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Membrane recruitment of effector proteins by Arf and Rab GTPases

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In their GTP-bound form, Arf and Rab family GTPases associate with distinct organelle membranes, to which they recruit specific sets of effector proteins that regulate vesicular transport. The Arf GTPases are involved in the formation of coated carrier vesicles by recruiting coat proteins. On the other hand, the Rab GTPases are involved in the tethering, docking and fusion of transport vesicles with target organelles, acting in concert with the tethering and fusion machineries. Recent structural studies of the Arf1–GGA and Rab5–Rabaptin-5 complexes, as well as other effector structures in complex with the Arf and Rab GTPases, have shed light on the mechanisms underlying the GTP-dependent membrane recruitment of these effector proteins.

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Current Opinion in Structural Biology 2005, **15**:681–689

This review comes from a themed issue on
Proteins
Edited by Edward N Baker and Guy G Dodson

Available online 9th November 2005

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2005.10.015

Introduction

Intracellular transport of proteins and lipids between membrane-bound organelles is mediated mainly by coated carrier vesicles. Three types of coated vesicles have been well characterized to date: COPI-, COPII- and clathrin-coated vesicles [1]. COPI- and COPII-coated vesicles are involved in retrograde and anterograde transport processes, respectively, between the endoplasmic reticulum (ER) and the Golgi apparatus. Clathrin-coated vesicles (CCVs) mediate diverse transport steps between the *trans*-Golgi network (TGN) and the plasma membrane. The coat of CCVs is composed of clathrin and adaptor proteins [2]. Adaptor protein (AP) complexes (AP-1, AP-2, AP-3 and AP-4) are heterotetrameric, whereas GGAs (Golgi-localizing, γ-adaptin ear domain homology, Arf-binding proteins) are monomeric adaptors.

ADP-ribosylation factor (Arf) family GTPases are crucial for assembling coat proteins during vesicle formation [3]. Arf1 recruits the COPI coat to the Golgi membranes, whereas a distantly related GTPase, Sar1, recruits the COPII coat to the ER membrane. Arfs also regulate the recruitment of clathrin adaptor proteins, AP-1, AP-3, AP-4 and GGAs, to the TGN and/or endosomes. Exceptionally, AP-2 is recruited to the plasma membrane through interaction with phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2]. Arf has a myristoylated amphipathic helix at its N terminus, which is folded into the molecule in its GDP-bound state. However, exchange of GDP for GTP on Arf, which is catalyzed by guanine nucleotide exchange factors (GEFs), exposes the myristoylated N-terminal helix for membrane anchoring. GDP/GTP exchange also causes a dramatic change in the conformation of the switch 1 and switch 2 regions of the GTPases, enabling only GTP-bound GTPases to bind specific sets of effector proteins. Thus, Arf•GTP recruits coat proteins to the membrane and, in turn, promotes vesicle budding. Upon GTP hydrolysis, which is stimulated by GTPase-activating proteins (GAPs), Arf then retracts its myristoylated N terminus and dissociates from the membrane. This step underlies the shedding of the coat from the vesicles before they fuse with target membranes.

Rab GTPases, on the other hand, mediate the tethering of transport vesicles to target membranes [4]. The tethered vesicles are then docked by the specific pairing of the SNARE proteins on the vesicle and target membranes, and finally the vesicle membrane fuses with the target membrane. In contrast to Arfs, Rab proteins undergo isoprenylation at the C terminus for membrane anchoring. The GTP-bound Rab GTPases are also recruited to membranes and interact with their specific effectors, which in turn regulate the vesicular tethering/fusion events.

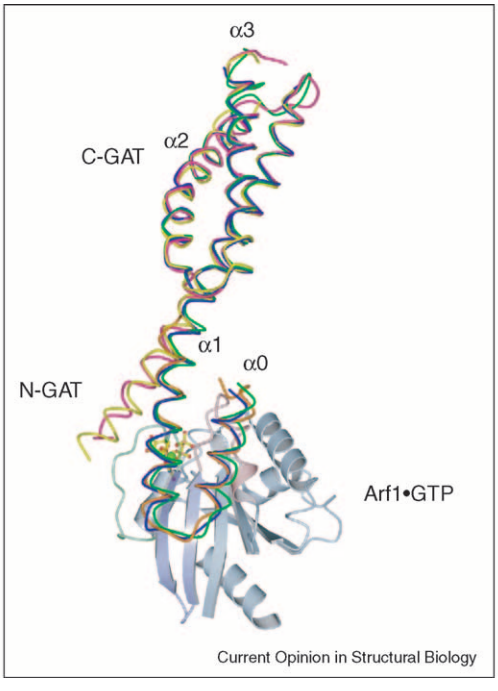
Membrane recruitment of effectors by these GTPases is therefore crucial to the regulation of vesicular transport events. We here review recent structural analyses of Arf and Rab GTPases in complex with their effectors, which revealed divergent but common mechanisms of their membrane recruitment. In most cases, the GTPase-binding regions of the effectors are composed of two α helices, which are aligned along the interswitch β strands between switch 1 and switch 2 of the GTPases, allowing their strict interaction with the two switch regions only in the GTP-bound state. We also highlight the GGA–Rabaptin-5 interaction as an example of crosstalk between the Arf and Rab pathways.

Arf/Arl–effector complexes

Arf1–N-GAT complex

GGAs (three isoforms in human and two in yeast) are monomeric clathrin adaptor proteins involved in the selective transport of lysosomal cargo receptors from the TGN to endosomes [5,6]. GGAs consist of four functional regions: an N-terminal VHS (Vps27/Hrs/Stam) domain; a GAT (GGA and Tom1) domain: a hinge region; and a C-terminal GAE (γ-adaptin ear) domain. The GAT domain of GGAs has been shown to bind Arf1, which is responsible for targeting GGAs to the TGN membrane. In 2003, four groups, including our own, reported the structure of the GGA1-GAT domain in the unbound form [7••,8,9,10••]. Two of the reported GAT structures are composed of four helices [7••,8], whereas the other two structures contain only three, lacking the N-terminal short helix of the other structures [9,10••] (Figure 1). The four-helix GAT structures consist of two independent subdomains: N-terminal helix-loop-helix (N-GAT) and C-terminal three-helix bundle (C-GAT). In the three-helix crystal structures, the N-GAT subdomain is disordered, while the C-GAT subdomain is intact. Mutational studies suggested that the N-GAT subdomain solely participates in Arf binding. Although Tom1 (target of Myb1) and Tom1L1 (Tom1-like 1) also have a GAT domain, neither binds Arf because they do not have the N-GAT subdomain.

