

Structural Biology of Arf and Rab GTPases' Effector Recruitment and Specificity

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Arf and Rab proteins, members of small GTPases superfamily, localize to specific subcellular compartments and regulate intracellular trafficking. To carry out their cellular functions, Arfs/Rabs interact with numerous and structurally diverse effector proteins. Over the years, a number of Arf/Rab:effector complexes have been crystallized and their structures reveal shared binding modes including α -helical packing, β - β complementation, and heterotetrameric assemblies. We review available structural information and provide a framework for in-depth analysis of complexes. The unifying features that we identify are organized into a classification scheme for different modes of Arf/Rab:effector interactions, which includes “all- α -helical,” “mixed α -helical,” “ β - β zipping,” and “bivalent” modes of binding. Additionally, we highlight structural determinants that are the basis of effector specificity. We conclude by expanding on functional implications that are emerging from available structural information under our proposed classification scheme.

ADP-ribosylation factor (Arf) and Rab proteins constitute two distinct families within the superfamily of small GTPases (Takai et al., 2001). These two families regulate various aspects of membrane trafficking in eukaryotic organisms. Arf proteins are required for vesicle budding via recruitment of cargo-sorting coat proteins (D'Souza-Schorey and Chavrier, 2006; Donaldson and Jackson, 2011). Rab proteins participate in various aspects of vesicle formation, transport, docking, and fusion (Zerial and McBride, 2001). To perform their biological functions, Arf/Rab proteins cycle between inactive (GDP-bound) and active (GTP-bound) states, and in this latter state, they localize to a specific membrane and recruit numerous and structurally diverse effectors.

The molecular basis for the biological activity of Arf/Rab proteins is a function of the conformational switch between the GDP-bound and GTP-bound states. Segments of polypeptide that are sensitive to the GDP/GTP cycle are called switch regions and are mainly localized to the so-called switch 1 and switch 2 (Figure 1; Stroupe and Brunger, 2000). The γ -phosphate of GTP interacts with elements of switch 1, a loop, and switch 2, a helix, and stabilizes their conformation. Thus, the switch regions are the main binding sites for effectors, as well as for guanine exchange factors (GEFs) and GTP-activating proteins (GAPs), which can discriminate between the GDP- and the GTP-bound conformations. Relative to other small GTPases, Arf proteins contain two additional regions that are structurally sensitive to the GDP/GTP cycle (Pasqualato et al., 2002): the intervening region between switch 1 and 2 (a β hairpin termed the interswitch; Figure 1); and an N-terminal α helix, unique to Arfs. Upon GTP binding, both the interswitch and the N-terminal α helix also move dramatically (Pasqualato et al., 2002). Thus, the effective switch region, broadly defined as the segments that are sensitive to the GDP versus GTP state, is larger in Arfs than in other small GTPases.

The mode of membrane attachment differs between Arf and Rab proteins, thus imposing distinct topological constraints in effector recruitment. Most of the Arf proteins are myristoylated

at a glycine residue in position 2 of their N termini (Figure 1). This site of lipid modification belongs to a region that differs slightly in size among Arfs and folds generally as an amphipathic α helix. Coupled to the myristate group, this α helix mediates direct membrane association (Franco et al., 1993). In contrast, Rab proteins are modified at one or two cysteine residues at their C termini by geranylgeranyl transferase (Leung et al., 2006). The prenylation sites are typically a CXC sequence with the second cysteine being the terminal residue. This conserved Rab motif is preceded by approximately 30 residues that are not conserved among the Rab family and are widely considered to be flexible. Given the close proximity of the N and C termini of the G protein fold, the identical faces of Arfs and Rabs are pointed toward the bilayer. However, Arfs reside proximal to the lipid membrane via their N-terminal amphipathic α helices, while Rabs presumably lie farther away and may have considerable mobility as a result of their flexible tails that precede the prenylation site. For example, Rab6 contains a 34-residue tail and has been modeled up to 105 Å away from the membrane surface (Burguete et al., 2008).

Altogether, these structural characteristics affect the manner in which effectors bind to Arf and Rab proteins. Recently, several newly determined crystal structures of Arfs and Rabs complexed to their effectors have revealed common binding modes. We begin our discussion by summarizing existing structural knowledge and highlighting individual features of currently available Arf:effector and Rab:effector complex structures. Then, we propose a new classification scheme for different modes of Arf/Rab:effector binding. Additionally, we highlight structural determinants that are the basis of Arf/Rab:effector specificity. We conclude by expanding on functional implications that are emerging from available structural information under our proposed classification scheme.

Arfs/Rabs and Their Effectors

The Arf protein family consists of about 30 members that can be divided into three groups: (1) the Arf GTPases, (2) the Arf-like

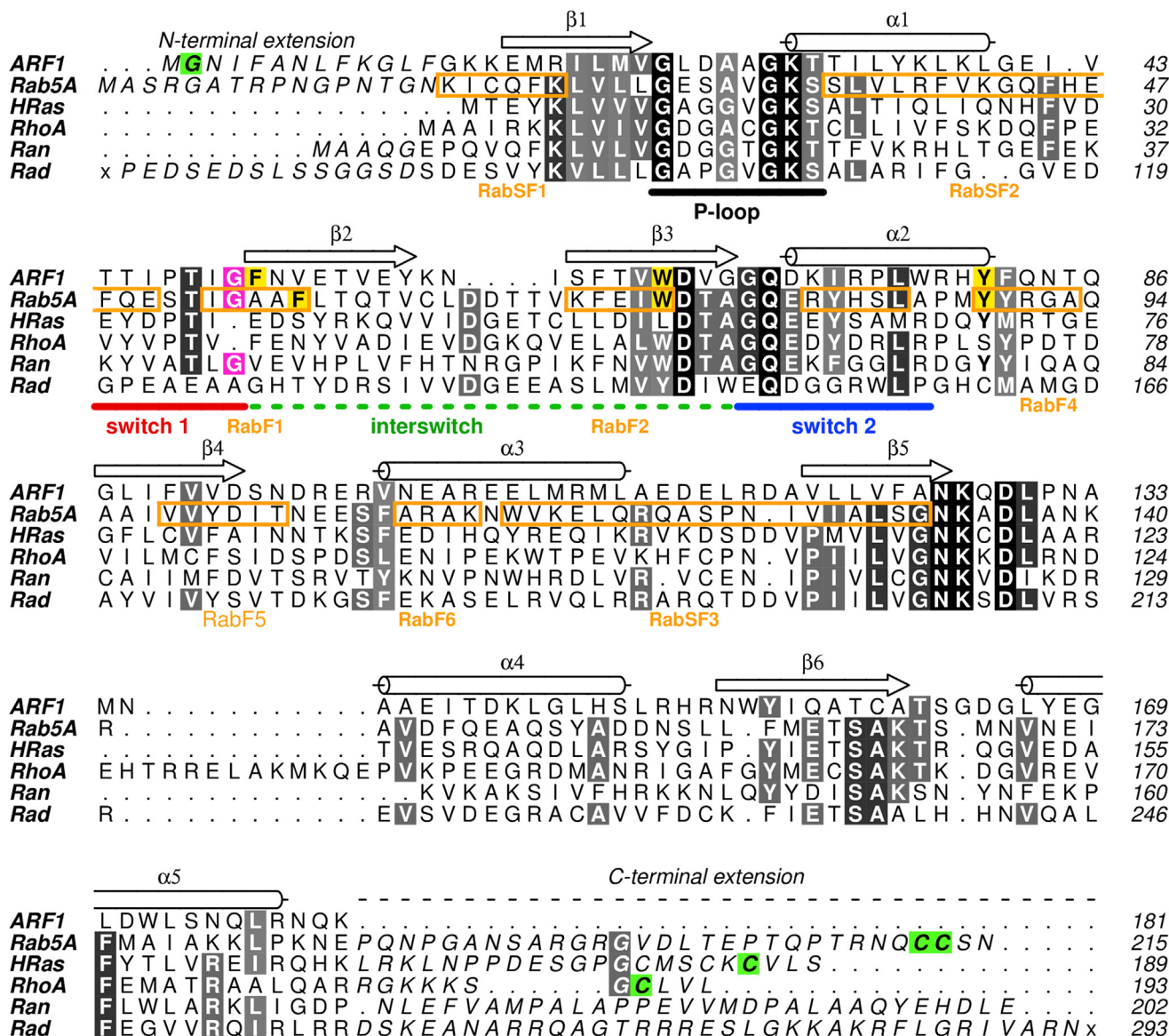


Figure 1. Structure-Based Sequence Alignment of Representative Arf, Rab, Ras, Rho, Ran, and RGK Proteins
The secondary structure of the minimal G domain of ARF1 is shown above the sequence alignment. The P loop is black, switch 1 is red, the interswitch is green, and switch 2 is blue. The glycine insertion in Arf, Rab, and Ran is highlighted in pink. The three residues that comprise the aromatic triad in Arfs and Rabs are highlighted in yellow. Residues that are modified by lipid enzymes to enable membrane attachment are highlighted in green. RabSF and RabF motifs are framed in orange.

