Published in final edited form as:

FEBS Lett. 2009 December 3; 583(23): 3872–3879. doi:10.1016/j.febslet.2009.10.066.

Toward a Model for Arf GTPases as Regulators of Traffic at the Golgi

Richard A. Kahn

Department of Biochemistry Emory University School of Medicine 1510 Clifton Rd. Atlanta, GA 30322 USA

Abstract

In this review I summarize the likely roles played by Arf proteins in the regulation of membrane traffic at the Golgi, from the perspective of the GTPase. The most glaring limitations to the development of a coherent molecular model are highlighted; including incomplete information on the initiation of Arf activation, identification of the “accessory proteins” required for carrier maturation and scission, and those required for directed traffic and fusion at the destination membrane. Though incomplete, the molecular model of carrier biogenesis has developed rapidly in recent years and promises richness in understanding this essential process.

Keywords

ADP-ribosylation factor (Arf); Arf-like (Arl); Arf-dependent adaptors; Arf GEFs; Arf GAPs

Background

The Arf family is an ancient family of regulatory GTPases, predicted to have arisen in prokaryotes and sharing a related lineage to the GTPases that regulate the synthesis of membrane proteins in the signal recognition complex at the rough endoplasmic reticulum (ER) [1,2]. The divergence of Ras/Rab/Rho/Ran proteins from the Arf/Sar/SR β family is the first branch in the evolution of this superfamily of cell regulators of membrane traffic and other essential cell functions. These early origins suggest that Arf family members were present for and likely facilitated or contributed directly to the endosymbiotic origins of the eukaryotes [1,2]. The Arf family may be viewed as evolving in parallel to the endomembrane system, as each increased in complexity and acquired novel functions over time. The Arf family is segregated into three sub-groups, the Arf, Sar, and Arf-like (Arl) GTPases. Although an earlier phylogenetic analysis failed to resolve the phylogenetic origins of Arfs vs Arls vs Sars [3], it is reasonable to propose that the Sar proteins were the earliest to arise as they have retained fundamental roles in export of proteins from the endoplasmic reticulum (ER) throughout eukaryotic evolution, and may have been operating prior to the origins of the Golgi. The development and elaboration of the Golgi and other organelles of the endomembrane system (see Dacks chapter) occurred in parallel to those of the Arf family; from the six members present in the earliest eukaryotes examined to thirty in mammals [3].

© 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved

Telephone: 1-404-727-3561 FAX: 1-404-727-3746 rkahn@emory.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The Golgi is the central site of action for most of the six mammalian Arfs and several of the more numerous (22 mammalian) Arls. Five of the six mammalian Arfs have been localized to or act at the Golgi, with Arf6 active instead at the cell surface. The actions of the Arf GTPases are the focus of this review, but it is important to bear in mind that other Arf family members also localize to the Golgi and that we have quite incomplete data on the extent of functional overlap or specificity for regulators and effectors between Arf family members. For example, Arl1 [4], Arfrp1 [5,6], Arl3 [7], and Arl5A, Arlf5B, Arl8A and Arl8B (Y. Li and R.A. Kahn, unpublished observations) have each been localized at least in part to Golgi membranes; though we have only limited information as to their functions there. With typically 40–60% primary sequence identity between Arfs and Arls we can expect to discover a number of instances in which the GTPases or their modulators are found to overlap in function and specificities. One recent example is the surprising observation that the first identified Arl2 GAP, ELMOD2, is also active against Arfs, despite the lack of the Arf GAP domain [8].

The ancient origins of the Arf family and its expansion in the number of genes/proteins and roles at Golgi membranes brings great challenges to the dissection of specific activities and functions. We have an embarrassment of riches in having more regulators and effectors of Arf activities than we can accommodate into models of Arf biology at the Golgi. Despite this, a couple of functions have emerged as central to Arf actions at the Golgi; the recruitment by activated Arf of protein adaptors or adaptor complexes to the site of carrier biogenesis, and the reciprocal regulation of Arf and lipid modifying enzymes. In this review, I summarize aspects of Arf biology as a regulatory GTPase that impact models of its actions at the Golgi to highlight key issues in the ongoing development of molecular models of the regulation of membrane traffic.

Arf as a Regulatory GTP binding protein

To develop models of the regulation of membrane traffic at the Golgi it is essential to understand the actions of regulatory GTPases and their history. Both the name ADP-ribosylation factor and the earliest means of assaying its activities grew out of studies of the regulation of adenyl cyclase and the discovery of heterotrimeric GTP-binding proteins (G proteins) and of G protein coupled receptors (GPCRs) as their immediate upstream activators [9,10]. All regulatory GTPases work in cells as molecular switches that undergo conformational changes that result in different sets of protein interactions, depending upon whether they bind GDP or GTP. Three sets of proteins interaction are central to defining the actions of any GTPase; (1) the activating, guanine nucleotide exchange factors (GEFs) that promote exchange of GDP for GTP, (2) GTPase activating proteins (GAPs) that promote hydrolysis of bound GTP and return to the GDP-bound state, (3) and effectors that bind the active conformation of the GTPase and carry out biological responses.

Arf GEFs

The GTP-bound conformation is referred to as the active state as it most commonly leads to a biological response, though this can be a bit ambiguous; e.g., in the case of a GTPase like Ran that acts bi-directionally to regulate nucleocytoplasmic transport of different sets of proteins, depending upon the nucleotide bound [11]. Proteins that promote the binding of GTP, typically as a result of stimulating the release of bound GDP, serve to activate the GTPase and are collectively referred to as GEFs. The binding of a ligand to a GPCR results in GEF activity that leads to increased production of activated G proteins. Thus, in the case of GPCRs, G protein signaling involves an external ligand leading to propagation of a signal inside the cell that can activate many G proteins, present on the inner leaflet of the plasma membrane, and downstream effectors. Transmission of a signal across a bilayer and signal amplification are keys to G protein signaling that probably do not translate to GEF activation of Arf GTPases. In addition, GPCRs are intrinsic membrane proteins, while Arf GEFs are extrinsic membrane proteins that

can cycle on and off the membrane surface (e.g., [12]) and are predicted to be capable of regulated recruitment, though mechanisms are incompletely defined.

