Chapter 5
Rab GEFs and GAPs: The Enigma Variations

Francis A. Barr

Abstract Rab GTPases are key regulators of membrane traffic activated on the surface of organelle and vesicle membranes during vesicle trafficking events, cell polarisation and autophagy. Rabs undergo a cycle of activation involving GTP binding and inactivation involving GTP hydrolysis in response to cellular regulators. Each Rab has a cognate GDP–GTP exchange factor (GEF) promoting release of GDP and subsequent binding of GTP, and a GTPase activating protein (GAP) stimulating the slow intrinsic GTP hydrolysis. Together these GEF and GAP regulators determine when and where a specific Rab is activated, and how long its activity will persist. Rab GEFs fall into a number of discrete families, the largest of which are the Vps9 domain, DENN and DENN domain-related proteins. Other Rab GEF families, including TRAPP, Ric1-Rgp1, Mon1-Ccz1 and Hps1-Hps4, are comprised of two or more polypeptide chains. By contrast, almost all known Rab GAPs possess a TBC1 domain. Here I will discuss the mechanisms by which these GEFs and GAPs regulate Rab GTPases, highlighting common themes and points of difference, and briefly outlining the cellular processes they regulate.

Keywords Vesicle traffic • Rab GTPase • GEF • GAP • TBC1 domain • Longin domain

5.1 Rab Function and the Need for Regulation

Rab GTPases form a large and highly conserved subfamily of the Ras-related GTPase in eukaryotic cells. Rabs are key regulators of membrane traffic activated at the surface of organelle and vesicle membranes during vesicle trafficking events, cell polarisation and autophagy (Zerial and McBride 2001; Pfeffer and Aivazian 2004).

F.A. Barr (✉)
Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK
e-mail: francis.barr@bioch.ox.ac.uk

A. Wittinghofer (ed.), *Ras Superfamily Small G Proteins: Biology and Mechanisms 2*,
DOI 10.1007/978-3-319-07761-1_5,

Careful analysis of Rab evolution indicates that the last common eukaryotic ancestor possessed 12 Rabs that we know to be involved in ER–Golgi trafficking, endocytosis, endocytic recycling, lysosome-related organelle formation, lipid storage and cilium function (Elias et al. 2012; Klopper et al. 2012). The size of the Rab family differs between species, but generally increases in metazoan lineages and is reduced in some single cell eukaryotes such as yeasts. Human cells express a subset of 66 Rabs, while budding yeast express 11 Rab/Ypts. The increased number of Rabs in higher eukaryotes is thought to reflect the greater number of cellular compartments, and increased complexity of cell polarisation in metazoans compared to single cell eukaryotes. Like other Ras superfamily proteins, Rabs switch between two states: an inactive GDP-bound state and an active GTP-bound state (Wittinghofer and Vetter 2011). This cycle of activation and inactivation is under the control of cellular regulators promoting GDP–GTP exchange during Rab activation and GTP hydrolysis during Rab inactivation (Barr and Lambright 2010; Barr 2013). Accordingly, each Rab has a cognate GDP–GTP exchange factor (GEF) promoting release of GDP and subsequent binding of GTP, and a GTPase activating protein (GAP) stimulating the slow intrinsic GTP hydrolysis. This cycle of activation and inactivation is linked to a cycle of membrane binding and release. Rabs are prenylated at the C-terminus and this modification is required for targeting to membrane surfaces (Khosravi-Far et al. 1991; Peter et al. 1992). When in the GDP-bound inactive form Rabs rapidly partition between a cytoplasmic pool bound to a protein termed GDI (guanine nucleotide dissociation inhibitor) and a membrane-associated pool that is accessible to GEFs (Soldati et al. 1993; Wu et al. 2010). GDI interacts with the GDP-bound form of the Rab nucleotide-binding domain, and also shields the hydrophobic prenylated tail of the Rab from the aqueous environment of the cytoplasm (Rak et al. 2003). Rabs accumulate at the membrane surface where their cognate GEF is located following GDP–GTP exchange, because GTP-bound Rabs have greatly reduced affinity for GDI and therefore do not partition into the cytoplasm (Soldati et al. 1993; Rak et al. 2003; Blumer et al. 2013). In this chapter I will focus on the mechanisms by which GEF and GAP regulatory factors act on their target Rabs.

5.2 Rab GEF Families and Mechanism

Rab GEFs initiate a kinetic proofreading system for vesicle and target organelle membrane surfaces by promoting accumulation of active Rabs only at the required sites (Barr 2013). Rab GEFs and their target Rabs are therefore key determinants of membrane trafficking. As for many other components of the membrane trafficking machinery the first candidate Rab GEFs were initially identified by means of genetic screens in budding yeast. Later biochemical characterisation then matched the GEFs to their targets Rab GTPases, and cell biological studies have confirmed these functional relationships (Mizuno-Yamasaki et al. 2012; Hutagalung and Novick 2011). As a result the identity of GEFs for the major Rabs controlling secretory and endocytic trafficking is known (Barr and Lambright 2010).

High-resolution X-ray crystallography has resulted in the elucidation of structures for five different Rab-GEF pairs. The cellular regulatory pairs Rab1-TRAPP, Sec4-Sec2 (equivalent to Rab8-Rabin8), Rab21-Rabex, Rab35-DENND1B and the Rab1-DrrA pair created upon infection by the intracellular pathogen *Legionella* (Delprato and Lambright 2007; Delprato et al. 2004; Cai et al. 2008; Dong et al. 2007; Sato et al. 2007b; Schoebel et al. 2009; Suh et al. 2010; Wu et al. 2011). These structures define different classes of Rab GEF in complex with a magnesium and nucleotide free target Rab, and therefore represent likely intermediates in the nucleotide exchange reaction. Details of how specificity in Rab interactions may be achieved have been discussed elsewhere (Lee et al. 2009), and here I will focus on the mechanism by which these different groups of GEF promote GDP–GTP exchange and contrast this with the canonical mechanism used by Ras.

Before explaining the mechanism of nucleotide exchange it is first important to explain some details of the mode of GDP/GTP binding in Rab GTPases. Here, Rab1 in complex with the non-hydrolysable GTP analogue GMP-PNP (Fig. 5.1) is taken as representative of Rabs in general, and the role of three regions termed switch I, switch II and the phosphate interaction site or P-loop will be explained. In Rab1 a magnesium ion makes direct contact with serine 22 in the GKS motif of the P-loop and threonine 40 in the conserved TIGVD RabF1 motif at the end of the switch I region. The beta- and gamma-phosphates of the bound GTP contact the metal ion. All these interactions fall in one plane around the magnesium ion. In addition the conserved lysine in the P-loop GKS motif interacts with the gamma-phosphate of GTP. Finally, there are two water molecules positioned above and below the metal ion. One of these water molecules interacts with the alpha-phosphate of the bound nucleotide. Aspartate 63 in the switch II region may influence the environment of and possibly contact these water molecules as well as the P-loop serine 22. The same water molecule is also predicted to interact with the main chain carbonyl oxygen of threonine 65 in the switch II region. Many studies of Ras superfamily GTPases use a serine to asparagine mutation in the P-loop, S22N in Rab1, since this prevents magnesium binding, and hence stable GDP or GTP binding. As well as these contacts to the beta- and gamma-phosphates of the nucleotide, a conserved aromatic residue in the switch I region, tyrosine 33 in Rab1, makes a potential ring stacking interaction with the guanine ring. Finally, aspartate 124 makes end on interactions with the guanine ring and is important for nucleotide specificity, while lysine 122 contacts the ribose sugar forming the guanosine moiety of the bound nucleotide. Following hydrolysis of GTP to GDP and release of inorganic phosphate, this interaction network is altered (Stroupe and Brunger 2000; Huber and Scheidig 2005; Dumas et al. 1999). First, the interactions of the threonine in the TIGID Rab F1 motif with the gamma-phosphate are lost, resulting in large conformational change in the switch I region. The P-loop lysine now interacts with the beta-phosphate of the bound nucleotide. As in other GTP-binding proteins in the GDP-bound form, water molecules replace the interactions of this conserved threonine and the gamma-phosphate with the magnesium ion. Depending on the Rab, one of these water molecules may be coordinated by an interaction with the

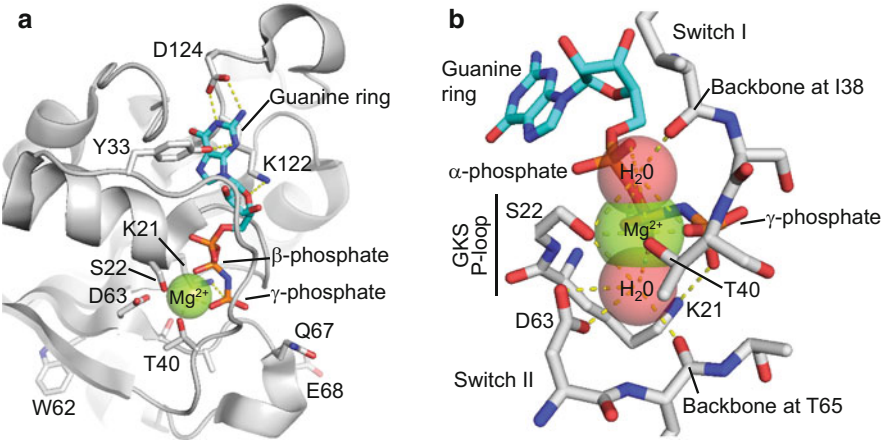

Fig. 5.1 The Rab1 nucleotide-binding site. (**a**) The structure of Rab1 bound to Mg^{2+} GMP-PNP (PDB: 1YZN) is shown in the figure. The polypeptide backbone is drawn in *grey* ribbon form, and only amino acid side chains involved in interaction with the nucleotide or magnesium ion are displayed. The switch II region W62 to E68 runs from left to right at the bottom of the structure. Switch I Y33 to T40 runs from top to bottom. The guanine ring, beta and gamma-phosphates of GMP-PNP are highlighted. (**b**) An enlarged view of the magnesium and phosphate-binding region of Rab1 is shown. In this view the two water molecules sitting above and below the magnesium ion are shown

conserved switch II glutamate. This has been observed for Rab4a, but not Sec4 GDP structures (Huber and Scheidig 2005; Stroupe and Brunger 2000).

Rab GEFs interact with Mg^{2+} and GDP-bound Rabs promoting conformational rearrangements in the switch I and II regions and nucleotide exchange. However, the order of events and intermediates in the exchange reaction cannot be precisely defined based on current data, and further studies of reaction intermediates for multiple Rab-GEF pairs are needed. GDP release occurs because switch I and II rearrangements displace the conserved aromatic residue making contacts with the guanine ring and disrupt the Mg^{2+} and phosphate-binding site. Previously it has been suggested that rearrangement of switch II and disruption of the Mg^{2+} binding site occurs only after or in concert with GDP release, whereas switch I displacement occurs during formation of the initial GEF-Rab-GDP intermediate. More recent evidence is consistent with the view that disruption of the Mg^{2+} binding site and rearrangement of switch II may be a primary event in nucleotide release (Langemeyer et al. 2014; Uejima et al. 2010). For Ras, Ran, Rho and Arf GTPases a highly conserved acidic residue typically glutamate intrinsic to the switch II region contacts the P-loop lysine (Gasper et al. 2008; Wittinghofer and Vetter 2011). This is thought to stabilise the GEF-bound nucleotide-free form of the GTPase (Boriack-Sjodin et al. 1998). However, this glutamate does not play the same role in Rab GEFs suggesting there are crucial mechanistic differences in the activation mechanism (Langemeyer et al. 2014; Gasper et al. 2008). The details are described in more detail for each of the Rab-GEF pairs in the subsequent sections.

