Role of Rab GTPases in Membrane Traffic and Cell Physiology

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I. Introduction

The cytoplasm of a typical eukaryotic cell is populated with a variety of membranous organelles, and a vast array of factors traffic between these organelles by vesicular transport. Despite the complexity of interconnected pathways and the large flux and diversity of transported components, appropriate and accurate delivery of cargo is stringently maintained. The molecular mechanisms by which this traffic is regulated to ensure both the fidelity and efficiency of transport has been, and will continue to be, a significant focus of research. Contributions from a multitude of laboratories have described mechanisms of cargo selection, the budding and scission of vesicles from their donor membranes, the assortment of coats that associate with these vesicles, the mechanism by which...
these vesicles are transported along cytoskeletal components such as actin filaments or microtubules, the association of the vesicles with the correct target membrane through diverse “tethering” complexes, and finally the mechanism of vesicle fusion with the target membrane through the action of soluble NSF attachment protein receptors (SNAREs) and their associated regulatory machinery. Each step requires a specific set of components to control not only the process itself, but also the transition from one step to the next. Many questions remain concerning the details relating to each of the above steps, for example: How is the transport cargo identified? How do vesicle coats associate and dissociate in a manner consistent with transport? How do vesicles move along cytoskeletal elements? What is the molecular mechanism of a “tethering” complex? What factors ensure appropriate SNARE-mediated membrane fusion? Perhaps the most perplexing question is how the identity of each organelle is maintained such that their assigned functions are conserved? What factors ensure appropriateness and directionality of transport? How do vesicles move along cytoskeletal elements? What is the molecular mechanism of vesicle fusion with the target membrane through diverse “tethering” complexes, and finally the insertion of the vesicles with the correct target membrane membrane transport pathways. We discuss how altering the functions needed at each step on their respective membranes. Replacement of the Rab5 hypervariable region with the hypervariable region of Rab7 targets the chimera to late endosomes that are normally marked by Rab7 (65).

II. THE CONSERVED STRUCTURE OF Rabs

Rabs constitute the largest family of small Ras-like GTPases with 11 identified in yeast and more than 60 members in humans that can be classified in several phylogenetic and functional groups (316, 367). The structures of at least 16 different Rab proteins in either their active (GTP-bound) or inactive (GDP-bound) state have been solved (140, 160). Almost every group has at least one member represented as a crystal structure, allowing for some generalization regarding the specific structural features that contribute to Rab function (319). Rabs generally possess the GTPase fold, composed of a six-stranded β-sheet flanked by five α-helices, common to all members of the Ras superfamily. COOH-terminal to the GTPase fold is the hypervariable region of the Rab followed by the CAAX boxes that normally contains two cysteine residues to which geranylgeranyl moieties are covalently attached. These geranylgeranyl tails allow for regulated membrane insertion of the Rab that will be discussed in greater detail below. Because of the overall structural conservation, the differences between the active and inactive states must define the regions that determine the specific functions of each Rab. The switch I and II regions of Rabs are the primary determinants of nucleotide-dependent Rab function, and both switch regions make contact with the γ phosphate of GTP. When GDP-bound, the switch regions tend to be disordered and undergo major changes to adopt a structurally well-ordered state upon binding GTP (140). Superimpositions of Rab structures in their active state show the greatest structural heterogeneity in their switch domains and the a3/β5 loop (a loop that connects α helix 3 with β sheet 5) that lies adjacent to the switch II domain, with little change elsewhere in the structure. These structural differences explain how different Rab proteins recruit specific sets of effectors to regulate their respective pathways (115, 140, 319). There are additional features of Rabs that contribute to their interactions with effector proteins and their mechanism of targeting to specific membranes. A multiple sequence alignment of all known Rabs led to the identification of conserved stretches of amino acids, named F1-F5, that distinguishes Rabs from other members of the Ras superfamily (317). The analysis also led to the identification of Rab subfamily-conserved sequences, named SF1–4, that allowed for grouping of Rabs into various subfamilies and were predicted to define the sites of interactions with their respective effectors (317). The crystal structure of Rab3A with its effector, Rabphilin, identified three complementarity-determining regions (CDRs) of Rab3A that made contact with Rabphilin, and these CDRs essentially overlap three of the four SF motifs (305). It should be noted that the switch domains contain F1, F3, and F4 and the a3/β5 loop is equivalent to CDR2, which has also been called SF3.

