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Structural mechanisms for regulation of membrane traffic by Rab GTPases

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Abstract

In all eukaryotic organisms, Rab GTPases function as critical regulators of membrane traffic, organelle biogenesis and maturation, and related cellular processes. The numerous Rab proteins have distinctive yet overlapping sub-cellular distributions throughout the endomembrane system. Intensive investigation has clarified the underlying molecular and structural mechanisms for several ubiquitous Rab proteins that control membrane traffic between tubular-vesicular organelles in the exocytic, endocytic and recycling pathways. In this review, we focus on structural insights that inform our current understanding of the organization of the Rab family as well as the mechanisms for membrane targeting and activation, interaction with effectors, deactivation, and specificity determination.

Keywords

Membrane traffic; Rab GTPase; GEF; GAP; effector; GDI; REP; GDF; TBC domain; Vps9 domain; Structure

Introduction

As critical regulators of membrane traffic, Rab GTPases cycle between GTP-bound (active) and GDP-bound (inactive) conformations (1–3). Prenylation of C-terminal cysteine motifs anchors Rab GTPases to membranes and is essential for function (4). Transfer between membranes is mediated through a soluble complex of GDP-bound Rab proteins with RabGDI (GDP dissociation inhibitor) and further facilitated by GDI displacement factors (GDFs) (5, 6). Following GDP to GTP exchange catalyzed by guanine nucleotide exchange factors (GEFs), Rab GTPases interact with effectors including sorting complexes, motor proteins, tethering factors/complexes, and lipid metabolic enzymes (1, 3, 7). Deactivation through GTP hydrolysis is accelerated by GTPase activating proteins (GAPs) (8). The spatiotemporal distributions of Rab GTPases depend on GEFs and GAPs as well as effectors (3, 9). There is also evidence for organization of organelle membranes into overlapping Rab domains with distinct functional properties (1). The functions of Rab GTPases, as well as other GTPases that regulate membrane traffic (e.g., Arf and Arl GTPases), can be

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coordinated through multivalent effectors with distinct binding domains (7). In addition, one Rab GTPase can recruit a GEF for a second Rab GTPase that will subsequently replace the first (3). This process is well documented for Rab5-to-Rab7 conversion during endosome maturation (10).

Over the last several years, our understanding of the structural basis for regulation of membrane traffic by Rab GTPases has expanded considerably. This review highlights structural insights regarding the mechanisms for membrane targeting, regulation of the GTPase cycle, interaction with effectors, and specificity determination.

Conserved molecular switch mechanism

As indicated in Figure 1A, Rab proteins possess a characteristic GTPase fold with two 'switch regions' that contact the γ phosphate of GTP and exhibit large conformational differences between the inactive and active states (11, 12). An essential Mg^{2+} cofactor is required for high affinity nucleotide binding and hydrolysis. Interactions with guanine nucleotides involve mainly residues from five 'G motifs' that are broadly conserved in GTPases (13, 14). The GxxxxGK(S/T) motif in the 'P-loop' ($\beta 1/\alpha 1$ loop) provides phosphate contacts and supplies a Ser/Thr that is coordinated by the Mg^{2+} ion. A threonine in switch I contacts the γ phosphate and is coordinated by the Mg^{2+} ion in the active state. The DxxGQ motif in switch II provides an aspartate that stabilizes the Mg^{2+} ion through interaction with a water ligand, a glycine that contacts the γ phosphate, and a glutamine that functions as a catalytic residue for the intrinsic GTP hydrolytic reaction. Partial-loss-of-function variants with altered nucleotide-binding and GTP hydrolytic properties are easily generated by mutation of conserved G motif residues. The two most widely utilized mutations involve asparagine substitution of the P-loop Ser/Thr (SN or TN mutant) and leucine substitution of the glutamine in the DxxGQ motif (QL mutant). The SN or TN substitution disrupts the Mg^{2+} binding site, resulting in greatly reduced affinity for guanine nucleotides. *In vitro* at low Mg^{2+} concentrations, the affinity for GTP is reduced 100-fold without affecting the affinity for GDP, suggesting that the mutants are 'GDP-locked' (15, 16). The effects *in vivo* are more complicated, given relatively high cellular concentrations of free Mg^{2+} and GTP. In any case, the SN or TN mutant are expected to shift the equilibrium towards the GDP/nucleotide free states and likely exert dominant negative effects by sequestration of endogenous GEFs due to low GTP-affinity.

Phylogeny and structural diversity

Encoded by 11 genes in budding yeast and ~60 in humans, the Rab family represents the largest and most diverse branch of the GTPase superfamily (17). The majority can be hierarchically organized into phylogenetic groups (PGs) that contain functionally distinct proteins in addition to subfamilies of highly similar isoforms (18). Subfamily members tend to be functionally similar if not redundant (although differences in tissue and possibly sub-cellular distribution might be important) whereas group members typically have partially or completely distinguishable functions. Nevertheless, group members are more likely to function in related processes and have overlapping subcellular distributions than members of different groups.

Crystal structures are available for at least 16 Rab GTPases in the active state and 17 in the inactive state, with one or more structures for 7 of the 8 PGs. The coverage is sufficient to allow some generalization with respect to conformational switching, characteristic family features, and similarity/diversity within and between PGs (19). In the inactive conformation, the switch regions are disordered or adopt ordered conformations that depend on crystal packing (Figure 1B). Both observations support the view that the switch regions are natively unfolded in the GDP-bound state. Conversely, in the active state, the switch regions adopt well defined conformations (Figure 1C), with the exception of Rab21 where switch II remains mobile.

Tertiary structural differences between Rab GTPases are generally small within conserved elements required for nucleotide and Mg^{2+} binding but strikingly large in switch II, interswitch, and the $\alpha 3/\beta 5$ loop (Figure 1C). The active conformation of switch I and, in particular, switch II varies substantially between and even within PGs (19). For example, Rab4 and Rab11 from PG2 have dissimilar active conformations as do Rab7 and Rab9 from PG7. In PG5, the active conformation of Rab21 is dissimilar to Rab5 and Rab22, which have nearly identical active conformations. Furthermore, the active conformation of Rab4 is more similar to Rab5 and Rab22 than to Rab11. Structural diversity in the active switch conformation may be due in part to non-conservative substitutions within the relatively well conserved switch regions; however, substitutions within the hydrophobic core involving residues proximal to the switch II region likely contribute as well. From an evolutionary perspective, these differences presumably reflect selective pressure to achieve functional specificity.

Membrane targeting

Membrane targeting requires prenylation and is dependent on two evolutionarily conserved paralogs, Rab escort protein (REP) and RabGDI (5, 20). REP presents nascent Rab GTPases to Rab geranylgeranyl transferase (RabGGTase) for addition of one or (in most cases) two 20 carbon prenyl groups and subsequently delivers Rab proteins to membranes (21). Transfer between membranes is facilitated by RabGDI, which extracts Rab GTPases after GTP hydrolysis (22), and by GDFs, which catalyze release of Rab GTPases from GDI (23). Crystallographic studies of RabGDI and REP, alone and in complexes with prenylated Rab GTPases, have delineated common structural features as well as key differences between the two proteins in addition to providing detailed snapshots of the protein-protein and protein-prenyl interfaces (24–29). RabGDI and REP share a similar overall architecture with two domains (Domain I and II) as compared in Figure 2A and 2B. Domain I conserves several features involved in Rab binding, including the ‘Rab-binding platform’ that interfaces with the switch/interswitch regions, the ‘C-terminal binding region’ (CBR) that engages an aliphatic-X-aliphatic (AXA) motif present in the otherwise hypervariable C-terminal extension of many Rab proteins, and the ‘mobile effector loop’ (MEL), which also contacts the C-terminal hypervariable extension. REP engages the α subunit of RabGGTase exclusively through Domain II (Figure 2C). The ability of REP but not RabGDI to bind RabGGTase reflects two non-conservative substitutions in the REP binding epitope of Domain II (30).