5.2.1 *Vps9 Family GEFs for the Rab5/21 Subfamily*

Rab5 subfamily GTPases, including Rab5A-C, Rab17, Rab21 and Rab22A/B, regulate trafficking in the early endocytic pathway. Vps9/Rabex the founder member of the Rab5 GEF family was identified through biochemical characterization of mammalian Rab5 and budding yeast screens for defects in vacuolar protein sorting (Vps) (Burd et al. 1996; Hama et al. 1999; Horiuchi et al. 1997). A family of GEF proteins with the Vps9 domain exists in higher eukaryotes, and humans (Carney et al. 2006). The best characterised is the mammalian Vps9 orthologue Rabex which is thought to be a major activator of Rab5 during trafficking into early endosomes from the plasma membrane (Horiuchi et al. 1997). Screens in *C. elegans* have shown that a second Vps9 domain GEF receptor-mediated endocytosis defective 6 (RME-6) is also involved in Rab5 regulation in the clathrin-mediated endocytic pathway, and this has been confirmed in mammalian cell lines (Sato et al. 2005; Semerdjieva et al. 2008). Interestingly, Vps9 domain GEFs can act on multiple although not necessarily all Rab5 subfamily members. Rabex activates Rab5 and Rab21 with equal efficiency, k_{cat}/K_m 2.3 and $3.2 \times 10^4\ M^{-1}\ s^{-1}$, but shows two orders of magnitude lesser activity towards Rab22 (Delprato et al. 2004). While there is no crystal structure for Rab5 in complex with a Vps9 domain GEF, a high-resolution X-ray crystal structure of a complex of the closely related GTPase Rab21 together with the Vps9 domain of Rabex has been solved (Delprato and Lambright 2007). This structure shows that a conserved aspartate finger residue in the Vps9 domain is inserted into the Mg^{2+} and phosphate-binding site where it contacts the P-loop lysine. The Vps9 domain engages the Rab switch regions stabilising an open conformation for switch I. More details of the exchange mechanism have come from the *Arabidopsis thaliana* Rab5 family GTPase ARA7 structure in both nucleotide-free and nucleotide-bound forms in complex with its Vps9 domain exchange factor (Uejima et al. 2010). These structures indicate that GEF interaction and insertion of the aspartate finger promote movement of the P-loop lysine away from the beta-phosphate of GDP and towards the conserved aspartate residue at the base of switch II. This would lead to release of the magnesium ion. It is proposed that these changes result in deprotonation of the beta-phosphate of GDP and destabilise GDP binding due to repulsion between this oxygen anions and the aspartate finger (Uejima et al. 2010). Once magnesium and GDP have left the binding pocket the aspartate finger of the Vps9 GEF also contacts the P-loop lysine and stabilises the nucleotide-free form of the Rab-GEF complex. Other evidence supporting this picture comes from analysis of the human Rab5-Rabex GEF system. Mutation of the conserved switch II aspartate leads to a form of Rab5 refractory to Rabex-stimulated GDP release (Langemeyer et al. 2014). Expulsion of the magnesium and GDP as a consequence of GEF binding inevitably results in a loss of defined switch I structure and movement to a more open position. Vps9 domain GEFs may therefore promote nucleotide exchange primarily though interactions with the P-loop lysine and switch II rather than promoting rearrangements in switch I.

5.2.2 Sec2/Rabin Is the GEF for Sec4/Rab8

Groundbreaking genetic screens in budding yeast generated the first protein secretion (Sec) mutants (Novick et al. 1980; Salminen and Novick 1987). Sec4, Rab8 in humans, was one of the first Rab family GTPases linked to vesicle transport as a result of such screens. Sec4 was shown to function in the delivery of Golgi-derived transport vesicles to the bud tip, the terminal step of the secretory pathway in budding yeast (Salminen and Novick 1987; Goud et al. 1988). Another of the Sec mutants, Sec2, was then shown to encode the exchange factor for Sec4 (Walch-Solimena et al. 1997). Sec4 cannot be activated and recruited to secretory vesicles in the absence of Sec2 function. Structural studies show that Sec2 forms an asymmetric coiled coil that interacts with and distorts the Sec4 switch regions (Dong et al. 2007; Sato et al. 2007a). A hydrophobic segment or platform formed by Sec2 leucine 104/108 and phenylalanine 109 interacts with hydrophobic residues in the Sec4 switch I region, and two aromatic residues phenylalanine 57 and tryptophan 74 (Dong et al. 2007; Sato et al. 2007a). Phenylalanine 57 lies immediately after the TIGID RabF1 motif, while tryptophan 74 falls at the start of the WDTAGQE switch II sequence. GDP release may be primarily due to the altered Sec4 switch I structure (Dong et al. 2007; Sato et al. 2007b). It is notable that in this structure Sec4 adopts a radically different P-loop conformation where lysine 33 interacts with a serine at position 161 (Dong et al. 2007). However in one of the Sec2-Sec4 structures the electron density for the P-loop is poorly defined, and there is insufficient density to be entirely certain of the P-loop lysine side chain position (Dong et al. 2007). In a second structure a phosphate occupies the P-loop site, which may not be present during the normal exchange process (Sato et al. 2007a). Because of these caveats it is difficult to draw any firm conclusions about the precise mechanism employed by Sec2/Rabin family GEFs. In summary, while both these known structures support the view that GDP release is promoted by switch I and II rearrangement more work is needed to define the intermediates leading to GDP release.

5.2.3 Multisubunit TRAPP Complexes form GEFs for Rab1

TRAPP I is a multisubunit GEF for Rab1/Ypt1 GTPases that function primarily in the COP II pathway of ER to Golgi trafficking (Plutner et al. 1991; Allan et al. 2000; Segev et al. 1988; Wang et al. 2000; Sacher et al. 2001; Kim et al. 2006; Cai et al. 2008). Rab1 has also been implicated in autophagy together with TRAPP III, a form of TRAPP I containing an additional targeting subunit Trs85 (Kakuta et al. 2012; Lynch-Day et al. 2010; Montpetit and Conibear 2009; Tan et al. 2013; Taussig et al. 2013). The catalytic core of the TRAPP I complex is comprised of five subunits: Trs31, Bet5, Trs23 and two copies of Bet3 (Cai et al. 2008). Trs23, Bet5 and one copy of Bet3 make contact with the Rab1 switch

regions and amino-terminus (Kim et al. 2006; Cai et al. 2008). Bet3 and Bet5 are both longin domain proteins and form a dimeric platform for Rab binding (Kinch and Grishin 2006; Levine et al. 2013) (Fig. 5.2). Similarly to Sec2, TRAPP makes contacts with residues in its target Rab in and adjacent to the TIGID RabF1 motif, and the conserved tryptophan at the start of the WDTAGQE switch II sequence (Cai et al. 2008). In addition, the extreme C-terminus of Bet3 has a highly conserved di-acidic motif, DE or EE that inserts into the phosphate-binding site. The last residue of Bet3 E192 interacts with the Rab1 P-loop lysine 21 and therefore perturbs the interactions needed for magnesium ion and phosphate binding. In the GEF-bound conformation the switch II region of Rab1 is restructured and the conserved glutamine residue at position 67 rotates and also makes contact with the P-loop lysine 21. Mutational analysis confirms that both the Bet3 acidic finger residue E192 and Rab1 Q67 in switch II are required for TRAPP-stimulated GDP release from Rab1 (Cai et al. 2008; Langemeyer et al. 2014). The nucleotide-free Rab1 switch I region is only partially structured in the TRAPP complex, and the density between residues 31 and 37 is lost (Cai et al. 2008). It is important to note that switch I is not physically displaced from its normal position by TRAPP. Rather Bet3 perturbs the interactions needed for magnesium ion and phosphate binding. This suggests that loss of stabilisation of switch I position occurs after magnesium ion and GDP release. Additional support for this view comes from the behaviour of the Rab1 Q67A mutant. This shows normal GDP and GTP-binding properties, but cannot release GDP in response to TRAPP (Langemeyer et al. 2014). If switch I displacement occurred prior to switch II rearrangement, then GDP release should have already occurred before the switch II glutamine 67 made contact with the P-loop lysine 21.

5.2.4 DrrA: A Pathogen-Encoded Rab1 GEF

DrrA/SidM is a *Legionella* protein with Rab1 GDP–GTP exchange activity required for Rab1 recruitment to the pathogen-containing intracellular vacuole (Machner and Isberg 2006; Murata et al. 2006; Ingmundson et al. 2007; Machner and Isberg 2007). Like the cellular GEFs discussed here, DrrA interacts with the switch regions and P-loop of its target Rab, and therefore promotes nucleotide exchange (Schoebel et al. 2009; Suh et al. 2009). Interestingly, there are obvious differences in the conformation of Rab1 in Rab1-DrrA complexes when compared to Rab1-TRAPP complexes. In the Rab1-DrrA complex the Rab1 P-loop lysine 21 contacts the Rab switch II aspartate 63 and glutamine 67. This is different to the Rab1-TRAPP complex where no contacts between the Rab1 switch II region and the P-loop lysine 21 are observed. It also diverges from the geometry seen in the Ras-SOS GEF complex where the Ras P-loop lysine interacts with a conserved glutamate intrinsic to the Ras active site switch II region (Wittinghofer and Vetter 2011). Mutation of this glutamate in Ras therefore reduces GEF-stimulated GDP release (Gasper et al. 2008) In Rab1, an equivalent switch II glutamine mutation

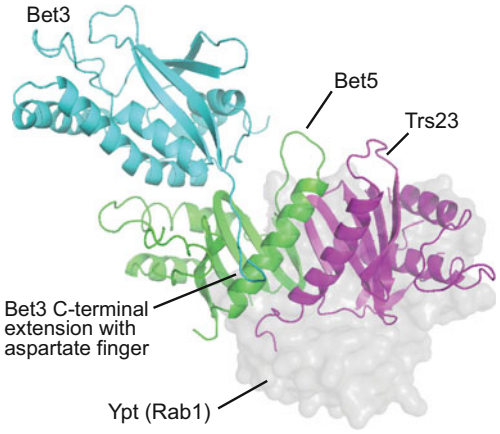

Fig. 5.2 The TRAPP longin core domains and Bet3 aspartate finger subunit. The polypeptide backbones of the Bet5 (*green*) and Trs23 (*magenta*) longin domain core subunits of TRAPP are shown in ribbon form. The longin domain is formed by two alpha-helices that form a hairpin overlaid by 5 beta-sheets. The final long alpha-helix lies over this platform and forms the interaction with the adjacent longin subunit which is related by a 180° rotation. The target Rab, Ypt1, sits on top of this platform slightly offset towards Trs23. The Bet3 subunit (*cyan*) contributes an extended C-terminal region carrying the aspartate finger residue needed for GEF activity

does not result in a reduction in either TRAPP- or DrrA-mediated GDP-release (Langemeyer et al. 2014). The defining feature of the Rab1-DrrA complex therefore appears to be a ternary interaction involving the Rab P-loop lysine 21 and switch II aspartate 63 and glutamine 67. Mutational analysis indicates that glutamine 67 although not required for nucleotide binding is important for DrrA-stimulated GDP release. This mutation has no effect on TRAPP-stimulated GDP release consistent with the structural data suggesting that the P-loop lysine interacts with the aspartate finger residue in the Bet3 C-terminus (Cai et al. 2008). DrrA and TRAPP therefore promote different conformations in Rab1, both of which lead to GDP release. This indicates there is considerable plasticity in the Rab nucleotide-binding domain, and that Rab GEFs can promote exchange by mechanistically separable pathways (Langemeyer et al. 2014).