GTPases (Arfs), and (3) secretion-associated and Ras-related GTPases. Previous analyses of seven Arf:effector complexes provided a structural understanding of effector recruitment by the Arf family (Chavrier and Ménétrey, 2010). More recent studies have increased the number of known crystal structures to 11 Arf:effector complexes (Table S1 available online). Notably, four of these effectors consist of a single globular domain (BART, CTA1, PDEδ, and UNC119a) and have been crystallized in their entirety as complexes with their cognate Arfs. A gallery of Arf:effector complex structures is shown in Figure 2, and more detailed descriptions of their features are given in the following sections.

ARF1:GGA Structure

Golgi-localized, gamma-ear-containing, ARF-binding (GGA) proteins are monomeric clathrin-associated adaptor proteins involved in the trafficking of cargo between the trans-Golgi network and endosomes (Bonifacino, 2004). GGAs are multidomain proteins containing a central GGA and TOM1 (GAT) domain that has been identified as the key region interacting with Arf. One interesting study identified GGA proteins as modulators of retrovirus release and Arf proteins as cellular cofactors in retroviral trafficking (Joshi et al., 2008). The crystal structure of a complex between ARF1 and the N-terminal part of the GAT domain (N-GAT) shows that N-GAT folds as a helix-loop-helix structure

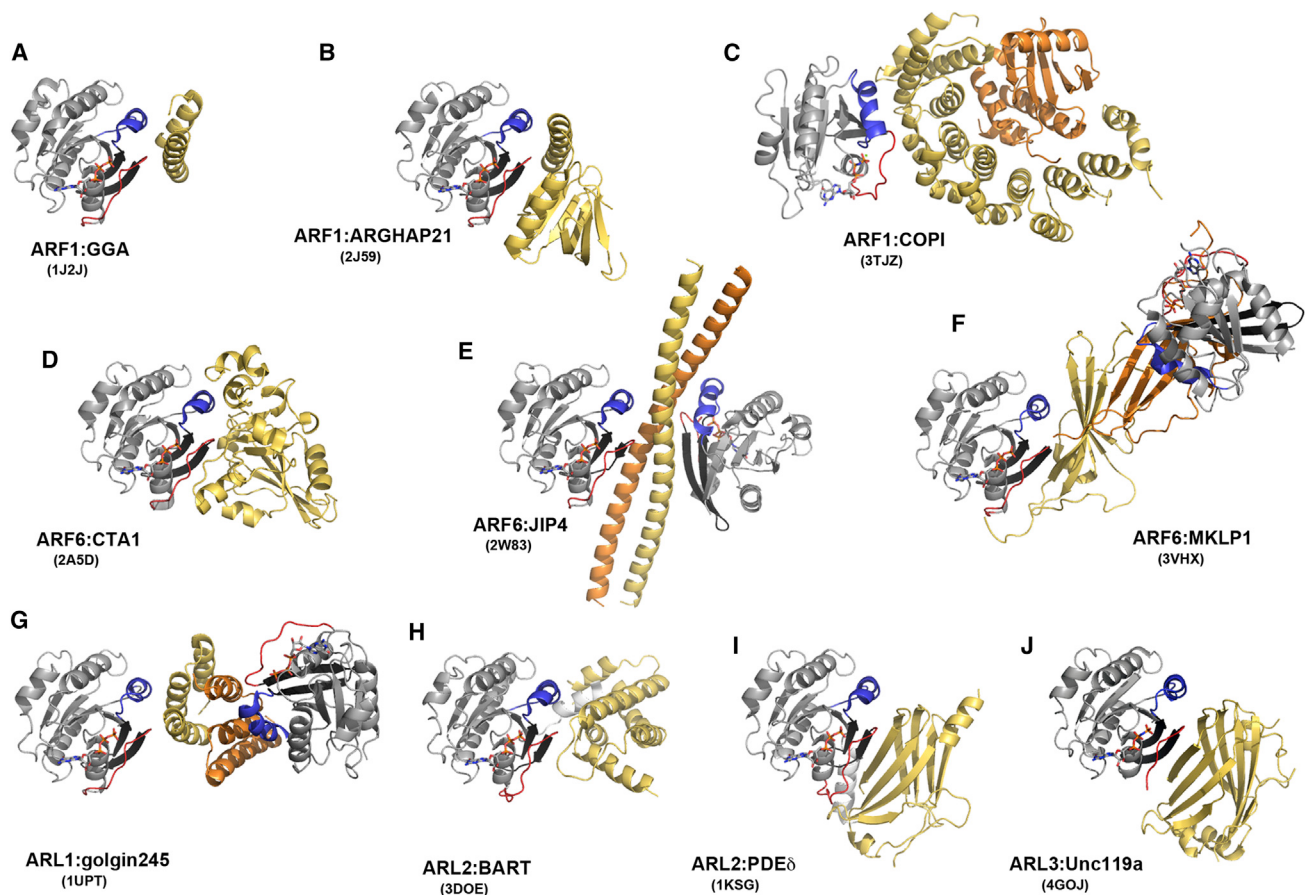


Figure 2. Gallery of 3D Structure of Arf:Effector Complexes

Arf proteins are in the same orientation and are colored in gray with the switch 1 in red, the interswitch in black, and the switch 2 in blue. All the effectors are shown in yellow and orange to indicate the second molecule of dimeric effectors. See Table S1 for structural information on the Arf:effector complexes.

(Shiba et al., 2003). The two helices of N-GAT are positioned against the β sheet of the interswitch and interact with switches 1 and 2 of ARF1. The crystal structure supports previous mutational data that highlight Asn194 of GGA1_{N-GAT} as a critical residue for the interaction with ARF1 (Puertollano et al., 2001).

ARF1:ARHGAP21 Structure

ARHGAP21 (also named ARHGAP10) is a large multidomain protein that exhibits RhoGAP activity. ARHGAP21 also interacts with several other proteins including β -arrestin-1, which inhibits its GAP activity and regulates the temporal activation of RhoA (Anthony et al., 2011). ARHGAP21 binding to α -tubulin is involved in remodeling of cell-cell junctions (Barcellos et al., 2013). ARHGAP21 is also recruited by ARF1 to the Golgi complex where it regulates F-actin dynamics by controlling nucleation through Cdc42 and Arp2/3 complexes (Dubois et al., 2005). The Arf-binding domain (ArfBD) of ARHGAP21 consists of a PH domain and a subsequent C-terminal α helix (α Cter helix) (Dubois et al., 2005), which bind to ARF1 and ARF6 (Ménétrey et al., 2007). The crystal structure of ARF1:ARHGAP21_{ArfBD} reveals that the α Cter helix of ArfBD aligns along the interswitch, making hydrophobic interactions with switches 1 and 2 of ARF1, while the PH domain interacts mainly with switch 1 (Ménétrey et al., 2007). Both the PH domain and the α Cter helix are essen-

tial for the binding of ArfBD to ARF1 and for recruitment of ARHGAP21 to Golgi membranes, as shown by in vitro and in vivo studies (Ménétrey et al., 2007).

ARF1:COPI Structure

COPI or coatamers belong to cytosolic coat protein complexes (COPs) that self-assemble into spherical cages to sculpt the membrane, capture cargo, and form vesicles (Lee et al., 2004). COPI is a 550 kDa protein complex of seven COPs: α -, β -, β' -, γ -, δ -, ϵ -, and ζ -COP. COPI-coated vesicles are involved in retrograde traffic from the Golgi and their assembly is initiated by the activation of Arfs on the membrane (Lee et al., 2004). Recently, the structure of γ ζ -COP subunits bound to ARF1 has been determined (Yu et al., 2012). The first 315 residues of γ -COP adopt a curved superhelix of 15 α helices (an α -solenoid fold) that binds as an arc around the ζ -COP subunit, which adopts a globular α/β fold. Switches 1 and 2 of ARF1 interact with helices α 4 and α 6 on the outer surface of the γ -COP arc, while making no contact with ζ -COP. Apart from two hydrogen bonds in the center of the interface, the interactions are mainly hydrophobic.

ARF6:CT Structure

The cholera toxin (CT) of *Vibrio cholerae*, the major bacterial agent of severe diarrheal diseases, is composed of a host cell-translocation B subunit and a globular A subunit that is cleaved

to give rise to an enzymatic A1 domain. CT-A1 by itself has relatively low activity *in vitro*, but its affinity and enzymatic activity toward the stimulatory G protein A subunit ($G_{\text{S}\alpha}$) is increased upon interaction with GTP-bound Arfs, but not Arls in host cells (Kahn and Gilman, 1986). CT-A1 is a single-domain protein with a mixed α/β fold comprising a β sandwich core of two perpendicular β sheets surrounded by helices (O'Neal et al., 2004). The crystal structure of ARF6 complexed to the full A1 domain of CT shows that the Arf-binding site of CT-A1 consists of loop regions with little secondary structure that rearrange to form an amphipathic helix upon ARF6 binding (O'Neal et al., 2005). CT-A1 binds to the switch and interswitch regions of ARF6 predominantly through hydrophobic interactions.