The location of the prenyl group binding pocket, which is not exposed in the absence of Rab GTPases, was initially suggested to be located under the Rab binding platform in Domain I based on the complex of RabGDI with a di-geranyl geranyl peptide (28). Curiously, however, subsequent structures of RabGDI and REP in complex with mono-geranyl geranyl Ypt1p and Rab7 revealed a distinct prenyl group pocket in Domain II (24–27). A similar binding modality is observed in the RabGDI complex with di-geranyl geranyl Ypt1p (Figure 2D), except that the first prenyl group inserts into the Domain II pocket in a different orientation while the second covers the first and remains partially exposed (25). The function of the second pocket in Domain I remains unclear; however, it might in principle serve as an intermediate docking site during prenyl group transfer (6) or perhaps as an alternative pocket for a subset of Rab GTPases. The structural observations and interaction analyses suggest a sequential allosteric model for extraction of Rab GTPases from membranes by RabGDI in which docking of the switch/interswitch regions followed by the C-terminal AXA motif exposes the non-polar pocket for prenyl group transfer from the membrane (24). In contrast to RabGDI, which has ~3–4 orders of magnitude higher affinity for di-geranyl geranylated vs. unprenylated Rab GTPases, REP has high affinity for both (31–34). Thus, despite very similar overall tertiary structures, the driving force for binding of REP to prenylated Rab GTPases predominantly reflects the protein-protein interface, allowing REP to facilitate presentation to RabGGTase and subsequent transfer to membranes at the expense of efficient membrane extraction (31).

Although both REP and RabGDI have broad Rab specificity, the affinity for Rab GTPases varies (6). These differences evidently contribute to the etiology of choroideremia, a retinopathy linked to a loss of function mutation in the REP-1 isoform. In cells from choroideremia patients, the majority of Rab27A is unprenylated suggesting that REP-2, which has lower affinity for Rab GTPases than REP-1, cannot compensate for loss of REP-1 function (35). The underprenylation of Rab27A is likely due to weaker affinity for REP-2 compared with other Rab proteins (26). Finally, it is interesting to note that the known eukaryotic GDFs, PRA1 and the yeast ortholog YIP3, are polytypic integral membrane proteins (23). Two other integral membrane proteins, YIP4 and YIP5, also bind doubly prenylated Rab GTPases but are not known to catalyze GDI displacement (36, 37). In depth of discussion related to membrane targeting by REP, GDI and GDF can be found in other reviews (4–6).

Activation by GEFs

Rab GEFs are structurally diverse, ranging in size and complexity from small proteins to large modular proteins and multiprotein complexes. Kinetic and thermodynamic analyses of GEFs for different GTPase families support an allosteric competition mechanism for acceleration of nucleotide exchange, whereby GEFs compete with nucleotides for binding to GTPases (38–40). Key intermediates are the nucleotide free GEF-GTPase complex and the ternary GEF-GTPase-GXP complexes, where GXP is GDP or GTP. GEFs by necessity substantially increase the rate of GDP-release, which is rate limiting for the intrinsic exchange reaction at cellular GTP levels. The rate of GTP-binding can also be accelerated as illustrated by a recent kinetic analysis of nucleotide exchange catalyzed by the TRAPPI complex (41). Despite a common underlying reaction mechanism, Rab GEFs exhibit a broad

range of catalytic efficiencies and substrate specificities, differ markedly regarding recognition of GDP- vs. GTP-bound conformations, and are subject to distinct intramolecular and intermolecular mechanisms for regulation of catalytic output. Structures of the catalytic cores of four Rab GEFs have been determined in complex with target Rab GTPases (Figure 3). Discussed below, these studies illustrate the remarkable degree to which Rab GEFs have evolved not only to recognize different Rab substrates but also to facilitate nucleotide exchange through largely unrelated structural mechanisms.

The 17 kDa protein Mss4 and its yeast ortholog Dss4 were amongst the first Rab GEFs identified (42, 43). Mss4 has weak exchange activity and an atypical ability to recognize both GDP- and GTP-bound forms of several exocytic Rab GTPases from different PGs, including Rab1/Ypt1p, Rab3, Rab8/Sec4p and Rab10 (44–46). As these Rab GTPases have other GEFs with higher catalytic efficiencies, Mss4/Dss4 have been suggested to function *in vivo* as molecular chaperones for nucleotide free Rab GTPases (46, 47). The structure of the nucleotide free Mss4-Rab8 complex (Figure 3A) indicates that Mss4 interacts with the N-terminal CDR, switch I, and the interswitch region, promoting nucleotide release by an indirect mechanism whereby Mss4-induced rearrangement of switch I causes the $\alpha 1$ helix to unfold (46).

Compared with Mss4/Dss4p, Sec2p has 100–1000 fold higher exchange activity for Sec4p, and yet the exchange domain in Sec2p is a deceptively simple asymmetric coiled coil. In the structures of nucleotide free Sec2p-Sec4p complexes (48, 49), Sec2p engages the switch and interswitch regions of Sec4p (Figure 3B). Although Sec2 does not intrude directly into the nucleotide binding site, comparison with the Sec4p-GDP structure (11) suggests that Sec2p stabilizes a switch I conformation that is incompatible with nucleotide binding. Interestingly, Rabin8 and Rab8 (the mammalian orthologs of Sec2p and Sec4p) are associated with Bardet-Biedl syndrome (BBS), a genetic disorder whose symptoms include obesity, retinal degeneration, and nephropathy (50–52). Rabin8 is required for targeting of Rab8 to the primary cilium and disruption of Rab8 in Zebrafish results in BBS phenotypes (52).

Defined by homology with yeast Vps9p, which was identified in a screen for vacuolar protein sorting defects, the Vps9 domains of eight modular mammalian proteins facilitate nucleotide exchange by a more direct mechanism analogous to the Sec7 domain of Arf GEFs (53, 54). The Vps9 domain protein Rabex-5 has a catalytic core consisting of a helical bundle (HB)-Vps9 domain tandem with equivalently high exchange activity for the PG5 Rab GTPases Rab5 and Rab21 (54, 55). In the structure of the Rabex-5 HB-Vps9 tandem in complex with nucleotide free Rab21 (56), invariant aromatic residues from the switch and interswitch regions of Rab21 dock in a non-polar groove between adjacent helices in the Vps9 domain (Figure 3C). An invariant ‘aspartate finger’, analogous to the ‘glutamate finger’ in the Sec7 domain, stabilizes the nucleotide free intermediate by mimicking interactions of the γ phosphate with the invariant P-loop lysine and switch II backbone. Comparison with the structures of the HB-Vps9 tandem alone and nucleotide-bound forms of Rab21 suggests that the aspartate finger promotes GDP release through disruption of the Mg^{2+} binding site. The Vps9 and Sec7 domains also facilitate nucleotide egress by propping switch I in an open/flexible conformation (54, 57, 58). Finally, in contrast to Mss4/Dss4p, the weak exchange activity of full length Rabex-5 (45) is not a property of the catalytic core

but instead reflects potent autoinhibition by a helical region C-terminal to the Vps9 domain (56).

TRAPPI (transport protein particle I) activates Ypt1p/Rab1 during COPII-vesicle tethering (59, 60). Unlike most GEFs, the catalytic core of TRAPPI consists of four proteins with a stoichiometry of 2 Bet3p to 1 each of Bet5p, Trs23p and Trs31p (61). Structures of Bet3p alone and two catalytically inactive subcomplexes of mammalian TRAPPI revealed the overall architecture as well as a common α/β fold shared by the small subunits (61, 62). Insight into the exchange mechanism was provided by the structure of the yeast TRAPPI catalytic core in complex with nucleotide free Ypt1p (63). Residues from Trs23p, Bet5p and the C-terminus of Bet3p engage the $\beta 1$ strand, switch, interswitch, and P-loop regions of Ypt1p (Figure 3D). Trs31p interacts with Bet3p and Trs23p but does not contact Ypt1p directly; nevertheless, comparison with the inactive subcomplexes lacking Trs23p revealed a conformational change in Trs23p that propagates to the interface with Ypt1p. Although a glutamate from Bet3p appears to stabilize the nucleotide free conformation of the P-loop, double alanine substitution of this residue and an adjacent aspartate has a relatively modest (5 fold) effect on the exchange activity. This contrasts with much larger effects for alanine substitution of the aspartate and glutamate fingers in the Vps9 and Sec7 domains (54, 64). Evidently, other elements account for much of the observed 400 fold acceleration of GDP release by wild type TRAPPI. Most notably, the C-terminus of one Bet3p subunit intrudes into the nucleotide binding pocket, suggesting a role in stabilization of the nucleotide free intermediate and/or acceleration of GDP release through interaction with switch I. Consistent with this hypothesis, deletion of the Bet3p C-terminus reduces exchange by ~ 2 orders or magnitude. Further discussion of TRAPPI/II complexes can be found in recent reviews (65, 66).

