. [PubMed: 10212200]
37. Jones HD, Moss J, et al. BIG1 and BIG2, brefeldin A-inhibited guanine nucleotide-exchange factors for ADP-ribosylation factors. *Methods Enzymol.* 2005; 404:174–184. [PubMed: 16413268]
38. Manolea F, Claude A, et al. 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.* 2008; 19(2):523–535. [PubMed: 18003980]
39. Szul T, Grabski R, et al. Dissecting the role of the ARF guanine nucleotide exchange factor GBF1 in Golgi biogenesis and protein trafficking. *J Cell Sci.* 2007; 120(Pt 22):3929–3940. [PubMed: 17956946]
40. Volpicelli-Daley LA, Li Y, et al. Isoform-selective effects of the depletion of ADP-ribosylation factors 1-5 on membrane traffic. *Mol Biol Cell.* 2005; 16(10):4495–4508. [PubMed: 16030262]
41. Kawamoto K, Yoshida Y, et al. GBF1, a guanine nucleotide exchange factor for ADP-ribosylation factors, is localized to the cis-Golgi and involved in membrane association of the COPI coat. *Traffic.* 2002; 3(7):483–495. [PubMed: 12047556]
42. Zhao X, Lasell TK, et al. Localization of large ADP-ribosylation factor-guanine nucleotide exchange factors to different Golgi compartments: evidence for distinct functions in protein traffic. *Mol Biol Cell.* 2002; 13(1):119–133. [PubMed: 11809827]
43. Claude A, Zhao BP, et al. Characterization of alternatively spliced and truncated forms of the Arf guanine nucleotide exchange factor GBF1 defines regions important for activity. *Biochem Biophys Res Commun.* 2003; 303(1):160–169. [PubMed: 12646181]
44. Garcia-Mata R, Sztul E. The membrane-tethering protein p115 interacts with GBF1, an ARF guanine-nucleotide-exchange factor. *EMBO Rep.* 2003; 4(3):320–325. [PubMed: 12634853]
45. Niu TK, Pfeifer AC, et al. Dynamics of GBF1, a brefeldin A-sensitive Arf1 exchange factor at the Golgi. *Mol Biol Cell.* 2005; 16(3):1213–1222. [PubMed: 15616190]
46. Yamaji R, Adamik R, et al. Identification and localization of two brefeldin A-inhibited guanine nucleotide-exchange proteins for ADP-ribosylation factors in a macromolecular complex. *Proc Natl Acad Sci USA.* 2000; 97(6):2567–2572. [PubMed: 10716990]

47. Shinotsuka C, Yoshida Y, et al. 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.* 2002; 277(11):9468–9473. [PubMed: 11777925]
48. Shinotsuka C, Waguri S, et al. 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.* 2002; 294(2):254–260. [PubMed: 12051703]
49. Charych EI, Yu W, et al. 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.* 2004; 90(1):173–189. [PubMed: 15198677]
50. Shin HW, Morinaga N, et al. BIG2, a guanine nucleotide exchange factor for ADP-ribosylation factors: its localization to recycling endosomes and implication in the endosome integrity. *Mol Biol Cell.* 2004; 15(12):5283–5294. [PubMed: 15385626]
51. Shin HW, Shinotsuka C, et al. Expression of BIG2 and analysis of its function in mammalian cells. *Methods Enzymol.* 2005; 404:206–215. [PubMed: 16413271]
52. Mansour SJ, Skaug J, et al. p200 ARF-GEP1: a Golgi-localized guanine nucleotide exchange protein whose Sec7 domain is targeted by the drug brefeldin A. *Proc Natl Acad Sci USA.* 1999; 96(14):7968–7973. [PubMed: 10393931]
53. Christis C, Munro S. The small G protein Arl1 directs the trans-Golgi-specific targeting of the Arf1 exchange factors BIG1 and BIG2. *J Cell Biol.* 2012; 196(3):327–335. [PubMed: 22291037]
54. Lowery J, Szul T, et al. The Sec7 guanine nucleotide exchange factor GBF1 regulates membrane recruitment of BIG1 and BIG2 to the trans-Golgi network (TGN). *J Biol Chem.* 2013; 288(16):11532–11545. [PubMed: 23386609]
55. Monetta P, Slavin I, et al. Rab1b Interacts with GBF1, modulates both ARF1 dynamics and COPI association. *Mol Biol Cell.* 2007; 18(7):2400–2410. [PubMed: 17429068]
56. Wessels E, Duijsings D, et al. Molecular determinants of the interaction between coxsackievirus protein 3A and guanine nucleotide exchange factor GBF1. *J Virol.* 2007; 81(10):5238–5245. [PubMed: 17329336]
57. Grebe M, Gadea J, et al. A conserved domain of the arabidopsis GNOM protein mediates subunit interaction and cyclophilin 5 binding. *Plant Cell.* 2000; 12(3):343–356. [PubMed: 10715321]
58. Ramaen O, Joubert A, et al. Interactions between conserved domains within homodimers in the BIG1, BIG2, and GBF1 Arf guanine nucleotide exchange factors. *J Biol Chem.* 2007; 282(39):28834–28842. [PubMed: 17640864]
59. Park SK, Hartnell LM, et al. Mutations in a highly conserved region of the Arf1p activator GEA2 block anterograde Golgi transport but not COPI recruitment to membranes. *Mol Biol Cell.* 2005; 16(8):3786–3799. [PubMed: 15930122]
60. Szul T, Garcia-Mata R, et al. Dissection of membrane dynamics of the ARF-guanine nucleotide exchange factor GBF1. *Traffic.* 2005; 6(5):374–385. [PubMed: 15813748]
61. Anders N, Nielsen M, et al. Membrane association of the Arabidopsis ARF exchange factor GNOM involves interaction of conserved domains. *Plant Cell.* 2008; 20(1):142–151. [PubMed: 18203920]
62. Dehring DA, Adler AS, et al. A C-terminal sequence in the guanine nucleotide exchange factor Sec7 mediates Golgi association and interaction with the Rsp5 ubiquitin ligase. *J Biol Chem.* 2008; 283(49):34188–34196. [PubMed: 18832381]
63. Richardson BC, McDonold CM, et al. The Sec7 Arf-GEF is recruited to the trans-Golgi network by positive feedback. *Dev Cell.* 2012; 22(4):799–810. [PubMed: 22516198]
64. Armbruster K, Luschnig S. The *Drosophila* Sec7 domain guanine nucleotide exchange factor protein Gartenzwerg localizes at the cis-Golgi and is essential for epithelial tube expansion. *J Cell Sci.* 2012; 125(Pt 5):1318–1328. [PubMed: 22349697]
65. Bouvet S, Golinelli-Cohen MP, et al. Targeting of the Arf-GEF GBF1 to lipid droplets and Golgi membranes. *J Cell Sci.* 2013; 126(Pt 20):4794–4805. [PubMed: 23943872]
66. Soni KG, Mardones GA, et al. Coatomer-dependent protein delivery to lipid droplets. *J Cell Sci.* 2009; 122(Pt 11):1834–1841. [PubMed: 19461073]