ARF6:JIP4 Structure

JNK interacting proteins (JIPs) are scaffolds for JNK cascade kinases and thus are important for brain development, neuronal traffic, and apoptosis (Koushika, 2008). JIPs are also adaptors linking cargo to kinesin-1, a major molecular motor for axonal transport (Koushika, 2008). JIPs have been shown to play significant and complex roles in axonal transport via interaction with multiple proteins. JIP3 and JIP4 interact specifically with ARF6, which regulates JIP's interaction with kinesin-1 and dynein/dynactin motors, leading to the control of endosome recycling to the plasma membrane (Montagnac et al., 2009). The structure of the middle part of JIP4 adopts a leucine zipper (LZII) complexed to two ARF6 molecules in a heterotetrameric assembly (Isabet et al., 2009). This structure reveals that the long and rigid coiled coil of JIP4_{LZII} interacts with the interswitch and switch 2 regions of ARF6. The structure reveals for the first time how a specific effector can discriminate between two Arfs, i.e., ARF6 and ARF1.

ARF6:MKLP1 Structure

Mitotic kinesin-like protein 1 (MKLP1), also known as KIF23, together with MgcRacGAP/Cyk4, constitutes the centralspindlin complex at the Flemming body. MKLP1 recruits ARF6 to the Flemming body during cytokinesis (Makyio et al., 2012). MKLP1 is a kinesin motor that interacts directly with ARF6 through its terminal tail domain (Makyio et al., 2012). The crystal structure of the ARF6:MKLP1_{tail} complex reveals that MKLP1_{tail} folds as a central β sheet surrounded by several α helices and assembles as a dimer through the central β sheet (Makyio et al., 2012). The MKLP1_{tail} homodimer is flanked by two ARF6 molecules, forming a 2:2 heterotetramer that results in an extended β sheet of 22 β strands spanning the entire complex. ARF6 and MKLP1_{tail} make antiparallel interprotein β sheet interactions through their respective β 2 and β 5 strands, as well as hydrophobic contacts at the interswitch and switch 2 regions.

ARL1:Golgin-245 Structure

Golgins are a family of large Golgi-localized proteins with extended coiled-coil regions that play a role in tethering cisternae and transport vesicles to Golgi membranes, thus maintaining the overall architecture of the Golgi complex (Short et al., 2005; Munro, 2011). Golgin-245 associates with Golgi membranes by interacting specifically with ARL1 through a C-terminal GRIP domain (Panic et al., 2003; Gillingham et al., 2004). The crystal structure of ARL1:golgin-245_{GRIP} reveals that GRIP consists of an array of three antiparallel helices and forms a tight homodimer that binds in a dyad-symmetric fashion to two ARL1 molecules (Panic et al., 2003; Wu et al., 2004). The

interaction is mediated by two adjacent helices of one GRIP domain, which align along the β sheet of the interswitch and recognize switches 1 and 2, respectively, of ARL1 mainly through hydrophobic interactions.

ARL2:BART Structure

Binder of ARL2 (BART) was shown to be involved in mitochondria transport and apoptosis (Sharer et al., 2002). BART is also essential for nuclear retention of signal transducers and activators of transcription 3 (STAT3), and ARL2 binding to BART enhances the interaction of BART with STAT3 (Muromoto et al., 2008). Finally, BART is found to inhibit pancreatic cancer cell invasion by inhibiting ARL2-mediated RhoA inactivation (Taniuchi et al., 2011). The crystal structure of full-length ARL2 complexed to BART shows that BART consists of a six α -helix bundle and interacts with ARL2 through two interfaces (Zhang et al., 2009). The first interface involves both hydrophobic and hydrophilic interactions between the switch regions (primarily switch 1) of ARL2 with the helix α 3 and a subsequent loop of BART. The second interface involves extensive hydrophobic interactions between the N-terminal amphipathic α helix of ARL2 and the hydrophobic cleft formed by helices α 3, α 4, and α 5 of BART. The latter interaction is the first to demonstrate that the N-terminal helix can be a key determinant of Arf:effector recognition.

ARL2/3:PDE δ and UNC119a Structures

Delta subunit of phosphodiesterase (PDE δ) and UNC119 proteins (a and b isoforms) are homologous proteins that share a similar structural fold consisting of an immunoglobulin-like β sandwich (Hanzal-Bayer et al., 2002; Ismail et al., 2012). The β fold contains a hydrophobic pocket that accommodates lipid moieties of posttranslationally modified membrane-associated proteins (Hanzal-Bayer et al., 2002; Ismail et al., 2012; Wright et al., 2011; Zhang et al., 2011). These proteins act as solubilizing factors for lipidated cargoes and play a critical role in ciliary trafficking (Ismail et al., 2011, 2012). The structures of ARL2:PDE δ , ARL2:UNC119a, and ARL3:UNC119a were determined using full-length proteins (Hanzal-Bayer et al., 2002). Overall, the interfaces are similar and formed primarily by a parallel interprotein β sheet interaction involving β 2 of the interswitch region of ARL2/3 and β 7 of PDE δ /UNC119, resulting in a 10-stranded β sheet. Additionally, PDE δ /UNC119 interacts with the switch 2 and interswitch regions of ARL2/3 in a hydrophobic manner.

Interestingly, the switch regions of Arf proteins recruit effectors that exhibit diverse folds. The Arf-binding domains of effectors range from all- α -helical to all β sheet, or a combination of the two, as illustrated by a pleckstrin homology (PH) domain followed by a single helix found in a number of effectors.

The Rab proteins constitute the largest family of small GTPases with more than 60 members in mammalian cells. A total of 14 independent Rab:effector complexes has now been deposited in the Protein Data Bank (Table S2). Rab effectors that are structural homologs can be counted as a single complex; thus, there are effectively 9 distinct Rab:effector structures that are known. All of the effectors have been truncated from their full-length parents and generally contain the minimal Rab-binding domain (RBD) that imparts the full affinity and specificity for their cognate Rabs. A gallery of Rab:effector complex structures is shown in Figure 3, and more detailed descriptions of their features are provided below.

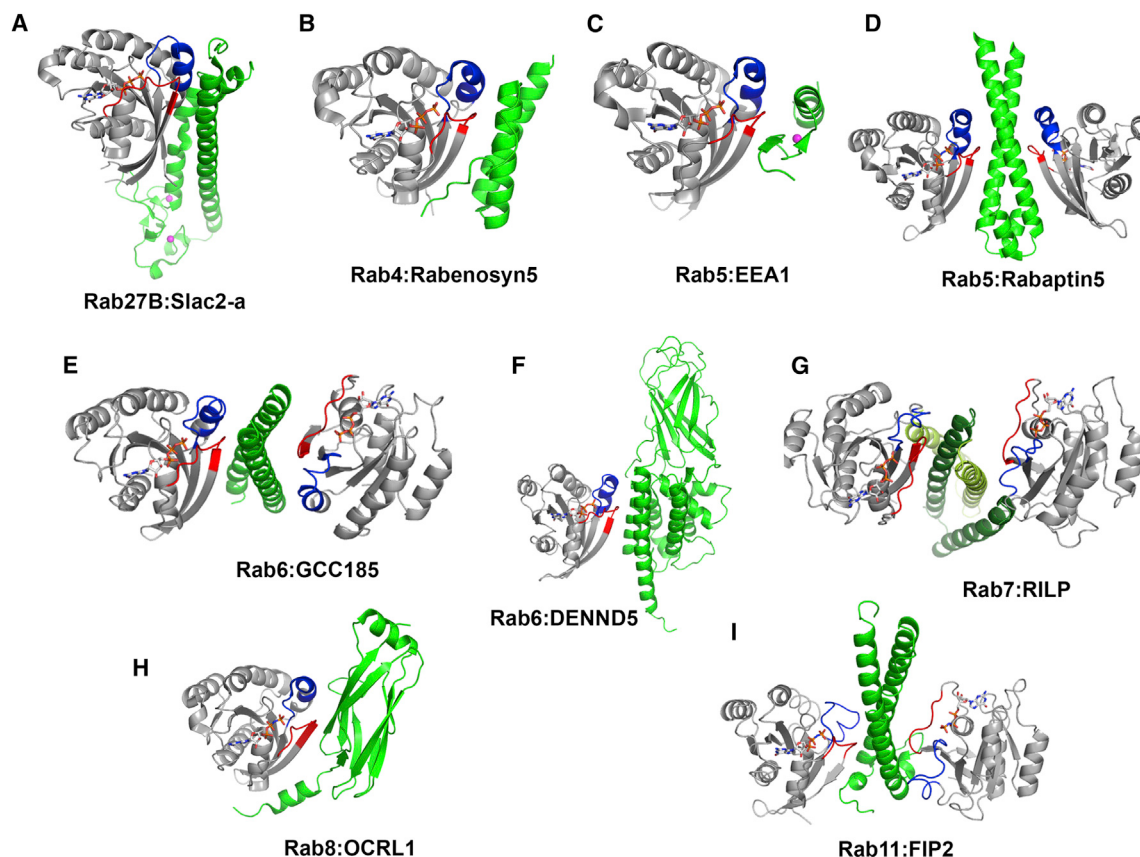


Figure 3. Gallery of 3D Structures of Rab:Effector Complexes

Rab proteins are in the same orientation and are colored in gray with the switch 1 in red and the switch 2 in blue. All the effectors are shown in light and dark green to indicate the second molecule of dimeric effectors. See Table S2 for structural information on the Rab:effector complexes.