Deactivation by GAPs

Most known Rab GAPs contain a catalytic TBC (Tre-2, Bub2 and Cdc16) domain (8). With the exception of Bub2 and Cdc16, which function as GAPs for specialized GTPases in the MEN/SIN pathway, the yeast TBC domain proteins, termed Gyps (GAPs for Ypt/Rab proteins), have GAP activity for one or more yeast Rab GTPases (67–69). The most extensively characterized TBC domain protein, Gyp1p, has high activity for the exocytic Rabs Ypt1p and Sec4p as well as the endocytic Rabs Ypt51p and Ypt7p (67, 68). Gyp1p localizes to the Golgi, negatively regulates Ypt1p, and is required for endocytic recycling. Mammalian genomes encode ~ 40 TBC domain proteins with widely varying domain architectures and Rab specificities (70–83). The structure of the Gyp1p TBC domain revealed a helical fold with two subdomains arranged such that one surface from each contributes to a distinctive L-shaped groove containing several conserved/exposed residues, including arginine and glutamine residues from the WxxxR, IxxDxxR and YxQ motifs in the N-terminal subdomain (84). Based on mutational analyses, the IxxDxxR arginine was proposed to function as a catalytic residue analogous to the ‘arginine fingers’ of Ras and Rho family GAPs (67). The TBC domains of two mammalian proteins have a similar overall structure with differences in the C-terminal subdomain that likely contribute to Rab specificity (85).

Unexpected insight into the catalytic mechanism was provided by the structure of Gyp1p in a ternary complex with GDP-bound Rab33 and the transition state mimic AlF₃ (77). Rab33 regulates retrograde Golgi trafficking (86), is a better Gyp1p substrate than Ypt1p with respect to catalytic efficiency, and forms a relatively stable ternary complex with Gyp1p and AlF₃ (77). As shown in Figure 4, the C-terminal subdomain of Gyp1p engages the switch and interswitch regions of Rab33 while the arginine and glutamine residues from the IxxDxxR and YxQ motifs mediate polar interactions with GDP-AlF₃-H₂O similar to the contacts mediated by the arginine finger supplied in trans by the GAP and DxxGQ glutamine supplied in cis by the GTPase in the analogous Ras/Rho GAP complexes (77, 87). Furthermore, the DxxGQ glutamine in Rab33 mediates polar interactions with the TBC domain backbone rather than GDP-AlF₃-H₂O. These observations suggest that TBC domains are dual finger GAPs that supply two different catalytic residues in trans, an arginine finger and a glutamine finger. In support of this conclusion, alanine substitution of either finger dramatically decreases k_{cat} with little effect on K_{m} . Conversely, substitution of the DxxGQ glutamine with alanine or leucine substantially increases K_{m} but has a negligible effect on k_{cat} (77), even though the leucine substitution substantially reduces the intrinsic hydrolytic rate (88). Thus, the DxxGQ glutamine plays the role of a binding residue in the context of TBC domain-accelerated hydrolysis. Finally, it is interesting to note that RapGAP accelerates Rap GTP hydrolysis in the absence of catalytic arginine or glutamine residues by supplying an ‘asparagine thumb’ analogous to the glutamine finger in the TBC domain (89).

Effector binding modalities

In the active state, Rab GTPases interact with diverse effectors to facilitate cargo sorting, couple vesicles or organelles to motor proteins for transport, and establish long range ‘tethering’ linkages preceding SNARE-mediated fusion (1–3). Apart from small families of homologous Rab binding domains (RBDs), the various Rab effectors share little sequence similarity. Crystallographic studies of RBDs in complex with Rab GTPases reveal instances of similarity and divergence at the tertiary structural level (Figure 5). In general, the binding interfaces include the switch/interswitch regions and are either centered on or overlap with a ‘hydrophobic triad’ of invariant aromatic residues (interswitch phenylalanine and tryptophan and switch II tyrosine) located near the switch/interswitch junction. In the Rabphilin-3A complex with Rab3, the binding interface extends from the switch/interswitch regions to an adjacent pocket formed by the $\alpha 3/\beta 5$ loop and N/C-terminal regions proximal to the GTPase domain (90). Termed ‘complementary determining regions’ (CDRs), the $\alpha 3/\beta 5$ loop and N/C-termini exhibit high sequence variability compared with the switch regions and were proposed to determine the specificity of Rab-effector interactions. Recent structures of Rab27 complexes with the RBDs of Slac2-a/Melanophilin (91) and Slp2-a/Exophilin4 (92) illustrate a similar binding modality for the core helical elements in the RBDs, despite substantial structural variations.

Several effectors for Rab GTPases from different PGs contain RBDs that consist of elaborations on a coiled coil core (19, 93–98). These include dyad symmetric parallel coiled coil homodimers with 2:2 Rab:effector binding stoichiometries (e.g., Rabaptin-5, GCC185, RILP and FIPs) as well as asymmetric helical hairpin monomers with 1:1 binding stoichiometries (e.g., Rabenosyn-5). In the structures determined to date, the coiled coil

cores align either roughly parallel to Rabphilin-3A (GCC185, RILP and FIP3) or are rotated by $\sim 45^\circ$ (Rabaptin-5 and Rabenosyn-5). In the RILP-Rab7 complex (97), the binding interface includes the N/C-terminal CDRs and, in this respect, resembles the Rab3/Rab27 effectors (90–92). In the Rab5-Rabaptin-5 (98), Rab11-FIP2/FIP3 (94–96), and Rab6-GCC185 (93) complexes as well as the Rab4 and Rab22 complexes with the central and C-terminal Rabenosyn-5 RBDs (19), the binding interfaces are restricted to the switch and interswitch regions. In the case of GCC185, however, the C-terminal hypervariable region was required for Rab6 binding even though it was not visible in the crystal structure (93).

A number of Rab effectors contain 2 or more distinct binding domains for small GTPases including, for example: i) Rabaptin-5 with N- and C-terminal coiled coil RBDs for Rab4 and Rab5, respectively (99); ii) Rabenosyn-5 with an N-terminal C_2H_2 Zn^{2+} finger that binds Rab5 in addition to central and C-terminal helical hairpin RBDs that bind Rab4/Rab14 and Rab5/Rab22/Rab24, respectively (19, 100, 101); iii) FIP3 with central and C-terminal binding domains for Arf6 and Rab11, respectively (102); iv) GCC185 with Rab6 and Rab9 RBDs proximal to a Grip domain that binds Arl1 with low affinity compared to other Grip domains (93); and v) Rab6IP1, which binds Rab11 in addition to Rab6 (103). Such multivalent effectors suggest a role in coordination or integration of Rab, Arf, and/or Arl inputs (7). Interestingly, Rab6 binding to GCC185 synergistically enhances binding of Arl1 whereas Rab9 has the opposite effect (93). The inhibitory effect of Rab9 is likely due to overlap of the Rab9 RBD with the Grip domain. The mechanism for Rab6-enhanced binding of Arl1 remains to be determined. In the Rab6 complex with the RUN-PLAT tandem of Rab6IP1, a coiled coil sub-structure in the RUN domain binds the switch/interswitch regions with a modality similar to the Rab6 RBD of GCC185 (104).

Finally, although most effectors recognize a largely pre-configured active conformation of the switch regions, a striking exception involves the Rab11 complexes with FIP2 or FIP3 (94–96). Here, switch II undergoes a large rearrangement coupled with FIP2/FIP3 binding. More subtle, though still significant changes occur in other Rab-effector complexes, including Rab3-Rabphilin-3A, Rab5-Rabaptin-5 and Rab6-Rab6IP1 (90, 98, 104).

Rab recognition

As noted above, the interfaces with effectors and regulatory factors generally involve one or more of the residues in the invariant hydrophobic triad and often other conserved switch/interswitch residues. These landmark features comprise a set of general recognition determinants characteristic of the Rab family. Several models have been suggested to explain the specificity of effectors or regulatory factors for particular Rab subsets. One model attributes specificity determination to the CDR regions (90). Another model identifies 5 subfamily motifs that tend to be conserved within subfamilies but are otherwise variable as potential determinants of functional specificity (18). Three of the subfamily motifs overlap with the CDRs. A third model highlights sequence variability within the switch/interswitch regions as a source of specificity (19, 48, 91). Finally, sequence variability in the protein core could indirectly contribute by influencing the conformation of the switch/interswitch regions (19, 105).