67. Ellong EN, Soni KG, et al. Interaction between the tri-glyceride lipase ATGL and the Arf1 activator GBF1. *PLoS One*. 2011; 6(7):e21889. [PubMed: 21789191]
68. Takashima K, Saitoh A, et al. GBF1-Arf-COPI-ArfGAP-mediated Golgi-to-ER transport involved in regulation of lipid homeostasis. *Cell Struct Funct*. 2011; 36(2):223–235. [PubMed: 22185782]
69. Chantalat S, Courbeyrette R, et al. A novel Golgi membrane protein is a partner of the ARF exchange factors Gea1p and Gea2p. *Mol Biol Cell*. 2003; 14(6):2357–2371. [PubMed: 12808035]
70. Eimer S, Gottschalk A, et al. Regulation of nicotinic receptor trafficking by the transmembrane Golgi protein UNC-50. *EMBO J*. 2007; 26(20):4313–4323. [PubMed: 17853888]
71. Jian X, Cavenagh M, et al. Modifications to the C-terminus of Arf1 alter cell functions and protein interactions. *Traffic*. 2010; 11(6):732–742. [PubMed: 20214751]
72. Manolea F, Chun J, et al. Arf3 is activated uniquely at the trans-Golgi network by brefeldin A-inhibited guanine nucleotide exchange factors. *Mol Biol Cell*. 2010; 21(11):1836–1849. [PubMed: 20357002]
73. Lefrancois S, McCormick PJ. The Arf GEF GBF1 is required for GGA recruitment to Golgi membranes. *Traffic*. 2007; 8(10):1440–1451. [PubMed: 17666033]
74. Wessels E, Duijsings D, et al. Effects of picornavirus 3A proteins on protein transport and GBF1-dependent COP-I recruitment. *J Virol*. 2006; 80(23):11852–11860. [PubMed: 17005635]
75. Wessels E, Duijsings D, et al. A viral protein that blocks Arf1-mediated COP-I assembly by inhibiting the guanine nucleotide exchange factor GBF1. *Dev Cell*. 2006; 11(2):191–201. [PubMed: 16890159]
76. Wessels E, Notebaart RA, et al. Structure-function analysis of the coxsackievirus protein 3A: identification of residues important for dimerization, viral rna replication, and transport inhibition. *J Biol Chem*. 2006; 281(38):28232–28243. [PubMed: 16867984]
77. Cohen LA, Honda A, et al. Active Arf6 recruits ARNO/cytohesin GEFs to the PM by binding their PH domains. *Mol Biol Cell*. 2007; 18(6):2244–2253. [PubMed: 17409355]
78. Hofmann I, Thompson A, et al. The Arl4 family of small G proteins can recruit the cytohesin Arf6 exchange factors to the plasma membrane. *Curr Biol*. 2007; 17(8):711–716. [PubMed: 17398095]
79. Stalder D, Barelli H, et al. Kinetic studies of the Arf activator Arno on model membranes in the presence of Arf effectors suggest control by a positive feedback loop. *J Biol Chem*. 2011; 286(5):3873–3883. [PubMed: 21118813]
80. Malaby AW, van den Berg B, et al. Structural basis for membrane recruitment and allosteric activation of cytohesin family Arf GTPase exchange factors. *Proc Natl Acad Sci USA*. 2013; 110(35):14213–14218. [PubMed: 23940353]
81. Stalder D, Antonny B. Arf GTPase regulation through cascade mechanisms and positive feedback loops. *FEBS Lett*. 2013; 587(13):2028–2035. [PubMed: 23684643]
82. Margarit SM, Sondermann H, et al. Structural evidence for feedback activation by Ras.GTP of the Ras-specific nucleotide exchange factor SOS. *Cell*. 2003; 112(5):685–695. [PubMed: 12628188]
83. Chen Z, Medina F, et al. Activated RhoA binds to the pleckstrin homology (PH) domain of PDZ-RhoGEF, a potential site for autoregulation. *J Biol Chem*. 2010; 285(27):21070–21081. [PubMed: 20430886]
84. Medina F, Carter AM, et al. Activated RhoA is a positive feedback regulator of the Lbc family of Rho guanine nucleotide exchange factor proteins. *J Biol Chem*. 2013; 288(16):11325–11333. [PubMed: 23493395]
85. Aizel K, Biou V, et al. Integrated conformational and lipid-sensing regulation of endosomal ArfGEF BRAG2. *PLoS Biol*. 2013; 11(9):e1001652. [PubMed: 24058294]
86. Folly-Klan M, Alix E, et al. A novel membrane sensor controls the localization and ArfGEF activity of bacterial RalF. *PLoS Pathog*. 2013; 9(11):e1003747. [PubMed: 24244168]
87. Chen KY, Tsai PC, et al. Syt1p promotes activation of Arl1p at the late Golgi to recruit Imh1p. *J Cell Sci*. 2010; 123(Pt 20):3478–3489. [PubMed: 20841378]
88. Chen KY, Tsai PC, et al. Competition between the Golgin Imh1p and the GAP Gcs1p stabilizes activated Arl1p at the late-Golgi. *J Cell Sci*. 2012; 125(Pt 19):4586–4596. [PubMed: 22767516]
89. Jones S, Jedd G, et al. Genetic interactions in yeast between Ypt GTPases and Arf guanine nucleotide exchangers. *Genetics*. 1999; 152(4):1543–1556. [PubMed: 10430582]