Rab3/27:RBD27-Containing Protein Structures

Rab3 and Rab27 mediate exocytosis in numerous cells including neurons, lymphocytes, and epithelial cells (Yi et al., 2002). Rab27-mediated transport pathways are compromised in patients with Griscelli disease, an inherited autosomal disorder associated with albinism, immune deficiency, and neurological disorders (Ménasché et al., 2000; reviewed in Khan, 2013). The structure of Rab3:Exophilin-1 (also called Rabphilin-3) was the first Rab:effector complex determined by X-ray crystallography (Ostermeier and Brunger, 1999). Recently, the structures of Rab27:Slp2-a and Rab27:Slac2-a (also known as melanophilin) have also been determined (Chavas et al., 2008; Kukimoto-Niino et al., 2008). All of these effectors contain a conserved N-terminal Rab27-binding domain (RBD27), although some of them are promiscuous. For example, Exophilin-1 can be recruited by Rab3 and Rab27 (Fukuda, 2006; Itzen and Goody, 2008; Kukimoto-Niino et al., 2008). The RBD27 consists of an α -helical hairpin motif that interacts with the switch and interswitch regions of Rabs. In many of these motifs such as Exophilin-1 and Slac2-a, a Zn^{2+} -binding globular domain is inserted between the two α helices.

Rab4/22:Rabenosyn-5 Structures

Rabenosyn-5 is implicated in the regulation of endocytic trafficking pathways (Nielsen et al., 2000). Its interaction with Rab4/Rab22 mediates the transport of clathrin-coated vesicles

from the plasma membrane into early endosomes (Navaroli et al., 2012). Rabenosyn-5 contains a Zn^{2+} -associated FYVE-finger domain, situated near its N terminus (Nielsen et al., 2000), which mediates lipid binding. Rab4 and Rab22 bind to discrete motifs of Rabenosyn-5, although the two motifs are structurally identical (Eathiraj et al., 2005). Crystal structures of the complexes Rab4:Rabenosyn-5 and Rab22:Rabenosyn-5 have been determined (Eathiraj et al., 2005). The structure of Rabenosyn-5 consists of two helices connected by a short loop (an α -helical hairpin), and binding to Rab4 is mediated by switch 1/2 and the interswitch regions.

Rab5:EEA1 Structure

Early endosomal autoantigen 1 (EEA1) enhances endosomal fusion throughout its interactions with Rab5 (Callaghan et al., 1999a) and several other soluble factors, including Rabenosyn-5, hVPS45, and Rabex-5, an exchange factor for Rab5 (Simonsen et al., 1998; Christoforidis et al., 1999; Nielsen et al., 2000; Ohya et al., 2009). EEA1 is composed of an N-terminal zinc finger, followed by coiled-coil regions and a C-terminal FYVE finger domain (Callaghan et al., 1999b). The N-terminal Zn^{2+} finger has high affinity for Rab5, and the crystal structure of Rab5 with this domain has recently been determined (Mishra et al., 2010). The Zn^{2+} finger motif (C_2H_2) adopts a $\beta\beta\alpha$ topology with the α helix and β 1 strand engaging the switch and interswitch regions of Rab5. The complex was the first example of

an RBD that is partly composed of a β sheet and provided insight into the structural basis for Rab5 specificity.

Rab5:Rabaptin5 Structure

Rabaptin-5 is a multidomain protein that regulates endocytic trafficking (Stenmark et al., 1995; Gournier et al., 1998). In addition to Rab5, Rabaptin-5 contains a separate binding site for Rab4 (Vitale et al., 1998); thus, it may provide a molecular link between two Rab trafficking pathways. The dynamics of Rab5 activity are also regulated by Rabex-5, a GEF for Rab5 that also interacts directly with Rabaptin-5 to promote endosome fusion and endocytosis (Zhu et al., 2007). The crystal structure of Rab5-GTP complexed to the RBD of Rabaptin-5 reveals a heterotetrameric assembly (Zhu et al., 2004). The RBD of Rabaptin-5 forms a central symmetric coiled coil, which binds to two Rab5 molecules on either side. The two Rab5 do not contact each other, and this is a recurring theme in Rab:effector oligomers mediated by symmetric coiled coils. Like most Rab:effector complexes, recruitment is mediated by the switch 1/2 and interswitch regions.

Rab6:GCC185 Structure

GRIP and coiled-coil domain-containing protein (GCC185) is a large protein that contains long stretches of coiled coils and mediates trafficking from late endosomes to the *trans*-Golgi network (Kooy et al., 1992; Fritzler et al., 1993; Barr and Short, 2003; Reddy et al., 2006; Derby et al., 2007). In addition to Rab6, GCC185 apparently contains a nonoverlapping site for ARL1 binding, termed the GRIP domain. Structural studies and in vitro assays suggest that Rab6 binding to GCC185 enables more efficient loading of ARL1 onto the GRIP domain (Burguete et al., 2008), although an alternative study finds no link between ARL1 and GCC185 localization (Houghton et al., 2009). More recently, GCC185 has been identified as an effector of ARL4, and the complex apparently maintains the integrity of Golgi compartments (Lin et al., 2011). The structure of Rab6 in complex with a short α -helical motif from GCC185 has been determined (Burguete et al., 2008). Like Rab5:Rabaptin-5, the complex of Rab6:GCC185 is a heterotetramer in which the effector forms a central symmetric coiled coil. Again, effector recruitment is mediated by the switch 1/2 and the interswitch regions.

Rab6:DENND5 Structure

DENND5 (alternatively, Rab6-interacting protein 1, or Rab6IP1) is a large multidomain protein that contains a series of differentially expressed in normal versus neoplastic cells (DENN) domains at its N terminus. DENND5 is recruited by Rab6 and regulates traffic into and out of the Golgi apparatus (Miserey-Lenkei et al., 2007). The C-terminal half of DENND5 consists of two RUN domains separated by a PLAT domain. The structure of Rab6 in complex with RUN1-PLAT domains of DENND5 has been determined and reveals that the first all- α -helical RUN domain mediates binding to the switch and interswitch regions of Rab6 (Recacha et al., 2009). The first and last α helices of the RUN1 domain (α 1 and α 8) stack in a parallel fashion and interact with Rab6. No direct interaction is observed between the PLAT domain of DENND5 and Rab6.

Rab7:RILP Structure

Rab-interacting lysosomal protein (RILP) recruitment by Rab7 mediates fusion of phagocytic vesicles with late endosomes and/or lysosomes (Harrison et al., 2003). More recently, RILP and RILP-related proteins have been identified as effectors

for Rab36 in mediating retrograde transport of melanosomes (Matsui et al., 2012). Rab7 interacts directly with a domain (RBD) situated in the middle of RILP, and the crystal structure of Rab7:RILP-RBD has been determined (Wu et al., 2005). The RBD adopts a helical hairpin motif that dimerizes and binds to two Rab7 molecules on equivalent and opposite sides of the effector. The RBD dimer is enabled by both α helices in the helical hairpin, such that the two helical hairpins interdigitate with each other. In addition to the switch and interswitch regions, the C-terminal helix of RBD engages CDR3 (C terminus) and the N-terminal helix engages CDR1 (N terminus) of Rab7, thus forming an extensive interface.

Rab8:OCRL1 Structure

Oculocerebrorenal syndrome of Lowe protein 1 (OCRL1) regulates vesicle trafficking from early endosomes to Golgi (Choudhury et al., 2005), endocytic recycling (Noakes et al., 2011), as well as cell migration (Coon et al., 2009). The Rab-binding region of OCRL1 was localized to a segment of the protein ASPM-SPD-2-Hydin, termed the ASH domain (Hyvola et al., 2006). The structure of Rab8 in complex with the RBD from OCRL1 has been determined (Hou et al., 2011). The RBD folds as an N-terminal α helix followed by a β sandwich that resembles immunoglobulin domains. Binding is mediated by β 9 of the ASH domain, which interacts with the switch and interswitch regions of Rab8. Additional interactions between switch 1/interswitch and the α helix/linker precede the ASH domain of OCRL1. The Rab:effector complex is unusual in the extent of interactions between OCRL1 and the interswitch.