From an experimental standpoint, Rab specificity determinants are most readily identified by mutational analyses aimed at understanding differences in binding affinities/catalytic activities for structurally similar effectors/regulatory factors or closely related Rab GTPases. For example, Rabex-5 has equivalent catalytic efficiency for Rab5 and Rab21 but 100 fold weaker activity for Rab22 (54). Compared with Rab5, it appears that the cumulative effect of multiple switch/interswitch substitutions renders Rab22 a poor substrate. This would also be the case for Rab21 were it not for a switch I substitution that replaces the otherwise invariant glycine with a glutamine. The Gln to Gly substitution in Rab21 decreases catalytic efficiency by ~50 fold whereas the reciprocal Gly to Gln substitution in Rab5 and Rab22 increases catalytic efficiency by ~10 fold. The Gly to Gln substitution has the opposite affect (~100 fold reduction in K_D) on Rab5 binding to Rabenosyn-5 (19). Replacing switch I of Ypt1p with that of Sec4p converts Ypt1p into a substrate for Sec2p, implicating one or more substitutions in switch I as the primary determinants of the specificity of Sec2p for Sec4p as compared with Ypt1p (48). Conversely, the specificity differences for Slac2-a, which selectively binds Rab27, and Rabphilin-3A, which binds both Rab27 and Rab3, are largely due to substitutions in switch II and the N-terminal CDR rather than insertions in the $\alpha 2/\beta 3$ or $\alpha 3/\beta 5$ loops (91).

Qualitative and semi-quantitative interaction profiles provide the information necessary to consider Rab specificity determinants from a family-wide perspective. To a first approximation, Rab GTPases can be grouped into strongly interacting vs. weakly or non-interacting subsets. Specificity determinants that are conserved in either the interacting or non-interacting subsets can then be identified by mutational analyses that convert conserved or similar residues in interacting subsets to the consensus residue(s) in non-interacting subsets. Rabex-5, for example, recognizes Rab GTPases in PG5 (Rabs 5, 21 and 22) but exhibits no detectable activity for 29 other Rab proteins (54). This is due in part to a strict requirement for a small non-acidic residue preceding the invariant phenylalanine at the switch I/interswitch junction. The Rabex-5 Vps9 domain enforces this requirement through a small non-polar pocket that can not accommodate the consensus aspartate/glutamate in the non-interacting subset (56). In Rabenosyn-5, an analogous selection against the same consensus aspartate/glutamate is imposed by the C-terminal RBD, which binds selectively to Rabs 5, 22 and 24 (19). The central RBD, on the other hand, utilizes variations within the helical hairpin core in combination with an N-terminal extension to achieve high selectivity for Rabs 4 and 14. In this case, the N-terminal extension compensates for weaker binding to the helical hairpin core, presumably due to the consensus aspartate/glutamate shared by Rabs 4 and 14. The interaction with the N-terminal extension requires a glycine residue following the invariant phenylalanine at the switch I/interswitch junction. The glycine packs against a leucine side chain in the N-terminal extension such that substitution of the glycine with alanine as in Rab11 or with the consensus leucine, which is shared by Rabs 5, 22, and 24, abrogates the contribution of the N-terminal extension to the binding affinity.

Conclusions and future perspectives

Over the last decade, our appreciation of the molecular and structural underpinnings of Rab-regulated membrane trafficking has matured considerably. Much has been learned regarding the structural basis for membrane targeting, regulation of the GTPase cycle, and interaction

with effectors. We are also beginning to understand the elaborate selection mechanisms that allow broad recognition by REP and RabGDI while maintaining the capacity for narrow recognition of Rab subfamilies or non-phylogenetic subsets by effectors, GEFs and GAPs. Nevertheless, many important questions remain. One obvious gap relates to the structural mechanisms by which GDFs facilitate release of prenylated Rab GTPases from RabGDI. Another concerns the higher order organization of the numerous proteins and multiprotein complexes that interact with Rab GTPases and the inter/intra-molecular mechanisms that modulate the catalytic output of Rab GEFs and GAPs in response to upstream signals or downstream feedback loops. In addition, there is increasing evidence for direct subversion of host Rab functions by pathogens (106). In the case of *Legionella pneumophila*, which replicates within an intracellular ER/Golgi-like vacuole, three of the proteins injected through the Dot/Icm secretion apparatus include a GEF/GDF (DrrA/SidM), a GAP (LepB) and a putative effector/tethering factor (LidA) for Rab1 (107–110). The *L. pneumophila* factors share no sequence homology with host Rab-interacting proteins and unlike known host GDFs, which are polytypic integral membrane proteins, DrrA/SidM is soluble. Consequently, the extent to which pathogenic factors recognize Rab GTPases and manipulate the GTPase cycle through structural mimicry of host proteins remains an open question.

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References

1. Zerial M, McBride H. Rab proteins as membrane organizers. *Nat Rev Mol Cell Biol.* 2001; 2:107–117. [PubMed: 11252952]
2. Pfeffer SR. Rab GTPases: specifying and deciphering organelle identity and function. *Trends Cell Biol.* 2001; 11:487–491. [PubMed: 11719054]
3. Grosshans BL, Ortiz D, Novick P. Rabs and their effectors: achieving specificity in membrane traffic. *Proc Natl Acad Sci U S A.* 2006; 103:11821–11827. [PubMed: 16882731]
4. Leung KF, Baron R, Seabra MC. Thematic review series: lipid posttranslational modifications. geranylgeranylation of Rab GTPases. *J Lipid Res.* 2006; 47:467–475. [PubMed: 16401880]
5. Goody RS, Rak A, Alexandrov K. The structural and mechanistic basis for recycling of Rab proteins between membrane compartments. *Cell Mol Life Sci.* 2005; 62:1657–1670. [PubMed: 15924270]
6. Pfeffer S, Aivazian D. Targeting Rab GTPases to distinct membrane compartments. *Nat Rev Mol Cell Biol.* 2004; 5:886–896. [PubMed: 15520808]
7. Segev N. Ypt and Rab GTPases: insight into functions through novel interactions. *Curr Opin Cell Biol.* 2001; 13:500–511. [PubMed: 11454458]
8. Bernards A. GAPs galore! A survey of putative Ras superfamily GTPase activating proteins in man and *Drosophila*. *Biochim Biophys Acta.* 2003; 1603:47–82. [PubMed: 12618308]
9. Pfeffer S. A model for Rab GTPase localization. *Biochem Soc Trans.* 2005; 33:627–630. [PubMed: 16042559]
10. Rink J, Ghigo E, Kalaidzidis Y, Zerial M. Rab conversion as a mechanism of progression from early to late endosomes. *Cell.* 2005; 122:735–749. [PubMed: 16143105]
11. Stroupe C, Brunger AT. Crystal structures of a Rab protein in its inactive and active conformations. *J Mol Biol.* 2000; 304:585–598. [PubMed: 11099382]
12. Dumas JJ, Zhu Z, Connolly JL, Lambright DG. Structural basis of activation and GTP hydrolysis in Rab proteins. *Structure.* 1999; 7:413–423. [PubMed: 10196122]