Figure 1



Comparison of the four crystal structures of the GGA1-GAT domain. The C-GAT subdomains of the free GGA1-GAT structures are superimposed: blue [7••], green [8], yellow [9] and red [10••]. The Arf1–N-GAT complex [10••] (N-GAT is shown as an orange line and Arf1 is drawn as a light-blue ribbon diagram, with switch 1 highlighted in light green and switch 2 in light red; GTP and Mg^{2+} are shown as ball-and-stick models) is superimposed on the N-GAT subdomains of the two four-helix structures (blue [7••] and green [8]).

Crystallization of the complex between Arf1 and the GAT domain was unsuccessful until the C-GAT subdomain was removed from the GAT construct [10••]. The Arf1–N-GAT complex structure revealed that N-GAT, which was unstructured in the three-helix structures of free GAT, adopts a helix-loop-helix structure similar to that seen in the four-helix structures of free GAT. These structures and circular dichroism (CD) data suggest that N-GAT is in equilibrium between unfolded and folded states, and is stabilized in the helix-loop-helix structure by Arf binding [10••]. The Arf-binding surface of N-GAT is on one side of helices α0 and α1, and is predominantly hydrophobic with several polar residues. The two helices of N-GAT are positioned against the antiparallel β sheet of the Arf interswitch. Helix α0 of N-GAT interacts mainly with switch 2 of Arf1, whereas α1 interacts with switch 1 (Figure 2a). Ile197 of N-GAT, located near the well-conserved Gly50 of switch 1, is crucial to Arf1 binding, because it interacts with both switch regions (Figure 2a). Given that the N terminus of Arf1•GTP is membrane anchored and that helix α1 of N-GAT continues to α1 of C-GAT (Figure 1), the long α1 helix of the GAT domain would protrude into the cytosol (Figure 3). The orientation of helix α1 would probably be perpendicular to rather than along the negatively charged membrane surface, because the C-GAT subdomain is predominantly negatively charged.

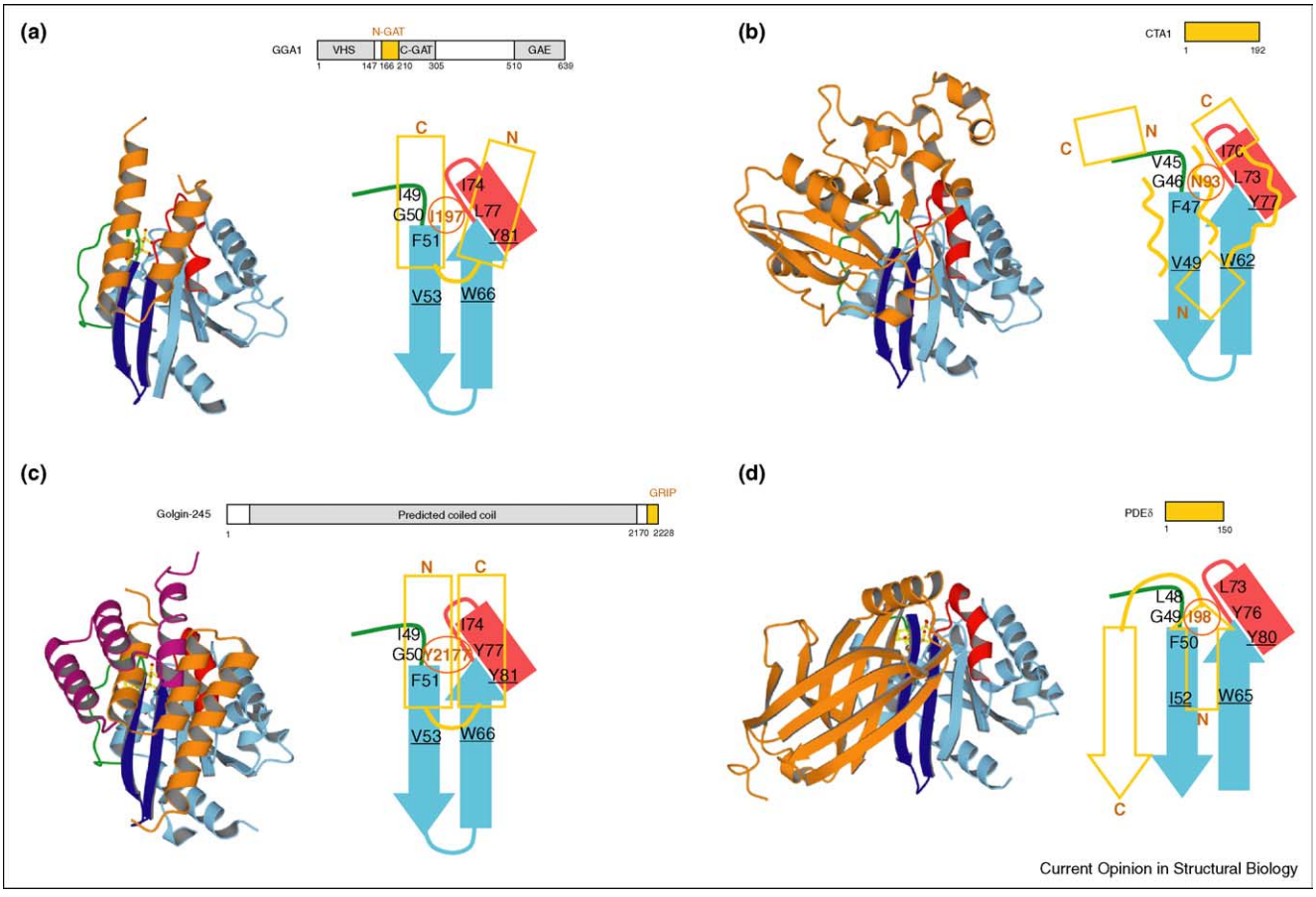
Arf6–CTA1 complex

Arf was first discovered as a cofactor of cholera-toxin-dependent ADP-ribosylation of the α subunit of the heterotrimeric G_s protein (hence designated ADP-ribosylation factor). The ADP-ribosyltransferase activity of the cholera toxin A1 subunit (CTA1) is allosterically increased by Arf binding. A very recent report describes an Arf6–CTA1 complex structure, in which the Arf-binding site of CTA1 consists of loop regions with little secondary structure [11]. CTA1 interacts with the switch and interswitch regions of Arf6, with Asn93 located between the two switches (Figure 2b). This structure suggests that a rigid tertiary structure is not a prerequisite for recognizing Arf•GTP; thus, CTA1 can pretend to be an Arf effector. Although the Arf-binding site of CTA1 is distant from the enzymatic active site, the conformation of the activation loop changes from a coil to an amphipathic helix upon Arf binding, resulting in the opening of the active site to bind the substrate NAD^+ [11].