5.2.5 DENN and DENN-Related Proteins Act on Diverse Rabs

DENN (differentially expressed normal versus neoplastic) domain proteins form the largest group of Rab GEFs (Levivier et al. 2001; Marat et al. 2011; Sato et al. 2008; Allaire et al. 2010; Yoshimura et al. 2010). In humans this family comprises seven subgroups DENND1–5, myotubularin-related proteins 5 and 13 (MTMR5/13) and MAP-kinase activating death domain protein (MADD), and

a divergent group of DENN-related proteins with four subgroups FAM116A/B, KIAA1147, AVL9 and FAM45A (Sato et al. 2008; Allaire et al. 2010; Yoshimura et al. 2010; Linford et al. 2012). DENN domain proteins were initially implicated as Rab GEFs when the protein MADD was purified as a Rab3 GEF from brain and subsequently the *C. elegans* orthologue AEX-3 found to act as a regulator of Rab3 in vivo (Wada et al. 1997; Brown and Howe 1998; Iwasaki et al. 1997; Figueiredo et al. 2008). Wider appreciation that DENN domain proteins were Rab GEFs came once it was shown that the *C. elegans* receptor-mediated endocytosis defective mutant rme-4 encoded a DENN domain protein homologous to mammalian DENND1A and regulated the Rab35 family GTPase RME-5 (Sato et al. 2008). Both RME-4 and DENND1A have specific Rab35 GEF activity (Sato et al. 2008; Allaire et al. 2010; Yoshimura et al. 2010). A family wide study of the human DENN domain proteins then defined the substrate specificity of this entire family of Rab GEFs. This defines the following Rab-GEF pairs: Rab35-DENND1A/B, Rab13-DENND1C, Rab2-DENND2A-D, Rab12-DENND3, Rab10-DENND4A/B, Rab39-DENND5A/B, Rab28-MTMR5/13 and Rab3/27A/B-MADD, respectively (Yoshimura et al. 2010). The best evidence confirming these biochemical assignments has been obtained for Rab3-MADD, Rab35-DENND1 and Rab10-DENND4. As already mentioned Rab35 and DENND1 are required for endocytic uptake in *C. elegans* and other work in human cells suggests they act at an early stage in the endocytic trafficking pathway and are needed for transport from the cell surface to the Golgi (Sato et al. 2008; Allaire et al. 2006, 2010; Yoshimura et al. 2010). Studies in *Drosophila melanogaster* provide compelling evidence that Rab10 and the DENND4 orthologue CRAG are required for polarised traffic of collagen and other cargo to the basolateral surface in epithelial cells (Denef et al. 2008; Lerner et al. 2013). These defects are reminiscent of those seen when Rab10 is inactivated in human cells (Denef et al. 2008; Schuck et al. 2007). Less is known about the function of the DENN-related GEFs and a defined Rab target and function has been found for only one subfamily member. FAM116A/B have been shown to be specific GEFs for Rab14, and to act in endocytic recycling by activation of Rab14. This pathway is important for the recycling of cell surface ADAM proteases and the control of cadherin family cell–cell adherens junctions in migrating cells (Linford et al. 2012).

A high-resolution X-ray crystal structure has been obtained for only one DENN domain protein, human DENND1B with Rab35 (Wu et al. 2011). This structure was a major advance, since it provided crucial new information about general themes in Rab GEF architecture and mechanism. Despite sharing no sequence similarity this structure revealed that the DENND1 has a longin fold similar to the Bet3 and Bet5 subunits of TRAPP, and a unique C-terminal domain (Levine et al. 2013; Cai et al. 2008; Wu et al. 2011). Both these domains make contacts with the Rab35 TIGID RabF1 motif, and the conserved tryptophan and polypeptide backbone of the WDTAGQE switch II sequence. The specificity of DENND1 for the GDP form of Rab35 may be explained by the availability of the TIGID RabF1 motif, since the threonine residue in this motif makes interactions with the magnesium ion in the GTP form of Rabs. Unlike TRAPP and Vps9 there are no insertions from the GEF

into the Rab nucleotide-binding pocket. Instead GEF binding promotes a conformation related to that seen in Rab1-DrrA complexes where the P-loop lysine 21 interacts with the switch II aspartate 63 and glutamine 67 (Schoebel et al. 2009; Suh et al. 2009). DENND1 fails to promote efficient nucleotide release from Rab35 glutamine 67 to alanine mutants (Langemeyer et al. 2014).

These observations suggest that switch II rearrangement is a primary cause of GDP release. Further support for this view comes from the analysis of mutations in the conserved aromatic residue phenylalanine 33 in switch I. Mutation of this residue to alanine gives rise to a form of Rab35 that shows rapid nucleotide release properties even in the absence of its DENND1 GEF (Langemeyer et al. 2014). If switch I rearrangement occurred as a primary consequence of GEF binding, then GDP release would still occur with Rab35 switch II glutamine mutant. Since GDP release is greatly reduced in the Rab35 glutamine 67 mutant, switch II rearrangement is likely to occur before that of switch I. The most parsimonious conclusion is that GEF binding promotes switch II rearrangement and release of magnesium and GDP, which then results in a loss of defined switch I structure and movement to a more open position.

5.2.6 HerMon GEFs for Rab7 Family GTPases

The different members of the Rab7/32/38 subfamily of GTPases are required for traffic to lysosomes and different classes of lysosome-related organelle such as the melanosomes and platelet granules. However, for a long time it was unclear how these Rabs are activated. Genetic screens for endocytic traffic defects revealed that yeast Mon1 and its *C. elegans* orthologue SAND-1 are important for Ypt7 and RAB-7 function (Poteryaev et al. 2010; Wang et al. 2003). Subsequently it was shown that the budding yeast Mon1-Ccz1 complex has Rab7 GEF activity consistent with its function in traffic to the vacuole and Ypt7-dependent vacuole tethering (Hoffman-Sommer et al. 2005; Wang et al. 2003; Nordmann et al. 2010). This was confirmed for the human Mon1-Ccz1 complex, which has specific activity towards Rab7 and does not act on the other closely related Rabs 32 and 38 (Gerondopoulos et al. 2012). Both Mon1 and Ccz1 subunits are required for this GEF activity, and neither subunit alone can activate Rab7. The Rab7-related Rabs Rab32 and Rab38 function in trafficking to lysosome-related organelles including the melanosome (Wasmeier et al. 2006). The Rab32/38 GEF is biogenesis of lysosome-related organelles complex-3 (BLOC-3), a complex of two proteins Hps1 and Hps4 distantly related to the Rab7 GEF Mon1-Ccz1 (Gerondopoulos et al. 2012). Both Hps1 and Hps4 are mutated in the human pigmentation and blood clotting disorder Hermansky Pudlak Syndrome, while Rab38 is altered in the ruby rat pigmentation mutant (Chiang et al. 2003; Martina et al. 2003; Nazarian et al. 2003; Oiso et al. 2004). Like Mon1-Ccz1, BLOC-3 has specific Rab GEF activity requiring the presence of both subunits (Gerondopoulos et al. 2012). Sequence analysis of the related Hps1 and Mon1 subunits of the HerMon GEFs suggest that these proteins

may have an N-terminal longin fold (Gerondopoulos et al. 2012). Despite this potential longin region there is no sequence similarity with TRAPP or DENND1, the GEFs known to contain longin domains from X-ray structure determination. Initial structural analysis has been performed on the BLOC-3 complex, which forms a 1:1 heterodimer, but it has not proven possible to obtain a structure possibly due to the presence of a long unstructured loop in the central region of Hps4 (Kloer et al. 2010). Mon1-Ccz1 and Hps1-Hps4 therefore form the founding members of the *Her*mansky Pudlak Syndrome and *Mon*1-Ccz1 (HerMon) GEF family acting on Rab7 and related GTPases.

5.2.7 Ric1-Rgp1 Is a Binary GEF Complex for Rab6

Rab6 family GTPases function in retrograde trafficking at or to the Golgi apparatus (White et al. 1999; Girod et al. 1999; Martinez et al. 1994). The best understood family member in terms of its regulation is budding yeast Ypt6, which functions in recycling of the exocytic SNARE Snc1 from endosomes back to the late Golgi where it is packaged into secretory vesicles targeted to the bud tip (Siniossoglou et al. 2000). Genetic screens in budding yeast identified a complex formed from Ric1 and Rgp1 that has Ypt6 GEF activity in vitro (Siniossoglou et al. 2000). Both Ypt6 and Ric1 are needed more generally for recycling of membrane proteins to the late Golgi in yeast (Bensen et al. 2001). In vivo both Ric1 and Rgp1 are required for correct localisation of Ypt6, consistent with the idea they act as its GEF. In human cells, the Ric1-Rgp1 complex acts as the GEF for Rab6 at the Golgi, and interestingly it is targeted via interactions with another Golgi Rab GTPase Rab33B (Pusapati et al. 2012). This is reminiscent of the BLOC-3 that interacts with, and may be targeted by, Rab9 (Kloer et al. 2010). It is tempting to speculate that the properties and dimeric form of Ric1-Rgp1 might indicate some relationship to the HerMon GEFs. However, Ric1-Rgp1 displays no homology to other Rab GEFs and lacks any conserved domains. It may therefore represent a unique class of Rab GEF for which structural data would be valuable.

5.2.8 Structural Themes in Rab Regulators

Based on the available evidence, Rab GEFs fall into one of two classes. Those that act by inserting an acidic finger residue into the Rab magnesium and phosphate-binding site, and those that induce restructuring of Rab switch I or II without insertion into the nucleotide-binding site. In both cases altered coordination of the P-loop lysine displacing the bound magnesium is likely to be central to the mechanism of GDP release. Likewise, specificity for the Rab GDP form may in part be due to the availability of the RabF1 TIGID motif in GDP-bound but not GTP-bound Rabs. Despite the lack of, or only limited sequence homology,

structural biology and highly sensitive sequence alignment tools based on hidden Markov modelling show that a number of Rab GEFs are united by longin/roadblock domains (Yoshimura et al. 2010; Wu et al. 2011; Levine et al. 2013). TRAPP, DENN family GEFs and the HerMon GEFs for Rab7-related GTPases all contain longin domains. These domains may therefore act as general platforms for GTPase regulation by promoting conformational change in the switch I and II regions, which represents a common theme in Rab activation. The longin/roadblock platform has also been found in other GTPase regulatory systems involving vesicle coat protein complexes, the ER signal sequence receptor, and a bacterial cell polarity MglA/MglB (Sun et al. 2007; Levine et al. 2013; Miertzschke et al. 2011). Therefore these domains may represent an ancient regulatory module used to control diverse GTPases and not only Rabs.