13. Vetter IR, Wittinghofer A. The guanine nucleotide-binding switch in three dimensions. *Science*. 2001; 294:1299–1304. [PubMed: 11701921]
14. Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: conserved structure and molecular mechanism. *Nature*. 1991; 349:117–127. [PubMed: 1898771]
15. Farnsworth CL, Feig LA. Dominant inhibitory mutations in the Mg(2+)-binding site of RasH prevent its activation by GTP. *Mol Cell Biol*. 1991; 11:4822–4829. [PubMed: 1922022]
16. Nuoffer C, Davidson HW, Matteson J, Meinkoth J, Balch WE. A GDP-bound of rab1 inhibits protein export from the endoplasmic reticulum and transport between Golgi compartments. *J Cell Biol*. 1994; 125:225–237. [PubMed: 8163542]
17. Pereira-Leal JB, Seabra MC. Evolution of the Rab family of small GTP-binding proteins. *J Mol Biol*. 2001; 313:889–901. [PubMed: 11697911]
18. Pereira-Leal JB, Seabra MC. The mammalian Rab family of small GTPases: definition of family and subfamily sequence motifs suggests a mechanism for functional specificity in the Ras superfamily. *J Mol Biol*. 2000; 301:1077–1087. [PubMed: 10966806]
19. Eathiraj S, Pan X, Ritacco C, Lambright DG. Structural basis of family-wide Rab GTPase recognition by rabenosyn-5. *Nature*. 2005; 436:415–419. [PubMed: 16034420]
20. Alory C, Balch WE. Organization of the Rab-GDI/CHM superfamily: the functional basis for choroideremia disease. *Traffic*. 2001; 2:532–543. [PubMed: 11489211]
21. Andres DA, Seabra MC, Brown MS, Armstrong SA, Smeland TE, Cremers FP, Goldstein JL. cDNA cloning of component A of Rab geranylgeranyl transferase and demonstration of its role as a Rab escort protein. *Cell*. 1993; 73:1091–1099. [PubMed: 8513495]
22. Ullrich O, Stenmark H, Alexandrov K, Huber LA, Kaibuchi K, Sasaki T, Takai Y, Zerial M. Rab GDP dissociation inhibitor as a general regulator for the membrane association of rab proteins. *J Biol Chem*. 1993; 268:18143–18150. [PubMed: 8349690]
23. Sivars U, Aivazian D, Pfeffer SR. Yip3 catalyses the dissociation of endosomal Rab-GDI complexes. *Nature*. 2003; 425:856–859. [PubMed: 14574414]
24. Ignatev A, Kravchenko S, Rak A, Goody RS, Pylypenko O. A structural model of the GDP dissociation inhibitor rab membrane extraction mechanism. *J Biol Chem*. 2008; 283:18377–18384. [PubMed: 18426803]
25. Pylypenko O, Rak A, Durek T, Kushnir S, Dursina BE, Thomae NH, Constantinescu AT, Brunsveld L, Watzke A, Waldmann H, Goody RS, Alexandrov K. Structure of doubly prenylated Ypt1:GDI complex and the mechanism of GDI-mediated Rab recycling. *Embo J*. 2006; 25:13–23. [PubMed: 16395334]
26. Rak A, Pylypenko O, Niculae A, Pyatkov K, Goody RS, Alexandrov K. Structure of the Rab7:REP-1 complex: insights into the mechanism of Rab prenylation and choroideremia disease. *Cell*. 2004; 117:749–760. [PubMed: 15186776]
27. Rak A, Pylypenko O, Durek T, Watzke A, Kushnir S, Brunsveld L, Waldmann H, Goody RS, Alexandrov K. Structure of Rab GDP-dissociation inhibitor in complex with prenylated YPT1 GTPase. *Science*. 2003; 302:646–650. [PubMed: 14576435]
28. An Y, Shao Y, Alory C, Matteson J, Sakisaka T, Chen W, Gibbs RA, Wilson IA, Balch WE. Geranylgeranyl switching regulates GDI-Rab GTPase recycling. *Structure*. 2003; 11:347–357. [PubMed: 12623022]
29. Schalk I, Zeng K, Wu SK, Stura EA, Matteson J, Huang M, Tandon A, Wilson IA, Balch WE. Structure and mutational analysis of Rab GDP-dissociation inhibitor. *Nature*. 1996; 381:42–48. [PubMed: 8609986]
30. Pylypenko O, Rak A, Reents R, Niculae A, Sidorovitch V, Cioaca MD, Bessolitsyna E, Thoma NH, Waldmann H, Schlichting I, Goody RS, Alexandrov K. Structure of Rab escort protein-1 in complex with Rab geranylgeranyltransferase. *Mol Cell*. 2003; 11:483–494. [PubMed: 12620235]
31. Wu YW, Tan KT, Waldmann H, Goody RS, Alexandrov K. Interaction analysis of prenylated Rab GTPase with Rab escort protein and GDP dissociation inhibitor explains the need for both regulators. *Proc Natl Acad Sci U S A*. 2007; 104:12294–12299. [PubMed: 17640890]
32. Alexandrov K, Simon I, Yurchenko V, Iakovenko A, Rostkova E, Scheidig AJ, Goody RS. Characterization of the ternary complex between Rab7, REP-1 and Rab geranylgeranyl transferase. *Eur J Biochem*. 1999; 265:160–170. [PubMed: 10491170]

33. Alexandrov K, Simon I, Iakovenko A, Holz B, Goody RS, Scheidig AJ. Moderate discrimination of REP-1 between Rab7 x GDP and Rab7 x GTP arises from a difference of an order of magnitude in dissociation rates. *FEBS Lett.* 1998; 425:460–464. [PubMed: 9563513]
34. Shapiro AD, Pfeffer SR. Quantitative analysis of the interactions between prenyl Rab9, GDP dissociation inhibitor-alpha, and guanine nucleotides. *J Biol Chem.* 1995; 270:11085–11090. [PubMed: 7744738]
35. Seabra MC, Mules EH, Hume AN. Rab GTPases, intracellular traffic and disease. *Trends Mol Med.* 2002; 8:23–30. [PubMed: 11796263]
36. Calero M, Chen CZ, Zhu W, Winand N, Havas KA, Gilbert PM, Burd CG, Collins RN. Dual prenylation is required for Rab protein localization and function. *Mol Biol Cell.* 2003; 14:1852–1867. [PubMed: 12802060]
37. Calero M, Winand NJ, Collins RN. Identification of the novel proteins Yip4p and Yip5p as Rab GTPase interacting factors. *FEBS Lett.* 2002; 515:89–98. [PubMed: 11943201]
38. Goody RS, Hofmann-Goody W. Exchange factors, effectors, GAPs and motor proteins: common thermodynamic and kinetic principles for different functions. *Eur Biophys J.* 2002; 31:268–274. [PubMed: 12122473]
39. Guo Z, Ahmadian MR, Goody RS. Guanine nucleotide exchange factors operate by a simple allosteric competitive mechanism. *Biochemistry.* 2005; 44:15423–15429. [PubMed: 16300389]
40. Klebe C, Prinz H, Wittinghofer A, Goody RS. The kinetic mechanism of Ran--nucleotide exchange catalyzed by RCC1. *Biochemistry.* 1995; 34:12543–12552. [PubMed: 7548002]
41. Chin HF, Cai Y, Menon S, Ferro-Novick S, Reinisch KM, De La Cruz EM. Kinetic Analysis of the Guanine Nucleotide Exchange Activity of TRAPP, a Multimeric Ypt1p Exchange Factor. *J Mol Biol.* 2009
42. Moya M, Roberts D, Novick P. DSS4-1 is a dominant suppressor of sec4–8 that encodes a nucleotide exchange protein that aids Sec4p function. *Nature.* 1993; 361:460–463. [PubMed: 8429886]
43. Burton J, Roberts D, Montaldi M, Novick P, De Camilli P. A mammalian guanine-nucleotide-releasing protein enhances function of yeast secretory protein Sec4. *Nature.* 1993; 361:464–467. [PubMed: 8429887]
44. Burton JL, Burns ME, Gatti E, Augustine GJ, De Camilli P. Specific interactions of Mss4 with members of the Rab GTPase subfamily. *Embo J.* 1994; 13:5547–5558. [PubMed: 7988552]
45. Esters H, Alexandrov K, Iakovenko A, Ivanova T, Thoma N, Rybin V, Zerial M, Scheidig AJ, Goody RS. Vps9, Rabex-5 and DSS4: proteins with weak but distinct nucleotide-exchange activities for Rab proteins. *J Mol Biol.* 2001; 310:141–156. [PubMed: 11419942]
46. Itzen A, Pylypenko O, Goody RS, Alexandrov K, Rak A. Nucleotide exchange via local protein unfolding--structure of Rab8 in complex with MSS4. *Embo J.* 2006; 25:1445–1455. [PubMed: 16541104]
47. Nuoffer C, Wu SK, Dascher C, Balch WE. Mss4 does not function as an exchange factor for Rab in endoplasmic reticulum to Golgi transport. *Mol Biol Cell.* 1997; 8:1305–1316. [PubMed: 9243509]
48. Dong G, Medkova M, Novick P, Reinisch KM. A catalytic coiled coil: structural insights into the activation of the Rab GTPase Sec4p by Sec2p. *Mol Cell.* 2007; 25:455–462. [PubMed: 17289591]
49. Sato Y, Fukai S, Ishitani R, Nureki O. Crystal structure of the Sec4p. Sec2p complex in the nucleotide exchanging intermediate state. *Proc Natl Acad Sci U S A.* 2007; 104:8305–8310. [PubMed: 17488829]
50. Walch-Solimena C, Collins RN, Novick PJ. Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles. *J Cell Biol.* 1997; 137:1495–1509. [PubMed: 9199166]
51. Hattula K, Furuhielm J, Arffman A, Peranen J. A Rab8-specific GDP/GTP exchange factor is involved in actin remodeling and polarized membrane transport. *Mol Biol Cell.* 2002; 13:3268–3280. [PubMed: 12221131]
52. Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peranen J, Merdes A, Slusarski DC, Scheller RH, Bazan JF, Sheffield VC, Jackson PK. A core complex of BBS proteins cooperates with the