Rab11:FIP Structures

Rab11-family interacting proteins (FIPs) regulate various aspects of endosomal trafficking including receptor recycling (Ren et al., 1998) and delivery of cargo to the growing cleavage furrow in cell division (Wilson et al., 2005). FIPs are multidomain effectors that comprise a highly conserved RBD11 at their extreme C termini. Rab25 also binds to FIPs and its activity has been linked to the aggressiveness of carcinomas in recent years (Cheng et al., 2004; Caswell et al., 2007; Goldenring and Nam, 2011). Three related structures of Rab11 in complex with members of FIPs have been crystallized (Eathiraj et al., 2006; Jagoe et al., 2006; Shiba et al., 2006). The butterfly-shaped complex is assembled as a heterotetramer with RBD11 forming a central coiled coil with equivalent Rab11-binding sites on each side. Unusually, the switch 2 conformation of Rab11 is nonhelical and undergoes a conformational rearrangement upon binding to FIPs. The nonhelical and flexible nature of switch 2 has been presumed to be a determinant of Rab11:FIP specificity.

Rab proteins also recruit, mainly through their switch regions, structurally diverse effector proteins. However, the vast majority of known RBDs adopt an α -helical conformation. Nevertheless, there are now two examples of RBDs that contain a nonhelical segment that binds to Rabs. Altogether, the preceding Arf/Rab:effector structures highlight recurrent modes of binding shared by these small GTPase families.

Arf/Rab:Effector Binding Modes

As indicated in the summary of structural data available from Arf/Rab:effector complexes we provided earlier, these complexes can be organized into distinct groups based on their mode of binding, despite unrelated sequences and folds of the effectors.

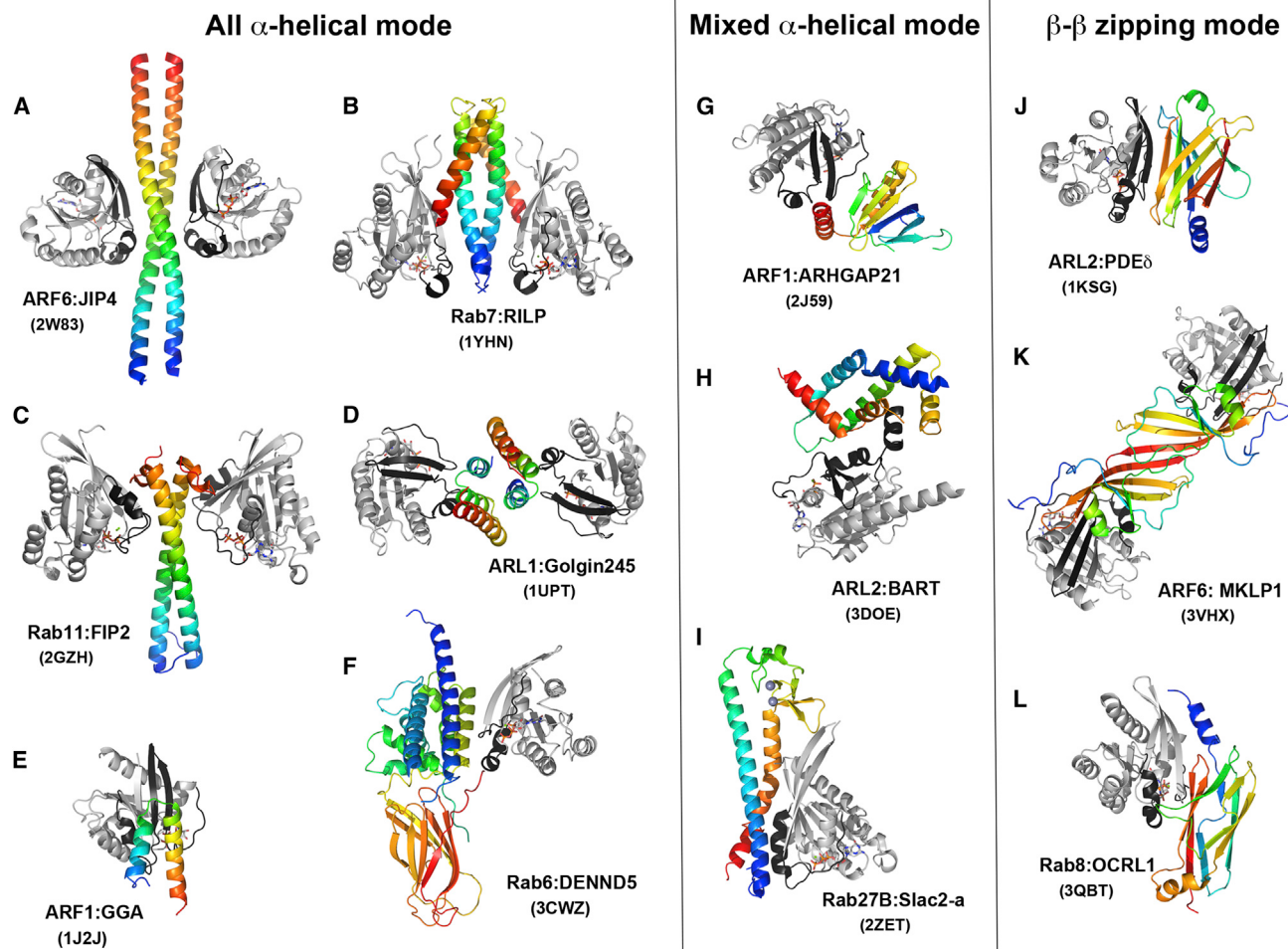


Figure 4. Arf/Rab Modes of Binding

(Left) All- α -helical mode of binding.

(Middle) Mixed α -helical mode of binding.

(Right) β - β zipping mode of binding. Arf/Rab proteins are shown in light gray with the switch regions in dark gray. Effectors are indicated in a rainbow spectrum from the N terminus in blue to the C terminus in red.

The major modes of binding that we put forward to reflect current structural insights are all- α -helical, mixed α -helical, β - β zipping, and bivalent modes of binding. We offer details of individual modes below and argue that such a conceptual organization can be useful in understanding the structural basis for specificity and biological function.

All- α -Helical Mode of Binding

Many Arf/Rab-binding domains adopt an all- α -helical conformation ranging from coiled-coil structures (JIP4, FIP2/3, GCC185, and Rabaptin-5) to α -helical bundles (GGA, γ -COP, golgin245, DENND5, and Rabenosyn5) or aspects of both (RILP). These binding domains interact mainly using two helices packed alongside the switch-interswitch junction of their Arf/Rab partners. Interestingly, the two helices arise either from different molecules of a dimer, like JIP4 (Figure 4A), RILP (Figure 4B), Rabaptin5, FIP2/3 (Figure 4C), and GCC185, or from the same molecule being either contiguous, like golgin245 (Figure 4D), GGA (Figure 4E), and Rabenosyn-5, or noncontiguous like γ -COP and DENND5 (Figure 4F). Differences are also observed in the

topology of the two helices relative to each other and to the Arf/Rab interface. Overall, the structural data suggest that two helices are the minimal motif necessary to bind Arf/Rab proteins at the switch junction.

Mixed α -Helical Mode of Binding

Some Arf/Rab binding domains exhibit a single α helix that is complementary to the switch-interswitch junction of the GTPase. Interestingly, in this case a second interaction site is observed outside the switch-interswitch junction that is critical for the full binding affinity of the effector for its Arf/Rab partners. This mode of binding is referred to as a mixed α -helical mode of binding (Figure 4, middle panel). The ArfBD of ARHGAP21 comprises a single C-terminal helix that interacts with the switch-interswitch junction of ARF1, but a second interaction is made between the PH domain of ArfBD and switch 1 of ARF1 (Figure 4G; Ménétrey et al., 2007). Mutagenesis studies have shown that neither the single helix nor the PH domain alone of ArfBD is sufficient for binding ARF1—both are required (Ménétrey et al., 2007). Also, two interfaces have been reported

between ARL2 and its effector BART (Figure 4H). One of them comprises a single helix ($\alpha 3$) of BART that packs against the switch-interswitch junction of ARL2. The second interface includes three helices ($\alpha 3$, $\alpha 4$, and $\alpha 5$) of BART that interact with the unique N-terminal helix of ARL2 (Zhang et al., 2009). Structural and biochemical data demonstrate that here, also, both interfaces are essential for the binding of ARL2 to BART (Zhang et al., 2009). Interaction of the Rab27 family of effectors (Slac2-a, Slp2-a, and Exophilin-1/Rabphilin-3) with Rab27 and Rab3 involves two discrete parts of the Rab (Figure 4I). In addition to the packing of a single helix to the switch-interswitch junction, the RBD consists of a globular zinc-stabilized subdomain that binds to a hydrophobic patch involving the complementarity-determining regions of Rab27/Rab3 (Ostermeier and Brunger, 1999; CDR1, N-terminal α helix; CDR2, the $\alpha 3$ - $\beta 5$ loop; and CDR3, the C-terminal α helix; Figure 1). Furthermore, the structures of Rab27:Slac2-a and Rab27:Slp2-a reveal that the second α helix in the hairpin interacts with the C-terminal end of the switch 2 α helix (Figure 4I). Presumably, both sets of interactions are necessary for the full affinity of the complexes, although detailed mutagenesis experiments have not been performed. Altogether, these various examples suggest that binding of a single α helix to switch-interswitch junctions alone is insufficient to provide the specificity and/or affinity necessary for effector recruitment, and thus a second interface is required.