Activation of Arfs in cells requires both a GEF and a membrane. Arfs (but not all Arls) are unique among the monomeric Ras superfamily in having their N-termini covalently coupled to the 14 carbon, saturated fatty acid myristate. This highly hydrophobic region is masked to solvent in the GDP-bound conformation but becomes exposed upon the protein assuming its active, GTP-bound conformation. Thus, activation of an Arf is coincident with exposure of its membrane anchor, consisting of both the myristate and N-terminal amphipathic α -helix, and translocation onto a membrane. It is probable that localized changes in the lipid composition or membrane curvature act to alter the kinetics of Arf activation as a result of changing the ability of the N-terminal myristate to insert into the bilayer (see below). But it is also likely that in practically all cases that a GEF is required for Arf activation at specific sites, because in at least a few instances knockdown of the GEF leads to loss of recruitment of Arf-dependent adaptors at that site [13,14].

Arf GEFs share the conserved Sec7 domain that has been found on at least 16 different proteins in humans [15,16]. Some beautiful insights into the mechanisms of action of Arf GEFs have come from detailed structural studies of different states of Arf GEFs, alone or complexed with the specific inhibitor of some Arf GEFs, brefeldin A [17]. Not all of these proteins have been localized in cells or studied to the extent of defining roles or locations in cells. But among those that have been studied it is clear that GBF1, BIG1, and BIG2 are each active at the Golgi (see Fig. 1). While it was initially thought that GBF1 localizes to the cis-Golgi and ER-Golgi intermediate compartment (ERGIC) [18] and the BIGs were at the trans-Golgi network (TGN) and endosomes [19], there is growing evidence that these GEFs likely act at more than one place and are perhaps even overlapping in their sites of action (see the Sztul review).

If Arf GEFs play the role of GPCRs in GTPase activation, what serves as the ligand or the initiator of Arf GEF activation? This is perhaps the largest gap in our understanding of the regulation of membrane traffic by Arfs. One possibility is that localized changes in lipid composition serve to initiate Arf signaling but this seems unlikely as it could result in wasted traffic of empty carriers. More likely it is the build-up of cargo at one site that leads to the recruitment of an Arf GEF and subsequent activation of Arf, with consequent adaptor recruitment (e.g., [20]). Publications that describe physical interactions between Arf GEFs and transmembrane protein cargos can be viewed as evidence supporting such a model [13,21]. But any model of Arf activation today must be broad enough to accommodate roles for lipid composition and post-translation modifications, e.g., protein phosphorylation, as additional regulatory aspects of Arf activation.

Arf GAPs

Like the Arf GEFs, the Arf GAPs are defined by the presence of a specific domain, the Arf GAP domain, which leads to the prediction of at least 23 genes/proteins sharing this activity in humans. Many of these Arf GAP genes encode proteins with multiple splice variants and most have not been localized in cells or defined functionally, but several have been implicated in the regulation of Arf activities and membrane traffic at or to the Golgi; including Arf GAP1–3 [22,23], ASAP2 [24], GIT2 short [25], ARAP1 [26], and SMAP2 [27] (Fig. 1). Most of these are large, multi-domain proteins that likely perform scaffolding functions in the regulated assembly and disassembly of protein complexes.

It is very likely that the Arf GAP activities of these proteins are regulated at multiple levels. Like most every aspect of Arf biology, the local lipid environment is important both as a result of allosteric regulation of GAP activity and through the ability of some Arf GAPs to “sense” the membrane curvature [28,29]. A number of Arf GAPs are highly dependent upon acid

phospholipids, typically phosphatidylinositol phosphates, for GAP activities *in vitro* [30]. Interestingly, activated Arfs have been identified as capable of the recruitment of two PI kinases and direct activation of at least one of these, PI(4)P 5-kinase [31]. Thus, it is possible that the half-life of activated Arf is limited, at least in part, by the generation of PIPs that activate Arf GAPs, resulting in inactivation of the Arf. Not only the lipid composition but also the surface geometry of the lipid interface has been proposed to regulate the activity of ArfGAP1, as a result of the presence of ArfGAP1 lipid-sensing packing sensor (ALPS) domains that interact with membranes and in so doing activate GAP activities [28].

The Arf-dependent recruitment of protein adaptors to the sites of carrier biogenesis is the mechanism of Arf action at the Golgi with the strongest experimental support, so it is intriguing that one such adaptor, the COPI complex, has been shown to stimulate the GAP activity of ArfGAP1 [32]. The simplest model that emerges from such data is that activated Arf recruits both an adaptor to couple it to cargo (the transmembrane protein to be concentrated and sorted for inclusion in the nascent carrier) and an Arf GAP through direct protein-protein interactions (see Fig. 2). Once the cargo-adaptor-GAP contacts have been made, and reinforced with subsequent protein interactions, Arf is no longer required and the Arf GAP promotes GTP hydrolysis and dissociation of Arf from the growing carrier. This model is attractive in several respects but requires considerable additional testing and should not (yet) be generalized to all cargo/adaptor/Arf GAPs. One prediction from this model that is supported by later results but runs counter to earlier predictions is that Arfs are not components of the mature carriers. The earliest work demonstrating that Arf and COPI were components of Golgi-derived transport carriers used the slowly hydrolyzable GTP analog, GTP γ S, in an *in vitro* system and later purification of vesicles carrying Arf and COPI [33]. This was interpreted as evidence that Arfs are stable components of carriers emanating from the Golgi and led to the proposal that GTP hydrolysis by Arf was the signal to uncoat the carrier at the acceptor site. However, we now have several instances in which Arf-dependent carriers have been purified from cells or tissues and in each case the Arfs are absent, consistent with their dissociation prior to carrier scission from the donor membrane [34,35].

Arf effectors

The identification of binding partners with higher affinity for the GTP-bound (over the GDP-bound) form of heterotrimeric G proteins led directly to molecular descriptions of the regulation of cAMP, IP₃, and other key signaling pathways in eukaryotic cells. The availability of ligands that acutely regulated those activities provided invaluable tools in the dissection of those pathways and, conversely, the lack of physiologically relevant acute activators of Arf activities has clearly slowed the development of models of Arf signaling in cells. In contrast to the situation with Arf GEFs and Arf GAPs there do not appear to exist any conserved Arf binding domains or motifs in Arf effectors that can be used to identify them by homology searching. Instead, Arf effectors have been discovered using a variety of techniques that typically depend upon the preferential binding to active Arfs over the GDP-bound protein. Most productive has been yeast two-hybrid screening of cDNA libraries with activating point mutations in the Arf, using the wild type Arf as a counter screen to ensure identification of effectors [36–38]. Those at the Golgi and presumed to be involved in one or more Arf-dependent functions include GGAs, Mints, Arfaptins, and Arfophilin (Fig. 1). The other key method used to identify Arf effectors was classical biochemical purification of a regulator of a specific enzymatic activity, including phospholipase D [39,40] and PI(4)P 5-kinase α [31], or reconstituted traffic-related assays, including COPI [33], AP-1 [41], and AP-3 [42]. Additional key Arf effectors acting at the Golgi are the PI(4)P binders FAPP1, FAPP2, OSBP (oxysterol binding protein), and GPBP/CERT. These proteins each contain unusual PH domains that bind both PI(4)P and Arfs [43]. In addition, FAPP2, OSBP, and GPBP contain lipid binding domains that are capable of transporting glycolipids, oxysterols, and ceramide, respectively, to and from the Golgi [44].