- GTPase Rab8 to promote ciliary membrane biogenesis. *Cell*. 2007; 129:1201–1213. [PubMed: 17574030]
53. Carney DS, Davies BA, Horazdovsky BF. Vps9 domain-containing proteins: activators of Rab5 GTPases from yeast to neurons. *Trends Cell Biol*. 2006; 16:27–35. [PubMed: 16330212]
 54. Delprato A, Merithew E, Lambright DG. Structure, exchange determinants, and family-wide rab specificity of the tandem helical bundle and Vps9 domains of Rabex-5. *Cell*. 2004; 118:607–617. [PubMed: 15339665]
 55. Horiuchi H, Lippe R, McBride HM, Rubino M, Woodman P, Stenmark H, Rybin V, Wilm M, Ashman K, Mann M, Zerial M. A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell*. 1997; 90:1149–1159. [PubMed: 9323142]
 56. Delprato A, Lambright DG. Structural basis for Rab GTPase activation by VPS9 domain exchange factors. *Nat Struct Mol Biol*. 2007; 14:406–412. [PubMed: 17450153]
 57. Renault L, Guibert B, Cherfils J. Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. *Nature*. 2003; 426:525–530. [PubMed: 14654833]
 58. Goldberg J. Structural basis for activation of ARF GTPase: mechanisms of guanine nucleotide exchange and GTP-myristoyl switching. *Cell*. 1998; 95:237–248. [PubMed: 9790530]
 59. Wang W, Sacher M, Ferro-Novick S. TRAPP stimulates guanine nucleotide exchange on Ypt1p. *J Cell Biol*. 2000; 151:289–296. [PubMed: 11038176]
 60. Jones S, Newman C, Liu F, Segev N. The TRAPP complex is a nucleotide exchanger for Ypt1 and Ypt31/32. *Mol Biol Cell*. 2000; 11:4403–4411. [PubMed: 11102533]
 61. Kim YG, Raunser S, Munger C, Wagner J, Song YL, Cygler M, Walz T, Oh BH, Sacher M. The architecture of the multisubunit TRAPP I complex suggests a model for vesicle tethering. *Cell*. 2006; 127:817–830. [PubMed: 17110339]
 62. Kim YG, Sohn EJ, Seo J, Lee KJ, Lee HS, Hwang I, Whiteway M, Sacher M, Oh BH. Crystal structure of bet3 reveals a novel mechanism for Golgi localization of tethering factor TRAPP. *Nat Struct Mol Biol*. 2005; 12:38–45. [PubMed: 15608655]
 63. Cai Y, Chin HF, Lazarova D, Menon S, Fu C, Cai H, Sclafani A, Rodgers DW, De La Cruz EM, Ferro-Novick S, Reinisch KM. The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. *Cell*. 2008; 133:1202–1213. [PubMed: 18585354]
 64. Beraud-Dufour S, Robineau S, Chardin P, Paris S, Chabre M, Cherfils J, Antonny B. A glutamic finger in the guanine nucleotide exchange factor ARNO displaces Mg²⁺ and the beta-phosphate to destabilize GDP on ARF1. *Embo J*. 1998; 17:3651–3659. [PubMed: 9649435]
 65. Sacher M, Kim YG, Lavie A, Oh BH, Segev N. The TRAPP Complex: Insights into its Architecture and Function. *Traffic*. 2008
 66. Cai H, Reinisch K, Ferro-Novick S. Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. *Dev Cell*. 2007; 12:671–682. [PubMed: 17488620]
 67. Albert S, Will E, Gallwitz D. Identification of the catalytic domains and their functionally critical arginine residues of two yeast GTPase-activating proteins specific for Ypt/Rab transport GTPases. *Embo J*. 1999; 18:5216–5225. [PubMed: 10508155]
 68. Du LL, Collins RN, Novick PJ. Identification of a Sec4p GTPase-activating protein (GAP) as a novel member of a Rab GAP family. *J Biol Chem*. 1998; 273:3253–3256. [PubMed: 9452439]
 69. Strom M, Vollmer P, Tan TJ, Gallwitz D. A yeast GTPase-activating protein that interacts specifically with a member of the Ypt/Rab family. *Nature*. 1993; 361:736–739. [PubMed: 8441469]
 70. Ishibashi K, Kanno E, Itoh T, Fukuda M. Identification and characterization of a novel Tre-2/Bub2/Cdc16 (TBC) protein that possesses Rab3A-GAP activity. *Genes Cells*. 2009; 14:41–52. [PubMed: 19077034]
 71. Yoshimura S, Egerer J, Fuchs E, Haas AK, Barr FA. Functional dissection of Rab GTPases involved in primary cilium formation. *J Cell Biol*. 2007; 178:363–369. [PubMed: 17646400]
 72. Sklan EH, Serrano RL, Einav S, Pfeffer SR, Lambright DG, Glenn JS. TBC1D20 is a Rab1 GTPase-activating protein that mediates hepatitis C virus replication. *J Biol Chem*. 2007; 282:36354–36361. [PubMed: 17901050]