At the COOH terminus of Rab proteins, upstream of the CAAX box, is the hypervariable region of 35–40 amino acids. As the name implies, this portion of the Rab shows the greatest divergence in primary sequence among the different phylogenetic groups. This region has been shown to play a role in targeting of the Rab to specific membranes. Replacement of the Rab5 hypervariable region with that of Rab7 targets the chimera to late endosomes that are normally marked by Rab7 (65).

In yeast, a similar chimera of Sec4 containing the hypervariable region of Ypt1 localized to Golgi structures that normally contain Ypt1 (40). Chimeras of Rab1 or Rab5...
FIG. 1. The intracellular localization of Rabs. A typical cell showing the intracellular localization and associated vesicle transport pathway(s) of several Rab GTPases. Rab1 regulates ER-Golgi traffic while Rab2 is involved in recycling, or retrograde traffic, from Golgi and the ERGIC back to the ER. Rab6 regulates intra-Golgi traffic. Several Rabs including Rab8, -10, and -14 regulate biosynthetic traffic from the trans-Golgi network (TGN) to the plasma membrane. The glucose transporter GLUT4 is found in vesicles that use these Rabs to reach the plasma membrane. Several secretory vesicles and granules use Rab3, -26, -27, and -37 to exocytose their cargo. Rab27 is well-studied in the melanosome transport that also relies on Rabs 32 and 38. There are numerous Rabs associated with endosomal traffic, and the most active site of localization is the early endosome. Most early endocytic steps rely on Rab5, which mediates fusion of endocytic vesicles to form the early endosome. Traffic can be directed towards the lysosome for degradation, which relies on action of Rab7, or to various recycling compartments to return factors to the plasma membrane. Rab15 directs membrane traffic from the early endosome to the recycling endosome. Rab4 and Rab11 regulate fast and slow endocytic recycling, respectively. Specialized Rab functions include Rab15-mediated regulation of lipid droplets, intracellular lipid storage sites. Rab24 and Rab33 mediate formation of the preautophagosomal structure that engulfs cellular components to form the autophagosome that is subsequently targeted to the lysosome/vacuole. Rab21 and Rab25 regulate transport of integrins to control cell adhesion and cytokinesis. Rab13 directs traffic to and regulates formation of tight junctions in polarized epithelial cells. Tight junctions define the boundary between the apical and basolateral regions of the polarized cell. Mutations in the mouse Rab23 gene lead to a severe developmental defect, open brain, because Rab23 acts downstream to negatively regulate Sonic hedgehog (Shh) signaling during dorsoventral development of the mouse spinal cord. It potentially interacts with the transcription factors activated by the Shh pathway. Rab40 also acts in a signaling pathway; it recruits components of the ubiquitination machinery to regulate Wnt signaling. There are several poorly characterized Rabs, such as Rab35. It controls plasma membrane recycling of an essential factor in cytokinesis. Rab34 and Rab39 are found on the Golgi, but it is unclear what role they play. AP, autophagosome; ERGIC, ER-Golgi intermediate compartment; ER, endoplasmic reticulum; EE, early endosome; LD, lipid droplet; LE, late endosome (multivesicular body); L/V, lysosome/vacuole; PAS, preautophagosomal structure; RE, recycling endosome; SV, secretory vesicle/granule.