90. Dumaesq-Doiron K, Savard MF, et al. The phosphatidylinositol 4-kinase PI4KIIIalpha is required for the recruitment of GBF1 to Golgi membranes. *J Cell Sci.* 2010; 123(Pt 13):2273–2280. [PubMed: 20530568]
91. Mazaki Y, Nishimura Y, et al. 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.* 2012; 23(13):2457–2467. [PubMed: 22573891]
92. Caster AH, Kahn RA. Recruitment of the Mint3 adaptor is necessary for export of the amyloid precursor protein (APP) from the Golgi complex. *J Biol Chem.* 2013; 288(40):28567–28580. [PubMed: 23965993]
93. Caster AH, Sztul E, et al. A role for cargo in Arf-dependent adaptor recruitment. *J Biol Chem.* 2013; 288(21):14788–14804. [PubMed: 23572528]
94. Lin S, Zhou C, et al. BIG1, a brefeldin A-inhibited guanine nucleotide-exchange protein modulates ATP-binding cassette transporter A-1 trafficking and function. *Arterioscler Thromb Vasc Biol.* 2013; 33(2):e31–e38. [PubMed: 23220274]
95. Li YC, Wang MJ, et al. Hyperdopaminergic modulation of inhibitory transmission is dependent on GSK-3beta signaling-mediated trafficking of GABAA receptors. *J Neurochem.* 2012; 122(2):308–320. [PubMed: 22676038]
96. Szul T, Burgess J, et al. The Garz Sec7 domain guanine nucleotide exchange factor for Arf regulates salivary gland development in *Drosophila*. *Cell Logist.* 2011; 1(2):69–76. [PubMed: 21686256]
97. Edeling M, Smith C, et al. Life of a clathrin coat: insights from clathrin and AP structures. *Nat Rev Mol Cell Biol.* 2006; 7(1):32–44. [PubMed: 16493411]
98. Szul T, Sztul E. COPII and COPI traffic at the ER-Golgi interface. *Physiology (Bethesda).* 2011; 26(5):348–364. [PubMed: 22013193]
99. Popoff V, Adolf F, et al. COPI budding within the Golgi stack. *Cold Spring Harb Perspect Biol.* 2011; 3(11):a005231. [PubMed: 21844168]
100. Deng Y, Golinelli-Cohen MP, et al. A COPI coat subunit interacts directly with an early-Golgi localized Arf exchange factor. *EMBO Rep.* 2009; 10(1):58–64. [PubMed: 19039328]
101. Richardson BC, Fromme JC. Autoregulation of Sec7 Arf-GEF activity and localization by positive feedback. *Small GTPases.* 2012; 3(4):240–243. [PubMed: 22996016]
102. DiNitto J, Delprato A, et al. Structural basis and mechanism of autoregulation in 3-phosphoinositide-dependent Grp1 family Arf GTPase exchange factors. *Mol Cell.* 2007; 28(4):569–583. [PubMed: 18042453]
103. Amor J, Swails J, et al. The structure of RalF, an ADP-ribosylation factor guanine nucleotide exchange factor from *Legionella pneumophila*, reveals the presence of a cap over the active site. *J Biol Chem.* 2005; 280(2):1392–1400. [PubMed: 15520000]
104. Chantalat S, Park SK, et al. The Arf activator Gea2p and the P-type ATPase Drs2p interact at the Golgi in *Saccharomyces cerevisiae*. *J Cell Sci.* 2004; 117(Pt 5):711–722. [PubMed: 14734650]
105. Natarajan P, Liu K, et al. Regulation of a Golgi flippase by phosphoinositides and an ArfGEF. *Nat Cell Biol.* 2009; 11(12):1421–1426. [PubMed: 19898464]
106. Daleke DL. Regulation of transbilayer plasma membrane phospholipid asymmetry. *J Lipid Res.* 2003; 44(2):233–242. [PubMed: 12576505]
107. Zhou X, Graham TR. Reconstitution of phospholipid translocase activity with purified Drs2p, a type-IV P-type ATPase from budding yeast. *Proc Natl Acad Sci USA.* 2009; 106(39):16586–16591. [PubMed: 19805341]
108. Costantino BF, Bricker DK, et al. A novel ecdysone receptor mediates steroid-regulated developmental events during the mid-third instar of *Drosophila*. *PLoS Genet.* 2008; 4(6):e1000102. [PubMed: 18566664]
109. Li CC, Kuo JC, et al. Effects of brefeldin A-inhibited guanine nucleotide-exchange (BIG) 1 and KANK1 proteins on cell polarity and directed migration during wound healing. *Proc Natl Acad Sci USA.* 2011; 108(48):19228–19233. [PubMed: 22084092]
110. Kakinuma N, Zhu Y, et al. Kank proteins: structure, functions and diseases. *Cell Mol Life Sci.* 2009; 66(16):2651–2659. [PubMed: 19554261]