Arl1–GRIP complex

Arf-like (Arl) GTPases are related to Arfs, with 40–60% sequence identity [12]. Through its N-terminal myristoyl moiety, Arl1 is anchored to the Golgi membranes, to which it recruits golgins. Golgins are a family of Golgi-

Figure 2



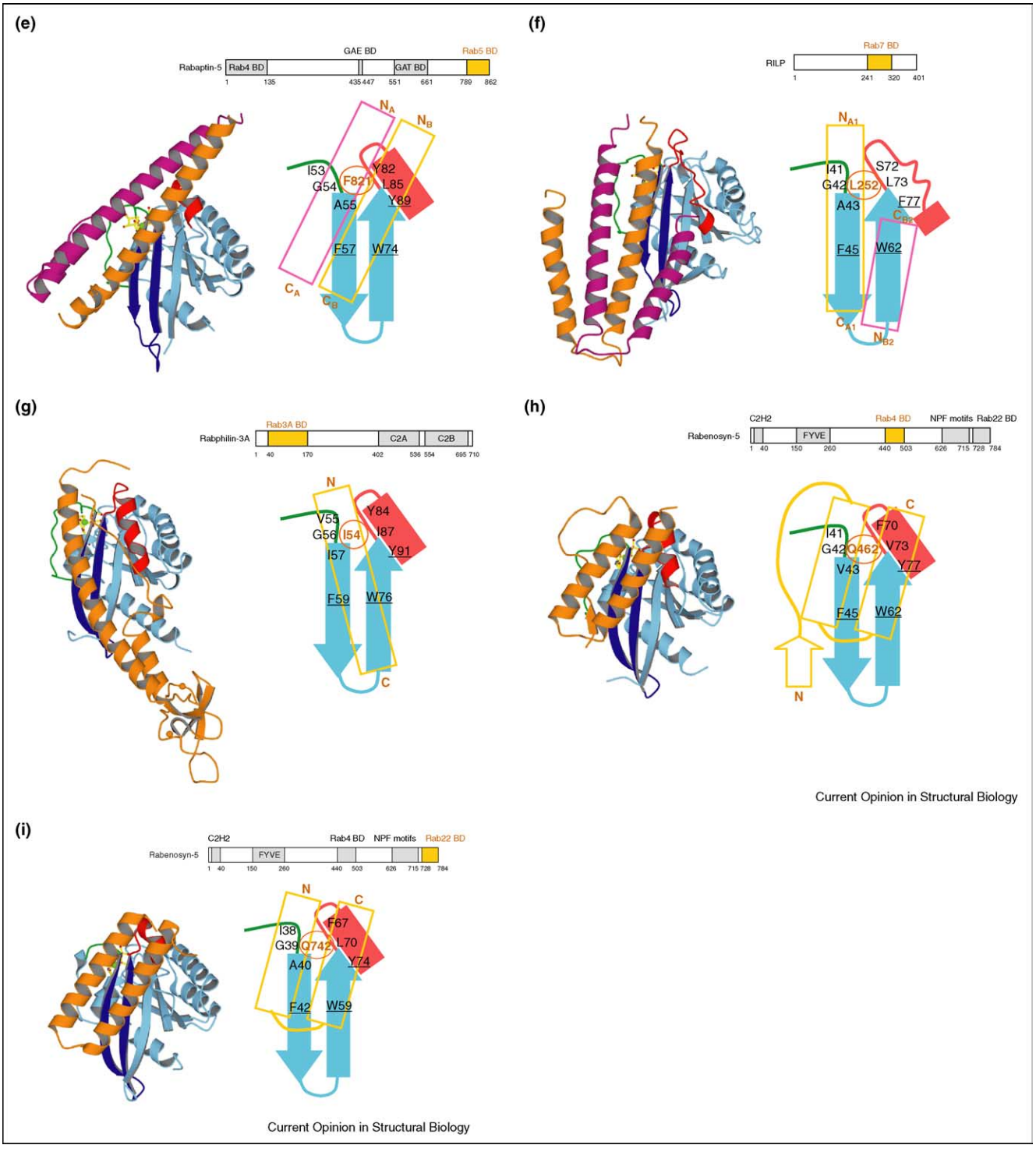
Structural comparison of the Arf/Rab–effector complexes. **(a)** Arf1–N-GAT complex [10••], **(b)** Arf6–CTA1 complex [11], **(c)** Arl1–GRIP complex [14••], **(d)** Arl2–PDEδ complex [16], **(e)** Rab5–Rabaptin-5 complex [18•], **(f)** Rab7–RILP complex [19•], **(g)** Rab3A–Rabphilin-3A complex [20], **(h)** Rab4–rabenosyn-5 complex [21••], **(i)** Rab22–rabenosyn-5 complex [21••]. The domain structure of the effectors is shown at the top of each panel. The GTPase-binding regions are colored orange. BD, binding domain. Left, ribbon diagram of the Arf/Rab–effector complex. The effector molecules are drawn in orange. The second effector molecule is shown in magenta in cases in which the effector forms a dimer (i.e. GRIP, Rabaptin-5 and RILP). The GTPase is shown in light blue, with switch 1 highlighted in green, switch 2 in red and the interswitch in blue. GTP and Mg^{2+} are shown as ball-and-stick models. Right, schematic diagram of the interaction of the effector (orange and magenta) with switch 1 (green), switch 2 (red) and the interswitch (blue) of the GTPase. α Helices, β sheets and loops are represented by boxes, arrows and lines, respectively. The effector residue located between the two switch regions is labeled in brown and circled, with surrounding residues of the switch regions labeled in black. The invariant triad of hydrophobic residues conserved in Rab GTPases [21••] and corresponding residues of Arfs are labeled in black and underlined.

