GGA proteins: new players in the sorting game

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Summary
The GGA proteins are a novel family of proteins that were discovered nearly simultaneously by several labs studying very different aspects of membrane trafficking. Since then, several studies have described the GGA proteins and their functions in yeast and mammalian cells. Four protein domains are present in all GGA proteins, as defined by sequence homology and function. These different domains interact directly with ARF proteins, cargo and clathrin. Alteration of the levels of GGA proteins by gene knockout or overexpression affects specific trafficking events between the trans-Golgi network and endosomes. These data suggest that GGAs function as ARF-dependent, monomeric clathrin adaptors to facilitate cargo sorting and vesicle formation at the trans-Golgi network.

Key words: Membrane traffic, trans-Golgi network, GGA, ARF, Clathrin adaptors

Introduction
An exciting flurry of papers within the past year has caught the attention of many in cell biology. These papers describe the discovery of the GGA proteins, a novel family of proteins that facilitate specific membrane trafficking events at the trans-Golgi network (TGN). The TGN is a major site of sorting and packaging of proteins and lipids to be delivered to distinct subcellular locations. At least four post-Golgi anterograde pathways appear to exist in most cell types: two pathways from the TGN to the plasma membrane (constitutive and regulated secretion), a pathway from the TGN directly to the lysosome, and at least one pathway from the TGN to the lysosome via endosomes. Transport between these compartments is generally believed to occur via protein-coated membrane vesicles that pinch off from the donor membrane. Protein coats both facilitate the sorting of cargo into the forming vesicle and help to produce the curvature of the vesicle. The first coat protein to be discovered was clathrin, visible in electron micrographs as a cage around buds and newly formed vesicles. Clathrin is required for several types of vesicle formation at the TGN and plasma membrane, different adaptor complexes being used for each type of vesicle. Four adaptor complexes are known in mammalian cells (AP-1 through AP-4), and three are present in yeast (AP-1 through AP-3). The adaptor complexes are heterotetramers that recognize cargo and recruit clathrin. In yeast, loss of clathrin is deleterious to cell growth because both endocytosis and TGN to vacuole traffic are inhibited. One puzzling observation is that deletion of all three adaptor complexes in yeast does not inhibit TGN-to-vacuole transport. This has led to the speculation that other adaptor-like complexes exist or that clathrin has an adaptor-independent function in TGN-to-endosome trafficking. The discovery of the GGA proteins may help solve this puzzle, their being likely coat proteins and/or clathrin adaptors.

Another long-standing question in the membrane traffic field is how ADP-ribosylation factor (ARF) proteins regulate vesicle formation. One hypothesis developed over the years is that GTP-bound ARF recruits coat proteins onto a site of vesicle formation through direct interaction. This has gained support in the past two years following reports of direct interaction between ARF and COP-I, a non-clathrin coat used in retrograde and/or anterograde traffic to and from the early Golgi (Zhao et al., 1999), and between ARF and AP-1 (Austin et al., 2000). ARF may also regulate the lipid content of membranes through direct regulation of phospholipase D and phosphoinositide kinases (Jones et al., 2000; Ktistakis et al., 1995). Whether coat recruitment and lipid modification are competing or complementary activities of ARF has not been resolved. If we are to gain a better understanding of the function of ARF, direct effectors of ARF that function in membrane trafficking events need to be identified and studied. Some of the most promising ARF effectors in this class are the GGA proteins.

The recent studies characterizing GGA proteins may provide answers to these two important questions in TGN trafficking. Here, I summarize the findings published on GGA proteins to date, which have provided a remarkable picture of the function of GGA proteins in a short period of time, with the goal of clarifying the known functions and identifying future directions of GGA research.