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<td>ER to Golgi</td>
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<td>Protein recycling/transport to plasma membrane</td>
<td>CD2AP, p-AKAP2, Rabip4, Rabip4', Rabaptin-5a, Rabaptin-5b, Syntaxin 4, Dynexin LIC-1, Rab coupling protein (RCP), Rabenosyn-5</td>
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<td>Effectors (32, 84, 85, 97, 118, 123, 237, 245, 291, 351, 448)</td>
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<td>Endosome to Golgi, intra-Golgi transport, Golgi to ER</td>
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<td>Intra-Golgi transport, preferentially expressed in neuronal cells</td>
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<td>Late endosome to lysosome</td>
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<td>Endosome to TGN</td>
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<td>Rab11B</td>
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ER, endoplasmic reticulum; CCV, clathrin-coated vesicles; TGN, trans-Golgi network; PM, plasma membrane; RE, recycling endosome.
with the hypervariable region of Rab9 (that interacts with TIP47) can be relocated from the Golgi (normal Rab1 localization) or the early endosome (normal Rab5 localization) to the late endosome (normal Rab9 localization) upon overexpression of TIP47 (1). More recent studies in mammalian cells show that certain F and SF regions of Rabs are more important than their hypervariable domains for membrane targeting and implicate interactions with effector proteins for proper localization (3, 307). It is important to note that the hypervariable region contains a motif that interacts with proteins that regulate the membrane-bound state of the Rab (see below). Therefore, the conflict in targeting mechanisms may reflect the different pathways being studied and the overall contribution of multiple Rab motifs and interacting partners to membrane localization.

III. Rab PROTEINS AS MOLECULAR SWITCHES

Rab proteins cycle between the cytosol and the membrane of its respective transport compartment (Fig. 1, Table 1). The nucleotide-bound state of the Rab influences its localization and activity (Fig. 2). Once the Rab protein is first translated, it associates with Rab escort protein (REP), which presents the Rab to Rab geranylgeranyl transferase (RabGGT) that catalyzes the addition of one or, in most cases, two geranylgeranyl lipid groups to the COOH terminus of the Rab (2, 14, 102). In its GDP-bound or “inactive” state, it is subsequently inserted into its respective membrane. A GDP dissociation inhibitor (GDI) dissociation factor (GDF) may assist in targeting and inserting the Rab in the appropriate membrane (80, 396). A guanine nucleotide exchange factor (GEF) acts on the membrane-inserted Rab to convert it to a GTP-bound or “active” state. The active Rab now interacts with effector proteins that specifically facilitate traffic in its respective pathway. A GTPase accelerating protein (GAP) binds to the Rab to catalyze hydrolysis of the bound GTP to GDP and thereby convert the Rab back to its inactive state (318, 372). The inactive Rab is then a substrate for GDI, which is able to extract the Rab in its GDP-bound conformation from the membrane (80, 360, 430, 470). REP and GDI, both of which bind to GDP-bound Rab proteins in the cytosol, are related proteins that are

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**Fig. 2.** The newly synthesized Rab protein associates with Rab escort protein (REP) that directs it to Rab geranylgeranyl transferase (RabGGT) to receive its prenyl tails (red wavy lines). REP delivers the Rab to its target membrane. Throughout this process, the Rab is GDP-bound. A guanine nucleotide exchange factor (GEF) catalyzes exchange of GDP for GTP to activate the Rab. The GTP-bound Rab interacts with effector proteins that mediate membrane traffic in the pathway regulated by its associated Rab. The Rab then interacts with its associated GTPase activating protein (GAP) that catalyzes hydrolysis of GTP to GDP by the Rab. The Rab is then removed from the membrane by guanine nucleotide dissociation inhibitor (GDI) in preparation for the next cycle. The insertion of the Rab into the target membrane is mediated by a GDI dissociation factor (GDF) that releases the Rab from GDI. Loss-of-function mutations at each of the above steps produce disease phenotypes as indicated by the red text boxes.
part of the GDI superfamily (7, 470). The Rab, bound to GDI, is now ready to be reinserted into a membrane and begin the cycle again.