5.3 Rab GAPs: The TBC Domain Proteins

TBC1 (Tre-2/Cdc16/Bub2) domain GTPase activating proteins (GAPs) were originally identified in genetic screens for modulators of yeast Rab/Ypt function (Strom et al. 1993; Du et al. 1998; Albert et al. 1999; Vollmer et al. 1999; Albert and Gallwitz 2000; Eitzen et al. 2000). Because of this they are referred to as GAPs for Ypts (Gyp) in yeasts. In higher eukaryotes, they are typically referred to as TBC1 domain proteins. Analysis of the evolutionary history of Rabs and Rab GAPs has failed to create a clear picture of their relationship (Gabernet-Castello et al. 2013). This type of investigation has suggested that there is an ancient complement of ten TBC domain proteins, yet matching this to the cognate Rabs has proven difficult. This difficulty may be in part due to the presence of other domains in addition to the Rab GAP activity encoding TBC domain. Because of this it is important to biochemically define the target specificity of each Rab GAP using purified proteins. Expression of Rab GAPs has been relatively widely used as a tool to inactivate Rabs in cellular trafficking pathways (Hsu et al. 2010; Yoshimura et al. 2010; Fuchs et al. 2007; Longatti et al. 2012). Once a specific GAP or set of GAPs blocking a transport pathway is known, then it is relatively simple to identify the target Rabs using biochemical assays for GTP hydrolysis. This methodology has been used to identify Rabs involved in endocytic and secretory trafficking, as well as cell polarisation during cilium formation. Caution is required when interpreting such screens, since in some cases the effect of the GAP may not always correlate with catalytic activity (Haas et al. 2007; Longatti et al. 2012). A counterscreen with a mutated catalytically inactive Rab GAP can be used to test for this. One complication that has arisen from such approaches is the promiscuous nature of some TBC domain proteins in terms of their Rab target specificity.

5.3.1 Rab GAP Mechanism

Pioneering structural studies of Rab GAPs have revealed that the catalytic mechanism for GTP hydrolysis differs from that used by Ras and Rho GAPs (Pan et al. 2006; Gavriljuk et al. 2012). For Ras, GTP hydrolysis requires a glutamine residue intrinsic to the Ras switch II DxxGQ sequence and a catalytic arginine finger residue from the GAP inserted into the Ras nucleotide-binding site. By contrast the TBC1 domain contains two signature motifs IxxDxxR and YxQ (Neuwald 1997), and both these are required for catalytic activity (Pan et al. 2006). Structures of Rab33 GDP-AlF_3 with the Gyp1 TBC1 domain and Rab1 GDP-BeF_3 with TBC1D20 reveal a dual finger mechanism involving the conserved arginine of the IxxDxxR motif, and the conserved glutamine of the YxQ motif (Pan et al. 2006; Gavriljuk et al. 2012) (Fig. 5.3). While the TBC1 domain arginine adopts an equivalent position to that seen for Ras GAPs, the Rab switch II glutamine plays no direct role in (GAP stimulated) GTP hydrolysis. Instead this function is taken by the conserved glutamine of the YxQ motif in the TBC1 domain. The Rab switch II glutamine projects away from the nucleotide-binding site and contacts the backbone of the GAP. Fitting with the idea that the switch II glutamine plays little direct role in GTP hydrolysis, mutation in this residue had little effect on the GAP activity of RUTBC3 towards Rab5 or RUTBC1 towards Rab33B (Nottingham et al. 2011). In the case of Rab5 the switch II glutamine resulted in a greater than 5-fold reduction in basal GTP hydrolysis (Langemeyer et al. 2014). This indicates that for some Rabs basal and GAP-stimulated GTP hydrolysis may occur via different mechanisms. Analysis of two other Rab-GAP pairs complicates this simple picture. Rab35 and Rab1 switch II glutamine mutants showed only a slight decrease in basal GTP hydrolysis, while GTP hydrolysis was greatly reduced when stimulated by TBC1D10A or TBC1D20, respectively (Langemeyer et al. 2014). By contrast, mutations of the catalytic arginine or glutamine residues in the TBC domain greatly reduced the catalytic efficiency of the respective GAPs (Pan et al. 2006; Haas et al. 2005; Haas et al. 2007). Thus, the major determinants of GAP-stimulated GTP hydrolysis are the catalytic residues contributed by the GAP, while the Rab switch II glutamine plays a greater or lesser role depending on the Rab under scrutiny.

As already described, unlike Ras the Rabs don't always require the conserved glutamine for hydrolysis, but may need it for the GEF reaction in some instances. This suggests there is a fundamental difference in the conformational plasticity in the switch regions of Ras and Rabs that has consequences for regulatory mechanisms.

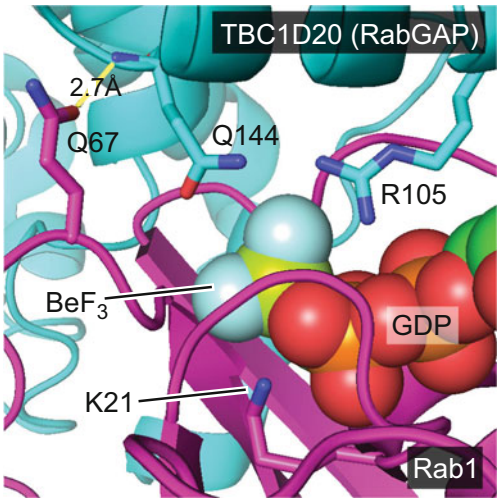

Fig. 5.3 Rab GAP mechanism and structure. TBC domain Rab GAPs utilise an arginine/glutamine dual finger mechanism. The interface between Rab1 (*magenta*) and its GAP TBC1D20 (*cyan*) shows the Rab1 switch II glutamine 67 residue contacting the GAP backbone. Glutamine 144 and arginine 105 finger residues contributed by the GAP are in close proximity to the gamma-phosphate position of the bound GTP, mimicked by GDP:berylium fluoride in this structure

5.3.2 Defining Cellular Functions for Rab GAPs

Although it has been relatively simple to identify the Rab GAP complement due to the presence of the conserved TBC1 domain in these proteins, elucidating specific cellular functions for these proteins has proven more difficult. In yeast the Gyps are not essential for growth under standard laboratory conditions, and biochemical analysis has provided conflicting reports on specificity. Here I will describe the functions of a subset of Rab-RabGAP pairs in secretion and cell polarisation, and endocytic trafficking and autophagy.

TBC1D20 and its yeast orthologue Gyp8 are ER-localised RabGAPs For Rab1 and Ypt1, respectively (De Antoni et al. 2002; Haas et al. 2007). Human TBC1D20 shows equivalent biochemical activity towards both Rab1 and Rab2, and some weak activity towards Rab18 (Haas et al. 2007). Cell biological studies have implicated both Rab1 and Rab2 in ER to Golgi trafficking (Tisdale et al. 1992; Schwaninger et al. 1992), while Rab18 plays a role in lipid droplet formation from the ER (Ozeki et al. 2005; Martin et al. 2005). Both overexpression and genome-wide siRNA screening support a physiologically relevant role for TBC1D20 in the regulation of secretion (Haas et al. 2007; Wendler et al. 2010), but have not definitively confirmed whether Rab1, 2 or 18 or a combination of these GTPases is the crucial cellular target. Loss-of-function mutations in TBC1D20 are found in the blind sterile mouse, and cause the human neurodegenerative and developmental

disorder Warburg Micro Syndrome (Liegel et al. 2013). TBC1D20 has also been implicated in other forms of neurodegenerative disorder (Gitler et al. 2008). This suggests that TBC1D20 function is essential for normal neuronal cell function, but may be less critical in other cell types. In budding yeast Gyp1 acts as a GAP for Ypt1 at the Golgi. In contrast to TBC1D20/Gyp8 which is an ER transmembrane protein, Gyp1 is a peripheral membrane protein found at the Golgi (Du and Novick 2001; De Antoni et al. 2002; Haas et al. 2007). Rab1/Ypt1 may therefore be regulated by a combination of both ER and Golgi GAPs.

Four TBC1 domain GAPs Gyp3/Msb3, Gyp4/Msb4, Gyl1/App2 and Gyp5 have been implicated in the regulation of Sec4 in polarised growth of budding yeast (Albert and Gallwitz 2000; Gao et al. 2003; Prigent et al. 2011; Chesneau et al. 2004; Chesneau et al. 2008). Why so many GAPs are required for Sec4 regulation during polarised growth is unclear. Biochemical characterisation paints a confusing picture of Gyp specificity, and Gyp3/Msb3 shows strong activity towards both Sec4 and Rab5-related GTPases. Furthermore, Gyp3/Msb3 has also been shown to act as a GAP for yeast Rab5-related GTPases during endosomal maturation (Nickerson et al. 2012; Lachmann et al. 2012). In this case the BLOC-1 complex recruits Gyp3/Msb3 to the yeast vacuole and promotes its activity towards Rab5 family GTPases (Nickerson et al. 2012; Lachmann et al. 2012). Therefore, in budding yeast it appears that Gyps may have more than one target GTPase and the specific site of action may be determined by additional regulatory factors. This may be due to the need to integrate regulation of different trafficking pathways, for example polarised growth with endocytosis; however this notion is speculative at best.

Overexpression screening and subsequent biochemical analysis have provided a clearer picture of the function of some human Rab-RabGAP pairs involved in formation of polarised cell structures. The primary cilium is an important site of signalling formed at the apical surface of many epithelial cell layers, and on fibroblast cell lines in culture. A series of Rab-RabGAP pairs was identified for the process of primary cilium formation in human cell lines (Yoshimura et al. 2007). TBC1D30 is a GAP for Rab8 enriched at primary cilia, while EVI5-like is the GAP for Rab23 known to modulate the Hedgehog signalling pathway at cilia during development (Yoshimura et al. 2007). A second GAP for Rab8, TBC1D17, has also been reported (Vaibhava et al. 2012). Intriguingly, TBC1D17 forms a complex with optineurin, and glaucoma-associated mutants in optineurin appear to potentiate TBC1D17 inhibition of Rab8 (Vaibhava et al. 2012). In addition to Rab8, Rab10 is an important regulator of transport in polarised cells and is controlled by two GAPs TBC1D1 and TBC1D4 (Peck et al. 2009; Miinea et al. 2005). These proteins are best characterised in terms of their function in the glucose transporter recycling pathway, and have different regulatory properties. TBC1D4 and TBC1D1 are regulated by the Akt and AMP-activated kinase signalling pathways, respectively (Peck et al. 2009; Miinea et al. 2005). Therefore in this instance multiple Rab GAPs are used so that specific signalling inputs can modulate the activity of the target GTPase, rather than to create redundancy.