Identification of GGA proteins
The GGA proteins were discovered nearly simultaneously by several labs studying very different aspects of membrane trafficking (Boman et al., 2000; Dell’Angelica et al., 2000; Hirst et al., 2000; Poussu et al., 2000). Together, these original studies provided a wealth of structural and functional information. Members of the Kahn laboratory were interested in the role of ARF proteins in membrane trafficking, particularly seeking direct effectors of ARF. Using a yeast two-hybrid screen of human cDNA libraries with activated ARF3 as the protein bait, they identified portions of two GGA...
proteins, GGA1 and GGA2 (Boman et al., 2000), and determined the full-length cDNAs through standard and computer-based 5' RACE. A third protein, GGA3, was identified in a BLAST search of GenBank as a full-length cDNA deposited by the Human cDNA Project at the Kazusa DNA Research Institute.

The Robinson and Bonifacino laboratories have long been interested in the composition and function of heterotetrameric clathrin adaptor complexes. The adaptor complexes each comprise four subunits: one highly conserved large subunit (β-adaptin), one less-conserved large subunit (α-, γ- or δ-adaptin, depending on the complex), one medium subunit (μ), and one small subunit (σ; Hirst and Robinson, 1998). The large subunits have three domains: the body, hinge and ear. In searching the sequence databases for potentially novel adaptor subunits, both groups found novel human ESTs that shared high homology with the ear domain of γ-adaptin (Dell’Angelica et al., 2000; Hirst et al., 2000). The Robinson and Bonifacino groups found three human GGA proteins. Bonifacino and co-workers also found an alternatively spliced form of GGA3 (long form) in addition to the shorter GGA3 clone in GenBank.

Members of the Lehto laboratory identified GGA2, which they named VEAR, through its sequence homology to proteins containing a VHS domain (Poussu et al., 2000). This domain was so named because it was initially found in three proteins: Vps27, Hrs, and STAM (Lohi and Lehto, 1998; Mao et al., 2000). The crystal structure of the VHS domain from another protein, Tom1 (target of Myb1), suggests that VHS domains mediate protein-protein or protein-membrane interactions (Misra et al., 2000).

The presence in sequence databases of GGA orthologs from other organisms indicates that GGA proteins are conserved throughout eukaryotes. ESTs of rat GGA proteins are nearly identical to the human proteins. The Drosophila, Caenorhabditis elegans and Schizosaccharomyces pombe genome projects each have revealed at least one GGA-related gene, and these share similar domain organization. Schizosaccharomyces cerevisiae has two GGA genes, which have now been studied in several laboratories. In all these organisms, the GGA proteins are ~65-80 kDa in size.

**Domain structure of GGA proteins**

Four domains are apparent when the amino acid sequences of human and yeast GGA proteins are aligned with each other and with other proteins (Fig. 1). An N-terminal domain of 150 residues resembles the VHS domain. The most highly conserved domain is ~170 residues in length (residues 150-320; 65% identity between GGA proteins) and contains two predicted coiled-coil domains. It has been named the GAT domain (Dell’Angelica et al., 2000) because it has some sequence homology to Tom1 (GGA and Tom1). The role of the coiled-coil domain is unclear, because GGA proteins appear to be monomeric in cytosol (Hirst et al., 2000). A ‘hinge’ region of variable lengths contains one or more clathrin-binding domains but no other significant homology to each other or other known proteins. A C-terminal domain of 120 residues (40% identity between human GGA proteins) has homology to the ear domain of γ-adaptin and has been named the gamma-adaptin ear (GAE) homology domain.

GGA proteins are monomeric in cytosol (Hirst et al., 2000) and hence unlikely to be members of a multi-subunit adaptor complex. Rather, evidence for the function of each domain, described below, suggests that GGA proteins as ARF-dependent monomeric clathrin adaptors involved in the sorting of cargo and recruitment of clathrin.