Localized changes in the phospholipid content of Golgi membranes by phospholipases or lipid kinases can also lead to the indirect recruitment to the Golgi of structural or other key proteins, including specific isoforms of spectrin and actin. Another Arf effector at the Golgi, and a reminder of the close functional ties between Arf family members is found in Gillingham, et al [45], in which the golgin GMAP-210 was shown to be recruited to Golgi membranes by Arf1, in a manner homologous to that of Arl1 recruiting other Golgins. With more than a dozen direct, downstream targets of activated Arfs at the Golgi, we have more than enough options to build models for regulating membrane traffic but we must add another group of effectors that more than doubles the total number of potential effectors, the Arf GAPs.

Though originally viewed as terminators of Arf signaling, it quickly became clear that Arf GAPs have many of the same properties as effectors in (i) having higher affinity for the activated form of the GTPase, (ii) being recruited to the site of Arf action [46], and (iii) binding to the conformationally-sensitive switch II domain [32]. Genetic studies in yeast provided early support for viewing at least some Arf GAPs as effectors, because all four Arf GAPs in the yeast *S. cerevisiae* were pulled out of a genomic screen as high-copy suppressors of a loss-of-function allele of *arf1* [47]. If solely terminating Arf signaling, these Arf GAPs could not rescue a loss-of-function mutation. It is not yet clear how the positive signaling functions and signaling termination functions are coordinated but it is likely that the Arf GAP activity of Arf GAP proteins can be regulated to allow the recruitment of an Arf GAP and associated partners to assemble onto a membrane containing activated Arf. One likely regulator (see above) is a change in the shape or lipid composition of the membrane to which they are bound that leads to activation of the GAP activity, but other mechanisms are also likely, including protein phosphorylation [48,49].

Arf-dependent adaptors as regulators of membrane traffic

The emergence of Arfs as essential regulators of membrane traffic at the Golgi parallels closely that of non-clathrin coats and the protein adaptors required for their assembly. We now know of three different families of adaptors or adaptor complexes that share homologous mechanisms of action in being recruited to the membrane by direct interaction with activated Arf and subsequently binding transmembrane cargos; the Golgi localized, γ -ear-containing, Arf binding (GGAs), Munc18-interacting (Mints), and adaptin (APs) families. Early studies in each of these families suggested common sites of action for the different members of each family, but more recent results support unique mechanisms of regulation and action for each member of each of these families, thus adding substantially to both the complexity and potential regulatory aspects of membrane traffic at the Golgi.

The three members of the GGA family are monomeric proteins with conserved VHS, GAT, hinge, and γ -adaptin homologous regions. GGAs bind to Arfs via GAT domains and to cargo containing DXXLL motifs through their VHS domains [50]. Immunofluorescent and immunogold labeling of NRK cells revealed that all three GGAs localize to endosomes and the TGN [51]. Cargos reported to use GGAs for traffic from the Golgi in mammalian cells include the mannose-6-phosphate receptor [52], β -secretase/memapsin 2 [53], and LR11/SorLA [54]. The three GGAs have been reported to work together in an inter-dependent fashion, though this is certainly not true in all cases. Similarly, the close proximity of GGAs and AP-1 on the same membranes and even in the same Golgi-derived budding carriers has led some to the belief that these different families of adaptors also work together. This is certainly not always true [50] and the extent to which GGAs and AP-1 may act jointly remains unknown. Indeed, in at least one experimental system (A. Caster, P. Shrivastava-Ranjan, and R. A. Kahn, manuscript in preparation) GGA and AP-1 binding at the Golgi appear to be competitive.

The Mint family is the most recent addition to the list of Arf-dependent adaptors acting at the Golgi and these proteins share common functional features with the others: direct binding to activated Arfs, the ability to be recruited to membranes by increased abundance of specific cargo, direct binding to that cargo, composition as multi-domain proteins that recruit other proteins to promote complex assembly, and sensitivity of Golgi localization to brefeldin A [55]. The Munc18-interacting proteins, Mint1–3, are also known as X11/X11 α /X11 β , or X11/X11-like (X11L1)/X11-like2 (X11L2). Mint1 and 2 are expressed predominantly in neurons, while Mint3 is ubiquitously expressed, including in neurons. Mints are diverse in overall size but share common PTB and dual, C-terminal PDZ domains. Arf binding to Mints requires both the C-terminal part of the PTB domain plus PDZ2 [55] while cargo can bind to any of these conserved domains. Mint-binding cargos include the Alzheimer's disease-related amyloid precursor protein [35,56], and presenilin1, a subunit of the γ -secretase complex, as well as several other transmembrane receptors. The most detailed studies of a Mint acting to regulate traffic of a specific cargo is that of Mint3 regulating export of the amyloid precursor protein from the Golgi, including its mis-sorting and delay in traffic to the cell surface in Mint3 siRNA cells [35].

Subunits of the tetrameric adaptin proteins (APs) share a common ancestry with subunits of the heptameric COPI complex (see Dacks review for details) and so are lumped together here in one family. Despite this common origin, COPI is involved in early secretory traffic, regulating bi-directional traffic between the ERGIC and cis-Golgi, and possibly within the Golgi. In contrast, most APs are thought to act at the late Golgi/TGN, except for AP2, which acts at the plasma membrane. COPI is a heptameric complex consisting of seven subunits: α , β , β' , γ , δ , ϵ , and ζ [57], that comprise the major protein components of the vesicles generated by treating enriched Golgi preparations with GTP γ S [33]. COPI is present in cells as a stable complex that cycles on and off membranes in an Arf-dependent and brefeldin A-sensitive manner. This important biochemical approach identified both COPI and Arf as essential components of Golgi-derived, non-clathrin coated vesicles but led to the mistaken belief that Arfs are stably incorporated into mature carriers. It is far more likely that the presence of activated Arfs in nascent buds or immature vesicles recruits an Arf GAP, which promotes GTP hydrolysis by Arf and dissociation from the maturing carrier (Fig. 2). An obligate role for ArfGAP1 [58] and closely related roles for ArfGAP2 and ArfGAP3 [23] are consistent with this later model and support a role for Arfs in initiating carrier biogenesis and the assembly of the protein coat but not in the mature vesicles or in the protein uncoating that is required for carrier fusion at the destination [59].