localized proteins with extensive coiled-coil regions [13]. Several golgins contain a C-terminal GRIP domain, whose interaction with Arl1 is responsible for their association with Golgi membranes. The GRIP domain of golgin-245 forms a homodimer and each protomer binds Arl1 [14••,15]. The GRIP domain is an array of three antiparallel helices. The hydrophobic concave surfaces of two GRIP domains face each other. The opposite convex side of each GRIP domain interacts with Arl1. The interaction with Arl1 is mediated by helices α1 and α2 of the GRIP domain, which recognize switches 1 and 2 of Arl1, respectively (Figure 2c). Panic *et al.* [14••] pointed out that Tyr2177 of the GRIP domain is accommodated by a 'selectivity pocket' sandwiched between switches 1 and 2 of Arl1 (Figure 2c). The position of Tyr2177 of GRIP corresponds to that of Ile197 of N-GAT in the Arf1–N-GAT complex. Thus, the mode of Arl1–GRIP interaction resembles that of Arf1–N-GAT; two antiparallel helices form predominantly hydrophobic interactions with switches 1 and 2 and the interswitch regions, although the directions of the helices are opposite.

Arl2–PDEδ complex

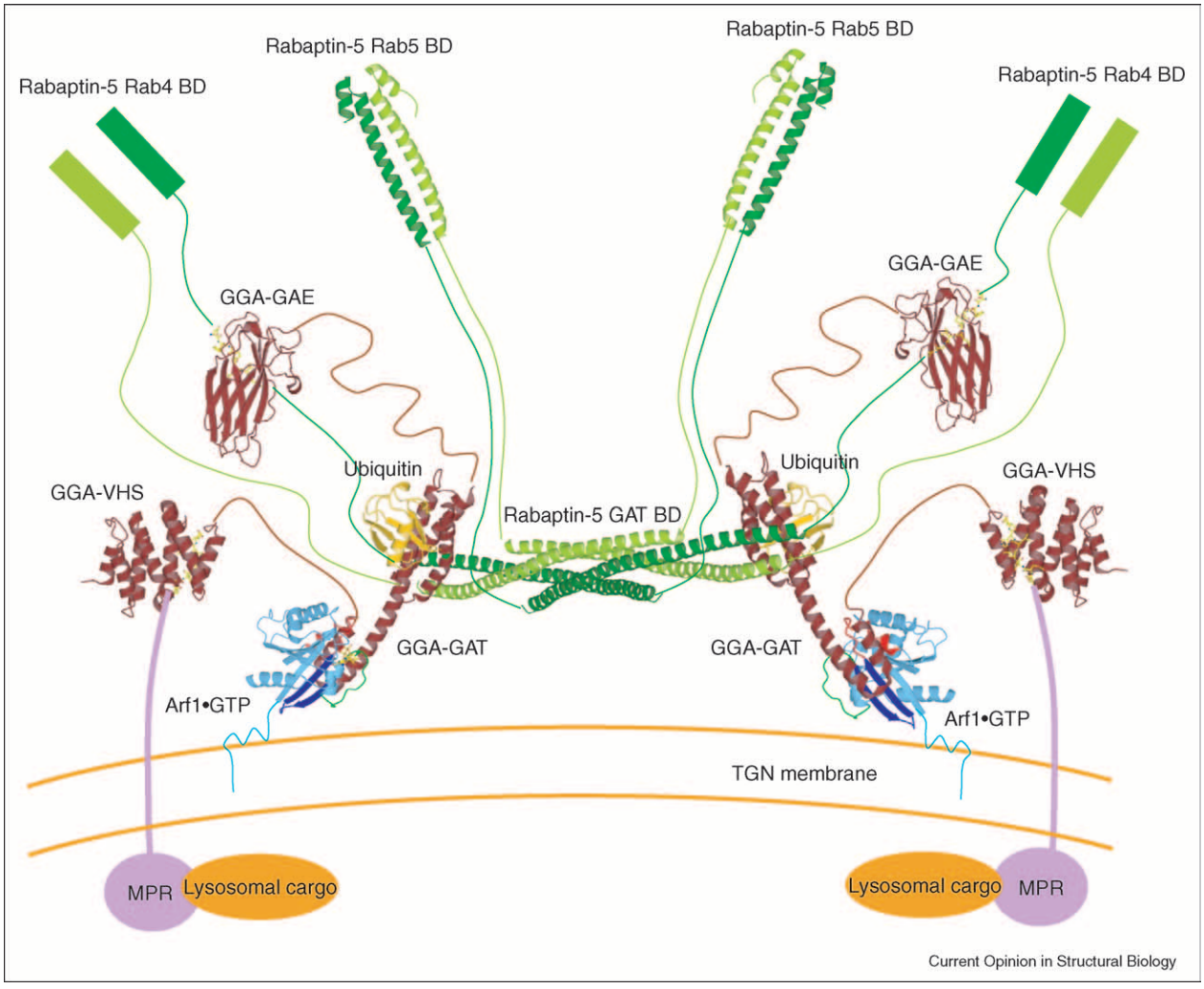
The δ subunit of rod-specific cyclic-GMP phosphodiesterase 6 (PDEδ) forms an immunogloblin-like β-sandwich fold, unlike other known structures of Arf and Rab effectors. Rather, PDEδ is structurally related to RhoGDI and possesses a hydrophobic pocket that could accom-

Figure 2 (*Continued*)



modate a lipid moiety [16]. In the Arl2–PDEδ complex, the β7 strand of PDEδ forms an intermolecular β sheet with the β2 strand of the interswitch region of Arl2 (Figure 2d). Additionally, the β6 strand of PDEδ interacts with the switch 2 and interswitch regions of Arl2 in a hydrophobic manner. Ile98 of PDEδ is located between the two switch regions. Unlike other Arf family members, Arl2 is not myristoylated. Nonetheless, Arl2 is supposed to interact with the membrane through its N-terminal amphipathic helix.