**GGA proteins are localized to the trans-Golgi network**

Several laboratories have examined the localization of GGA proteins in mammalian cells, using indirect immunofluorescence microscopy and immunoelectron microscopy. Endogenous GGA1, GGA2 and GGA3 localize predominantly to the trans-Golgi region in NRK, HeLa, Cos7 and human embryonic skin cells (Boman et al., 2000; Dell’Angelica et al., 2000; Hirst et al., 2000; Poussu et al., 2000; Takatsu et al., 2000), providing evidence for a function in the trans-Golgi region. The three human proteins show overlapping but subtle differences in staining patterns. In addition to the shared TGN staining, GGA1 shows a highly punctate pattern within the TGN and late Golgi region, GGA2 shows diffuse and cytosolic staining, and GGA3 stains larger puncta in the cytosol. Mammalian GGA proteins isolated following cell lysis are soluble (Boman et al., 2000; Hirst et al., 2000), which suggests that the membrane-associated proteins rapidly exchange with a cytosolic pool of GGA proteins, or that the membrane association is lost upon cell lysis. In yeast, GGA proteins also localize to Golgi-like puncta (A.L.B., unpublished); however, they fractionate with membranes rather than cytosol, which suggests that membrane association in yeast is stabilized to a greater extent than in mammalian cells, probably through protein-protein interactions.

**The function of GGA proteins in mammalian cells**

Transiently or stably overexpressed GGA proteins localize similarly to the endogenous proteins as long as the expression level is low. High levels of GGA proteins cause dramatic changes in the overall morphology of the Golgi (Boman et al., 2000; Poussu et al., 2000; Takatsu et al., 2000). Several phenotypes have been observed, including a compaction and ‘fizzling’ of the Golgi stacks and fragmented vacuolar-like blobs that contain Golgi markers. The underlying reason for this alteration is unknown.

Moderate levels of GGA protein overexpression cause changes in the localization of specific coat proteins and cargo without affecting Golgi morphology. Coat proteins that are recruited to the TGN, such as AP-1 and clathrin, are affected by GGA protein overexpression. AP1 staining at the TGN is reduced in cells overexpressing GGA proteins, suggesting that GGA proteins compete with AP-1 for binding sites or ARF (Dell’Angelica et al., 2000). In contrast, clathrin staining at the TGN is increased in cells overexpressing GGA proteins (Puertollano et al., 2001b), suggesting that GGA proteins recruit clathrin. The effect of moderate GGA expression on coats is limited to the TGN, because neither β-COP, used at the early Golgi, nor AP-2, used at the plasma membrane, shows altered localization in these cells (Boman et al., 2000; Dell’Angelica et al., 2000).

The steady-state localization of cargo proteins that cycle
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between the TGN, endosomes and the plasma membrane (mannose 6-phosphate receptor (M6PR) and TGN38) in cells overexpressing GGA3 displays a dramatic shift from the TGN to the plasma membrane (Boman et al., 2000). Cells overexpressing GGA1 exhibit a similar but less dramatic shift (Boman et al., 2000). Cargo perturbations are also limited to proteins trafficking through the TGN; mannosidase II, which is localized to the cis/medial Golgi, is unaffected by equivalent levels of overexpression. This shift in M6PR and TGN38 localization suggested that GGA proteins have a role in stimulating exit from the TGN. More recent data suggest that the accumulation of cargo at the plasma membrane in cells overexpressing GGA proteins is enhanced by sequestering clathrin away from sites of endocytosis (A.L.B., unpublished).

The levels of other proteins at the Golgi increase in response to GGA overexpression, including ARF (Zhu et al., 2000) and γ-synergin (Takatsu et al., 2000). In the case of ARF, this is due to stabilization of the GTP-bound form of ARF (see below). γ-synergin is likely to be recruited to the TGN through interaction with TGN-localized GGA proteins. These results in mammalian cells support the hypothesis that GGA proteins facilitate membrane trafficking events at the late Golgi, perhaps by sorting or recruiting cargo into forming vesicles.