There are four different tetrameric protein complexes that comprise the adaptin family (AP1–AP4), and all but AP2 are thought to function at the Golgi [60]. Each adaptin is composed of four subunits, sharing homology and consisting of two large (γ , α , δ , or ϵ and a β 1–4 subunit), one medium (μ 1–4), and one small (σ 1–4) subunit [61]. The μ -subunits bind the YXX ϕ sorting motif in the cytoplasmic tails of cargo and β -subunits recognize XXXLL motifs [60]. The adaptins were the first coat proteins identified because they are components of clathrin coated vesicles, which allowed their ready isolation and biochemical characterization [62]. Later proteomic and refined fractionation methods led to the identification of AP3 and AP4 [60]. The most recent diversification in this family is the presence of two tetramers, AP1a vs AP1b, which share three common subunits but different μ subunits [63]. AP1b is found only in epithelial cells and is involved in Golgi traffic to the basolateral membrane. AP1 and AP3 have been clearly identified as Arf-dependent adaptors but a direct role for an Arf in AP2 recruitment or assembly at the plasma membrane appears unlikely and one for AP4 is unknown. The adaptins are involved in the sorting and regulated traffic of a large number of cargos and can offer such cargo distinct destinations upon leaving the Golgi/TGN. Indeed, sources of specificity in sorting of cargo leaving the Golgi is one of the largest unresolved questions. The presence of so many different Arf-dependent adaptors, capable of specifying different

destinations, will require several more years to dissect. One source of confusion is that some models for sorting and export from the Golgi are built upon analyses of a single cargo or a single adaptor and generalizations from such data are risky. Another confounding issue that is surprisingly persistent is that we still struggle to cleanly define the *direction* of traffic of most adaptor containing carriers. There has been a growing appreciation in recent years for the essential contributions played by specific lipids as determinants of membrane traffic. As we continue to develop better methods of determining local changes in low abundance lipids our understanding of membrane traffic and determinants of specificity will grow in parallel.

Lipids and lipid modifying enzymes as Arf-dependent regulators of membrane traffic

The idea that the membrane to which Arfs are recruited is an integral part of carrier biogenesis is obvious and today the evidence supporting this assertion is overwhelming. But the details are lacking, or at least do not fit readily into a general model of Arf-dependent carrier budding. I summarize briefly here three different aspects of the interplay between Arf and lipid biology; lipid modifying enzymes as effectors of activated Arfs, localized changes in lipid composition impacts on Arf and its regulators, and membrane topology as a regulator of Arf activities.

The identification of Arf as the soluble mediator of GTP-stimulated phospholipase D activity [40,64] was a technical tour de force that initially appeared capable of providing tremendous insights into the mechanisms of action of Arf in membrane traffic. Unfortunately, today we still lack clear evidence that Arf activation of PLD is an obligate component of regulated membrane traffic at the Golgi. What role PLDs play in Arf signaling is thus still uncertain though expected to be important in certain cell types and sites. PLDs hydrolyze phosphatidylcholine to generate phosphatidic acid (PA) and choline. The lack of a head group in PA has led to speculation that it is concentrated at the site of membrane deformations, including nascent buds and the necks of more mature carriers. PA has also been shown to have effects on Arf related proteins (see below), suggesting the additional possibilities of less direct effects of PLD activity on Arf functions. Another lipid modifying enzyme for which there is strong evidence for a direct role for Arfs as allosteric activators is PI(4)P 5-kinase [31]. Interestingly, Arf-stimulated PI(4)P 5-kinase activation requires PA, suggesting the possibility of these two Arf-stimulated enzymes acting in concert. Activated Arfs have also been implicated in the recruitment of PI 4-kinase β to the Golgi, which would lead to increased abundance of PI(4)P at that site. The product of PI(4)P 5-kinase is PI(4,5)P₂, which has been implicated in a wide array of membrane activities. It is possible that PLD activation by Arfs plays an ancillary role to its more fundamental role in the regulation of PIPs. Thus, Arfs certainly have the potential to regulate in a spatially and temporally controlled fashion the generation of several signaling lipids at the Golgi, and other sites, and in the coming years we can expect the development of models that better integrate these activities with those of adaptor or other effector recruitment and assembly of coated carriers.

Almost since the requirement for lipids for Arf to bind GTP was first noted there has been disagreement on the optimal way to assay *in vitro* Arf activation and Arf GEF activities. Indeed, it can be difficult to resolve effects of specific lipids on an Arf GEF from those on the nucleotide exchange reaction by Arfs. It is clear that PI(4,5)P₂ can have dramatic and specific consequences to *in vitro* nucleotide binding by Arfs [65] but whether this occurs in cells is difficult to test and remains unproven. The dramatic and acute effects of brefeldin A or the slower acting siRNA of Arf GEFs on recruitment of Arf-dependent adaptors is consistent with the conclusion that Arf GEFs are required for Arf activation, but I still consider it likely that localized changes in lipids play ancillary roles in Arf activation and activities of at least some Arf GEFs. Signaling lipids (including PI(4,5)P₂, PI(3,4,5)P₃, and PA) have also been found to be potent allosteric activators of the multi-domain Arf GAPs ASAP1, ARAP1, and AGAP1. The lipid composition of any membrane also has dramatic effects on recruitment of Arf

effectors. For example, the β and μ subunits of APs bind specific phosphoinositides; $\mu 1$ binding to Yxx ϕ motifs is enhanced by PI(3)P and β -subunit binding to XXXLL motifs is inhibited by PI(3,4)P₂ but unaffected by PI or PI(4,5)P₂ [66]. Perhaps the best example of Arf effectors with close ties to lipids and lipid signaling are the FAPPs, OSBP, and CERT (see above). They each contain a PH domain that binds both activated Arfs and PI(4)P [43]. Recruitment to the late Golgi/TGN is brefeldin A sensitive, confirming a role for Arf, but also requires the PI(4)P. Thus, we see a clear example of dual regulation of effector recruitment by both Arf and a lipid. FAPPs play critical role(s) in export from the Golgi, though how they may be linked to other Arf-dependent adaptors or effectors is still under study. Thus, local lipid environments can certainly contribute, along with protein-protein interactions, to the specificity and affinity required for adaptor recruitment and subsequent cargo sorting (Fig. 2) [67–69].