The Rab cycle is critical for regulating traffic to and from particular organelles and thus helps to define their identity. Any perturbation in the steps described above can result in a variety of disease states (Fig. 2). Mutations in the human REP-1 gene lead to choroideremia, a disease characterized by progressive atrophy of the choroid, retinal pigment epithelium, and retina that lead to eventual blindness (365). The cause of the disease is most likely due to loss of Rab27A function, which accumulates in an unprenylated form in retinal tissue samples from patients with the disease. Although there is a second REP gene, REP-2, it apparently cannot compensate for loss of REP-1 in the prenylation of Rab27A (89, 366).

Modulation of RabGGT function has also been shown to play a role in several diseases. The mouse gunmetal mutant is a RabGGTa loss-of-function mutant that is phenotypically similar to patients with Hermansky-Pudlak syndrome, a disease marked by albinism, prolonged bleeding, and lysosomal defects (35, 104, 367). Bisphosphonate drugs that inhibit geranylgeranylation of Rab proteins have been used to remedy bone diseases characterized by excessive resorption, such as osteoporosis (88, 350). These drugs have also been shown to induce apoptosis in certain types of cancers (234). These data correlate well with the identification of several Rab proteins as cancer markers. This is discussed in detail below in this article.

Mutations in the human GDII gene lead to X-linked nonspecific mental retardation (91). Mice carrying a deletion of the Gdi1 gene have defects in short-term memory formation and social interaction patterns that are phenotypically similar to humans carrying GDII mutations (92). Analysis of brain extracts from mutant mice revealed an accumulation of membrane-bound Rap proteins, but Rab4 and Rab5, both of which regulate endosomal traffic, were more significantly affected than other Rab proteins analyzed (92).

Mutations in the genes encoding the regulatory and catalytic subunits of the Rab3GAP lead to Warburg Micro and Martosof syndromes, diseases characterized by developmental abnormalities of the eye, nervous system, and genitalia (4, 5). Rab3A is the most abundant Rab found in the brain and regulates exocytosis of synaptic vesicles (144, 145, 267). A Rab GEF has also been implicated in human disease. Mutations in the human SEDL gene, the homolog of the yeast TRAPP subunit Tsr20, lead to spondyloepiphysyeal dysplasia tarda, an X-linked disorder characterized by disproportionately short stature, a short neck and trunk, and degeneration of the spine and hips (142, 143, 162, 258, 425). Mutations in the human TRAPPC9 gene, the homolog of the yeast TRAPP subunit Tsr120, lead to nonsyndromic autosomal-recessive mental retardation, intellectual disability, and postnatal microcephaly (275, 279). The TRAPP complex is a GEF for Rab1/Ypt1 that performs a regulatory "tethering" step for endoplasmic reticulum (ER)-derived vesicles targeted to the Golgi (353). These are clear examples of physiological disorders that arise from disrupting the Rab cycle. Additional examples below highlight how interacting partners of Rab proteins are involved in diseases ranging from Huntington's to cancer and how intracellular pathogens manipulate Rab-regulated pathways to their advantage.

IV. STRUCTURAL DATA OF Rab REGULATORS

Although Rabs in general are strikingly similar in their overall structure, the proteins that interact with them, to either regulate their activity or carry out their downstream functions are not. Recent crystal structures illustrate several distinct mechanisms by which GAPs and GEFs regulate the nucleotide-bound state of Rab proteins. The structures of GDI and REP cocrystallized with Rabs show how they associate with Rab proteins and their hydrophobic geranylgeranyl tails that mediate membrane insertion.