Figure 3



Model of interactions involving Arf1, GGA, Rabaptin-5 and ubiquitin. This diagram exhibits dyad symmetry in the middle, including two GGA1 molecules (brown) and four Rabaptin-5 molecules (green and light green). Arf1•GTP (light blue, with switch 1 highlighted in green, switch 2 in red and the interswitch in blue) on the TGN membrane binds the N-terminal helix-loop-helix of the GGA1-GAT domain (brown) [10••]. The N-terminal VHS domain of GGA1 (brown) binds the cytoplasmic tail of lysosomal cargo receptors, such as mannose-6-phosphate receptor (MPR) (purple) [36]. Rabaptin-5 forms a homodimer (green and light green). GGA binds Rabaptin-5 in a bivalent manner; the GGA1-GAT domain binds the Rabaptin-5 GAT-binding domain (BD) [26••], whereas the C-terminal GAE domain of GGA1 (brown) binds the DFGPL sequence of Rabaptin-5 (modeled from the GGA3-GAE–Rabaptin-5 complex structure [34•]). Ubiquitin (yellow) can bind to another side of GGA1-GAT [31]. Two Rabaptin-5 homodimers form a tetramer comprising a four-helix bundle (middle of diagram). The C-terminal Rab5-binding domain (BD) of Rabaptin-5 binds Rab5•GTP (the Rab5 BD structure is taken from the Rab5–Rabaptin-5 complex structure [17]), whereas the N terminus of Rabaptin-5 (drawn as a rectangle) binds Rab4•GTP.

Rab–effector complexes

Rab5–Rabaptin-5 complex

Several Rab GTPases associate with endosomal compartments [4]. Rab5 is involved in the fusion of early endosomes and Rab4 regulates receptor recycling from endosomes back to the cell surface. Rabaptin-5 is a multivalent effector that interacts with Rab4 and Rab5 through distinct binding regions [17] (Figure 2e). It also interacts with GGAs in a bivalent manner (see below). Rabaptin-5 is recruited to early endosomes through a specific interaction between its C-terminal region and Rab5. Crystal structure analysis of the Rab5–Rabaptin-5 complex revealed that the Rabaptin-5 C-terminal region forms a parallel coiled-coil homodimer, to which two Rab5 molecules independently bind in a dyad-symmetrical manner [18•]. The switch 2 and interswitch regions, but not the variable regions (RabSF1, RabSF3 and RabSF4), of Rab5 are mainly involved in the interaction. The long helices of the Rabaptin-5 homodimer are placed along the β strands of the Rab5 interswitch (Figure 2e). Although the positions of the two helices partially overlap with those of N-GAT in complex with Arf1, the Rabaptin-

5 helices are shifted toward switch 1. Phe821 of Rabaptin-5, which is located at the position corresponding to that of Ile197 of N-GAT, plays a pivotal role in Rab5 binding by interacting with both switch regions (Figure 2e); a mutation of this residue (F821R) abolished the interaction [18•].

Rab7–RILP complex

Rab7 regulates vesicle traffic from early to late endosomes. Rab7-interacting lysosomal protein (RILP) is a Rab7 effector, although its function remains elusive. Its Rab7-binding domain contains two helices; one of them, α1, participates in the formation of a parallel coiled-coil homodimer (Figure 2f) [19•]. Two Rab7 molecules independently interact with either side of the RILP homodimer. The switch 1 and interswitch regions of Rab7 interact with helix α1 of one RILP molecule, whereas switch 2, which is an extended loop rather than a helical structure, interacts with helix α2 of the other molecule. Leu252 of RILP is located between the two switch regions and its mutation (L252A) abolishes Rab7 binding [19•]. In addition to these interactions, variable regions of Rab7 (RabSF1 and RabSF4) form signature interactions with helix α2. The C-terminal helix of Rab7 changes into a small β strand and is involved in the interaction with RILP. Similar interactions, which do not involve the switch or interswitch regions, are also observed in the Rab3A–Rabphilin-3A and Rab4–rabenosyn-5 complexes (see below), but not in the Rab5–Rabaptin-5 or Rab22–rabenosyn-5 complexes.

Rab3A–Rabphilin-3A complex

Rab3A regulates synaptic vesicle exocytosis together with Rabphilin-3A. In the Rab3A–Rabphilin-3A complex, the switch and interswitch regions of Rab3A interact with the long α1 helix of a Rabphilin-3A monomer (Figure 2g) [20]. Ile54 of Rabphilin-3A is located between the two switch regions. In addition, the C terminus of helix α2 and the subsequent short motif of Rabphilin-3A interact with the three variable regions (RabSF1, RabSF3 and RabSF4) of Rab3A. The Zn^{2+}-binding domain connecting the two helices of Rabphilin-3A is supposed to associate with the membrane.

Rab4–rabenosyn-5 and Rab22–rabenosyn-5 complexes

Rabenosyn-5 is a multivalent effector containing two independent Rab-binding domains that share significant sequence similarity [21••,22]. Despite this similarity, each Rab-binding domain recognizes distinct subsets of Rab family proteins: one (residues 440–503), in the middle region of rabenosyn-5, binds tightly to Rab4 and Rab14, whereas the other (residues 728–784), at the C terminus, prefers Rab5, Rab22 and Rab24. Crystal structures of the Rab4–rabenosyn-5(440–503) and Rab22–rabenosyn-5(728–784) complexes, recently determined by Eathiraj *et al.* [21••], revealed that both interaction modes resemble that of the Arf1–N-GAT complex (Figure 2h,i). Rabenosyn-5(728–784) mainly interacts with the switch 2 and interswitch regions of Rab22 (Figure 2i); Gln742 is a key residue that hydrogen bonds with the Ala40 mainchain of switch 1 and the Tyr74 sidechain of switch 2. In addition to the similar interactions of the two regions, the Rab4–rabenosyn-5(440–503) complex reveals an interaction that does not involve the switch or interswitch regions; the extended N-terminal region of rabenosyn-5(440–503) folds into a β strand to form an intermolecular β sheet with the Rab4 β2 strand and interacts with helix α1 of Rab4 (Figure 2h). In contrast to the N-GAT structure, which is stabilized by Arf1 binding, the helix-loop-helix structure of rabenosyn-5(440–503) in complex with Rab4 is essentially the same as that of the free form [21••].