A number of potentially important findings have emerged in recent years that point to membrane topology or curvature as a regulator of Arf activities and, conversely, that Arf activation may contribute to changes in membrane topology. Bigay, et al [28] provide evidence that changing the curvature of liposomes leads to changes in the activity of ArfGAP1 as a result of the membrane insertion of the ArfGAP1 lipid packing sensor (ALPS) domain. This very attractive hypothesis provides a novel means of regulating an enzymatic activity, specifically Arf GAP, and predicts that ArfGAP1 would be activated during carrier biogenesis, as the nascent carrier deforms the planar, donor membrane. A prediction from such a model would be that Arfs are absent from the mature carriers, which runs counter to earlier models in which Arfs are stably incorporated into mature carriers. Indirect support for the model comes from the observations in at least three different purified, coated carrier populations that Arfs are absent. Despite the attraction of the model of membrane curvature as a component in Arf signaling, it is quite difficult to cleanly resolve effects of membrane curvature from those of liposome composition, lipid packing, membrane fluidity, charge density, and others, so additional tests of this hypothesis should be devised. A related hypothesis that has gained a lot of attention recently is that the binding of activated Arf to a membrane leads directly to its deformation. This is based upon homologies to the actions of the Sar1 GTPase, the most distant member of the Arf family, as regulator of protein export from the ER and to dynamin as the GTPase involved in carrier scission [70].

An interesting extension of the membrane curvature model can be found in the work of Liu, et al [71], in which the first structure of an N-myristoylated Arf protein was described. This paper included the observations that the GDP bound form of yeast Arf1 appears to have a preference for interacting with larger, more planar membrane mimetics. Thus, the possibility was raised that Arf-GDP may be more prone to interact with planar membranes, where interaction with an Arf GEF would generate the activated Arf-GTP, with subsequent recruitment or activation of Arf effectors and ArfGAPs, many of which promote carrier biogenesis and membrane curvature, which could activate the ArfGAP activity and promote release of Arf from the carrier prior to its maturation and scission, as shown in Fig. 2.

Toward a model of the role of Arfs at the Golgi

The Arf family is an ancient group of regulatory proteins that has been expanded throughout the evolution of eukaryotes. This expansion in gene/protein number is predicted to have allowed greater complexity in cellular signaling and finer degrees of regulation, e.g., allowing distinctive regulation by the same or overlapping sets of GTPases at different sites of the endomembrane system or of different cargos within a common membrane site. We still have a very limited understanding of the extent of functional overlap between the members of the Arf family and even between the different isoforms of Arf at the Golgi (Arf1–5) [72]. An emerging theme in Golgi protein structural work is that many of the key proteins act as dimers. It has recently been argued that Arfs can homodimerize on membranes [73] and this raises the

additional possibility of different Arfs forming heterodimers, further increasing the number of potential signaling complexes [72]. The role of Sar1, the most divergent member of the Arf family, at the ER is simple in comparison, well defined and clearly distinguishable from Arfs in location. Yet Sars and Arfs are thought to play homologous roles in regulating carrier biogenesis through the recruitment of adaptor/coating proteins and promotion of membrane curvature [70]. We know that Arfs and Sars diverged very early in evolution, with a predicted shared function at that point, and thus it is reasonable to begin model building for Arfs as primary regulators of carrier biogenesis at the Golgi through recruitment of adaptors and localized changes in lipids, despite the large amount of evidence for important regulatory functions at other sites and with other effectors.

One model for Arf actions at the Golgi is shown in Fig. 2. It shows a cargo with one or more sorting motifs in the cytoplasmic tail as the initiator of Arf signaling, by facilitating the recruitment of an Arf GEF, which is then present to help “capture” an Arf by promoting GTP binding and consequent increase in stability on the membrane. Note that Arf GEFs are also known to bind other proteins on Golgi membranes [74] so this model is not intended to exclude the participation of other proteins in Arf GEF recruitment or cargo concentration. The activated Arf then recruits a number of types of proteins, including adaptors for cargo binding/concentration (4a), Arf GAP(s) (4b), lipid kinases (4c–d), and phospholipase D (4e). We do not know if all of these are involved in every Arf-dependent budding event or if subsets are used by Arf at different sites or for different cargos. In addition to changes in localization of proteins to the site of carrier biogenesis, figure 2 also highlights some predicted changes in phospholipids, resulting from recruitment and activation of enzymatic activities, depicted in green and shown incorporated into the mature carrier at the right. Carrier purification and lipidomics have not yet advanced to the point where we can actually determine the lipid content of donor, carrier, and acceptor membranes so these changes are speculative and inferred from the changes in enzyme activities shown. The mature carrier (step 5) is also enriched in cargo and the specific adaptor used in sorting. Thus, the overall cycle depicted is one in which the accumulation of a specific cargo at a donor membrane leads to Arf activation and subsequent recruitment of the protein machinery, in parallel with changes in the phospholipid content of the underlying membrane, that work together to generate the carrier, which can be identified based upon its cargo and/or adaptor content. I expect that variations on this theme will explain a large fraction of traffic from the Golgi. This model clearly omits some key elements of the process, e.g., regulators of scission (e.g., dynamins), binding of the carrier to cytoskeletal filaments (e.g., microtubules), motor proteins that will propel it along the filament (e.g., kinesins), and fusion machinery. Model systems have been in a number of laboratories that will allow many of the key questions mentioned above to be answered. We will need several such models for comparison as the diversity in signaling and regulation of membrane traffic is certain to be large. But it will also clearly be worth the effort if it allows us to better understand the key aspects of regulation of specific cargos at specific sites, and the generation of drugs capable of altering specific traffic toward beneficial clinical outcomes.

Acknowledgments

The author is supported by grants from the National Institutes of Health (R01 GM67226 and R01 GM61268). The author regrets the omission of numerous citations to original work, necessitated by limits on space. Discussions with colleagues and members of the Kahn lab, particularly Amanda Caster, and comments on early drafts were helpful to improving this review.

Abbreviations

Adaptin proteins, AP1–4; Arf, ADP-ribosylation factor; Arl, Arf-like; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GGA, Golgi localized; γ -ear-containing, Arf binding proteins; Mint, Munc18-interacting proteins.