Elucidation of GGA protein function in yeast

Work in S. cerevisiae has provided more specific evidence for the pathways in which GGA proteins act. Yeast express two GGA genes, GGA1 and GGA2, which share 50% amino acid identity. Deletion of either gene alone causes no (GGA1 deletion) or minor (GGA2 deletion) defects, whereas deletion of both genes causes notable trafficking defects in distinct post-Golgi events (Black and Pelham, 2000; Costaguta et al., 2001; Hirst et al., 2000; Zhdankina et al., 2001). Current evidence supports a role for GGA proteins in a TGN-to-early endosome or in a TGN-to-late endosome pathway, but there is no clear resolution as to which of these pathways GGA proteins function in. Several of the pathways tested for GGA protein involvement, shown in Fig. 2, are described below.

Two vacuolar enzymes that traffic via the early endosome and prevacuolar compartment, carboxypeptidase Y (CPY) and carboxypeptidase S (CPS), display trafficking defects in cells lacking both GGA1 and GGA2. CPY, a soluble protein, is normally delivered to the vacuole, where it is cleaved from a glycosylated precursor form (P2) to the smaller mature form. In cells lacking both GGA genes, ~40% of newly synthesized CPY is misrouted to the cell exterior as the uncleaved P2 form and an aberrantly cleaved pseudomature form (Dell’Angelica et al., 2000; Hirst et al., 2000; Zhdankina et al., 2001). Vacuolar delivery and cleavage of CPS, a transmembrane protein, is dramatically slowed in cells lacking both GGA genes (Costaguta et al., 2001), although whether CPS is mislocalized to the plasma membrane or retained in the TGN or endosome is not known.

The mating pheromone α-factor is synthesized as a large peptide, glycosylated as it moves through the Golgi apparatus, and cleaved to its mature form by three proteases. The first of these proteases, Kex2p, is localized to the TGN through both a retention mechanism and retrieval from the prevacuolar compartment (PVC). Defects in Kex2p localization can be observed indirectly in assays for α-factor secretion or vacuolar degradation of Kex2p. In cells lacking both GGA genes, ~60% of α-factor is secreted as a highly glycosylated precursor, which suggests that there is a defect in the localization of Kex2p protease (Costaguta et al., 2001). In some strain backgrounds, Kex2p stability is unaffected by loss of GGA1 and GGA2 (Costaguta et al., 2001). In our strains, Kex2p is unstable and probably missorted via the plasma membrane to the vacuole (A.L.B., unpublished). All these data suggest that GGA proteins are involved in a TGN-to-endosome pathway.

Pep12p is a syntaxin or SNARE that is normally delivered from the TGN to late endosomes (Black and Pelham, 2000). In strains lacking GGA1 and GGA2, a Pep12p chimera is misrouted to an early endosome and does not progress to the late endosome (Black and Pelham, 2000). Pelham concluded that GGA proteins are key components of a specific pathway from the TGN directly to the late endosome and that AP-1 mediates TGN-to-early endosome transport. This may explain...
the differential effects of GGA and AP-1 deletions on different cargo. Pelham’s proposal of a distinct GGA-dependent pathway to late endosomes may account for the effects of GGA depletion on CPY, CPS, and Kex2p cleavage and trafficking if the early endosome is sufficiently perturbed by the presence of late endosomal components and if exit of specific proteins from the early endosome is inhibited.

Genetic data from the Payne laboratory (Costaguta et al., 2001) may support both potential pathways. This group has found synthetic interactions between GGA genes and the large subunits of AP-1. When both GGA genes and either APL4 (γ subunit) or APL2 (β subunit) are deleted, the cells are severely growth deficient. When GGA2 and APL2 are deleted, the yeast grow slowly and exhibit more severe defects in CPS processing and α-factor secretion than when either gene alone is deleted. These data can be interpreted to indicate that GGA proteins and AP-1 facilitate distinct pathways, and elimination of both pathways causes severe defects, or that GGA proteins and AP-1 have different cargo selectivity but work together to form one vesicle in a TGN-to-endosome pathway.