Endocytic trafficking involves a complex network of trafficking between the cell surface, endocytic compartments and the Golgi apparatus. Accordingly, multiple Rabs are required for normal endocytic traffic including Rab5, Rab7 and Rab35. A series of studies have implicated different TBC1 domain Rab GAPs in the regulation of Rab5 during the plasma membrane to early endosome traffic. RabGAP-5/RUTBC3 has a high specificity for Rab5, and is required for growth factor receptor trafficking (Fuchs et al. 2007; Haas et al. 2005). Another GAP, RN-tre, was initially found as a GAP important for Rab5 inactivation during endocytosis and uptake of beta1-integrins at focal adhesion sites (Lanzetti et al. 2000; Palamidessi et al. 2013). RN-tre is also implicated in Rab43 regulation during endocytic trafficking of the Shiga toxin from the cell surface to the Golgi apparatus (Fuchs et al. 2007). In terms of its biochemical specificity, Rn-tre has some activity towards Rab5A-C, but shows a greater activity towards Rab43 (Fuchs et al. 2007; Haas et al. 2005). It may therefore act as a GAP for both Rab5 and Rab43 in vivo, and could be regulated by different regulatory signals depending on the tissue type of growth state of the cells. A further Rab5 GAP, TBC-2, has been identified from genetics screens for phagocytosis mutants in *C. elegans* (Li et al. 2009). However, the related mammalian protein TBC1D2/Armus appears to act as a GAP for Rab7 and regulate E-cadherin degradation (Frasa et al. 2010). Rab7 is inhibited by a second GAP TBC1D5 which binds to the retromer coat complex involved in the endosome to Golgi retrieval of the mannose-6-phosphate receptor (Seaman et al. 2009). In addition to the Rab5-Rab7 pathway, Rab35 is an important regulator of endocytosis in metazoa (Sato et al. 2008; Marat and McPherson 2010 ; Allaire et al. 2010). As well as the DENND1 GEF regulator already described, a Rab35 GAP TBC1D10A-C has been identified. TBC1D10A-C have been implicated in Rab35 regulation in trafficking from the cell surface to the Golgi as well as in the Rab35-dependent pathway for exosome formation (Fuchs et al. 2007; Patino-Lopez et al. 2008; Hsu et al. 2010). In *Drosophila melanogaster*, the TBC1D10 homologue whacked and Rab35 are important for the formation of cell surface protrusions called seamless tubes (Schottenfeld-Roames and Ghabrial 2012). These cell–cell connections become elaborated if whacked is lost or Rab35 dominant active mutations are expressed (Schottenfeld-Roames and Ghabrial 2012), suggesting whacked is important for inactivating Rab35 in this system. A neuronal TBC1 domain GAP for Rab35 has also been found in *Drosophila melanogaster* (Uytterhoeven et al. 2011). The *skywalker* protein has been implicated together with Rab35 in endocytic trafficking at the synapse (Uytterhoeven et al. 2011); however the biochemical specificity of this protein for Rab35 still needs to be confirmed.

TBC1 domain Rab GAPs have also been implicated in the regulation of endocytic traffic during autophagy (Popovic et al. 2012; Longatti et al. 2012; Carroll et al. 2013). A diverse set of 14 TBC1 domain proteins could be pulled down using GST-tagged LC3, and mapping experiments showed this is due to the presence of LC3 interaction (LIR) motifs in these GAPs (Popovic et al. 2012). TBC1D5 was found to possess two LIR motifs, and the LC3 interaction titrated out the interaction of TBC1D5 with the retromer complex. This suggests that TBC1D5

and RabGAPs more generally play roles in reprogramming membrane trafficking following starvation-induced autophagy (Popovic et al. 2012). Other evidence links Rab GAPs to the regulation of autophagy. TBC1D2/Armus may promote transient Rab7 inactivation during the early stages of autophagy (Carroll et al. 2013). TBC1D14 localises to recycling endosomes where it binds to Rab11 rather than acting as a Rab11 GAP (Longatti et al. 2012). TBC1D14 also interacts with the ULK1 autophagy kinase on recycling endosomes, and when overexpressed blocks autophagy. Under starvation conditions TBC1D14 relocalises from the recycling endosomes to the Golgi apparatus, while ULK1 localises to autophagosomes (Longatti et al. 2012). Together these findings indicate that transport from recycling endosomes is a key step in autophagy, and suggest that TBC1D14 is an important regulator of this process.

Finally, there are a number of Rab GAPs for which there is currently no specificity data, but evidence that suggests important physiological functions and are therefore worthy of some mention. TBC1D24 mutations are associated with epilepsy and deafness, and result in severe neurodegeneration (Afawi et al. 2013; Falace et al. 2010; Guven and Tolun 2013; Rehman et al. 2014). The target Rab regulated by TBC1D24 remains mysterious; however these data suggest that it functions in a trafficking pathway required for proper neuronal function. TBC1D23 was isolated by comparative genomic screens of mouse and *C. elegans* searching for regulators of innate immunity (De Arras et al. 2012; Alper et al. 2008).

5.4 Closing Remarks

Rabs are similar to but diverge from Ras in key aspects. Despite high levels of sequence homology in the regions needed for nucleotide binding there are crucial differences in the mechanism by which both GEF and GAP regulators control Rab activation and inactivation. While good evidence exists for the function of GEFs in driving Rab activation on specific organelles to promote specific transport events, the evidence relating to Rab GAP function is less clear. It seems most probable that combinations of Rab GAPs work in synergy in response to specific signalling inputs, rather than single GAPs acting in isolation. Further studies on the cell biological functions of Rab and their regulators, as well as careful analysis of the mechanism and structures of these proteins, are clearly required.

References

Afawi Z, Mandelstam S, Korczyn AD, Kivity S, Walid S, Shalata A, Oliver KL, Corbett M, Gecz J, Berkovic SF, Jackson GD (2013) TBC1D24 mutation associated with focal epilepsy, cognitive impairment and a distinctive cerebro-cerebellar malformation. Epilepsy Res 105(1–2):240–244. doi:10.1016/j.eplepsyres.2013.02.005

Albert S, Gallwitz D (2000) Msb4p, a protein involved in Cdc42p-dependent organization of the actin cytoskeleton, is a Ypt/Rab-specific GAP. Biol Chem 381(5–6):453–456. doi:10.1515/BC.2000.059

Albert S, Will E, Gallwitz D (1999) 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 18(19):5216–5225

Allaire PD, Ritter B, Thomas S, Burman JL, Denisov AY, Legendre-Guillemin V, Harper SQ, Davidson BL, Gehring K, McPherson PS (2006) Connecdenn, a novel DENN domain-containing protein of neuronal clathrin-coated vesicles functioning in synaptic vesicle endocytosis. J Neurosci 26(51):13202–13212. doi:10.1523/JNEUROSCI.4608-06.2006

Allaire PD, Marat AL, Dall'Armi C, Di Paolo G, McPherson PS, Ritter B (2010) The Connecdenn DENN domain: a GEF for Rab35 mediating cargo-specific exit from early endosomes. Mol Cell 37(3):370–382. doi:10.1016/j.molcel.2009.12.037

Allan BB, Moyer BD, Balch WE (2000) Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. Science 289(5478):444–448. doi:10.1126/science.289.5478.444, 8682 [pii]

Alper S, Laws R, Lackford B, Boyd WA, Dunlap P, Freedman JH, Schwartz DA (2008) Identification of innate immunity genes and pathways using a comparative genomics approach. Proc Natl Acad Sci U S A 105(19):7016–7021. doi:10.1073/pnas.0802405105

Barr FA (2013) Rab GTPases and membrane identity: causal or inconsequential? J Cell Biol 202(2):191–199. doi:10.1083/jcb.201306010, jcb.201306010 [pii]

Barr F, Lambright DG (2010) Rab GEFs and GAPs. Curr Opin Cell Biol 22(4):461–470. doi:10.1016/j.ceb.2010.04.007, S0955-0674(10)00059-1 [pii]

Bensen ES, Yeung BG, Payne GS (2001) Ric1p and the Ypt6p GTPase function in a common pathway required for localization of trans-Golgi network membrane proteins. Mol Biol Cell 12(1):13–26

Blumer J, Rey J, Dehmelt L, Mazel T, Wu YW, Bastiaens P, Goody RS, Itzen A (2013) RabGEFs are a major determinant for specific Rab membrane targeting. J Cell Biol 200(3):287–300. doi:10.1083/jcb.201209113

Boriack-Sjodin PA, Margarit SM, Bar-Sagi D, Kuriyan J (1998) The structural basis of the activation of Ras by Sos. Nature 394(6691):337–343. doi:10.1038/28548

Brown TL, Howe PH (1998) MADD is highly homologous to a Rab3 guanine-nucleotide exchange protein (Rab3-GEP). Curr Biol 8(6):R191

Burd CG, Mustol PA, Schu PV, Emr SD (1996) A yeast protein related to a mammalian Ras-binding protein, Vps9p, is required for localization of vacuolar proteins. Mol Cell Biol 16(5):2369–2377

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 (2008) The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. Cell 133(7):1202–1213. doi:10.1016/j.cell.2008.04.049

Carney DS, Davies BA, Horazdovsky BF (2006) Vps9 domain-containing proteins: activators of Rab5 GTPases from yeast to neurons. Trends Cell Biol 16(1):27–35. doi:10.1016/j.tcb.2005.11.001

Carroll B, Mohd-Naim N, Maximiano F, Frasa MA, McCormack J, Finelli M, Thoresen SB, Perdios L, Daigaku R, Francis RE, Futter C, Dikic I, Braga VM (2013) The TBC/RabGAP Armus coordinates Rac1 and Rab7 functions during autophagy. Dev Cell 25(1):15–28. doi:10.1016/j.devcel.2013.03.005

Chesneau L, Dupre S, Burdina A, Roger J, Le Panse S, Jacquet M, Cuif MH (2004) Gyp5p and Gyl1p are involved in the control of polarized exocytosis in budding yeast. J Cell Sci 117(Pt 20):4757–4767. doi:10.1242/jcs.01349

Chesneau L, Prigent M, Boy-Marcotte E, Daraspe J, Fortier G, Jacquet M, Verbavatz JM, Cuif MH (2008) Interdependence of the Ypt/RabGAP Gyp5p and Gyl1p for recruitment to the sites of polarized growth. Traffic 9(4):608–622. doi:10.1111/j.1600-0854.2007.00699.x

Chiang PW, Oiso N, Gautam R, Suzuki T, Swank RT, Spritz RA (2003) The Hermansky-Pudlak syndrome 1 (HPS1) and HPS4 proteins are components of two complexes, BLOC-3 and BLOC-4, involved in the biogenesis of lysosome-related organelles. J Biol Chem 278 (22):20332–20337. doi:10.1074/jbc.M300090200

De Antoni A, Schmitzova J, Trepte HH, Gallwitz D, Albert S (2002) Significance of GTP hydrolysis in Ypt1p-regulated endoplasmic reticulum to Golgi transport revealed by the analysis of two novel Ypt1-GAPs. J Biol Chem 277(43):41023–41031. doi:10.1074/jbc.M205783200, M205783200 [pii]

De Arras L, Yang IV, Lackford B, Riches DW, Prekeris R, Freedman JH, Schwartz DA, Alper S (2012) Spatiotemporal inhibition of innate immunity signaling by the Tbc1d23 RAB-GAP. J Immunol 188(6):2905–2913. doi:10.4049/jimmunol.1102595

Delprato A, Lambright DG (2007) Structural basis for Rab GTPase activation by VPS9 domain exchange factors. Nat Struct Mol Biol 14(5):406–412. doi:10.1038/nsmb1232