A. GDI and REP

Several features distinguish the functions of GDI and REP and thereby allow them to play different roles in the life cycle of a Rab protein. Although both GDI and REP are members of the GDI superfamily, REP associates with RabGGT to facilitate the addition of geranylgeranyl lipid moieties to the COOH termini of Rabs while GDI extracts inactive, prenylated Rabs from membranes. They are structurally similar and related in function by their affinity for the GDP-bound form of Rabs and their ability to interact with the Rab geranylgeranyl tails. However, REP binds with high affinity to the GDP-bound Rab protein either prenylated or unprenylated, while GDI binds tightly to the Rab with its prenyl groups and binds poorly to the unprenylated Rab protein (326, 473). The interaction of REP with unprenylated Rabs is consistent with its role in facilitating Rab prenylation by RabGGT, while the main function of GDI is to extract Rabs from membranes as part of the Rab cycle. The structures of GDI bound to mono- and di-geranylgeranylated Ypt1 and REP bound to mono-geranylated Rab7 help to distinguish their functions (7, 11, 140, 326, 330–332). Both GDI and REP are composed of two domains: domain I interacts with the GDP-bound Rab, while domain 2 contains the pocket that binds the geranylgeranyl motifs. The structures show strong conservation in their interaction with the switch and interswitch domains of their associated Rab, maintaining it in a GDP-bound state. Domain I also contains the binding site for the aliphatic-X (polar)-aliphatic (AXA) motif in the

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Rab hypervariable region while in domain 2, both geranylgeranyl motifs bind in the same prenyl-binding pocket. REP binds RabGGT exclusively through domain 2 and contains two critical amino acid substitutions in domain 2 that mediate its interaction with RabGGT, differentiating it from GDI. Insight into the differences in their functions comes from binding studies of REP and GDI with prenylated forms of Rab7 (473). REP binds with high affinity to unprenylated Rab7 ($K_d = 0.22$ nM) and even higher affinity to monoprenylated Rab7 ($K_d = 0.061$ nM). It binds with less affinity to diprenylated Rab7 ($K_d = 1.3$ nM) compared with monoprenylated Rab7. The more constricted prenyl-binding pocket of REP compared with GDI suggests the second prenyl group may bind outside of the pocket and partially displace the first geranylgeranyl moiety to reduce its overall affinity for REP. The higher affinity for monoprenylated Rab7 may ensure a second geranylgeranyl group is attached to the Rab as Rabs with only one prenyl group tend to be retained at the ER and do not move to their normal intracellular location (152). In the case of GDI, it binds poorly to unprenylated Rabs but with high affinity to mono- and diprenyalted Rab7. There is little difference in the affinity of GDI for monoversus diprenylated Rab7, unlike REP. The structure of GDI with di-geranylgeranylated Ypt1 shows both groups in an overlapped arrangement in the prenyl-binding pocket (326). Although the above data describe the interactions of prenylated Rabs with GDI or REP, it remains unclear how GDI extracts Rab proteins from membranes or how GDFs dissociate GDI or REP to insert Rabs into membranes. The ~1,000-fold higher affinity of GDI for prenylated versus unprenylated Rabs provides a potential explanation for how GDI might remove a membrane-bound Rab by masking its hydrophobic prenyl tails from the aqueous environment (473). This implies that the opposite reaction would require additional factors, such as a GDF or the molecular chaperone Hsp90, to efficiently break the stable Rab-GDI interaction (67, 140, 153, 197). The GDF Yip3, an integral membrane protein found on endosomes, has been shown to catalyze dissociation of GDI from Rab9 through an as yet uncharacterized mechanism (110, 396). The opposing GDI-mediated Rab extraction and GDF-mediated Rab insertion mechanisms are undoubtedly related, and uncovering one mechanism will likely shed light on the other.

B. Rab GAP Proteins

All characterized Rab GAP proteins to date contain a conserved TBC (Tre2/Bub2/Cdc16) domain that confers GAP activity (338). The crystal structure of the TBC domain of Gyp1, the GAP for Ypt1, revealed the mechanism to be dependent on a conserved arginine finger that interfaces with the Rab nucleotide binding pocket to stimulate GTP hydrolysis (114, 329). On the basis of the crystal structure, the fundamental GAP mechanism of Gyp1 was expected to be the same as that of GAP proteins for Ras or Cdc42, despite significant overall structural differences. However, the more recent crystal structures of Gyp1 with several different Rab proteins revealed an additional glutamine “finger” substitues for a glutamine from the Rab to mediate GTP hydrolysis (311, 329). This GAP mechanism is likely to be conserved among all Rab-GAP combinations, but additional structures will be needed to test this prediction.