Because both GGA genes must be deleted for one to see the defects described, they were not identified in the screens for vacuolar protein sorting (VPS) mutants (Robinson et al., 1988; Rothman and Stevens, 1986). Yet it is intriguing that the double GGA deletion phenotypes do not fit into any of the six classes of VPS gene that are based on mutant phenotypes (Raymond et al., 1992). In class E mutants, a fraction of CPY is missorted to the cell exterior; as is seen in GGA deletions. However, class E mutants are also characterized by an enlarged prevacuolar compartment and by cleavage of the CPY receptor Vps10p to a smaller but still functional peptide. Neither of these phenotypes is found in cells lacking GGA genes (Costaguta et al., 2001; Hirst et al., 2000; Zhdankina et al., 2001). In addition, only the P2 form of CPY is secreted in class E mutants, whereas P2 and pseudomature forms are both secreted in GGA deletions. Hence the GGA genes are not class E genes. Particularly surprising is the fact that the receptor for CPY, Vps10p, is localized normally in cells lacking GGA1 and GGA2, as determined by immunofluorescence (Hirst et al., 2000) and by pulse-chase experiments (Costaguta et al., 2001), and yet CPY is significantly missorted. One scenario consistent with these data is that GGA proteins are important for sorting of Vps10p into PVC-bound vesicles. In the absence of GGA proteins, a portion of Vps10p might get trapped in the TGN rather than transported with bound CPY to the early endosome or PVC. The excess CPY in the TGN would then get secreted, but the transmembrane Vps10p would stay in the TGN. The portion of Vps10p that does arrive at the PVC would release its CPY for vacuolar delivery and be retrieved normally, giving a normal pattern of staining and stability.

In contrast to the pathways described above, other trafficking pathways are unaffected by loss of both GGA genes: delivery of alkaline phosphatase to the vacuole via the AP-3 pathway is normal (Costaguta et al., 2001; Hirst et al., 2000); invertase is secreted at normal rates (A.L.B., unpublished); and endocytosis of the lipophilic dye FM 4-64 to the vacuole occurs at normal rates (Hirst et al., 2000; Zhdankina et al., 2001).

Together, these data point to the involvement of GGA proteins in a specific TGN-to-endosome pathway rather than in a general TGN exit pathway or in maintaining normal TGN morphology. In vitro analysis of vesicle formation from purified components and identification of cargo that requires GGA proteins and not AP-1 (or vice versa) for proper sorting will help to distinguish between the possible TGN-to-endosome pathways.

### Interactions between GGAs and other proteins

GGA proteins have been predicted to act by interacting directly with one or more proteins, such as cargo, coats and ARFs. Indeed, each domain of GGA proteins has now been shown to interact directly with one or more proteins (Fig. 3). These interactions have shed much light on the function of GGA proteins, especially in mammalian cells.

As discussed above, GGAs were originally identified through their interaction with ARF proteins (Boman et al., 2000). This interaction has been studied both in vitro and in vivo, and all data indicate that GGAs are effectors of ARF. All three human GGA proteins and both yeast GGA proteins interact strongly with GTP-bound ARF in two-hybrid assays. Purified GST-GGA proteins (mammalian and yeast) interact specifically in vitro with GTP-bound ARF, but not GDP-bound ARF, which confirms the two-hybrid results (Boman et al., 2000; Zhdankina et al., 2001). The region of GGA proteins that interacts with ARF has been mapped to residues 170-330 (Boman et al., 2000; Zhdankina et al., 2001), which span the GAT domain. Mutations of ARF3 that cannot interact with GGA1 occur in the switch I region (Kuai et al., 2000), a known effector-binding region in other small GTP-binding proteins. These mutants were later shown to fail to interact with the GAT domain of GGA3, as expected (Puertollano et al., 2001b). As in the case of other ARF effectors, purified GST-GGA1 stabilizes the GTP-bound form of ARF in vitro (Zhu et al., 2000). The rate of GTP-binding to ARF is not affected; hence GGA proteins are not exchange factors. GGA proteins have no GTPase-activating protein (GAP) activity (Boman et al., 2000; Puertollano et al., 2001b); nor do they enhance the GAP activity of ARF-GAP1 (Puertollano et al., 2001b), as has been expected.