References

1. Jekely G. Small GTPases and the evolution of the eukaryotic cell. *Bioessays* 2003;25:1129–1138. [PubMed: 14579253]
2. Dong JH, Wen JF, Tian HF. Homologs of eukaryotic Ras superfamily proteins in prokaryotes and their novel phylogenetic correlation with their eukaryotic analogs. *Gene* 2007;396:116–124. [PubMed: 17449198]
3. Li Y, Kelly WG, Logsdon JM Jr, Schurko AM, Harfe BD, Hill-Harfe KL, Kahn RA. Functional genomic analysis of the ADP-ribosylation factor family of GTPases: phylogeny among diverse eukaryotes and function in *C. elegans*. *Faseb J* 2004;18:1834–1850. [PubMed: 15576487]
4. Lowe SL, Wong SH, Hong W. The mammalian ARF-like protein 1 (Arl1) is associated with the Golgi complex. *J Cell Sci* 1996;109:209–220. [PubMed: 8834805]
5. Panic B, Whyte JR, Munro S. The ARF-like GTPases Arl1p and Arl3p Act in a Pathway that Interacts with Vesicle-Tethering Factors at the Golgi Apparatus. *Curr Biol* 2003;13:405–410. [PubMed: 12620189]
6. Setty SR, Shin ME, Yoshino A, Marks MS, Burd CG. Golgi Recruitment of GRIP Domain Proteins by Arf-like GTPase 1 Is Regulated by Arf-like GTPase 3. *Curr Biol* 2003;13:401–404. [PubMed: 12620188]
7. Zhou C, Cunningham L, Marcus AI, Li Y, Kahn RA. Arl2 and Arl3 regulate different microtubule-dependent processes. *Mol Biol Cell* 2006;17:2476–2487. [PubMed: 16525022]
8. Bowzard JB, Cheng D, Peng J, Kahn RA. ELMOD2 is an Arl2 GTPase-activating protein that also acts on Arfs. *J Biol Chem* 2007;282:17568–17580. [PubMed: 17452337]
9. Kahn RA, Gilman AG. ADP-ribosylation of Gs promotes the dissociation of its alpha and beta subunits. *J Biol Chem* 1984;259:6235–6240. [PubMed: 6327672]
10. 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;259:6228–6234. [PubMed: 6327671]
11. Melchior F, Gerace L. Two-way trafficking with Ran. *Trends Cell Biol* 1998;8:175–179. [PubMed: 9695834]
12. Szul T, Garcia-Mata R, Brandon E, Shestopal S, Alvarez C, Sztul E. Dissection of membrane dynamics of the ARF-guanine nucleotide exchange factor GBF1. *Traffic* 2005;6:374–385. [PubMed: 15813748]
13. Lefrançois S, McCormick PJ. The Arf GEF GBF1 is required for GGA recruitment to Golgi membranes. *Traffic* 2007;8:1440–1451. [PubMed: 17666033]
14. Manolea F, Claude A, Chun J, Rosas J, Melancon P. 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;19:523–535. [PubMed: 18003980]
15. Cherfils J, Melancon P. On the action of Brefeldin A on Sec7-stimulated membrane-recruitment and GDP/GTP exchange of Arf proteins. *Biochem Soc Trans* 2005;33:635–638. [PubMed: 16042561]
16. Casanova JE. Regulation of Arf activation: the Sec7 family of guanine nucleotide exchange factors. *Traffic* 2007;8:1476–1485. [PubMed: 17850229]
17. Renault L, Guibert B, Cherfils J. Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. *Nature* 2003;426:525–530. [PubMed: 14654833]
18. Claude A, Zhao BP, Kuziemy CE, Dahan S, Berger SJ, Yan JP, Arnold AD, Sullivan EM, Melancon P. GBF1: A novel Golgi-associated BFA-resistant guanine nucleotide exchange factor that displays specificity for ADP-ribosylation factor 5. *J Cell Biol* 1999;146:71–84. [PubMed: 10402461]
19. Zhao X, Lasell TK, Melancon P. 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;13:119–133. [PubMed: 11809827]
20. Hirst J, Seaman MN, Buschow SI, Robinson MS. The role of cargo proteins in GGA recruitment. *Traffic* 2007;8:594–604. [PubMed: 17451558]

21. Le Borgne R, Griffiths G, Hoflack B. Mannose 6-phosphate receptors and ADP-ribosylation factors cooperate for high affinity interaction of the AP-1 Golgi assembly proteins with membranes. *J Biol Chem* 1996;271:2162–2170. [PubMed: 8567674]
22. Lanoix J, Ouwendijk J, Stark A, Szafer E, Cassel D, Dejgaard K, Weiss M, Nilsson T. Sorting of Golgi resident proteins into different subpopulations of COPI vesicles: a role for ArfGAP1. *J Cell Biol* 2001;155:1199–1212. [PubMed: 11748249]
23. Saitoh A, Shin HW, Yamada A, Waguri S, Nakayama K. Three homologous ArfGAPs participate in coat protein I-mediated transport. *J Biol Chem* 2009;284:13948–13957. [PubMed: 19299515]
24. Andreev J, Simon JP, Sabatini DD, Kam J, Plowman G, Randazzo PA, Schlessinger J. Identification of a new Pyk2 target protein with Arf-GAP activity. *Mol Cell Biol* 1999;19:2338–2350. [PubMed: 10022920]
25. Mazaki Y, Hashimoto S, Okawa K, Tsubouchi A, Nakamura K, Yagi R, Yano H, Kondo A, Iwamatsu A, Mizoguchi A, Sabe H. An ADP-ribosylation factor GTPase-activating protein Git2-short/KIAA0148 is involved in subcellular localization of paxillin and actin cytoskeletal organization. *Mol Biol Cell* 2001;12:645–662. [PubMed: 11251077]
26. Miura K, Jacques KM, Stauffer S, Kubosaki A, Zhu K, Hirsch DS, Resau J, Zheng Y, Randazzo PA. ARAP1: a point of convergence for Arf and Rho signaling. *Mol Cell* 2002;9:109–119. [PubMed: 11804590]
27. Natsume W, Tanabe K, Kon S, Yoshida N, Watanabe T, Torii T, Satake M. SMAP2, a novel ARF GTPase-activating protein, interacts with clathrin and clathrin assembly protein and functions on the AP-1-positive early endosome/trans-Golgi network. *Mol Biol Cell* 2006;17:2592–2603. [PubMed: 16571680]
28. Bigay J, Gounon P, Robineau S, Antonny B. Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature. *Nature* 2003;426:563–566. [PubMed: 14654841]
29. Bigay J, Casella JF, Drin G, Mesmin B, Antonny B. ArfGAP1 responds to membrane curvature through the folding of a lipid packing sensor motif. *EMBO J* 2005;24:2244–2253. [PubMed: 15944734]
30. Randazzo PA, Kahn RA. GTP hydrolysis by ADP-ribosylation factor is dependent on both an ADP-ribosylation factor GTPase-activating protein and acid phospholipids. *J Biol Chem* 1994;269:10758–10763. [PubMed: 8144664] published erratum appears in *J Biol Chem* 1994 Jun 10;269(23):16519
31. Honda A, Nogami M, Yokozeki T, Yamazaki M, Nakamura H, Watanabe H, Kawamoto K, Nakayama K, Morris AJ, Frohman MA, Kanaho Y. Phosphatidylinositol 4-phosphate 5-kinase alpha is a downstream effector of the small G protein ARF6 in membrane ruffle formation. *Cell* 1999;99:521–532. [PubMed: 10589680]
32. Goldberg J. Structural and functional analysis of the ARF1-ARFGAP complex reveals a role for coatomer in GTP hydrolysis. *Cell* 1999;96:893–902. [PubMed: 10102276]
33. Serafini T, Orci L, Amherdt M, Brunner M, Kahn RA, Rothman JE. ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for a GTP-binding protein. *Cell* 1991;67:239–253. [PubMed: 1680566]
34. Salazar G, Craig B, Wainer BH, Guo J, De Camilli P, Faundez V. Phosphatidylinositol-4-kinase type II alpha is a component of adaptor protein-3-derived vesicles. *Mol Biol Cell* 2005;16:3692–3704. [PubMed: 15944223]
35. Shrivastava-Ranjan P, Faundez V, Fang G, Rees H, Lah JJ, Levey AI, Kahn RA. Mint3/X11 gamma is an ADP-ribosylation factor-dependent adaptor that regulates the traffic of the Alzheimer's Precursor protein from the trans-Golgi network. *Mol Biol Cell* 2008;19:51–64. [PubMed: 17959829]
36. Boman AL, Zhang C, Zhu X, Kahn RA. A family of ADP-ribosylation factor effectors that can alter membrane transport through the trans-Golgi. *Mol Biol Cell* 2000;11:1241–1255. [PubMed: 10749927]
37. Van Valkenburgh H, Shern JF, Sharer JD, Zhu X, Kahn RA. ADP-ribosylation factors (ARFs) and ARF-like 1 (ARL1) have both specific and shared effectors: characterizing ARL1-binding proteins. *J Biol Chem* 2001;276:22826–22837. [PubMed: 11303027]
38. Kanoh H, Williger BT, Exton JH. Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes. *J Biol Chem* 1997;272:5421–5429. [PubMed: 9038142]