Modes of membrane association of Arf and Rab effectors

Based on the above-described interactions, Arf and Rab effectors can be classified into three groups: stable 'coiled-coil structural scaffolds', such as golgins; flexible 'mooring cables', such as GGAs; and a combination of the two, such as Rabaptin-5. The GTPase-binding sites of these effectors are generally composed of two helices that interact with hydrophobic surfaces formed by the switch and interswitch regions of the GTPases. Notably, one effector residue located between the two switch regions of the GTPase, near the well-conserved glycine of switch 1, is crucial to the recognition of the GTP-bound form of the GTPase. The area of binding interface is largest when the two helices align along the two β strands of the interswitch. The orientation of the helices relative to the interswitch β strands, however, varies according to the shape of the hydrophobic surface of the switch and interswitch regions of each GTPase.

The Arl1–GRIP, Rab5–Rabaptin-5 and Rab7–RILP complexes show dyad symmetry of GTPase binding. These effectors dimerize and two GTPase molecules independently bind to opposite sides of the dimer. As pointed out by Panic *et al.* [14••] this bivalent binding would increase the residence time of the effector on the membrane, because GTP hydrolysis on both GTPase molecules would be required for effector release. The GTPase-binding sites of GRIP, Rabaptin-5, and RILP form homodimeric coiled-coil structures. If the binding site is located in the middle of the long straight section of the coiled-coil structure, it has to lie along the membrane and cannot stand perpendicular to the membrane. In order to project into the cytosol and fulfill their functions, the effectors must have their GTPase-binding sites at one end of the molecule or, alternatively, in the middle of the molecule with a helix-loop-helix structure. In the Arl1–GRIP, Rab5–Rabaptin-5 and Rab22–rabenosyn-5 complexes, the GTPase-binding regions are located at the C termini of the effectors, whereas the Rab3A-binding site of Rabphilin-3A is located near the N terminus. On the other hand, in the Arf1–N-GAT, Rab7–RILP and Rab4–

rabenosyn-5 complexes, the GTPase-binding regions adopt helix-loop-helix structures, as they are located in the middle of the effector sequence. A spectacular exception, as described above, is CTA1, which falls outside of these interaction patterns but still forms an effective hydrophobic interaction surface for Arf6.

Crosstalk between the Arf and Rab pathways

Bivalent Arf and Rab effectors

Rabaptin-5 and rabenosyn-5 are bivalent Rab effectors that interact with both Rab4 and Rab5 [17,22]. Rab-coupling protein (RCP), a member of the Rab11-family interacting proteins (FIPs), is also a bivalent Rab effector, which binds both Rab4 and Rab11 [23]. Because Rab4, Rab5 and Rab11 have been demonstrated to associate with distinct compartments/subdomains of the endocytic pathway [4], these bivalent Rab effectors are suggested to mediate communication between individual Rab-specific compartments. Notably, Arfophilin-1/FIP3 and Arfophilin-2/FIP4 are dual Arf/Rab effectors, both of which interact with both Arfs (Arf5 and Arf6) and Rab11 [24], suggesting possible roles in integrating signals from Arfs and Rab11 in order to regulate endosomal trafficking.

GGA-GAT–Rabaptin-5 complex

Crosstalk between the Arf and Rab pathways also occurs as a result of the interaction between the Arf effector GGA and the Rab effector Rabaptin-5. Rabaptin-5 interacts with GGAs in a bivalent manner; one of the predicted coiled-coil regions, covering residues 551–661, binds the GAT domain of GGA1 and GGA3, whereas an FGPLV sequence (residues 439–443) binds the GAE domain of GGAs [25]. The interaction between Rabaptin-5 and GGAs might promote the tethering or fusion of TGN-derived CCVs with endosomes. In the crystal structure of Rabaptin-5(551–661) in complex with GGA1 C-GAT, two Rabaptin-5 molecules form a parallel coiled coil at the N terminus [26••]. GGA1 C-GAT binds one side of the N terminus of the Rabaptin-5 coiled coil (Figure 3). Helices α2 and α3 of C-GAT form a hydrophobic patch for Rabaptin-5 binding. The Arf-binding site of N-GAT and the Rabaptin-5-binding site of C-GAT are about 35 Å apart. Furthermore, two symmetry-related Rabaptin-5 dimers form a four-helix bundle using the C-terminal 50 amino acids (Figure 3).

The C-GAT subdomain of GGAs interacts with ubiquitin [27–29] and Tsg101, in addition to Rabaptin-5. The Rabaptin-5-binding site was expected to overlap with the ubiquitin-binding site. However, in the crystal structure of the GGA3 C-GAT–ubiquitin complex, ubiquitin was unexpectedly bound on the opposite side of the three-helix bundle [30,31]. Biochemical and NMR data suggest that the GAT domain has two ubiquitin-binding surfaces [31,32], one of which indeed overlaps with the Rabaptin-5-binding site. Thus, it is conceivable that GGA1-GAT, Rabaptin-5 and ubiquitin form a ternary complex [30] (Figure 3), whereas GGA2 and GGA3 cannot because they are unable to bind ubiquitin and Rabaptin-5, respectively.

GGA-GAE–Rabaptin-5 complex

The C-terminal GAE domain of GGA recruits various accessory proteins, including Rabaptin-5 (Figure 3). The GAE domain has an immunoglobulin-like fold [33] and interacts with an extended acidic phenylalanine peptide (DFGXΦ, where the first D is designated as position –1 and Φ is a bulky hydrophobic residue) found in the accessory proteins [34•,35]. In the crystal structure of GGA3-GAE in complex with a Rabaptin-5 peptide (DFGPLVGA), each of the hydrophobic residues, Phe(0) and Leu(3), is accommodated by a hydrophobic pocket composed of aliphatic portions of the conserved basic and hydrophobic residues [34•]. In addition, there are four hydrogen bonds between the mainchain atoms of the ligand peptide and the β4 strand of GGA3-GAE, resulting in intermolecular β-sheet formation. The bivalent interaction between GGA and Rabaptin-5 produces an extensive network of effectors and their binding partners (Figure 3).