Delprato A, Merithew E, Lambright DG (2004) Structure, exchange determinants, and family-wide rab specificity of the tandem helical bundle and Vps9 domains of Rabex-5. Cell 118 (5):607–617. doi:10.1016/j.cell.2004.08.009

Denef N, Chen Y, Weeks SD, Barcelo G, Schupbach T (2008) Crag regulates epithelial architecture and polarized deposition of basement membrane proteins in Drosophila. Dev Cell 14 (3):354–364. doi:10.1016/j.devcel.2007.12.012

Dong G, Medkova M, Novick P, Reinisch KM (2007) A catalytic coiled coil: structural insights into the activation of the Rab GTPase Sec4p by Sec2p. Mol Cell 25(3):455–462. doi:10.1016/j.molcel.2007.01.013

Du LL, Novick P (2001) Yeast rab GTPase-activating protein Gyp1p localizes to the Golgi apparatus and is a negative regulator of Ypt1p. Mol Biol Cell 12(5):1215–1226

Du LL, Collins RN, Novick PJ (1998) Identification of a Sec4p GTPase-activating protein (GAP) as a novel member of a Rab GAP family. J Biol Chem 273(6):3253–3256

Dumas JJ, Zhu Z, Connolly JL, Lambright DG (1999) Structural basis of activation and GTP hydrolysis in Rab proteins. Structure 7(4):413–423

Eitzen G, Will E, Gallwitz D, Haas A, Wickner W (2000) Sequential action of two GTPases to promote vacuole docking and fusion. EMBO J 19(24):6713–6720. doi:10.1093/emboj/19.24.6713

Elias M, Brighouse A, Gabernet-Castello C, Field MC, Dacks JB (2012) Sculpting the endomembrane system in deep time: high resolution phylogenetics of Rab GTPases. J Cell Sci 125(Pt 10):2500–2508. doi:10.1242/jcs.101378

Falace A, Filipello F, La Padula V, Vanni N, Madia F, De Pietri Tonelli D, de Falco FA, Striano P, Dagna Bricarelli F, Minetti C, Benfenati F, Fassio A, Zara F (2010) TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. Am J Hum Genet 87 (3):365–370. doi:10.1016/j.ajhg.2010.07.020

Figueiredo AC, Wasmeier C, Tarafder AK, Ramalho JS, Baron RA, Seabra MC (2008) Rab3GEP is the non-redundant guanine nucleotide exchange factor for Rab27a in melanocytes. J Biol Chem 283(34):23209–23216

Frasa MA, Maximiano FC, Smolarczyk K, Francis RE, Betson ME, Lozano E, Goldenring J, Seabra MC, Rak A, Ahmadian MR, Braga VM (2010) Armus is a Rac1 effector that inactivates Rab7 and regulates E-cadherin degradation. Curr Biol 20(3):198–208. doi:10.1016/j.cub.2009.12.053

Fuchs E, Haas AK, Spooner RA, Yoshimura S, Lord JM, Barr FA (2007) Specific Rab GTPase-activating proteins define the Shiga toxin and epidermal growth factor uptake pathways. J Cell Biol 177(6):1133–1143. doi:10.1083/jcb.200612068, jcb.200612068 [pii]

Gabernet-Castello C, O'Reilly AJ, Dacks JB, Field MC (2013) Evolution of Tre-2/Bub2/Cdc16 (TBC) Rab GTPase-activating proteins. Mol Biol Cell 24(10):1574–1583. doi:10.1091/mbc.E12-07-0557

Gao XD, Albert S, Tcheperegine SE, Burd CG, Gallwitz D, Bi E (2003) The GAP activity of Msb3p and Msb4p for the Rab GTPase Sec4p is required for efficient exocytosis and actin organization. J Cell Biol 162(4):635–646. doi:10.1083/jcb.200302038

Gasper R, Thomas C, Ahmadian MR, Wittinghofer A (2008) The role of the conserved switch II glutamate in guanine nucleotide exchange factor-mediated nucleotide exchange of GTP-binding proteins. J Mol Biol 379(1):51–63. doi:10.1016/j.jmb.2008.03.011, S0022-2836(08)00302-1 [pii]

Gavriljuk K, Gazdag EM, Itzen A, Kotting C, Goody RS, Gerwert K (2012) Catalytic mechanism of a mammalian Rab.RabGAP complex in atomic detail. Proc Natl Acad Sci U S A 109 (52):21348–21353. doi:10.1073/pnas.1214431110, 1214431110 [pii]

Gerondopoulos A, Langemeyer L, Liang JR, Linford A, Barr FA (2012) BLOC-3 mutated in Hermansky-Pudlak syndrome is a Rab32/38 guanine nucleotide exchange factor. Curr Biol 22 (22):2135–2139. doi:10.1016/j.cub.2012.09.020

Girod A, Storrie B, Simpson JC, Johannes L, Goud B, Roberts LM, Lord JM, Nilsson T, Pepperkok R (1999) Evidence for a COP-I-independent transport route from the Golgi complex to the endoplasmic reticulum. Nat Cell Biol 1(7):423–430. doi:10.1038/15658

Gitler AD, Bevis BJ, Shorter J, Strathearn KE, Hamamichi S, Su LJ, Caldwell KA, Caldwell GA, Rochet JC, McCaffery JM, Barlowe C, Lindquist S (2008) The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis. Proc Natl Acad Sci U S A 105(1):145–150. doi:10.1073/pnas.0710685105, 0710685105 [pii]

Goud B, Salminen A, Walworth NC, Novick PJ (1988) A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. Cell 53(5):753–768. doi:10.1016/0092-8674(88)90093-1, 0092-8674(88)90093-1 [pii]

Guven A, Tolun A (2013) TBC1D24 truncating mutation resulting in severe neurodegeneration. J Med Genet 50(3):199–202. doi:10.1136/jmedgenet-2012-101313

Haas AK, Fuchs E, Kopajtich R, Barr FA (2005) A GTPase-activating protein controls Rab5 function in endocytic trafficking. Nat Cell Biol 7(9):887–893. doi:10.1038/ncb1290, ncb1290 [pii]

Haas AK, Yoshimura S, Stephens DJ, Preisinger C, Fuchs E, Barr FA (2007) Analysis of GTPase-activating proteins: Rab1 and Rab43 are key Rabs required to maintain a functional Golgi complex in human cells. J Cell Sci 120(Pt 17):2997–3010. doi:10.1242/jcs.014225, jcs.014225 [pii]

Hama H, Tall GG, Horazdovsky BF (1999) Vps9p is a guanine nucleotide exchange factor involved in vesicle-mediated vacuolar protein transport. J Biol Chem 274(21):15284–15291

Hoffman-Sommer M, Migdalski A, Rytka J, Kucharczyk R (2005) Multiple functions of the vacuolar sorting protein Ccz1p in Saccharomyces cerevisiae. Biochem Biophys Res Commun 329(1):197–204. doi:10.1016/j.bbrc.2005.01.107

Horiuchi H, Lippe R, McBride HM, Rubino M, Woodman P, Stenmark H, Rybin V, Wilm M, Ashman K, Mann M, Zerial M (1997) A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. Cell 90(6):1149–1159. doi:10.1016/S0092-8674(00)80380-3, S0092-8674(00)80380-3 [pii]

Hsu C, Morohashi Y, Yoshimura S, Manrique-Hoyos N, Jung S, Lauterbach MA, Bakhti M, Gronborg M, Mobius W, Rhee J, Barr FA, Simons M (2010) Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. J Cell Biol 189(2):223–232. doi:10.1083/jcb.200911018, jcb.200911018 [pii]

Huber SK, Scheidig AJ (2005) High resolution crystal structures of human Rab4a in its active and inactive conformations. FEBS Lett 579(13):2821–2829. doi:10.1016/j.febslet.2005.04.020

Hutagalung AH, Novick PJ (2011) Role of Rab GTPases in membrane traffic and cell physiology. Physiol Rev 91(1):119–149. doi:10.1152/physrev.00059.2009, 91/1/119 [pii]

Ingmundson A, Delprato A, Lambright DG, Roy CR (2007) Legionella pneumophila proteins that regulate Rab1 membrane cycling. Nature 450(7168):365–369

Iwasaki K, Staunton J, Saifee O, Nonet M, Thomas JH (1997) aex-3 encodes a novel regulator of presynaptic activity in C. elegans. Neuron 18(4):613–622

Kakuta S, Yamamoto H, Negishi L, Kondo-Kakuta C, Hayashi N, Ohsumi Y (2012) Atg9 vesicles recruit vesicle-tethering proteins Trs85 and Ypt1 to the autophagosome formation site. J Biol Chem 287(53):44261–44269. doi:10.1074/jbc.M112.411454

Khosravi-Far R, Lutz RJ, Cox AD, Conroy L, Bourne JR, Sinensky M, Balch WE, Buss JE, Der CJ (1991) Isoprenoid modification of rab proteins terminating in CC or CXC motifs. Proc Natl Acad Sci U S A 88(14):6264–6268

Kim YG, Raunser S, Munger C, Wagner J, Song YL, Cygler M, Walz T, Oh BH, Sacher M (2006) The architecture of the multisubunit TRAPP I complex suggests a model for vesicle tethering. Cell 127(4):817–830. doi:10.1016/j.cell.2006.09.029

Kinch LN, Grishin NV (2006) Longin-like folds identified in CHiPS and DUF254 proteins: vesicle trafficking complexes conserved in eukaryotic evolution. Protein Sci 15(11):2669–2674. doi:10.1110/ps.062419006

Kloer DP, Rojas R, Ivan V, Moriyama K, van Vlijmen T, Murthy N, Ghirlando R, van der Sluijs P, Hurley JH, Bonifacino JS (2010) Assembly of the biogenesis of lysosome-related organelles complex-3 (BLOC-3) and its interaction with Rab9. J Biol Chem 285(10):7794–7804. doi:10.1074/jbc.M109.069088

Klopper TH, Kienle N, Fasshauer D, Munro S (2012) Untangling the evolution of Rab G proteins: implications of a comprehensive genomic analysis. BMC Biol 10:71. doi:10.1186/1741-7007-10-71, 1741-7007-10-71 [pii]

Lachmann J, Barr FA, Ungermann C (2012) The Msb3/Gyp3 GAP controls the activity of the Rab GTPases Vps21 and Ypt7 at endosomes and vacuoles. Mol Biol Cell 23(13):2516–2526. doi:10.1091/mbc.E11-12-1030

Langemeyer L, Bastos RN, Cai Y, Itzen A, Reinisch K, Barr FA (2014) Diversity and plasticity in Rab GTPase nucleotide release mechanism has consequences for Rab activation and inactivation. eLife 3:e01623

Lanzetti L, Rybin V, Malabarba MG, Christoforidis S, Scita G, Zerial M, Di Fiore PP (2000) The Eps8 protein coordinates EGF receptor signalling through Rac and trafficking through Rab5. Nature 408(6810):374–377. doi:10.1038/35042605

Lee MT, Mishra A, Lambright DG (2009) Structural mechanisms for regulation of membrane traffic by rab GTPases. Traffic 10(10):1377–1389