39. Brown HA, Gutowski S, Kahn RA, Sternweis PC. Partial purification and characterization of Arf-sensitive phospholipase D from porcine brain. *J Biol Chem* 1995;270:14935–14943. [PubMed: 7797473]
40. Cockcroft S, Thomas GM, Fensome A, Geny B, Cunningham E, Gout I, Hiles I, Totty NF, Truong O, Hsuan JJ. Phospholipase D: a downstream effector of ARF in granulocytes. *Science* 1994;263:523–526. [PubMed: 8290961]
41. Stamnes MA, Rothman JE. The binding of AP-1 clathrin adaptor particles to Golgi membranes requires ADP-ribosylation factor, a small GTP-binding protein. *Cell* 1993;73:999–1005. [PubMed: 8500185]
42. Ooi CE, Dell'Angelica EC, Bonifacino JS. ADP-Ribosylation factor 1 (ARF1) regulates recruitment of the AP-3 adaptor complex to membranes. *J Cell Biol* 1998;142:391–402. [PubMed: 9679139]
43. Godi A, Di Campli A, Konstantakopoulos A, Di Tullio G, Alessi DR, Kular GS, Daniele T, Marra P, Lucocq JM, De Matteis MA. FAPPs control Golgi-to-cell-surface membrane traffic by binding to ARF and PtdIns(4)P. *Nat Cell Biol* 2004;6:393–404. [PubMed: 15107860]
44. Hanada K, Kumagai K, Yasuda S, Miura Y, Kawano M, Fukasawa M, Nishijima M. Molecular machinery for non-vesicular trafficking of ceramide. *Nature* 2003;426:803–809. [PubMed: 14685229]
45. Gillingham AK, Tong AH, Boone C, Munro S. The GTPase Arf1p and the ER to Golgi cargo receptor Erv14p cooperate to recruit the golgin Rud3p to the cis-Golgi. *J Cell Biol* 2004;167:281–292. [PubMed: 15504911]
46. Cukierman E, Huber I, Rotman M, Cassel D. The ARF1 GTPase-activating protein: zinc finger motif and Golgi complex localization. *Science* 1995;270:1999–2002. [PubMed: 8533093]
47. Zhang CJ, Cavenagh MM, Kahn RA. A family of Arf effectors defined as suppressors of the loss of Arf function in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* 1998;273:19792–19796. [PubMed: 9677411]
48. Pulvirenti T, Giannotta M, Capestrano M, Capitani M, Pisanu A, Polishchuk RS, San Pietro E, Beznoussenko GV, Mironov AA, Turacchio G, Hsu VW, Sallese M, Luini A. A traffic-activated Golgi-based signalling circuit coordinates the secretory pathway. *Nat Cell Biol* 2008;10:912–922. [PubMed: 18641641]
49. McKay MM, Kahn RA. Multiple phosphorylation events regulate the subcellular localization of GGA1. *Traffic* 2004;5:102–116. [PubMed: 14690499]
50. Hirst J, Lindsay MR, Robinson MS. GGAs: Roles of the Different Domains and Comparison with AP-1 and Clathrin. *Mol Biol Cell* 2001;12:3573–3588. [PubMed: 11694590]
51. Hirst J, Lui WW, Bright NA, Totty N, Seaman MN, Robinson MS. A family of proteins with gamma-adaptin and VHS domains that facilitate trafficking between the trans-Golgi network and the vacuole/lysosome. *J Cell Biol* 2000;149:67–80. [PubMed: 10747088]
52. Puertollano R, Aguilar RC, Gorshkova I, Crouch RJ, Bonifacino JS. Sorting of mannose 6-phosphate receptors mediated by the GGAs. *Science* 2001;292:1712–1716. [PubMed: 11387475]
53. He X, Chang WP, Koelsch G, Tang J. Memapsin 2 (beta-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: implications on the endocytosis mechanism of memapsin 2. *FEBS Lett* 2002;524:183–187. [PubMed: 12135764]
54. Jacobsen L, Madsen P, Nielsen MS, Geraerts WP, Gliemann J, Smit AB, Petersen CM. The sorLA cytoplasmic domain interacts with GGA1 and -2 and defines minimum requirements for GGA binding. *FEBS Lett* 2002;511:155–158. [PubMed: 11821067]
55. Hill K, Li Y, Bennett M, McKay M, Zhu X, Shern J, Torre E, Lah JJ, Levey AI, Kahn RA. Munc18 interacting proteins: ADP-ribosylation factor-dependent coat proteins that regulate the traffic of beta-Alzheimer's precursor protein. *J Biol Chem* 2003;278:36032–36040. [PubMed: 12842896]
56. Borg JP, Yang Y, De Taddeo-Borg M, Margolis B, Turner RS. The X11alpha protein slows cellular amyloid precursor protein processing and reduces Abeta40 and Abeta42 secretion. *J Biol Chem* 1998;273:14761–14766. [PubMed: 9614075]
57. Waters MG, Serafini T, Rothman JE. 'Coatomer': a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. *Nature* 1991;349:248–251. [PubMed: 1898986]