111. Saeki N, Tokuo H, et al. BIG1 is a binding partner of myosin IXb and regulates its Rho-GTPase activating protein activity. *J Biol Chem.* 2005; 280(11):10128–10134. [PubMed: 15644318]
112. Le K, Li CC, et al. Arf guanine nucleotide-exchange factors BIG1 and BIG2 regulate nonmuscle myosin IIA activity by anchoring myosin phosphatase complex. *Proc Natl Acad Sci USA.* 2013; 110(34):E3162–E3170. [PubMed: 23918382]
113. Sapperstein SK, Walter DM, et al. p115 is a general vesicular transport factor related to the yeast endoplasmic reticulum to Golgi transport factor Uso1p. *Proc Natl Acad Sci USA.* 1995; 92(2): 522–526. [PubMed: 7831323]
114. Alvarez C, Fujita H, et al. ER to Golgi transport: requirement for p115 at a pre-Golgi VTC stage. *J Cell Biol.* 1999; 147(6):1205–1222. [PubMed: 10601335]
115. Seemann J, Jokitalo EJ, et al. The role of the tethering proteins p115 and GM130 in transport through the Golgi apparatus in vivo. *Mol Biol Cell.* 2000; 11(2):635–645. [PubMed: 10679020]
116. Sztul E, Lupashin V. Role of vesicle tethering factors in the ER-Golgi membrane traffic. *FEBS Lett.* 2009; 583(23):3770–3783. [PubMed: 19887069]
117. Ungar D, Oka T, et al. Characterization of a mammalian Golgi-localized protein complex, COG, that is required for normal Golgi morphology and function. *J Cell Biol.* 2002; 157(3):405–415. [PubMed: 11980916]
118. Fotso P, Koryakina Y, et al. Cog1p plays a central role in the organization of the yeast conserved oligomeric Golgi complex. *J Biol Chem.* 2005; 280(30):27613–27623. [PubMed: 15932880]
119. Zolov SN, Lupashin VV. Cog3p depletion blocks vesicle-mediated Golgi retrograde trafficking in HeLa cells. *J Cell Biol.* 2005; 168(5):747–759. [PubMed: 15728195]
120. Shestakova A, Suvorova E, et al. Interaction of the conserved oligomeric Golgi complex with t-SNARE Syntaxin5a/Sed5 enhances intra-Golgi SNARE complex stability. *J Cell Biol.* 2007; 179(6):1179–1192. [PubMed: 18086915]
121. Smith RD, Willett R, et al. The COG complex, Rab6 and COPI define a novel Golgi retrograde trafficking pathway that is exploited by SubAB toxin. *Traffic.* 2009; 10(10):1502–1517. [PubMed: 19678899]
122. Chen S, Cai H, et al. Trs65p, a subunit of the Ypt1p GEF TRAPP2, interacts with the Arf1p exchange factor Gea2p to facilitate COPI-mediated vesicle traffic. *Mol Biol Cell.* 2011; 22(19): 3634–3644. [PubMed: 21813735]
123. Cai Y, Chin HF, et al. The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. *Cell.* 2008; 133(7):1202–1213. [PubMed: 18585354]
124. Yip CK, Berscheminski J, et al. Molecular architecture of the TRAPP2 complex and implications for vesicle tethering. *Nat Struct Mol Biol.* 2010; 17(11):1298–1304. [PubMed: 20972447]
125. Choi C, Davey M, et al. Organization and assembly of the TRAPP2 complex. *Traffic.* 2011; 12(6):715–725. [PubMed: 21453443]
126. Yamasaki A, Menon S, et al. mTrs130 is a component of a mammalian TRAPP2 complex, a Rab1 GEF that binds to COPI-coated vesicles. *Mol Biol Cell.* 2009; 20(19):4205–4215. [PubMed: 19656848]
127. Zhang CJ, Bowzard JB, et al. Genetic interactions link ARF1, YPT31/32 and TRS130. *Yeast.* 2002; 19(12):1075–1086. [PubMed: 12210902]
128. Whyte J, Munro S. The Sec34/35 Golgi transport complex is related to the exocyst, defining a family of complexes involved in multiple steps of membrane traffic. *Dev Cell.* 2001; 1(4):527–537. [PubMed: 11703943]
129. Spelbrink R, Nothwehr S. The yeast GRD20 gene is required for protein sorting in the trans-Golgi network/endosomal system and for polarization of the actin cytoskeleton. *Mol Biol Cell.* 1999; 10(12):4263–4281. [PubMed: 10588657]
130. Oka T, Vasile E, et al. Genetic analysis of the subunit organization and function of the conserved oligomeric golgi (COG) complex: studies of COG5- and COG7-deficient mammalian cells. *J Biol Chem.* 2005; 280(38):32736–32745. [PubMed: 16051600]
131. Sacher M, Barrowman J, et al. TRAPP I implicated in the specificity of tethering in ER-to-Golgi transport. *Mol Cell.* 2001; 7(2):433–442. [PubMed: 11239471]
132. Cai H, Zhang Y, et al. Mutants in trs120 disrupt traffic from the early endosome to the late Golgi. *J Cell Biol.* 2005; 171(5):823–833. [PubMed: 16314430]