Lerner DW, McCoy D, Isabella AJ, Mahowald AP, Gerlach GF, Chaudhry TA, Horne-Badovinac S (2013) A Rab10-dependent mechanism for polarized basement membrane secretion during organ morphogenesis. Dev Cell 24(2):159–168. doi:10.1016/j.devcel.2012.12.005

Levine TP, Daniels RD, Wong LH, Gatta AT, Gerondopoulos A, Barr FA (2013) Discovery of new Longin and Roadblock domains that form platforms for small GTPases in Ragulator and TRAPP-II. Small GTPases 4(2):62–69

Levivier E, Goud B, Souchet M, Calmels TP, Mornon JP, Callebaut I (2001) uDENN, DENN, and dDENN: indissociable domains in Rab and MAP kinases signaling pathways. Biochem Biophys Res Commun 287(3):688–695. doi:10.1006/bbrc.2001.5652

Li W, Zou W, Zhao D, Yan J, Zhu Z, Lu J, Wang X (2009) C. elegans Rab GTPase activating protein TBC-2 promotes cell corpse degradation by regulating the small GTPase RAB-5. Development 136(14):2445–2455. doi:10.1242/dev.035949

Liegel RP, Handley MT, Ronchetti A, Brown S, Langemeyer L, Linford A, Chang B, Morris-Rosendahl DJ, Carpanini S, Posmyk R, Harthill V, Sheridan E, Abdel-Salam GM, Terhal PA, Faravelli F, Accorsi P, Giordano L, Pinelli L, Hartmann B, Ebert AD, Barr FA, Aligianis IA, Sidjanin DJ (2013) Loss-of-function mutations in TBC1D20 cause cataracts and male infertility in blind sterile mice and Warburg micro syndrome in humans. Am J Hum Genet. doi:10.1016/j.ajhg.2013.10.011

Linford A, Yoshimura S, Nunes Bastos R, Langemeyer L, Gerondopoulos A, Rigden DJ, Barr FA (2012) Rab14 and its exchange factor FAM116 link endocytic recycling and adherens junction stability in migrating cells. Dev Cell 22(5):952–966. doi:10.1016/j.devcel.2012.04.010

Longatti A, Lamb CA, Razi M, Yoshimura S, Barr FA, Tooze SA (2012) TBC1D14 regulates autophagosome formation via Rab11- and ULK1-positive recycling endosomes. J Cell Biol 197(5):659–675. doi:10.1083/jcb.201111079

Lynch-Day MA, Bhandari D, Menon S, Huang J, Cai H, Bartholomew CR, Brumell JH, Ferro-Novick S, Klionsky DJ (2010) Trs85 directs a Ypt1 GEF, TRAPPIII, to the phagophore to promote autophagy. Proc Natl Acad Sci U S A 107(17):7811–7816. doi:10.1073/pnas.1000063107

Machner MP, Isberg RR (2006) Targeting of host Rab GTPase function by the intravacuolar pathogen Legionella pneumophila. Dev Cell 11(1):47–56

Machner MP, Isberg RR (2007) A bifunctional bacterial protein links GDI displacement to Rab1 activation. Science 318(5852):974–977

Marat AL, McPherson PS (2010) The connecdenn family, Rab35 guanine nucleotide exchange factors interfacing with the clathrin machinery. J Biol Chem 285(14):10627–10637. doi:10.1074/jbc.M109.050930

Marat AL, Dokainish H, McPherson PS (2011) DENN domain proteins: regulators of Rab GTPases. J Biol Chem 286(16):13791–13800. doi:10.1074/jbc.R110.217067

Martin S, Driessen K, Nixon SJ, Zerial M, Parton RG (2005) Regulated localization of Rab18 to lipid droplets: effects of lipolytic stimulation and inhibition of lipid droplet catabolism. J Biol Chem 280(51):42325–42335. doi:10.1074/jbc.M506651200, M506651200 [pii]

Martina JA, Moriyama K, Bonifacino JS (2003) BLOC-3, a protein complex containing the Hermansky-Pudlak syndrome gene products HPS1 and HPS4. J Biol Chem 278(31):29376–29384. doi:10.1074/jbc.M301294200

Martinez O, Schmidt A, Salamero J, Hoflack B, Roa M, Goud B (1994) The small GTP-binding protein rab6 functions in intra-Golgi transport. J Cell Biol 127(6 Pt 1):1575–1588

Miertzschke M, Koerner C, Vetter IR, Keilberg D, Hot E, Leonardy S, Sogaard-Andersen L, Wittinghofer A (2011) Structural analysis of the Ras-like G protein MglA and its cognate GAP MglB and implications for bacterial polarity. EMBO J 30(20):4185–4197. doi:10.1038/emboj.2011.291, emboj2011291 [pii]

Miinea CP, Sano H, Kane S, Sano E, Fukuda M, Peranen J, Lane WS, Lienhard GE (2005) AS160, the Akt substrate regulating GLUT4 translocation, has a functional Rab GTPase-activating protein domain. Biochem J 391(Pt 1):87–93

Mizuno-Yamasaki E, Rivera-Molina F, Novick P (2012) GTPase networks in membrane traffic. Annu Rev Biochem 81:637–659. doi:10.1146/annurev-biochem-052810-093700

Montpetit B, Conibear E (2009) Identification of the novel TRAPP associated protein Tca17. Traffic 10(6):713–723. doi:10.1111/j.1600-0854.2009.00895.x

Murata T, Delprato A, Ingmundson A, Toomre DK, Lambright DG, Roy CR (2006) The Legionella pneumophila effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. Nat Cell Biol 8(9):971–977

Nazarian R, Falcon-Perez JM, Dell'Angelica EC (2003) Biogenesis of lysosome-related organelles complex 3 (BLOC-3): a complex containing the Hermansky-Pudlak syndrome (HPS) proteins HPS1 and HPS4. Proc Natl Acad Sci U S A 100(15):8770–8775. doi:10.1073/pnas.1532040100

Neuwald AF (1997) A shared domain between a spindle assembly checkpoint protein and Ypt/Rab-specific GTPase-activators. Trends Biochem Sci 22(7):243–244

Nickerson DP, Russell MR, Lo SY, Chapin HC, Milnes JM, Merz AJ (2012) Termination of isoform-selective Vps21/Rab5 signaling at endolysosomal organelles by Msb3/Gyp3. Traffic 13(10):1411–1428. doi:10.1111/j.1600-0854.2012.01390.x

Nordmann M, Cabrera M, Perz A, Brocker C, Ostrowicz C, Engelbrecht-Vandre S, Ungermann C (2010) The Mon1-Ccz1 complex is the GEF of the late endosomal Rab7 homolog Ypt7. Curr Biol 20(18):1654–1659. doi:10.1016/j.cub.2010.08.002

Nottingham RM, Ganley IG, Barr FA, Lambright DG, Pfeffer SR (2011) RUTBC1 protein, a Rab9A effector that activates GTP hydrolysis by Rab32 and Rab33B proteins. J Biol Chem 286(38):33213–33222. doi:10.1074/jbc.M111.261115, M111.261115 [pii]

Novick P, Field C, Schekman R (1980) Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell 21(1):205–215. doi:10.1016/0092-8674(80)90128-2, 0092-8674(80)90128-2 [pii]

Oiso N, Riddle SR, Serikawa T, Kuramoto T, Spritz RA (2004) The rat Ruby (R) locus is Rab38: identical mutations in Fawn-hooded and Tester-Moriyama rats derived from an ancestral Long Evans rat sub-strain. Mamm Genome 15(4):307–314. doi:10.1007/s00335-004-2337-9

Ozeki S, Cheng J, Tauchi-Sato K, Hatano N, Taniguchi H, Fujimoto T (2005) Rab18 localizes to lipid droplets and induces their close apposition to the endoplasmic reticulum-derived membrane. J Cell Sci 118(Pt 12):2601–2611. doi:10.1242/jcs.02401

Palamidessi A, Frittoli E, Ducano N, Offenhauser N, Sigismund S, Kajiho H, Parazzoli D, Oldani A, Gobbi M, Serini G, Di Fiore PP, Scita G, Lanzetti L (2013) The GTPase-activating protein RN-tre controls focal adhesion turnover and cell migration. Curr Biol 23(23):2355–2364. doi:10.1016/j.cub.2013.09.060

Pan X, Eathiraj S, Munson M, Lambright DG (2006) TBC-domain GAPs for Rab GTPases accelerate GTP hydrolysis by a dual-finger mechanism. Nature 442(7100):303–306. doi:10.1038/nature04847, nature04847 [pii]

Patino-Lopez G, Dong X, Ben-Aissa K, Bernot KM, Itoh T, Fukuda M, Kruhlak MJ, Samelson LE, Shaw S (2008) Rab35 and its GAP EPI64C in T cells regulate receptor recycling and immunological synapse formation. J Biol Chem 283(26):18323–18330. doi:10.1074/jbc.M800056200, M800056200 [pii]

Peck GR, Chavez JA, Roach WG, Budnik BA, Lane WS, Karlsson HK, Zierath JR, Lienhard GE (2009) Insulin-stimulated phosphorylation of the Rab GTPase-activating protein TBC1D1 regulates GLUT4 translocation. J Biol Chem 284(44):30016–30023. doi:10.1074/jbc.M109.035568

Peter M, Chavrier P, Nigg EA, Zerial M (1992) Isoprenylation of rab proteins on structurally distinct cysteine motifs. J Cell Sci 102(Pt 4):857–865

Pfeffer S, Aivazian D (2004) Targeting Rab GTPases to distinct membrane compartments. Nat Rev Mol Cell Biol 5(11):886–896. doi:10.1038/nrm1500, nrm1500 [pii]

Plutner H, Cox AD, Pind S, Khosravi-Far R, Bourne JR, Schwaninger R, Der CJ, Balch WE (1991) Rab1b regulates vesicular transport between the endoplasmic reticulum and successive Golgi compartments. J Cell Biol 115(1):31–43

Popovic D, Akutsu M, Novak I, Harper JW, Behrends C, Dikic I (2012) Rab GTPase-activating proteins in autophagy: regulation of endocytic and autophagy pathways by direct binding to human ATG8 modifiers. Mol Cell Biol 32(9):1733–1744. doi:10.1128/MCB.06717-11

Poteryaev D, Datta S, Ackema K, Zerial M, Spang A (2010) Identification of the switch in early-to-late endosome transition. Cell 141(3):497–508. doi:10.1016/j.cell.2010.03.011

Prigent M, Boy-Marcotte E, Chesneau L, Gibson K, Dupre-Crochet S, Tisserand H, Verbavatz JM, Cuif MH (2011) The RabGAP proteins Gyp5p and Gyl1p recruit the BAR domain protein Rvs167p for polarized exocytosis. Traffic 12(8):1084–1097. doi:10.1111/j.1600-0854.2011.01218.x

Pusapati GV, Luchetti G, Pfeffer SR (2012) Ric1-Rgp1 complex is a guanine nucleotide exchange factor for the late Golgi Rab6A GTPase and an effector of the medial Golgi Rab33B GTPase. J Biol Chem 287(50):42129–42137. doi:10.1074/jbc.M112.414565, M112.414565 [pii]

Rak A, Pylypenko O, Durek T, Watzke A, Kushnir S, Brunsveld L, Waldmann H, Goody RS, Alexandrov K (2003) Structure of Rab GDP-dissociation inhibitor in complex with prenylated YPT1 GTPase. Science 302(5645):646–650. doi:10.1126/science.1087761, 302/5645/646 [pii]