C. Rab GEF Proteins

Unlike Rab GAP proteins, there are, to date, no clear motifs that define Rab GEF proteins. However, the structures of several GEF proteins indicate that they directly insert into, or indirectly alter, the Rab nucleotide or magnesium-binding site to cause displacement of the bound nucleotide (38). The recent crystal structures of Sec2, the GEF for Sec4, and the TRAPP complex, the GEF for Ypt1, highlight the diversity in mechanisms of Rab nucleotide exchange compared with the structures of other Rab GEF proteins: Rabex5, the GEF for Rab5, Rab21 and Rab22 and Mss4, a protein that stimulates nucleotide dissociation from Rab8 (54, 101, 112, 202, 354).

1. Sec2

Sec2 is the GEF for Sec4 and is recruited to secretory vesicles as an effector of the Rab GTPase Ypt32 (304, 449). The crystal structure of Sec2-Sec4 complex was recently solved and revealed the mechanism by which the coiled coil Sec2 dimer facilitates nucleotide exchange on the Rab Sec4 (112). Sec2 interacts with residues in the switch I and switch II domains of Sec4 to induce structural changes in the nucleotide binding pocket that reduce its affinity for nucleotide. No part of Sec2 directly inserts into the nucleotide binding pocket of Sec4, unlike the Rabex5 mechanism of nucleotide exchange (101). Sec4 is the closest yeast homolog of Rab8, and Rabin8 is a GEF for Rab8 that shares a region of homology with the catalytic site of Sec2p (174).

2. Mss4 and Dss4

Sec4 and Rab8 interact with two other related proteins, Dss4 and Mss4, respectively. These are much less efficient than Sec2 and Rabin8 in catalyzing exchange (203), and in the case of Dss4, it only stimulates dissociation of GDP, not the subsequent binding of GTP (283). The structure of Rab8 complexed with Mss4 indicates that Mss4 forms a stable binary association with Rab8 through its switch I and interswitch domains, resulting in an intermolecular β-sheet (202). Mss4 has also been shown to
form a stable association with other Rab proteins on both exocytic and endocytic pathways, and this activity of Mss4 may relate to its proposed function as a general chaperone for misfolded Rab proteins rather than a specific GEF (47–49, 283, 299, 409).

3. TRAPP complex

The TRAPP protein complex is interesting in that it is a multisubunit vesicle tethering complex (see below), yet it is also a GEF for Ypt1 (353, 452). The crystal structure of the TRAPP complex with and without bound Ypt1 revealed how the interplay of several TRAPP subunits facilitates the exchange of GDP for GTP in the Rab GTPase (54, 223). The crystallized complex contains two copies of Bet3 and one copy each of Bet5, Trs23, and Trs31. Within the complex, Bet5, Trs23, and both copies of Bet3 interact with regions of Ypt1 that include the switch I, II, and P-loop domains. These interactions of TRAPP with Ypt1 stabilize the open form of its nucleotide binding pocket, i.e., nucleotide-free form, in preparation for binding GTP. Although the COOH terminus of one of the Bet3 subunits inserts into the Ypt1 nucleotide binding pocket, it is mechanistically different from the Rabex 5 “aspartate finger” that wedges into the magnesium binding site of Rab21 to catalyze nucleotide release (101).

Other subunits in TRAPP do not make contact with Ypt1 but are important for allosteric regulation of the TRAPP subunits that directly interact with Ypt1.