133. Xu KF, Shen X, et al. Interaction of BIG2, a brefeldin A-inhibited guanine nucleotide-exchange protein, with exocyst protein Exo70. *Proc Natl Acad Sci USA*. 2005; 102(8):2784–2789. [PubMed: 15705715]
134. Colanzi A, Corda D. Mitosis controls the Golgi and the Golgi controls mitosis. *Curr Opin Cell Biol*. 2007; 19(4):386–393. [PubMed: 17689238]
135. Wei JH, Seemann J. Mitotic division of the mammalian Golgi apparatus. *Semin Cell Dev Biol*. 2009; 20(7):810–816. [PubMed: 19508856]
136. Corda D, Barretta ML, et al. Golgi complex fragmentation in G2/M transition: an organelle-based cell-cycle check-point. *IUBMB Life*. 2012; 64(8):661–670. [PubMed: 22730233]
137. Preisinger C, Barr FA. Kinases regulating Golgi apparatus structure and function. *Biochem Soc Symp*. 2005; 72:15–30. [PubMed: 15649126]
138. Levine TP, Rabouille C, et al. Binding of the vesicle docking protein p115 to Golgi membranes is inhibited under mitotic conditions. *J Biol Chem*. 1996; 271(29):17304–17311. [PubMed: 8663393]
139. Nakamura N, Lowe M, et al. The vesicle docking protein p115 binds GM130, a cis-Golgi matrix protein, in a mitotically regulated manner. *Cell*. 1997; 89(3):445–455. [PubMed: 9150144]
140. Sohda M, Misumi Y, et al. Phosphorylation of the vesicle docking protein p115 regulates its association with the Golgi membrane. *J Biol Chem*. 1998; 273(9):5385–5388. [PubMed: 9478999]
141. Dell KR, Turck CW, et al. Mitotic phosphorylation of the dynein light intermediate chain is mediated by cdc2 kinase. *Traffic*. 2000; 1(1):38–44. [PubMed: 11208058]
142. Dirac-Svejstrup AB, Shorter J, et al. Phosphorylation of the vesicle-tethering protein p115 by a casein kinase II-like enzyme is required for Golgi reassembly from isolated mitotic fragments. *J Cell Biol*. 2000; 150(3):475–488. [PubMed: 10931861]
143. Jesch SA, Lewis TS, et al. Mitotic phosphorylation of Golgi reassembly stacking protein 55 by mitogen-activated protein kinase ERK2. *Mol Biol Cell*. 2001; 12(6):1811–1817. [PubMed: 11408587]
144. Wang Y, Seemann J, et al. A direct role for GRASP65 as a mitotically regulated Golgi stacking factor. *EMBO J*. 2003; 22(13):3279–3290. [PubMed: 12839990]
145. Mao L, Li N, et al. AMPK phosphorylates GBF1 for mitotic Golgi disassembly. *J Cell Sci*. 2013; 126(Pt 6):1498–1505. [PubMed: 23418352]
146. Morohashi Y, Balklava Z, et al. Phosphorylation and membrane dissociation of the ARF exchange factor GBF1 in mitosis. *Biochem J*. 2010; 427(3):401–412. [PubMed: 20175751]
147. Miyamoto T, Oshiro N, et al. AMP-activated protein kinase phosphorylates Golgi-specific brefeldin A resistance factor 1 at Thr1337 to induce disassembly of Golgi apparatus. *J Biol Chem*. 2008; 283(7):4430–4438. [PubMed: 18063581]
148. Kuroda F, Moss J, et al. Regulation of brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1) and BIG2 activity via PKA and protein phosphatase 1gamma. *Proc Natl Acad Sci USA*. 2007; 104(9):3201–3206. [PubMed: 17360629]
149. Citterio C, Jones HD, et al. Effect of protein kinase A on accumulation of brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1) in HepG2 cell nuclei. *Proc Natl Acad Sci USA*. 2006; 103(8):2683–2688. [PubMed: 16467138]
150. Lowery J, Szul T, et al. Novel C-terminal motif within Sec7 domain of guanine nucleotide exchange factors regulates ADP-ribosylation factor (ARF) binding and activation. *J Biol Chem*. 2011; 286(42):36898–36906. [PubMed: 21828055]
151. Feng Y, Walsh CA. The many faces of filamin: a versatile molecular scaffold for cell motility and signalling. *Nat Cell Biol*. 2004; 6(11):1034–1038. [PubMed: 15516996]
152. Kawauchi T, Hoshino M. Molecular pathways regulating cytoskeletal organization and morphological changes in migrating neurons. *Dev Neurosci*. 2008; 30(1-3):36–46. [PubMed: 18075253]
153. Zhang J, Neal J, et al. Brefeldin A-inhibited guanine exchange factor 2 regulates filamin A phosphorylation and neuronal migration. *J Neurosci*. 2012; 32(36):12619–12629. [PubMed: 22956851]