β - β Zipping Mode of Binding

Another common mode of binding observed for Arf/Rab effectors is the formation of β - β zipping interactions made with the $\beta 2$ strand of the interswitch. Three examples highlight this mode of binding in the Arf/Rab families (Figure 4, right panel) (Hanzal-Bayer et al., 2002; Hou et al., 2011; Makyio et al., 2012). In ARL2:PDE δ and ARF6:MKLP1 structures (Figures 4J and 4K), interactions take place via an interprotein β sheet interaction with the $\beta 2$ strand of the interswitch either in a parallel (PDE δ) or antiparallel (MKLP1) orientation (Hanzal-Bayer et al., 2002; Makyio et al., 2012). ARL2:PDE δ and ARF6:MKLP1 complexes exhibit seven and five hydrogen bonds, respectively, in this β - β zipping interaction from each molecule of the complex. The third example is the recent crystal structure of Rab8 in complex with OCRL1 (Figure 4L; Hou et al., 2011). The β - β zipping interaction between Rab8 and OCRL1 is antiparallel, like that of ARF6:MKLP1, but it is shorter and involves three hydrogen bonds. Interestingly, both PDE δ and OCRL1 feature an N-terminal helix followed by an immunoglobulin-like β sandwich domain (Figures 4J and 4L). Although both PDE δ and OCRL1 use their respective $\beta 7$ strand to make β - β zipping interactions with the $\beta 2$ strand of their partners, they do so differently. Indeed, the β - β zipping interaction of ARL2:PDE δ is parallel, whereas that of OCRL1 is antiparallel (Figures 4J and 4L). This difference situates the N-terminal helix of OCRL1 ahead of the β sandwich and in contact with the tip of the interswitch of Rab8, while that of PDE δ makes no contact with ARL2. Another interesting point in these three Arf/Rab effectors is that the strand involved in the β - β zipping interaction is part of a β sheet, thus resulting in a contiguous β sheet that extends over both molecules.

Bivalent Mode of Binding

Several Arf/Rab:effector complexes assemble as heterotetramers with the Arf/Rab-binding domains forming a central symmetric homodimer (Figure 5). The homodimeric effectors

bind to two Arf/Rab molecules on equivalent and nonoverlapping sides. Given that all known active Arf/Rab proteins are monomers, symmetric dimers enable the oligomerization of Arf/Rab:effector complexes that may be relevant to biological activity. This assembly has previously been called a bivalent mode of binding (Kawasaki et al., 2005; Panic et al., 2003). Among the 10 Arf:effector complexes, 3 have been characterized as heterotetramers—ARL1:golgin245, ARF6:JIP4, and ARF6:MKLP1 (Figures 5A–5C). A survey of this mode of binding in the Arf family reveals several variants that are adopted by the complexes. The GRIP domain of golgin245 dimerizes as a three-helix bundle, the LZII of JIP4 is a coiled-coil structure, and the tail of MKLP1 is mainly an all β sheet structure that dimerizes through a β - β zipping interaction (Figures 5A–5C). Whereas each monomer of golgin245-GRIP and MKLP1-tail interacts with one molecule of ARL1 and ARF6, respectively, each monomer from the JIP4-LZII interacts with the two ARF6 molecules. Of note, γ -COP and β -COP subunits of coatomer are evolutionarily related and will likely have a similar overall structure. Biochemical data and mutagenesis strongly support a model in which ARF1 binds not only to γ -COP, but also to β -COP in a similar fashion, although the atomic details differ somewhat (Yu et al., 2012). Taken together with the finding that $\alpha\beta'\epsilon$ -COP does not bind to ARF1, these results imply that heptameric coatomer has two binding sites for ARF1 and thus can be recruited in a bivalent manner to membranes (Yu et al., 2012). In the case of Rab:effector complexes, four belong to this category—Rab5:Rabaptin-5, Rab11:FIPs, Rab6:GCC185, and Rab7:RILP (Figures 5D–5G). In contrast to the structural variety observed for Arf:effector structures, all of these Rab-binding domains are coiled coils. However, the orientation of effector α helices is variable, with the long axis of Rabaptin5 rotated by 60° relative to FIPs and GCC185 (Figures 5D–5F). Moreover, despite a similar orientation of the long axis, the parallel α helices of FIPs and GCC185 are topologically inverted relative to each other (Figures 5E and 5F).

As previously discussed, such a bivalent mode of binding with two Arf/Rab molecules interacting with a dimeric effector at the membrane would increase the effector residence time at the membrane (Panic et al., 2003; Kawasaki et al., 2005). Indeed, one of the Arf/Rab molecules could remain associated with the membrane without inactivation, while the second could in theory dissociate from the effector, be inactivated, and be replaced by a new active Arf/Rab. Increasing residence time of the effectors on the membrane may be essential for Arf/Rab-mediated trafficking processes. However, such a bivalent mode of recruitment also imposes a restricted orientation to the dimeric effectors relative to the membrane. Notably, because Arfs are more closely attached to the membrane bilayer than Rab proteins are, the orientation of their dimeric effectors is more restricted. To prevent steric hindrances between the membrane and polypeptide, the ArfBD of these effectors must be situated either at the N/C termini or in the middle of the polypeptide with a “U-turn” kink, thus allowing the effectors to project into the cytosol to fulfill their functions.

Thus far, the structures of all known homodimeric effectors conform to these structural constraints. Golgin245, Rabaptin5, and FIP2/FIP3 (Figures 5A, 5D, and 5E) have their Arf/Rab-binding site at their extreme C termini, whereas the centrally located Rab7-binding site of RILP (Figure 5G) adopts a helix-loop-helix