73. Mukhopadhyay A, Pan X, Lambright DG, Tissenbaum HA. An endocytic pathway as a target of tubby for regulation of fat storage. *EMBO Rep.* 2007; 8:931–938. [PubMed: 17762880]
74. Haas AK, Yoshimura S, Stephens DJ, Preisinger C, Fuchs E, Barr FA. Analysis of GTPase-activating proteins: Rab1 and Rab43 are key Rabs required to maintain a functional Golgi complex in human cells. *J Cell Sci.* 2007; 120:2997–3010. [PubMed: 17684057]
75. Fuchs E, Haas AK, Spooner RA, Yoshimura S, Lord JM, Barr FA. Specific Rab GTPase-activating proteins define the Shiga toxin and epidermal growth factor uptake pathways. *J Cell Biol.* 2007; 177:1133–1143. [PubMed: 17562788]
76. Dabbeek JT, Faltar SL, Dufresne CP, Cowell JK. The EVI5 TBC domain provides the GTPase-activating protein motif for RAB11. *Oncogene.* 2007; 26:2804–2808. [PubMed: 17099728]
77. Pan X, Eathiraj S, Munson M, Lambright DG. TBC-domain GAPs for Rab GTPases accelerate GTP hydrolysis by a dual-finger mechanism. *Nature.* 2006; 442:303–306. [PubMed: 16855591]
78. Itoh T, Satoh M, Kanno E, Fukuda M. Screening for target Rabs of TBC (Tre-2/Bub2/Cdc16) domain-containing proteins based on their Rab-binding activity. *Genes Cells.* 2006; 11:1023–1037. [PubMed: 16923123]
79. Itoh T, Fukuda M. Identification of EPI64 as a GTPase-activating protein specific for Rab27A. *J Biol Chem.* 2006; 281:31823–31831. [PubMed: 16923811]
80. Zhang XM, Walsh B, Mitchell CA, Rowe T. TBC domain family, member 15 is a novel mammalian Rab GTPase-activating protein with substrate preference for Rab7. *Biochem Biophys Res Commun.* 2005; 335:154–161. [PubMed: 16055087]
81. Miinea CP, Sano H, Kane S, Sano E, Fukuda M, Peranen J, Lane WS, Lienhard GE. AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. *Biochem J.* 2005; 391:87–93. [PubMed: 15971998]
82. Lanzetti L, Rybin V, Malabarba MG, Christoforidis S, Scita G, Zerial M, Di Fiore PP. The Eps8 protein coordinates EGF receptor signalling through Rac and trafficking through Rab5. *Nature.* 2000; 408:374–377. [PubMed: 11099046]
83. Cuif MH, Possmayer F, Zander H, Bordes N, Jollivet F, Couedel-Courteille A, Janoueix-Lerosey I, Langsley G, Bornens M, Goud B. Characterization of GAPCenA, a GTPase activating protein for Rab6, part of which associates with the centrosome. *Embo J.* 1999; 18:1772–1782. [PubMed: 10202141]
84. Rak A, Fedorov R, Alexandrov K, Albert S, Goody RS, Gallwitz D, Scheidig AJ. Crystal structure of the GAP domain of Gyp1p: first insights into interaction with Ypt/Rab proteins. *Embo J.* 2000; 19:5105–5113. [PubMed: 11013213]
85. Tempel W, Tong Y, Dimov S, Bochkarev A, Park H. First crystallographic models of human TBC domains in the context of a family-wide structural analysis. *Proteins.* 2008; 71:497–502. [PubMed: 18186464]
86. Valsdottir R, Hashimoto H, Ashman K, Koda T, Storrie B, Nilsson T. Identification of rabaptin-5, rabex-5, and GM130 as putative effectors of rab33b, a regulator of retrograde traffic between the Golgi apparatus and ER. *FEBS Lett.* 2001; 508:201–209. [PubMed: 11718716]
87. Bos JL, Rehmann H, Wittinghofer A. GEFs and GAPs: critical elements in the control of small G proteins. *Cell.* 2007; 129:865–877. [PubMed: 17540168]
88. De Antoni A, Schmitzova J, Trepte HH, Gallwitz D, Albert S. Significance of GTP hydrolysis in Ypt1p-regulated endoplasmic reticulum to Golgi transport revealed by the analysis of two novel Ypt1-GAPs. *J Biol Chem.* 2002; 277:41023–41031. [PubMed: 12189143]
89. Scrima A, Thomas C, Deaconescu D, Wittinghofer A. The Rap-RapGAP complex: GTP hydrolysis without catalytic glutamine and arginine residues. *Embo J.* 2008; 27:1145–1153. [PubMed: 18309292]
90. 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. [PubMed: 10025402]
91. Kukimoto-Niino M, Sakamoto A, Kanno E, Hanawa-Suetsugu K, Terada T, Shirouzu M, Fukuda M, Yokoyama S. Structural basis for the exclusive specificity of Slac2-a/melanophilin for the Rab27 GTPases. *Structure.* 2008; 16:1478–1490. [PubMed: 18940604]

92. Chavas LM, Ihara K, Kawasaki M, Torii S, Uejima T, Kato R, Izumi T, Wakatsuki S. Elucidation of Rab27 recruitment by its effectors: structure of Rab27a bound to Exophilin4/Slp2-a. *Structure*. 2008; 16:1468–1477. [PubMed: 18940603]
93. Burguete AS, Fenn TD, Brunger AT, Pfeffer SR. Rab and Arl GTPase family members cooperate in the localization of the golgin GCC185. *Cell*. 2008; 132:286–298. [PubMed: 18243103]
94. Jagoe WN, Lindsay AJ, Read RJ, McCoy AJ, McCaffrey MW, Khan AR. Crystal structure of rab11 in complex with rab11 family interacting protein 2. *Structure*. 2006; 14:1273–1283. [PubMed: 16905101]
95. Eathiraj S, Mishra A, Prekeris R, Lambright DG. Structural basis for Rab11-mediated recruitment of FIP3 to recycling endosomes. *J Mol Biol*. 2006; 364:121–135. [PubMed: 17007872]
96. Shiba T, Koga H, Shin HW, Kawasaki M, Kato R, Nakayama K, Wakatsuki S. Structural basis for Rab11-dependent membrane recruitment of a family of Rab11-interacting protein 3 (FIP3)/Arfophilin-1. *Proc Natl Acad Sci U S A*. 2006; 103:15416–15421. [PubMed: 17030804]
97. 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. [PubMed: 15933719]
98. 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. [PubMed: 15378032]
99. 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. [PubMed: 9524117]
100. Merithew E, Stone C, Eathiraj S, Lambright DG. Determinants of Rab5 interaction with the N terminus of early endosome antigen 1. *J Biol Chem*. 2003; 278:8494–8500. [PubMed: 12493736]
101. 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. [PubMed: 11788822]
102. Fielding AB, Schonteich E, Matheson J, Wilson G, Yu X, Hickson GR, Srivastava S, Baldwin SA, Prekeris R, Gould GW. Rab11-FIP3 and FIP4 interact with Arf6 and the exocyst to control membrane traffic in cytokinesis. *Embo J*. 2005; 24:3389–3399. [PubMed: 16148947]
103. Miserey-Lenkei S, Waharte F, Boulet A, Cuif MH, Tenza D, El Marjou A, Raposo G, Salamero J, Heliot L, Goud B, Monier S. Rab6-interacting protein 1 links Rab6 and Rab11 function. *Traffic*. 2007; 8:1385–1403. [PubMed: 17725553]
104. Recacha R, Boulet A, Jollivet F, Monier S, Houdusse A, Goud B, Khan AR. Structural Basis for Recruitment of Rab6-Interacting Protein 1 to Golgi via a RUN Domain. *Structure*. 2009; 17:21–30. [PubMed: 19141279]
105. Merithew E, Hatherly S, Dumas JJ, Lawe DC, Heller-Harrison R, Lambright DG. Structural plasticity of an invariant hydrophobic triad in the switch regions of Rab GTPases is a determinant of effector recognition. *J Biol Chem*. 2001; 276:13982–13988. [PubMed: 11278565]
106. Brumell JH, Scidmore MA. Manipulation of rab GTPase function by intracellular bacterial pathogens. *Microbiol Mol Biol Rev*. 2007; 71:636–652. [PubMed: 18063721]
107. Machner MP, Isberg RR. A bifunctional bacterial protein links GDI displacement to Rab1 activation. *Science*. 2007; 318:974–977. [PubMed: 17947549]
108. Ingmundson A, Delprato A, Lambright DG, Roy CR. Legionella pneumophila proteins that regulate Rab1 membrane cycling. *Nature*. 2007; 450:365–369. [PubMed: 17952054]
109. Murata T, Delprato A, Ingmundson A, Toomre DK, Lambright DG, Roy CR. The Legionella pneumophila effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nat Cell Biol*. 2006; 8:971–977. [PubMed: 16906144]
110. Machner MP, Isberg RR. Targeting of host Rab GTPase function by the intravacuolar pathogen Legionella pneumophila. *Dev Cell*. 2006; 11:47–56. [PubMed: 16824952]
111. Huber SK, Scheidig AJ. High resolution crystal structures of human Rab4a in its active and inactive conformations. *FEBS Lett*. 2005; 579:2821–2829. [PubMed: 15907487]
112. Chattopadhyay D, Langsley G, Carson M, Recacha R, DeLucas L, Smith C. Structure of the nucleotide-binding domain of Plasmodium falciparum rab6 in the GDP-bound form. *Acta Crystallogr D Biol Crystallogr*. 2000; 56:937–944. [PubMed: 10944329]

113. Constantinescu AT, Rak A, Alexandrov K, Esters H, Goody RS, Scheidig AJ. Rab-subfamily-specific regions of Ypt7p are structurally different from other RabGTPases. *Structure*. 2002; 10:569–579. [PubMed: 11937061]
114. Wittmann JG, Rudolph MG. Crystal structure of Rab9 complexed to GDP reveals a dimer with an active conformation of switch II. *FEBS Lett*. 2004; 568:23–29. [PubMed: 15196914]
115. Pasqualato S, Senic-Matuglia F, Renault L, Goud B, Salamero J, Cherfils J. The structural GDP/GTP cycle of Rab11 reveals a novel interface involved in the dynamics of recycling endosomes. *J Biol Chem*. 2004; 279:11480–11488. [PubMed: 14699104]
116. Esters H, Alexandrov K, Constantinescu AT, Goody RS, Scheidig AJ. High-resolution crystal structure of *S. cerevisiae* Ypt51(DeltaC15)-GppNHp, a small GTP-binding protein involved in regulation of endocytosis. *J Mol Biol*. 2000; 298:111–121. [PubMed: 10756108]

Figure 1. Structural similarity and diversity within the Rab family

A) Overall structure of a representative Rab GTPase (Rab3) with functional regions colored as indicated. B) Comparison of inactive GDP-bound Rab GTPase structures after superposition with Rab2. Note that the switch regions are either poorly ordered or adopt ordered conformations as a result of crystal packing. C) Comparison of active GppNHp-bound Rab GTPase structures after superposition with Rab3. Although both switch regions adopt stable active conformations, switch II exhibits large conformational differences between Rab GTPases. All figures were rendered using PyMOL and coordinates as indicated in Table 1.