154. Naydenov NG, Harris G, et al. Loss of a membrane trafficking protein alphaSNAP induces non-canonical autophagy in human epithelia. *Cell Cycle*. 2012; 11(24):4613–4625. [PubMed: 23187805]
155. Plutner H, Cox AD, et al. Rab1b regulates vesicular transport between the endoplasmic reticulum and successive Golgi compartments. *J Cell Biol*. 1991; 115(1):31–43. [PubMed: 1918138]
156. Tisdale EJ, Bourne JR, et al. GTP-binding mutants of rab1 and rab2 are potent inhibitors of vesicular transport from the endoplasmic reticulum to the Golgi complex. *J Cell Biol*. 1992; 119(4):749–761. [PubMed: 1429835]
157. Nuoffer C, Davidson HW, et al. A GDP-bound of rab1 inhibits protein export from the endoplasmic reticulum and transport between Golgi compartments. *J Cell Biol*. 1994; 125(2): 225–237. [PubMed: 8163542]
158. Wilson BS, Nuoffer C, et al. A Rab1 mutant affecting guanine nucleotide exchange promotes disassembly of the Golgi apparatus. *J Cell Biol*. 1994; 125(3):557–571. [PubMed: 8175881]
159. Romero N, Dumur CI, et al. Rab1b overexpression modifies Golgi size and gene expression in HeLa cells and modulates the thyrotrophin response in thyroid cells in culture. *Mol Biol Cell*. 2013; 24(5):617–632. [PubMed: 23325787]
160. Wang S, Meyer H, et al. GBF1 (Gartenzweig)-dependent secretion is required for *Drosophila tubulogenesis*. *J Cell Sci*. 2012; 125(Pt 2):461–472. [PubMed: 22302994]
161. Abrams EW, Andrew DJ. CrebA regulates secretory activity in the *Drosophila* salivary gland and epidermis. *Development*. 2005; 132(12):2743–2758. [PubMed: 15901661]
162. Fox RM, Hanlon CD, et al. The CrebA/Creb3-like transcription factors are major and direct regulators of secretory capacity. *J Cell Biol*. 2010; 191(3):479–492. [PubMed: 21041443]
163. Grzmil P, Enkhbaatar Z, et al. Early embryonic lethality in gene trap mice with disruption of the *Arfgef2* gene. *Int J Dev Biol*. 2010; 54(8-9):1259–1266. [PubMed: 20857375]
164. Shen X, Meza-Carmen V, et al. Interaction of brefeldin A-inhibited guanine nucleotide-exchange protein (BIG) 1 and kinesin motor protein KIF21A. *Proc Natl Acad Sci USA*. 2008; 105(48): 18788–18793. [PubMed: 19020088]
165. Peyroche A, Jackson CL. Functional analysis of ADP-ribosylation factor (ARF) guanine nucleotide exchange factors Gea1p and Gea2p in yeast. *Methods Enzymol*. 2001; 329:290–300. [PubMed: 11210546]
166. Padilla PI, Uhart M, et al. Association of guanine nucleotide-exchange protein BIG1 in HepG2 cell nuclei with nucleolin, U3 snoRNA, and fibrillarin. *Proc Natl Acad Sci USA*. 2008; 105(9): 3357–3361. [PubMed: 18292223]
167. Xu Z, Gong Q, et al. A role of histone H3 lysine 4 methyltransferase components in endosomal trafficking. *J Cell Biol*. 2009; 186(3):343–353. [PubMed: 19651892]
168. Padilla PI, Pachecho-Rodriguez G, et al. Nuclear localization and molecular partners of BIG1, a brefeldin A-inhibited guanine nucleotide-exchange protein for ADP-ribosylation factors. *Proc Natl Acad Sci USA*. 2004; 101(9):2752–2757. [PubMed: 14973189]
169. Shen X, Hong MS, et al. BIG1, a brefeldin A-inhibited guanine nucleotide-exchange protein, is required for correct glycosylation and function of integrin beta1. *Proc Natl Acad Sci USA*. 2007; 104(4):1230–1235. [PubMed: 17227842]
170. Ishizaki R, Shin HW, et al. AMY-1 (associate of Myc-1) localization to the trans-Golgi network through interacting with BIG2, a guanine-nucleotide exchange factor for ADP-ribosylation factors. *Genes Cells*. 2006; 11(8):949–959. [PubMed: 16866877]
171. Puxeddu E, Uhart M, et al. Interaction of phosphodiesterase 3A with brefeldin A-inhibited guanine nucleotide-exchange proteins BIG1 and BIG2 and effect on ARF1 activity. *Proc Natl Acad Sci USA*. 2009; 106(15):6158–6163. [PubMed: 19332778]
172. Padilla PI, Chang MJ, et al. Interaction of FK506-binding protein 13 with brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1): effects of FK506. *Proc Natl Acad Sci USA*. 2003; 100(5):2322–2327. [PubMed: 12606707]
173. Belov GA, Habbersett C, et al. Activation of cellular Arf GTPases by poliovirus protein 3CD correlates with virus replication. *J Virol*. 2007; 81(17):9259–9267. [PubMed: 17567696]