Rehman AU, Santos-Cortez RL, Morell RJ, Drummond MC, Ito T, Lee K, Khan AA, Basra MA, Wasif N, Ayub M, Ali RA, Raza SI, University of Washington Center for Mendelian Genomics, Nickerson DA, Shendure J, Bamshad M, Riazuddin S, Billington N, Khan SN, Friedman PL, Griffith AJ, Ahmad W, Riazuddin S, Leal SM, Friedman TB (2014) Mutations in TBC1D24, a Gene Associated With Epilepsy, Also Cause Nonsyndromic Deafness DFNB86. Am J Hum Genet 94(1):144–152. doi:10.1016/j.ajhg.2013.12.004

Sacher M, Barrowman J, Wang W, Horecka J, Zhang Y, Pypaert M, Ferro-Novick S (2001) TRAPP I implicated in the specificity of tethering in ER-to-Golgi transport. Mol Cell 7(2):433–442

Salminen A, Novick PJ (1987) A ras-like protein is required for a post-Golgi event in yeast secretion. Cell 49(4):527–538. doi:10.1016/0092-8674(87)90455-7, 0092-8674(87)90455-7 [pii]

Sato M, Sato K, Fonarev P, Huang CJ, Liou W, Grant BD (2005) Caenorhabditis elegans RME-6 is a novel regulator of RAB-5 at the clathrin-coated pit. Nat Cell Biol 7(6):559–569. doi:10.1038/ncb1261, ncb1261 [pii]

Sato Y, Fukai S, Ishitani R, Nureki O (2007a) Crystal structure of the Sec4p.Sec2p complex in the nucleotide exchanging intermediate state. Proc Natl Acad Sci U S A 104(20):8305–8310. doi:10.1073/pnas.0701550104

Sato Y, Shirakawa R, Horiuchi H, Dohmae N, Fukai S, Nureki O (2007b) Asymmetric coiled-coil structure with Guanine nucleotide exchange activity. Structure 15(2):245–252. doi:10.1016/j.str.2007.01.003

Sato M, Sato K, Liou W, Pant S, Harada A, Grant BD (2008) Regulation of endocytic recycling by C. elegans Rab35 and its regulator RME-4, a coated-pit protein. EMBO J 27(8):1183–1196. doi:10.1038/emboj.2008.54

Schoebel S, Oesterlin LK, Blankenfeldt W, Goody RS, Itzen A (2009) RabGDI displacement by DrrA from Legionella is a consequence of its guanine nucleotide exchange activity. Mol Cell 36(6):1060–1072. doi:10.1016/j.molcel.2009.11.014, S1097-2765(09)00828-4 [pii]

Schottenfeld-Roames J, Ghabrial AS (2012) Whacked and Rab35 polarize dynein-motor-complex-dependent seamless tube growth. Nat Cell Biol 14(4):386–393. doi:10.1038/ncb2454, ncb2454 [pii]

Schuck S, Gerl MJ, Ang A, Manninen A, Keller P, Mellman I, Simons K (2007) Rab10 is involved in basolateral transport in polarized Madin-Darby canine kidney cells. Traffic 8(1):47–60. doi:10.1111/j.1600-0854.2006.00506.x

Schwaninger R, Plutner H, Bokoch GM, Balch WE (1992) Multiple GTP-binding proteins regulate vesicular transport from the ER to Golgi membranes. J Cell Biol 119(5):1077–1096

Seaman MN, Harbour ME, Tattersall D, Read E, Bright N (2009) Membrane recruitment of the cargo-selective retromer subcomplex is catalysed by the small GTPase Rab7 and inhibited by the Rab-GAP TBC1D5. J Cell Sci 122(Pt 14):2371–2382. doi:10.1242/jcs.048686

Segev N, Mulholland J, Botstein D (1988) The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. Cell 52(6):915–924. doi:10.1016/0092-8674(88)90433-3, 0092-8674(88)90433-3 [pii]

Semerdjieva S, Shortt B, Maxwell E, Singh S, Fonarev P, Hansen J, Schiavo G, Grant BD, Smythe E (2008) Coordinated regulation of AP2 uncoating from clathrin-coated vesicles by rab5 and hRME-6. J Cell Biol 183(3):499–511. doi:10.1083/jcb.200806016

Siniossoglou S, Peak-Chew SY, Pelham HR (2000) Ric1p and Rgp1p form a complex that catalyses nucleotide exchange on Ypt6p. EMBO J 19(18):4885–4894. doi:10.1093/emboj/19.18.4885

Soldati T, Riederer MA, Pfeffer SR (1993) Rab GDI: a solubilizing and recycling factor for rab9 protein. Mol Biol Cell 4(4):425–434

Strom M, Vollmer P, Tan TJ, Gallwitz D (1993) A yeast GTPase-activating protein that interacts specifically with a member of the Ypt/Rab family. Nature 361(6414):736–739

Stroupe C, Brunger AT (2000) Crystal structures of a Rab protein in its inactive and active conformations. J Mol Biol 304(4):585–598. doi:10.1006/jmbi.2000.4236

Suh HY, Lee DW, Lee KH, Ku B, Choi SJ, Woo JS, Kim YG, Oh BH (2009) Structural insights into the dual nucleotide exchange and GDI displacement activity of SidM/DrrA. EMBO J 29(2):496–504

Suh HY, Lee DW, Lee KH, Ku B, Choi SJ, Woo JS, Kim YG, Oh BH (2010) Structural insights into the dual nucleotide exchange and GDI displacement activity of SidM/DrrA. EMBO J 29(2):496–504. doi:10.1038/emboj.2009.347

Sun Z, Anderl F, Frohlich K, Zhao L, Hanke S, Brugger B, Wieland F, Bethune J (2007) Multiple and stepwise interactions between coatomer and ADP-ribosylation factor-1 (Arf1)-GTP. Traffic 8(5):582–593. doi:10.1111/j.1600-0854.2007.00554.x, TRA554 [pii]

Tan D, Cai Y, Wang J, Zhang J, Menon S, Chou HT, Ferro-Novick S, Reinisch KM, Walz T (2013) The EM structure of the TRAPPIII complex leads to the identification of a requirement for COPII vesicles on the macroautophagy pathway. Proc Natl Acad Sci U S A 110(48):19432–19437. doi:10.1073/pnas.1316356110

Taussig D, Lipatova Z, Segev N (2013) Trs20 is required for TRAPP III complex assembly at the PAS and its function in autophagy. Traffic. doi:10.1111/tra.12145

Tisdale EJ, Bourne JR, Khosravi-Far R, Der CJ, Balch WE (1992) GTP-binding mutants of rab1 and rab2 are potent inhibitors of vesicular transport from the endoplasmic reticulum to the Golgi complex. J Cell Biol 119(4):749–761

Uejima T, Ihara K, Goh T, Ito E, Sunada M, Ueda T, Nakano A, Wakatsuki S (2010) GDP-bound and nucleotide-free intermediates of the guanine nucleotide exchange in the Rab5.Vps9 system. J Biol Chem 285(47):36689–36697. doi:10.1074/jbc.M110.152132

Uytterhoeven V, Kuenen S, Kasprowicz J, Miskiewicz K, Verstreken P (2011) Loss of skywalker reveals synaptic endosomes as sorting stations for synaptic vesicle proteins. Cell 145(1):117–132. doi:10.1016/j.cell.2011.02.039, S0092-8674(11)00191-7 [pii]

Vaibhava V, Nagabhushana A, Chalasani ML, Sudhakar C, Kumari A, Swarup G (2012) Optineurin mediates a negative regulation of Rab8 by the GTPase-activating protein TBC1D17. J Cell Sci 125(Pt 21):5026–5039. doi:10.1242/jcs.102327

Vollmer P, Will E, Scheglmann D, Strom M, Gallwitz D (1999) Primary structure and biochemical characterization of yeast GTPase-activating proteins with substrate preference for the transport GTPase Ypt7p. Eur J Biochem 260(1):284–290

Wada M, Nakanishi H, Satoh A, Hirano H, Obaishi H, Matsuura Y, Takai Y (1997) Isolation and characterization of a GDP/GTP exchange protein specific for the Rab3 subfamily small G proteins. J Biol Chem 272(7):3875–3878

Walch-Solimena C, Collins RN, Novick PJ (1997) Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles. J Cell Biol 137(7):1495–1509

Wang W, Sacher M, Ferro-Novick S (2000) TRAPP stimulates guanine nucleotide exchange on Ypt1p. J Cell Biol 151(2):289–296

Wang CW, Stromhaug PE, Kauffman EJ, Weisman LS, Klionsky DJ (2003) Yeast homotypic vacuole fusion requires the Ccz1-Mon1 complex during the tethering/docking stage. J Cell Biol 163(5):973–985. doi:10.1083/jcb.200308071

Wasmeier C, Romao M, Plowright L, Bennett DC, Raposo G, Seabra MC (2006) Rab38 and Rab32 control post-Golgi trafficking of melanogenic enzymes. J Cell Biol 175(2):271–281. doi:10.1083/jcb.200606050

Wendler F, Gillingham AK, Sinka R, Rosa-Ferreira C, Gordon DE, Franch-Marro X, Peden AA, Vincent JP, Munro S (2010) A genome-wide RNA interference screen identifies two novel components of the metazoan secretory pathway. EMBO J 29(2):304–314. doi:10.1038/emboj.2009.350

White J, Johannes L, Mallard F, Girod A, Grill S, Reinsch S, Keller P, Tzschaschel B, Echard A, Goud B, Stelzer EH (1999) Rab6 coordinates a novel Golgi to ER retrograde transport pathway in live cells. J Cell Biol 147(4):743–760

Wittinghofer A, Vetter IR (2011) Structure-function relationships of the G domain, a canonical switch motif. Annu Rev Biochem 80:943–971. doi:10.1146/annurev-biochem-062708-134043

Wu YW, Oesterlin LK, Tan KT, Waldmann H, Alexandrov K, Goody RS (2010) Membrane targeting mechanism of Rab GTPases elucidated by semisynthetic protein probes. Nat Chem Biol 6(7):534–540. doi:10.1038/nchembio.386, nchembio.386 [pii]

Wu X, Bradley MJ, Cai Y, Kummel D, De La Cruz EM, Barr FA, Reinisch KM (2011) Insights regarding guanine nucleotide exchange from the structure of a DENN-domain protein complexed with its Rab GTPase substrate. Proc Natl Acad Sci U S A 108(46):18672–18677. doi:10.1073/pnas.1110415108

Yoshimura S, Egerer J, Fuchs E, Haas AK, Barr FA (2007) Functional dissection of Rab GTPases involved in primary cilium formation. J Cell Biol 178(3):363–369. doi:10.1083/jcb.200703047, jcb.200703047 [pii]

Yoshimura S, Gerondopoulos A, Linford A, Rigden DJ, Barr FA (2010) Family-wide characterization of the DENN domain Rab GDP-GTP exchange factors. J Cell Biol 191(2):367–381. doi:10.1083/jcb.201008051

Zerial M, McBride H (2001) Rab proteins as membrane organizers. Nat Rev Mol Cell Biol 2(2):107–117. doi:10.1038/35052055