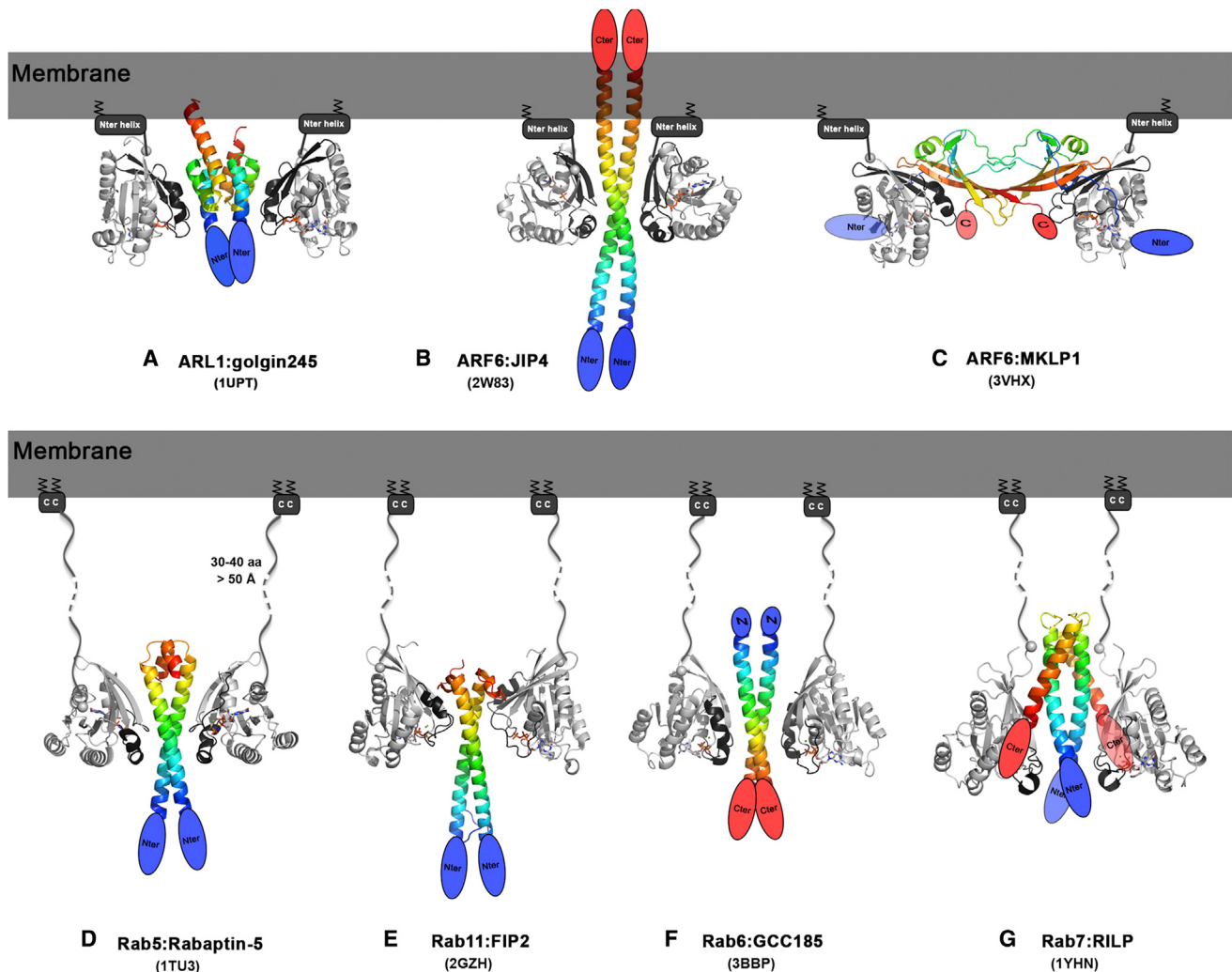


Figure 5. The Bivalent Mode of Binding of Arf/Rab:Effector Complexes

All the Arf/Rab:effector complexes are modeled relative to the membrane bilayers. Arf/Rab proteins are shown in light gray with the switch regions in dark gray. Effectors are indicated in a rainbow spectrum from the N terminus in blue to the C terminus in red. Lipid modification of Arf/Rab proteins as well as N/C terminus domains (not crystallized) of the effectors are schematized.

hairpin that directs both N- and C-terminal parts of the molecule opposite to the membrane. MKLP1 and GCC185 (Figures 5C and 5F) have their respective Arf/Rab-binding domains close to the extreme C terminus, although the N-terminal 100 residues are missing from these structures. In the case of MKLP1, this extreme C-terminal segment should not induce membrane steric hindrance because both C termini extend away from the membrane. In the case of GCC185, the hypervariable C-terminal extension of Rab6 has been proposed to be long enough to accommodate the remaining C-terminal part of GCC185 without membrane steric hindrance (Burguete et al., 2008). One exception is the extended coiled coil of JIP4, which contains the ARF6-binding domain in the middle part of the protein. In this case, we have suggested that the formation of a heterotetramer of ARF6:JIP4 will probably induce severe steric conflicts between JIP4 and the membrane. Thus, JIP4 will likely be recruited to the membrane by a single ARF6 molecule (Isabet et al., 2009).

In summary, Arfs and Rabs complexed to their effectors share four distinct modes of binding. Of note, the bivalent mode is not restricted to α -helical coiled coils—the β - β zipping mode in the ARF6:MKLP1 complex enables assembly of a heterotetrameric complex. Bivalent modes likely enable longer lifetimes of complexes, which may be relevant for tethering and vesicle biogenesis/fusion. Binary complexes with large surface areas of interaction, such as Rab27:effector complexes, are sufficiently strong to enable both specificity and motility via myosins.

Structural Basis for Effector Specificity

A global analysis of the crystal structures of Arf/Rab:effectors and comparisons with other small GTPases in the Ras superfamily highlight structural determinants of effector specificity that distinguish the Arf/Rab families. The aromatic triad is a well-established structural hallmark shared by Arf and Rab proteins. Also, a contiguous hydrophobic interface at the junction of switches 1 and 2 as well as sequence variability at the periphery

of switch regions also play critical roles in effector specificity of Arf/Rab GTPases.

The Aromatic Triad

The hydrophobic triad was first identified and described in Rab proteins and was proposed to be a major structural determinant for effector binding and specificity (Merithew et al., 2001). The hydrophobic triad is composed of an invariant tryptophan residue in the interswitch and a phenylalanine and tyrosine/phenylalanine residue situated adjacent to switch 1 and switch 2, respectively (Figure 1). Arf proteins also share the sequence and position of the hydrophobic triad with Rab proteins, except for the switch-1-proximal phenylalanine, which is found two residues upstream (Figure 1). Because the hydrophobic triad in Arf and Rab proteins consists of aromatic residues, we refer hereafter to this motif as an “aromatic triad.” Like Rabs, the aromatic triad in Arf proteins is proposed to be a structural determinant for effector specificity (Chavrier and Ménétreay, 2010). Global analyses of Arf/Rab:effector complexes reveal that the manner by which effectors discriminate their Arf versus Rab partners appears to be divergent. In Arf proteins, sequence variability is found among subgroups, thus enabling effectors to discriminate among them. In contrast, Rab sequences are more highly conserved, but conformational variability in side-chain rotamers is observed that may partly confer specificity to effector recognition between the various Rab proteins (Merithew et al., 2001). Of note, no such aromatic triad is found at the same location in Ras, Rho, Ran, and RGK families (Figure 1).

In Arf proteins, the aromatic triad has been identified as an important structural determinant for effector binding. In the case of the ARL1:golgin-245 complex, the aromatic triad of ARL1 makes interactions with the Met2194 of golgin-245; its mutation to alanine abolishes ARL1-mediated targeting of golgin-245 to Golgi membranes (Wu et al., 2004). Interestingly, UNC119a has been crystallized pairwise with ARL2 and ARL3 proteins, which share 62% sequence identities in their switch regions. The structure of these complexes reveals differences at their interface. In ARL2:UNC119a, the Trp62 side chain adopts a novel rotamer, distinct from all other Arf:effector complex structures, including that of ARL3:UNC119a. More specifically, Trp62 of ARL2 is involved in hydrogen bonds with the main-chain carbonyl of Phe179 and Phe181 from UNC119a, while that of ARL3 makes only one hydrogen bond (Ismail et al., 2012). Although ARL2 and ARL3 bind UNC119a with similar affinities, only ARL3 allosterically displaces cargo by accelerating its release by three orders of magnitude (Ismail et al., 2012). The structural difference observed at the aromatic triad of ARL2 may account for the difference in cargo displacement. Thus, in addition to sequence variability in the aromatic triad as a mechanism of effector discrimination (Chavrier and Ménétreay, 2010), conformational variability also plays a role in Arf:effector specificity, as observed in Rab proteins (see below).

In Rabs, the aromatic triad forms a hydrophobic interface with all known effector complexes except for Rab8:OCRL1. Despite sequence conservation, the side-chain conformations of these residues are variable and have been linked with effector specificity (Merithew et al., 2001; Grosshans et al., 2006). The Rab6 interfaces with GCC185 and DENND5, two structurally unrelated effectors, reveal comparable determinants of hydrophobicity (aromatic triad) and polar/electrostatic parity, suggesting a con-

servation of chemistry at the Rab6 interface (Burguete et al., 2008; Recacha et al., 2009). However, the interface promiscuity between Rab6 and these two effectors is associated with structural plasticity of the aromatic triad, particularly variant χ_1 dihedral angles for Phe50 (switch 1) and Trp67 (interswitch). Such a conformational variability in the aromatic triad of Rab6 probably accounts for the ability of these two structurally unrelated effectors to interact at the same location (Burguete et al., 2008; Recacha et al., 2009). One exception is the structure of Rab8:OCRL1 in which the aromatic triad has only a peripheral involvement in effector binding. Given that OCRL1 also binds to Rab1, Rab5, and Rab6, which are evolutionarily divergent Rabs, it has been suggested that abolition of an interface at the aromatic triad may lead to effector promiscuity (Hou et al., 2011). An alternative (and somewhat correlated) view is that the low affinities observed in these complexes (K_d of 1–4 μ M) implicate the aromatic triad as a significant component of the affinity in effector binding.

Hydrophobic Surface at the Junction of Switches 1 and 2

In the active GTP-bound state of small GTPases, the C terminus of switch 1 and the N terminus of switch 2 are brought into spatial proximity with the γ -phosphate of GTP via conserved hydrogen bonds. These interactions enable a contiguous switch 1/2 binding surface for effector recruitment. In the case of Arf/Rab proteins, the switch binding surfaces share structural properties that are not observed in Rho and Ras family proteins. First, Arf/Rab proteins have a glycine insertion at the C terminus of switch 1 relative to Ras/Rho proteins (Figure 1). Notably, Ran protein also exhibits this glycine insertion at the same position. The insertion facilitates the close apposition of the C-terminal part of switch 1 to the N-terminal segment of switch 2. Second, Arf/Rab proteins exhibit a hydrophobic patch that spans the switch 1/2 interface. This hydrophobic surface is adjacent to the invariant glycine and extends toward the interswitch region encompassing the aromatic triad. Thus, a contiguous hydrophobic interface, spanning elements from switch 1 to interswitch to switch 2, appears to be critical for the formation of a complementary interface with Arf/Rab effectors (Figure 6). Altogether, the glycine insertion and hydrophobic/aromatic residues from switch regions lead to similar structural features adopted by Arf and Rab proteins (Figures 6A and 6B) compared to Ras and Rho proteins (Figures 6C and 6D), which likely accounts for shared modes of effector binding.