174. Li H, Adamik R, et al. Protein kinase A-anchoring (AKAP) domains in brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2). *Proc Natl Acad Sci USA*. 2003; 100(4):1627–1632. [PubMed: 12571360]
175. Islam A, Shen X, et al. The brefeldin A-inhibited guanine nucleotide-exchange protein, BIG2, regulates the constitutive release of TNFR1 exosome-like vesicles. *J Biol Chem*. 2007; 282(13): 9591–9599. [PubMed: 17276987]
176. Islam A, Jones H, et al. cAMP-dependent protein kinase A (PKA) signaling induces TNFR1 exosome-like vesicle release via anchoring of PKA regulatory subunit RIIbeta to BIG2. *J Biol Chem*. 2008; 283(37):25364–25371. [PubMed: 18625701]
177. Wolf JR, Lasher RS, et al. The putative membrane anchor protein for yeast Sec7p recruitment. *Biochem Biophys Res Commun*. 1996; 224(1):126–133. [PubMed: 8694799]
178. Deitz SB, Rambourg A, et al. Sec7p directs the transitions required for yeast Golgi biogenesis. *Traffic*. 2000; 1(2):172–183. [PubMed: 11208097]

Fig. 1.

Domain arrangement within mammalian large GEFs. The position of distinct domains within the sequences of the human large GEFs is shown. *DCB* dimerization and cyclophilin-binding domain, *HUS* homology upstream of Sec7d, *HDS1–4* homology downstream of Sec7 1–4. The position of the catalytic E794 “glutamic finger” residue within the Sec7d is indicated. The location of the highly conserved HUS box within the HUS domain is indicated

Fig. 2.
 Sites of partner interactions within GBF1 and Gea2. Regions of human GBF1 (*upper panel*) or the yeast *S. cerevisiae* ortholog Gea2 (*lower panel*) shown to bind the indicated partner protein are shown below the full-length GEFs

Fig. 3. Mitotic and developmental regulation of GBF1. **a** HeLa cells at different stages of mitosis were processed for IF to visualize GBF1 (*green*) dissociation from Golgi fragments marked with GM130 (*red*). **b** Ventral view of a stage 13–16 *Drosophila* larva showing high levels of Garz expression within the salivary glands (BDGP—in situ 45008; image ID FBim9076831; <http://insitu.fruitfly.org/cgi-bin/ex/report.pl?ftype=0&ftext=garz>)

Table 1

Interactors of members of the GBF1/Gea family of large GEFs (ARFGEF binding partners)