V. EFFECTORS OF Rab PROTEINS

Rab proteins regulate their respective pathways by interacting with various effector proteins. Effectors are generally defined as proteins that preferentially interact with the GTP-bound form of their respective Rab, although there are examples, such as protrudin, that interact preferentially with the GDP-bound form of Rab11 (382). Different Rab effectors act during vesicle formation, movement, tethering, and fusion, with each pathway having its own unique set of effectors (Fig. 3). We begin

![Diagram showing Rab effectors and their functions](image-url)

**FIG. 3. Rab effectors.** Rabs perform their regulatory function by recruiting a variety of effectors to mediate different functions in membrane transport. These functions are as follows: 1) cargo selection/budding/coat formation, 2) vesicle transport, 3) vesicle uncoating/tethering, and 4) vesicle fusion. Below each function are examples of Rab effectors that perform said function. Mutations in Rab effectors also lead to disease phenotypes: Griscelli Syndrome is caused by mutations in either Rab27A, the Rab27A effector protein melanophilin, or myosin VA, while congenital disorders of glycosylation and spondyloepiphyseal dysplasia tarda (SEDT) are caused by mutations in several COG subunits (COG1, COG7, and COG8) and the TRAPP subunit Trs20, respectively.
by highlighting some of the best-characterized Rab effectors and their specific functions in membrane traffic.

A. Rab Proteins and Cargo Selection/Vesicle Formation

A significant portion of intracellular membrane traffic utilizes coated vesicles of the COPI, COPII, or clathrin variety. Vesicle cargo selection is determined by components of each coat complex that recognize specific elements of the cargo to be transported. The Sar/Arf family of GTPases plays a major role in recruiting the coat complexes as well as additional effectors that facilitate vesicle formation. However, several Rab proteins also have been shown to participate in this process.

The best example of this involves Rab9, which regulates membrane traffic between late endosomes and the trans-Golgi network (TGN) (250). TIP47 is a Rab9 effector that interacts with the cytoplasmic domain of mannose-6-phosphate receptors and is required for them to be recycled from endosomes to the TGN (1, 60, 107). The interaction of Rab9 with TIP47 enhances the interaction between the mannose-6-phosphate receptor and TIP47 during the formation of the transport vesicle.

Another example of a complex that acts to appropriately select cargo is the retromer complex. It is required for retrieval of transmembrane proteins from endosomes to the TGN (36, 370). The retromer is composed of a dimer of sorting nexins (SNXs; Vps5 and Vps17 in yeast) associated with the Vps26-Vps29-Vps35 trimer (187, 370). The SNXs contain a PX (phox homology) domain that interacts with phosphoinositides and a BAR domain that can serve as a multimerization interface to induce membrane curvature (58, 130). The Vps26-Vps29-Vps35 trimer is responsible for cargo binding, and the sequential actions of Rab5 and Rab7 are required for recruiting this trimer complex (345). Rab5 is important for phosphoinositide regulation through its effector, phosphatidylinositol 3-kinase, while the retromer trimer is an effector of Rab7. It is not known if Rab7 influences the interaction of retromer with cargo. Traffic in the opposing direction relies on the AP-3 pathway that is required for the movement of alkaline phosphatase from the Golgi to the vacuole/lysosome (87). The protein Vps41, a component of the HOPS complex that is an effector of Ypt7, binds to the AP-3 subunit Apl5, and this step is essential for AP-3-dependent traffic (50, 335, 339, 369).

B. Rab Proteins and Vesicle Movement

In addition to selecting cargo, Rab proteins recruit effectors that are critical for vesicle movement along actin- or microtubule-based cytoskeletal structures. There are several outstanding examples of such effectors, Ypt31/32, yeast homologs of Rab11, recruit the type V myosin Myo2 as an effector to transport secretory vesicles to sites of secretion (30, 62, 247). Rab11 in mammalian cells interacts with myosin Vb through its effector, Rab11 family interacting protein 2 (Rab11-FIP2), to regulate plasma membrane recycling (171). Rab27a regulates transport of melanosomes, melanin-containing organelles found in melanocytes, to the plasma membrane through recruitment of its effector melanophilin/Slac2-a that couples it to myosin Va (20, 192, 265, 410, 471, 472). These tripartite complexes are physiologically important because mutations in any one member lead to the rare autosomal recessive disorder Griscelli syndrome (GS), first identified by the mouse mutants dilute, leaden, and ashen (Myo5a, Rab27a, and melanophilin, respectively) (438). These patients display a range of symptoms from hypopigmentation (GS3, melanophilin mutation) to immunological defects (GS2, Rab27a mutations) and neurological impairments (GS1, Myo5a, Rab27a mutations).