![Fig. 3. Model of GGA interactions with other proteins. Each domain of GGA proteins has been shown to interact with one or more proteins. These interactions are depicted to occur at the TGN membrane, facilitating cargo sorting and vesicle formation in response to GTP exchange on ARF.](image-url)
been shown for COP-I (Goldberg, 1999). Rather, the GGA proteins can compete with ARF-GAPs for binding to ARF (Puertollano et al., 2001b). In the presence of GGA proteins, therefore, GTP hydrolysis on ARF is slowed. Because ARF has no intrinsic GTpase activity, the stabilization of GTP-bound ARF and the reduction of GAP-dependent GTP hydrolysis are separate effects of GGA proteins, although they will certainly act synergistically in vivo.

Several in vivo studies have shown that human GGA proteins are ARF effectors. First, stable expression of a dominant activating mutant of ARF, [Q71L]ARF1, in NRK cells causes dramatic expansion of the Golgi lumen through an unknown effector (Zhang et al., 1994). Overexpression of GGA1 in these cells prevented the [Q71L]ARF1-induced expansion of the Golgi apparatus (Boman et al., 2000), indicating a direct interaction between GGA1 and ARF in vivo and a function unrelated to Golgi expansion. Second, the membrane-associated, GTP-bound form of ARF is stabilized in vivo, as expected from the in vitro experiments described above. An increased amount of ARF is associated with Golgi membranes in cells overexpressing GGA1, as visualized by immunofluorescence (Zhu et al., 2000). Similarly, overexpression of GGA3 slows the BFA-induced dissociation of ARF1 from Golgi membranes (Puertollano et al., 2001b). Finally, a number of experiments show that the localization of GGA proteins at the Golgi is due to interaction with ARF. First, treatment with brefeldin A causes rapid translocation of GGA proteins to the cytosol in a time frame indistinguishable from that of ARF itself (Boman et al., 2000; Dell’Angelica et al., 2000; Hirst et al., 2000; Poussu et al., 2000). Second, the interaction between the GAT domain of GGA proteins and ARF is strong enough to drive a reporter construct (GFP) onto the Golgi, even in the absence of the VHS, hinge and ear domains (Dell’Angelica et al., 2000). Third, and most convincingly, point mutations within the ARF-binding domain that cause loss of ARF interaction also cause loss of Golgi localization (Puertollano et al., 2001b) (A.L.B., unpublished). Together, these data suggest that ARF-GTP recruits GGA proteins from the cytosol onto the late Golgi membrane by interacting with the GAT domain.

A major question that remains is how GGA proteins can be recruited by ARF only to the TGN despite the fact that ARF proteins are localized to many (or all) organelle membranes. In two-hybrid and in vitro binding experiments, all of the human ARFs bind equally well to GGA proteins, which suggests that isoform specificity is not the answer. Perhaps, in the cell, however, different ARF isoforms are actually recruited to distinct membranes, and GGA proteins are only recruited by one (or more) ARF isoform. Alternatively, other proteins described below might stabilize the interaction between ARF and GGA proteins at the TGN, whereas this interaction at other locations might be extremely transient.