In Arf proteins, the area that encompasses the hydrophobic surface formed at the switch 1/2 junction and the aromatic triad has been previously termed the common hydrophobic area (CHA; Chavrier and Ménétreay, 2010). One difference with Rab proteins is that the CHA of Arf proteins exhibits a hydrophobic pocket (Figure 6A) where one residue from the effector can enter more or less deeply. This hydrophobic pocket carries specificity to discriminate among the different Arf subgroups (Chavrier and Ménétreay, 2010). The recently solved ARF6:MKLP1 structure (Makyio et al., 2012) reveals that MKLP1 also interacts with ARF6 through its CHA using the hydrophobic pocket. It is interesting to note that in contrast to the two other structures of effectors complexed to ARF6, ARF6:JIP4 (Montagnac et al., 2009) and ARF6:CTA1 (O'Neal et al., 2005), MKLP1 directs a voluminous hydrophobic residue (phenylalanine) into the hydrophobic pocket of ARF6 as deeply as the key tyrosine residue of the

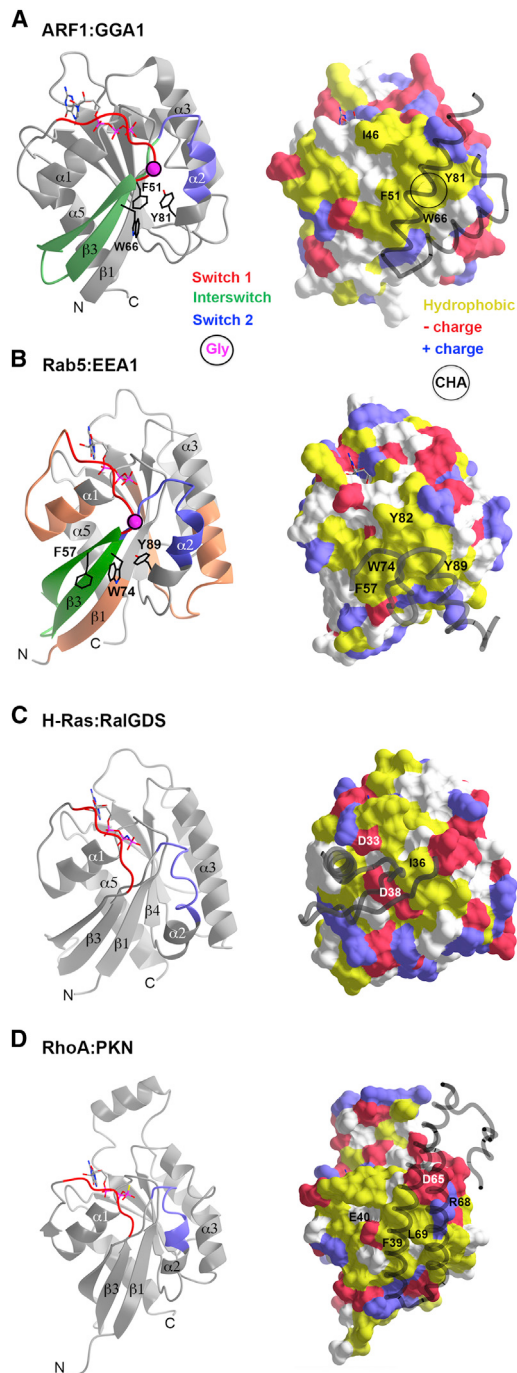


Figure 6. Structural Determinants of Effector Recruitment

Selected small GTPases are shown.

(Left) Ribbon models indicate the orientation of the effector interface relative to the GTP. The glycine residue in switch 1 of ARF1 and Rab5 is indicated by a magenta sphere to highlight its position relative to the hydrophobic interface. (Right) The effector-binding interface is represented as water-accessible surface. Basic and acid residues are indicated, respectively, in blue and red, hydrophobic residues in yellow, and remaining residues are white. The common hydrophobic area of ARF1 is indicated with a circle. Effectors are represented as transparent gray worms and have been truncated to reduce complexity and enable visualization of small GTPase surfaces.

GRIP domain of golgin-245 complexed to ARL1 (Panic et al., 2003; Wu et al., 2004).

Sequence Variability at the Edge of Switch Regions

One intriguing aspect in effector specificity is the ability of some effectors to discriminate between two small GTPases that belong to the same subgroup and thus are virtually identical in sequence and structure at the switch junction. This observation raises the question of how specificity takes place in such cases.

In the case of ARF1 and ARF6, which belong to the same Arf subgroup and are structurally identical at the CHA region (except for one conserved difference between switch 1 and the interswitch), the determination of the ARF6:JIP4 complex structure has revealed for the first time the structural determinants of such specificity (Montagnac et al., 2009). In addition to the CHA of ARF6, JIP4 makes specific interactions at the edge of the switch regions, proximal to the CHA. At these positions, clear sequence differences between ARF1 and ARF6 are sufficient to encode specificity, as shown by mutagenesis and binding assays (Montagnac et al., 2009). Interestingly, because these positions technically belong to the switch regions, they could be sufficient to allow the effector to recognize the GTP-bound form of the Arf protein without resorting to interactions with the CHA. This would enable two distinct effectors, such as GGA and FIP3 (Schonteich et al., 2007), to bind simultaneously to a single Arf protein—one at the CHA and the other at the edge of the switch regions. In contrast to the Arf CHA, the switch regions are narrower in Rab proteins, and therefore GTP-specific recruitment of two effectors simultaneously would be unlikely.

In the Rab family, sequence analyses have identified mammalian-specific sequence motifs (RabF1–RabF5, Rab family) that cluster within and adjacent to the switch regions (Figure 1; Pereira-Leal and Seabra, 2000). Beyond the switch regions, it was suggested that additional elements of the primary sequence mediate effector specificity and biological function (Moore et al., 1995; Ostermeier and Brunger, 1999). These regions were termed the Rab subfamily motifs (RabSF1–RabSF3; Pereira-Leal and Seabra, 2000), which partially overlap with the previously termed CDRs (Figures 1 and 4). Phylogenetic analyses of Rabs suggest that sequence determinants in RabF and RabSF motifs are conserved among functional groups (Pereira-Leal et al., 2001), suggesting an evolution of distinct structural frameworks for effector recruitment to mediate trafficking. The contribution of RabSF motifs to effector binding may be direct—such as RabSF1 (CDR1) in Rab27:effectors—or they may affect the conformation of switch 1 (RabF1) and switch 2 (RabF2–RabF4), thus indirectly influencing effector specificity (Figure 6). Taken together, Rab sequences and Rab:effector complexes have revealed subtle variations in active (GTP-bound) switch 1 and 2 conformations that encode effector specificity (Eathiraj et al., 2005; Mishra et al., 2010). Thus, specificity is achieved despite high sequence conservation in the switch regions and a relatively narrow binding face in Rab proteins.

Conclusions

Although Arf and Rab families are relatively distant on the evolutionary timeline, they jointly regulate vesicular trafficking, sometimes via a common effector. Despite their differences in sequence and mechanism of membrane attachment, Arf and

Rab proteins share structural determinants at the molecular level that lead to common effector modes of binding. Two recurring themes for Arf/Rab effector recruitment are all- α -helical and bivalent modes of binding. More recent themes include mixed α -helical modes, as well as β - β zipping that bridges two β sheets. These binding modes are underpinned by a shared continuous hydrophobic interface at the switch 1/2 junction that includes the aromatic triad. Finally, for Arf and Rab families, the molecular basis for effector specificity is a function of modest sequence and structural variability situated at the aromatic triad and the periphery of switch regions. In the future, it will be interesting to examine how new Arf/Rab:effector structures follow these general themes or whether new binding modes are exploited to mediate trafficking in eukaryotic cells.

The long, extended conformations of many ArfBDs and RabBDs appear suited for the microenvironment of membrane surfaces. As shown by a proteomics analysis of the synaptic vesicle (Takamori et al., 2006; Jahn and Fasshauer, 2012), the surface of trafficking vesicles is dense and composed of large transmembrane and membrane-associated glycoproteins. Extended coiled coils would enable access to Rabs in a crowded space, while also freeing the other globular domains to regulate trafficking. It will be particularly exciting to observe developments in our understanding of Arf/Rab interactions with motor proteins, such as myosins and kinesins, which regulate tethering, motility, and fusion.

A limitation from structural studies of Arf/Rab:effector complexes is the inability to wholly mimic the cellular environment when extrapolating biological function. A full-length structural model of a Rab effector remains to be determined, and in vivo, cellular trafficking is affected by local concentrations of proteins in lipid microdomains (Wang et al., 2002; Pfeffer, 2003; Heo et al., 2006; Mizuno-Yamasaki et al., 2010). Also, posttranslational modifications of effectors may affect the kinetics and affinity of recruitment by Arf/Rab GTPases. Overall, these studies suggest additional layers of regulation in a cellular context; integrative structural, cellular, and biophysical approaches are required to fully understand the molecular basis for trafficking by Arf and Rab GTPases.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.str.2013.06.016>.

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