Conclusions

Vesicle transport is a series of orchestrated events regulated by Arf and Rab GTPases. They directly bind to membranes and function as mooring posts for their effectors. The GTPase-binding sites of the Arf and Rab effectors are located at the end of a coiled-coil sequence or, alternatively, are found in the middle of a sequence with a helix-loop-helix structure. Effectors may function as stable coiled-coil scaffolds or flexible mooring cables, making an extensive network of proteins that regulate vesicle transport. Several effectors show bivalent Arf/Rab binding, facilitating crosstalk between the Arf and Rab pathways.

Acknowledgements

We thank Tomoo Shiba for preparing the figures, and Kentaro Ihara, Satoshi Hirano and Mariko Kawasaki for helpful discussions.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. McMahon HT, Mills IG: **COP and clathrin-coated vesicle budding: different pathways, common approaches**. *Curr Opin Cell Biol* 2004, **16**:379-391.

2. Owen DJ, Collins BM, Evans PR: **Adaptors for clathrin coats: structure and function**. *Annu Rev Cell Dev Biol* 2004, **20**:153-191.

3. Nie Z, Hirsch DS, Randazzo PA: **Arf and its many interactors**. *Curr Opin Cell Biol* 2003, **15**:396-404.

4. Zerial M, McBride H: **Rab proteins as membrane organizers**. *Nat Rev Mol Cell Biol* 2001, **2**:107-117.

5. Nakayama K, Wakatsuki S: **The structure and function of GGAs, the traffic controllers at the TGN sorting crossroads**. *Cell Struct Funct* 2003, **28**:431-442.

6. Bonifacino JS: **The GGA proteins: adaptors on the move**. *Nat Rev Mol Cell Biol* 2004, **5**:23-32.

7. •• Collins BM, Watson PJ, Owen DJ: **The structure of the GGA1-GAT domain reveals the molecular basis for ARF binding and membrane association of GGAs**. *Dev Cell* 2003, **4**:321-332.

The authors report the crystal structure of the GGA1-GAT domain. It consists of four helices, which can be divided into two independent subdomains: N-terminal helix-loop-helix and C-terminal three-helix bundle. They propose a 'beads on a string' model for full-length GGA1. GGA1 could extend over 400 Å as a result of long random coils that loosely connect the folded domains (VHS, GAT and GAE).

8. Zhu G, Zhai P, He X, Terzyan S, Zhang R, Joachimiak A, Tang J, Zhang XC: **Crystal structure of the human GGA1 GAT domain**. *Biochemistry* 2003, **42**:6392-6399.

9. Suer S, Misra S, Saidi LF, Hurley JH: **Structure of the GAT domain of human GGA1: a syntaxin amino-terminal domain fold in an endosomal trafficking adaptor**. *Proc Natl Acad Sci USA* 2003, **100**:4451-4456.

10. •• Shiba T, Kawasaki M, Takatsu H, Nogi T, Matsugaki N, Igarashi N, Suzuki M, Kato R, Nakayama K, Wakatsuki S: **Molecular mechanism of membrane recruitment of GGA by ARF in lysosomal protein transport**. *Nat Struct Biol* 2003, **10**:386-393.

This crystal structure of the free GGA1-GAT domain includes only the C-terminal three-helix bundle (C-GAT), whereas the N-terminal helix-loop-helix subdomain (N-GAT) is almost invisible. It was demonstrated that the helix-loop-helix structure of N-GAT is stabilized upon Arf•1GTP binding and the complex structure was determined.

11. O'Neal CJ, Jobling MG, Holmes RK, Hol WG: **Structural basis for the activation of cholera toxin by human ARF6-GTP**. *Science* 2005, **309**:1093-1096.

12. Burd CG, Strochlic TI, Gangi Setty SR: **Arf-like GTPases: not so Arf-like after all**. *Trends Cell Biol* 2004, **14**:687-694.

13. Short B, Haas A, Barr FA: **Golgins and GTPases, giving identity and structure to the Golgi apparatus**. *Biochim Biophys Acta* 2005, **1744**:383-395.

14. •• Panic B, Perisic O, Veprintsev DB, Williams RL, Munro S: **Structural basis for Arl1-dependent targeting of homodimeric GRIP domains to the Golgi apparatus**. *Mol Cell* 2003, **12**:863-874.

This structure provides the first example of the dyad-symmetrical interaction of a small GTPase with its effector. The authors point out that Tyr2177 of the golgin-245 GRIP domain, which interacts with the switch and interswitch regions of Arl1•GTP, is an important determinant of binding specificity. Their insightful schematic diagram of the interaction between effectors and switch regions prompted us to review all reported Arf/Rab–effector interactions.

15. Wu M, Lu L, Hong W, Song H: **Structural basis for recruitment of GRIP domain golgin-245 by small GTPase Arl1**. *Nat Struct Mol Biol* 2004, **11**:86-94.

16. Hanzal-Bayer M, Renault L, Roversi P, Wittinghofer A, Hillig RC: **The complex of Arl2-GTP and PDE delta: from structure to function**. *EMBO J* 2002, **21**:2095-2106.

17. Vitale G, Rybin V, Christoforidis S, Thornqvist P, McCaffrey M, Stenmark H, Zerial M: **Distinct Rab-binding domains mediate the interaction of Rabaptin-5 with GTP-bound Rab4 and Rab5**. *EMBO J* 1998, **17**:1941-1951.

18. • Zhu G, Zhai P, Liu J, Terzyan S, Li G, Zhang XC: **Structural basis of Rab5-Rabaptin5 interaction in endocytosis**. *Nat Struct Mol Biol* 2004, **11**:975-983.

Rabaptin-5 is a bivalent Rab effector that binds to Rab4 through its N terminus and to Rab5 through its C terminus. The authors revealed that the C-terminal Rab5-binding domain forms a coiled-coil homodimer, with two Rab5 molecules binding independently on either side. Dimerization of Rabaptin-5 is essential for Rab5 binding, because Rab5 interacts with both protomers of the Rabaptin-5 homodimer

19. • Wu M, Wang T, Loh E, Hong W, Song H: **Structural basis for recruitment of RILP by small GTPase Rab7**. *EMBO J* 2005, **24**:1491-1501.