GEF	Binding partner	Interacting region in GEF	References
GBF/Gea subfamily			
GBF1 (<i>H.s</i>)	GGA3 (<i>H.s</i>)	N-terminus	[73]
GBF1 (<i>H.s</i>)	p115 (<i>H.s</i>)	1762–1859	[44]
GBF1 (<i>H.s</i>)	Rab1b (<i>H.s</i>)	1–380	[55]
GBF1 (<i>H.s</i>)	3A (poliovirus) B3 (Coxsackievirus)	1–566	[58, 75, 76]
GBF1 (<i>H.s</i>)	ATGL (<i>H.s</i>)	DCB Sec7 HDS1 HDS2	[67]
GBF1 (<i>H.s</i>)	AMPK (<i>H.s</i>)	nd	[147]
Gea1p (<i>S.c</i>) GBF1 (<i>H.s</i>)	γ -COP (<i>S.c</i> and <i>H.s</i>)	434–521 of Gea1p N-terminus of GBF1	[100]
Gea1p (<i>S.c</i>)	Gmh1p (<i>S.c</i> , <i>C.e</i> , and <i>H.s</i>)	749–1263	[69, 70]
Gea1/2 (<i>S.c</i>)	Scp160p (<i>S.c</i>)	nd	[165]
Gea2p (<i>S.c</i>)	Cog4 (<i>S.c</i>)	1–550	
Gea2p (<i>S.c</i>)	Drs2p (<i>S.c</i>)	Sec7	[105]
Gea2p (<i>S.c</i>)	Trs65 (<i>S.c</i>)	759–1460	[122]
GNOM (<i>A.t</i>)	CYP5 (<i>A.t</i>)	1–146	[57]
BIG/Sec7 subfamily			
BIG1 (<i>H.s</i>)	ABCA1 (<i>H.s</i>)	nd	[94]
BIG1 (<i>H.s</i>)	Fibrillarin (<i>H.s</i>)	nd	[166]
BIG1 (<i>H.s</i>)	KANK1 (<i>H.s</i>)	nd	[109]
BIG1 (<i>H.s</i>)	KIF21A (<i>H.s</i>)	886–1849	[164]
BIG1 (<i>H.s</i>)	La (<i>H.s</i>)	nd	[166]
BIG1 (<i>H.s</i>)	mDpy-30 (<i>H.s</i>)	nd	[167]
BIG1 (<i>H.s</i>)	Myosin IXb (<i>H.s</i>)	1305–1849	[111]
BIG1 (<i>H.s</i>)	Nucleolin (<i>H.s</i>)	nd	[168]
BIG1 (<i>H.s</i>)	Nucleoporin p62 (<i>H.s</i>)	nd	[166]
BIG1 (<i>H.s</i>)	Integrin β 1 (<i>H.s</i>)	nd	[169]
BIG1/2 (<i>H.s</i>)	AMY-1 (<i>H. sapiens</i>)	283–301 (BIG1) 320–341 (BIG2)	[170]
BIG1/2 (<i>H.s</i>)	ARF4 (<i>H.s</i>)	DCB-HUS	[54]
BIG1/2 (<i>H.s</i>)	ARF5 (<i>H.s</i>)	DCB-HUS	[54]
BIG1/2 (<i>H.s</i>)	Myosin IIa (<i>H.s</i>)	nd	[112]
BIG1/2 (<i>H.s</i>)	PD3A (<i>H.s</i>)	nd	[171]
BIG1/2 (<i>H.s</i>)	FKBP13 FK506-binding (<i>H.s</i>)	1–331	[172]
BIG1/2 (<i>H.s</i>)	PP1 γ (<i>H.s</i>)	nd	[148]
BIG1/2 (<i>H.s</i>)	3CD poliovirus	nd	[173]
BIG2 (<i>R.n</i>)	β 3IL subunit, GABA _A receptor (<i>R.n</i>)	682–1791	[49]
BIG2 (<i>H.s</i>)	Exo70 (<i>H.s</i>)	1–643	[133]
BIG2 (<i>H.s</i>)	PKA subunits (RI α , RI β , RII α , RII β) (<i>H.s</i>)	1–643	[174]

GEF	Binding partner	Interacting region in GEF	References
BIG2 (<i>H.s</i>)	TNFR1 (<i>H.s</i>)	nd	[175, 176]
Sec71 (<i>D.m</i>)	ARL1 (<i>D.m</i>)	DCB-HUS	[53]
Sec7p (<i>S.c</i>)	P90(<i>S.c</i>)	nd	[177]
Sec7p (<i>S.c</i>)	ARF1 (<i>S.c</i>)	HDS1	[63]
Sec7p (<i>S.c</i>)	Sec21 γ -COPI (<i>S.c</i>)	493–1196	[178]
Sec7p (<i>S.c</i>)	Sec24 (<i>S.c</i>)	1196–2010	[178]
All			
BIG1/2 (<i>H.s</i>)	Self	DCB–DCB	[57, 58]
GBF1 (<i>H.s</i>)		DCB-HUS	
Gea1/2p (<i>S.c</i>)			
GNOM (<i>A.t</i>)			

Some of the Listed interactions have been shown to be direct while others may involve linker proteins (see the referenced manuscript for details)

H.s *Homo sapiens*, *S.c* *Saccharomyces cerevisiae*, *R.n* *Rattus norvegicus*, *C.e* *Caenorhabditis elegans*, *D.m* *Drosophila melanogaster*, *M.m* *Mus musculus*, *A.t* *Arabidopsis thaliana*, *nd* not determined