Figure 2. Structures of RabGDI and REP complexes with prenylated Rab GTPases or RabGGTase illuminate the structural mechanisms for membrane targeting

A) Structure of RabGDI in complex with mono-geranyl geranylated Ypt1p. B) Structure of REP in complex with mono-geranyl geranylated Rab7. Note overall similarity to the RabGDI-Ypt1p complex with respect to the protein-protein interface and location of the prenyl binding pocket. C) Structure of REP in complex with RabGGTase. Docking of Domain II with the α subunit of RabGGTase orients the Rab binding platform in Domain I of REP such that the flexible C-terminus of bound Rab GTPases can readily extend to the active site in the β subunit. D) Comparison of mono- and di-geranyl geranyl binding to domain II of RabGDI in the complexes with prenylated Ypt1p.

Figure 3. Structures of GEFs in complex with nucleotide free Rab GTPases suggest largely distinct structural mechanisms for catalysis of nucleotide exchange

A) Mss4 promotes nucleotide release by a local unfolding mechanism in which switch I of Rab8 is contorted into a conformation that forces unfolding of the $\alpha 1$ helix. B) The Sec2p catalytic core is an asymmetric coiled coil that indirectly facilitates nucleotide release by stabilizing switch I of Sec4p in a conformation that conflicts with nucleotide binding. C) The Rabex-5 catalytic core accelerates nucleotide exchange on Rab5 and Rab21 by supplying an ‘aspartate finger’ to destabilize Mg^{2+}/GDP binding and subsequently stabilize the nucleotide free intermediate through interactions that mimic the γ phosphate of GTP. D) The multi-subunit catalytic core of yeast TRAPPI relies on the C-terminus of Bet3p to stimulate GDP release from Ypt1p and stabilize the nucleotide free conformation.

Figure 4. The structure of the Gyp1p TBC domain in a ternary complex with GDP-bound Rab33 and the transition state mimic AlF₃ suggests that TBC domains are dual finger GAPs

A) Overall structure of the Gyp1p TBC domain-Rab33-GDP-AlF₃ ternary complex. B) The N-terminal subdomain of the Gyp1p TBC domain stabilizes the AlF₃ transition state mimetic complex by supplying two catalytic residues in trans, an ‘arginine finger’ from the IxxDxxR motif and a ‘glutamine finger’ from the YxQ motif. The DxxGQ glutamine in Rab33 interacts with the Gyp1p backbone but does not directly participate in stabilization of the AlF₃ transition state mimetic.

Figure 5. Structures of effector RBDs in complex with active Rab GTPases illustrate similarity and diversity in Rab binding modalities

A) The structurally related RBDs of Rabphilin-3A, Slac2-a, and Slp2-a form an extended interface with Rab3 or Rab27 that includes the switch/interswitch regions and all three CDRs. B) Interleaved helical hairpins in the RILP RBD support 2:2 binding of a dyad symmetric coiled coil-like dimer to the switch/interswitch regions and N-terminal CDR of Rab7. C) The RUN domain of Rab6IP1 uses a coiled-coil like arrangement of adjacent helices to engage the switch/interswitch regions of Rab6 in a manner analogous to the dyad symmetric coiled coil RBD of GCC185, which supports 2:2 binding. D) The dyad symmetric coiled coil RBD of FIP3 flares apart at the C-terminus to facilitate 2:2 binding to the switch/interswitch regions of Rab11. FIP3 binding induces a large change in the active conformation of switch II. E) The central and C-terminal RBDs of Rabenosyn-5 combine a conserved helical hairpin core with variable N-terminal extensions to engage the switch/interswitch regions of Rab22 or Rab4, respectively. F) The C-terminal dyad symmetric coiled coil RBD of Rabaptin-5 supports 2:2 binding to the switch/interswitch regions of

Rab5 with an orientation similar to the asymmetric antiparallel helical hairpins in Rabenosyn-5.

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Table 1

Summary of structures presented in Figures 1–5

Protein or Complex	Ligand	Rab construct	PDB ID	References
Rab GTPases (Figure 1)				
Rab2A	GDP	G domain	1Z0A	(19)
Rab4A	GDP	G domain	2BMD	(111)
Rab5A	GDP	G domain	1TU4	(98)
Rab6	GDP	G domain	1D5C	(112)
Rab7	GDP	G domain	1VG1	(26)
Ypt7p	GDP	G domain	1KY3	(113)
Sec4p	GDP	G domain	1G16	(11)
Rab9	GDP	G domain	1S8F	(114)
Rab11A	GDP	G domain	1OIV	(115)
Rab14	GDP	G domain	1Z0F	(19)
Rab21	GDP	G domain	1Z0I	(19)
Rab23	GDP	G domain	1Z22	(19)
Rab25	GDP	G domain	2OIL	
Rab43	GDP	G domain	2HUP	
Ypt1p	GppNHp	G domain	1YZN	(19)
Rab3A	GppNHp	G domain	3RAB	(12)
Rab4A	GppNHp	G domain	1YU9	(19)
Rab5C	GppNHp	G domain	1HUQ	(105)
Ypt51p	GppNHp	G domain	1EK0	(116)
Rab6A	GppNHp	G domain	1YZQ	(19)
Rab7	GppNHp	FL	1VG8	(26)
Ypt7p	GppNHp	G domain	1KY2	(113)
Sec4p	GppNHp	G domain	1G17	(11)
Rab9	GppNHp	G domain	1YZL	(19)
Rab11A	GppNHp	G domain	1YZK	(19)
Rab21	GppNHp	G domain	1YZU	(19)
Rab22A	GppNHp	G domain	1YVD	(19)
Rab33B	GppNHp	G domain	1Z06	(19)
Accessory factors/GDP-bound Rab GTPases (Figure 2)				
REP1/RabGGTase			1LTX	(30)
REP1/Rab7_G	GDP	FL	1VG0	(26)
GDI/Ypt1_G	GDP	FL	1UKV	(27)
GDI/Ypt1_GG	GDP	FL G204C	2BCG	(25)
GEF catalytic cores/Nucleotide free Rab GTPases (Figure 3)				
Mss4/Rab8		G domain	2FU5	(46)
Sec2p/Sec4p		G domain	2OCY	(48)

Protein or Complex	Ligand	Rab construct	PDB ID	References
Rabex-5/Rab21		G domain	2OT3	(56)
Ypt1p/TRAPPI		FL	3CUE	(63)
TBC domain GAP/Rab GTPase/Transition state mimic (Figure 4)				
Gyp1p/Rab33B/AlF ₃	GDP	G domain	2G77	(77)
Effector Rab Binding domains/Active Rab GTPases (Figure 5)				
Rabphilin-3A/Rab3A	GTP	G domain Q81L	1ZBD	(90)
Slac2-a/Rab27B	GTP	G domain Q78L	2ZET	(91)
Slp2-a/Rab27a	GppNHp	G domain	3BC1	(92)
RILP/Rab7	GTP	FL Q67L	1YHN	(97)
Rab6IP1/Rab6A	GTP	G domain Q72L	3CWZ	(104)
GCC185/Rab6A	GTP	FL Q72L SC207-208LL	3BBP	(93)
FIP3/Rab11A	GTP	G domain Q70L	2HV8	(95)
Rabenosyn-5/Rab22A	GTP	G domain Q64L V22M	1Z0J	(19)
Rabenosyn-5/Rab4	GTP	G domain Q67L	1Z0K	(19)
Rabaptin-5/Rab5A	GppNHp	G domain	1TU3	(98)

Abbreviations: FL, Full length; G domain, GTPase Domain; _G, geranyl geranyl; _GG, di-geranyl geranyl.