The Rab7-binding domain of RILP forms a homodimer, with each protomer adopting a helix-loop-helix structure. Two Rab7 molecules symmetrically interact with both protomers. The interaction is mediated not only by the switch and interswitch regions, but also by the hypervariable RabSF1 and RabSF4 regions.

20. Ostermeier C, Brunger AT: **Structural basis of Rab effector specificity: crystal structure of the small G protein Rab3A complexed with the effector domain of rabphilin-3A**. *Cell* 1999, **96**:363-374.

21. •• Eathiraj S, Pan X, Ritacco C, Lambright DG: **Structural basis of family-wide Rab GTPase recognition by rabenosyn-5**. *Nature* 2005, **436**:415-419.

In this recent tour de force, the authors determined crystal structures of eight Rabs bound to GppNHp (Ypt1, Rab4A, Rab6A, Rab9, Rab11A, Rab21, Rab22A and Rab33) and five Rabs bound to GDP (Rab2A, Rab5C, Rab14, Rab21 and Rab23). To assess the different specificities of rabenosyn-5 for individual Rabs, they determined the structure of the Rab4–rabenosyn-5(440–503) and Rab22–rabenosyn-5(728–784) complexes. The structures revealed highly selective recognition by structurally similar effector regions

22. de Renzis S, Sonnichsen B, Zerial M: **Divalent Rab effectors regulate the sub-compartmental organization and sorting of early endosomes**. *Nat Cell Biol* 2002, **4**:124-133.

23. Lindsay AJ, Hendrick AG, Cantalupo G, Senic-Matuglia F, Goud B, Bucci C, McCaffrey MW: **Rab coupling protein (RCP), a novel Rab4 and Rab11 effector protein**. *J Biol Chem* 2002, **277**:12190-12199.

24. Hickson GR, Matheson J, Riggs B, Maier VH, Fielding AB, Prekeris R, Sullivan W, Barr FA, Gould GW: **Arfophilins are dual Arf/Rab 11 binding proteins that regulate recycling endosome distribution and are related to *Drosophila* nuclear fallout**. *Mol Biol Cell* 2003, **14**:2908-2920.

25. Mattera R, Arighi CN, Lodge R, Zerial M, Bonifacino JS: **Divalent interaction of the GGAs with the Rabaptin-5-Rabex-5 complex**. *EMBO J* 2003, **22**:78-88.

26. •• Zhu G, Zhai P, He X, Wakeham N, Rodgers K, Li G, Tang J, Zhang XC: **Crystal structure of human GGA1 GAT domain complexed with the GAT-binding domain of Rabaptin5**. *EMBO J* 2004, **23**:3909-3917.

The GAT-binding domain of Rabaptin-5 is a 90-residue single helix that forms a parallel coiled-coil homodimer. The GGA1-GAT domain 'asymmetrically' binds to one side of the N terminus of the Rabaptin-5 homodimer, in contrast to the dyad-symmetrical binding of two Rab5 molecules to the C-terminal end of the Rabaptin-5 homodimer. The two homodimers of the GAT-binding domain of Rabaptin-5 unexpectedly formed a homotetramer at their C termini.

27. Shiba Y, Katoh Y, Shiba T, Yoshino K, Takatsu H, Kobayashi H, Shin HW, Wakatsuki S, Nakayama K: **GAT (GGA and Tom1) domain responsible for ubiquitin binding and ubiquitination**. *J Biol Chem* 2004, **279**:7105-7111.

28. Puertollano R, Bonifacino JS: **Interactions of GGA3 with the ubiquitin sorting machinery**. *Nat Cell Biol* 2004, **6**:244-251.

29. Scott PM, Bilodeau PS, Zhdankina O, Winistorfer SC, Hauglund MJ, Allaman MM, Kearney WR, Robertson AD, Boman AL, Piper RC: **GGA proteins bind ubiquitin to facilitate sorting at the trans-Golgi network**. *Nat Cell Biol* 2004, **6**:252-259.

30. Prag G, Lee S, Mattera R, Arighi CN, Beach BM, Bonifacino JS, Hurley JH: **Structural mechanism for ubiquitinated-cargo recognition by the Golgi-localized, γ-ear-containing, ADP-ribosylation-factor-binding proteins**. *Proc Natl Acad Sci USA* 2005, **102**:2334-2339.

31. Kawasaki M, Shiba T, Shiba Y, Yamaguchi Y, Matsugaki N, Igarashi N, Suzuki M, Kato R, Kato K, Nakayama K, Wakatsuki S: **Molecular mechanism of ubiquitin recognition by GGA3 GAT domain**. *Genes Cells* 2005, **10**:639-654.

32. Bilodeau PS, Winistorfer SC, Allaman MM, Surendhran K, Kearney WR, Robertson AD, Piper RC: **The GAT domains of clathrin-associated GGA proteins have two ubiquitin binding motifs**. *J Biol Chem* 2004, **279**:54808-54816.

33. Lui WW, Collins BM, Hirst J, Motley A, Millar C, Schu P, Owen DJ, Robinson MS: **Binding partners for the COOH-terminal appendage domains of the GGAs and gamma-adaptin**. *Mol Biol Cell* 2003, **14**:2385-2398.

34. • Miller GJ, Mattera R, Bonifacino JS, Hurley JH: **Recognition of accessory protein motifs by the gamma-adaptin ear domain of GGA3**. *Nat Struct Biol* 2003, **10**:599-606.
In contrast to the ear domains of α-adaptin and β2-adaptin of the AP2 complex, which are composed of two subdomains (the platform and the β sandwich), the GAE (β-adaptin ear) domain consists solely of the β-sandwich domain. GGA-GAE interacts with various accessory proteins, including Rabaptin-5, in a different manner to the α and β2 ears.

35. Collins BM, Praefcke GJ, Robinson MS, Owen DJ: **Structural basis for binding of accessory proteins by the appendage domain of GGAs**. *Nat Struct Biol* 2003, **10**:607-613.

36. Shiba T, Takatsu H, Nogi T, Matsugaki N, Kawasaki M, Igarashi N, Suzuki M, Kato R, Earnest T, Nakayama K, Wakatsuki S: **Structural basis for recognition of acidic-cluster dileucine sequence by GGA1**. *Nature* 2002, **415**:937-941.