58. Yang J-S, Lee SY, Gao M, Bourgoïn S, Randazzo PA, Premont RT, Hsu VW. ARFGAP1 promotes the formation of COPI vesicles, suggesting function as a component of the coat. *J Cell Biol* 2002;159:69–78. [PubMed: 12379802]
59. Hsu VW, Lee SY, Yang JS. The evolving understanding of COPI vesicle formation. *Nat Rev Mol Cell Biol* 2009;10:360–364. [PubMed: 19293819]
60. Robinson MS, Bonifacino JS. Adaptor-related proteins. *Curr Opin Cell Biol* 2001;13:444–453. [PubMed: 11454451]
61. Kirchhausen T. Adaptors for clathrin-mediated traffic. *Annu Rev Cell Dev Biol* 1999;15:705–732. [PubMed: 10611976]
62. Keen JH. Clathrin assembly proteins: affinity purification and a model for coat assembly. *J Cell Biol* 1987;105:1989–1998. [PubMed: 2890644]
63. Folsch H, Ohno H, Bonifacino JS, Mellman I. A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. *Cell* 1999;99:189–198. [PubMed: 10535737]
64. Brown HA, Gutowski S, Moomaw CR, Slaughter C, Sternweis PC. ADP-ribosylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. *Cell* 1993;75:1137–1144. [PubMed: 8261513]see comments
65. Terui T, Kahn RA, Randazzo PA. Effects of acid phospholipids on nucleotide exchange properties of ADP-ribosylation factor 1. Evidence for specific interaction with phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 1994;269:28130–28135. [PubMed: 7961749]
66. Rapoport I, Chen YC, Cupers P, Shoelson SE, Kirchhausen T. Dileucine-based sorting signals bind to the beta chain of AP-1 at a site distinct and regulated differently from the tyrosine-based motif-binding site. *Embo J* 1998;17:2148–2155. [PubMed: 9545228]
67. Munro S. Organelle identity and the targeting of peripheral membrane proteins. *Curr Opin Cell Biol* 2002;14:506–514. [PubMed: 12383804]
68. Simonsen A, Wurmser AE, Emr SD, Stenmark H. The role of phosphoinositides in membrane transport. *Curr Opin Cell Biol* 2001;13:485–492. [PubMed: 11454456]
69. De Matteis MA, Godi A. PI-loting membrane traffic. *Nat Cell Biol* 2004;6:487–492. [PubMed: 15170460]
70. Pucadyil TJ, Schmid SL. Conserved functions of membrane active GTPases in coated vesicle formation. *Science* 2009;325:1217–1220. [PubMed: 19729648]
71. Liu Y, Kahn RA, Prestegard JH. Structure and Membrane Interaction of Myristoylated ARF1. *Structure* 2009;17:79–87. [PubMed: 19141284]
72. Volpicelli-Daley LA, Li Y, Zhang CJ, Kahn RA. Isoform-selective effects of the depletion of ADP-ribosylation factors 1–5 on membrane traffic. *Mol Biol Cell* 2005;16:4495–4508. [PubMed: 16030262]
73. Beck R, Sun Z, Adolf F, Rutz C, Bassler J, Wild K, Sinning I, Hurt E, Brugger B, Bethune J, Wieland F. Membrane curvature induced by Arf1-GTP is essential for vesicle formation. *Proc Natl Acad Sci U S A* 2008;105:11731–11736. [PubMed: 18689681]
74. Garcia-Mata R, Sztul E. The membrane-tethering protein p115 interacts with GBF1, an ARF guanine-nucleotide-exchange factor. *EMBO Rep* 2003;4:320–325. [PubMed: 12634853]

Fig. 1. Summary of Arf interactions at the Golgi

Arfs are present in cytosol of all eukaryotic cells and interact with (predominantly planar) membranes, where the actions of Arf GEFs (GBF1, Big1, and Big2 are implicated at Golgi) promote binding of GTP and activation of Arf that stabilizes binding to the bilayer. The Arf-GTP assumes the activated conformation, which has increased affinity for multiple effectors that include protein adaptors for coupling to cargos, lipid modifying enzymes for generating local changes in membrane and additional binding sites for other proteins, and Arf GAPs (e.g., ArfGAP1–3) that may act as effectors to recruit other components to a nascent bud, or promote GTP hydrolysis by Arf and return to its less active, GDP-bound conformation. The activation of Arf on the membrane also is accompanied by insertion of the myristate into the bilayer,

binding of the amphipathic α -helix with surface head groups and, along with effector recruitment and localized changes in lipids, promotes membrane curvature and budding of nascent carriers.

Fig. 2. Building a model for Arf regulation of carrier biogenesis at the Golgi

Almost every single step shown has been documented, but the specific contributions of most steps to carrier biogenesis at the Golgi remain somewhat uncertain. Most speculative is step 1, in which the accumulation of cargo is shown as the initiating event in coat protein assembly, through recruitment of an Arf GEF. The cargo is shown as a transmembrane protein with cytoplasmic tail that contains sorting motifs, such as those shown on the left most cargo. Soluble Arfs are proposed to “sample” planar membranes (step 2) independently of other events, but when an Arf GEF is encountered, the Arf is activated (step 3) and becomes more stably bound to the membrane as a result of insertion of the myristate into the bilayer and binding of the N-terminal helix to lipid head groups. The different steps 4 represent Arf recruitment (4a–d) and activation (4d–e) of various effectors that have each been implicated in aspects of carrier biogenesis at the Golgi. The deformation of the planar membrane is not shown, but the mature severed carrier is shown at the right (step 5). Note the absence of Arf in the mature carrier and predicted presence of cargo, adaptor, Arf GAP, and lipid products. The components depicted are not to scale, but rather each is shown as similar in size, to highlight its likely role in Arf actions at the Golgi.