The VHS domain of GGA proteins interacts with a defined subset of sorting receptors that traffic between the TGN and lysosomes. Sortilin is a sorting receptor for cytoplasmic cargo such as neurotensin, is a major component of GLUT4 vesicles and is the closest mammalian homolog of Vps10p (Morris et al., 1998; Nielsen et al., 1999). The VHS domain of GGA2 interacts with a dileucine sorting motif present in the cytoplasmic tail of sortilin (Nielsen et al., 2001; Takatsu et al., 2001). Similarly, both the cation-independent and cation-dependent M6PRs interact through an acidic-cluster-dileucine motif with the VHS domain of GGA proteins (Puertollano et al., 2001a; Takatsu et al., 2001; Zhu et al., 2001). Many other transmembrane proteins lacking such a motif do not interact with GGA proteins, which indicates their specificity for a subset of transported proteins. These findings suggest that GGA proteins interact directly with sorting signals in cargo to facilitate sorting into vesicles. The interaction with cargo is not strong enough to drive Golgi localization of the GGA proteins: constructs containing only the VHS domain are soluble (Dell’Angelica et al., 2000). Hence the interaction with ARF is a prerequisite for cargo interaction in mammalian cells. It will be very interesting to determine which other cargo specifically interacts with GGA proteins and to define the binding sites on both cargo and GGA proteins.

The hinge domain of mammalian (Puertollano et al., 2001b) and yeast (Costaguta et al., 2001) GGA proteins interacts directly with clathrin in vitro. The presence of the ear domain strengthens the interaction, and the ear domains of certain GGA proteins can interact with clathrin on their own. Although direct interaction in vivo has not been shown, indirect evidence is strongly in favor of this conclusion. In mammalian cells, overexpression of GGA3 recruits clathrin to the TGN, as previously mentioned. A truncated GGA3 construct lacking the clathrin-binding hinge and ear domains causes M6PR to compact at the TGN rather than be packaged into vesicles, which suggests that the GGA-clathrin interaction is required for vesicle formation (Puertollano et al., 2001b). In yeast, a temperature-sensitive clathrin heavy chain mutant, chc1-ts, is synthetically lethal when combined with a GGA2 deletion (Costaguta et al., 2001). Fractionation of vesicles on a sizing column shows co-elution of GGA proteins, AP-1 and clathrin, which suggests that GGA proteins are components of clathrin-coated vesicles.

The ear domain of human GGA proteins interacts with γ-synergin (Takatsu et al., 2000), a Golgi-localized protein partner of γ-adaptin that has an unknown function (Page et al., 1999). γ-synergin has no homolog in yeast, and the ear domains of yeast and human GGA proteins are not very well conserved; hence the role of the ear domain in yeast is even less clear. However, a binding partner shared by the mammalian GGA ear and γ-adaptin ear shows that these two domains indeed share similar functions.

Conclusions

In conclusion, the GGA proteins appear to be monomeric, ARF-dependent clathrin adaptors that are conserved throughout eukaryotes. Different domains on the GGA proteins are involved in ARF interaction, cargo interaction, and clathrin interaction. A model depicting these interactions is shown in Fig. 3. This model raises several questions about the function of GGA proteins. First, is interaction with ARF required in all organisms and for all GGA protein functions? Second, do GGA proteins bind to ARF, clathrin, and cargo at the same time, or are the interactions sequential? If simultaneous, what are the conformational changes that occur in GGA proteins upon binding to ARF that allow or stabilize interactions with cargo and clathrin? Similarly, do GGA proteins interact with clathrin in the cytosol or only at the membrane? Third, if GGA proteins help to form a vesicle, do they remain with the vesicle or
dissociate upon budding? Fourth, which specific cargos utilize a GGA-dependent pathway in yeast and mammalian cells? Fifth, what is the function of the ear domain, and how does this relate to the function of AP-1?

These and other questions raised throughout this Commentary will surely be addressed in future studies. In addition, work with Drosophila and C. elegans knockouts may reveal interesting developmental roles for GGA proteins. The recent publications on GGA proteins represent an exciting discovery that affects how we view post-Golgi trafficking. Hold on, there is more to come!

References