Rab proteins are also involved in movement of organelles. Yeast cells utilize different pathways, some of which share factors in common, to ensure that the daughter cell acquires the full complement of organelles necessary for survival. Organelles generally utilize the actin cytoskeleton, appropriately polarized through the action of formin proteins, as a track for transport by a type V myosin from the mother cell to the bud (123). The Rab Ypt11 has been shown to regulate the inheritance of both mitochondria and Golgi in yeast by recruiting the type V myosin Myo2 as an effector (17, 34, 201). Although Golgi appears to travel by associating with Myo2, mitochondrial movement may be powered in part by actin polymerization, and the recruitment of Myo2 by Ypt11 is necessary for retaining mitochondria at the poles of mother and daughter cell during the cell cycle (34).

In animal cells, many membrane traffic pathways rely on microtubules, and Rabs have been shown to interact with microtubule-based motors to regulate these pathways. Microtubules are generally organized with their minus ends at microtubule organizing centers, such as the centrosome, and direct their plus ends into the cytoplasm and towards the cell periphery. Rab proteins can regulate traffic in either direction by interacting with members of the kinesin (plus-end directed motors) or dynein (minus-end directed motors) family. Dynein is normally in a complex with dynactin that couples the motor to and stimulates vesicle motility along microtubules (219, 268, 445, 458). Rab6 localizes to the Golgi and primarily regulates retrograde traffic between endosomes, Golgi, and the ER but has recently been shown to also be involved in exocytic traffic to the plasma membrane (99, 148, 158, 207, 259, 261, 262, 303, 433, 463). Rab6 interacts directly with Rabkinesin-6 (kinesin family member 20A) to facilitate intra-Golgi transport (116, 262). Rab6 also indirectly regulates microtubule motors through the effector pro-
teins Bicaudal D1/D2 that link Rab6-containing vesicles to the dynein-dynactin motor complex and, more recently, kinesin for exocytosis (116, 158, 184, 264, 481). Rab7, which coordinates traffic between late endosomes and the lysosome, interacts with Rab-interacting lysosomal protein (RILP) to recruit the dynein-dynactin motor complex to transport late endosomes towards centrosomes and the lysosome (213, 214). This particular Rab-effector interaction is of interest because it is manipulated by several intracellular pathogens. The Salmonella SifA protein prevents the recruitment of RILP by Rab7 to facilitate growth of the membrane-bound compartment in which the bacterium can replicate (163, 173). Helicobacter pylori

**C. Rab Proteins and Vesicle Uncoating**

Most membrane traffic pathways utilize coated vesicles of one sort or another, and these coats must be shed to allow the vesicles to fuse with their target membrane. In addition to playing a role in coat formation, Rabs may also play a role in uncoating. Rab5 regulates the early endocytic pathway and is found on clathrin-coated vesicles (CCVs). Recruitment of clathrin to newly forming endocytic vesicles is primarily through the assembly polypeptide 2 (AP-2) clathrin adaptor complex that recognizes and binds to both cargo (i.e., transferrin receptor) destined for internalization and clathrin triskelions to facilitate coat formation (31, 308, 307). The \( \mu_2 \) subunit of AP-2 recognizes cargo, and it must be phosphorylated by \( \mu_2 \) kinase, which is recruited by clathrin, to perform this function (204). Phosphatidylinositol-4,5-bisphosphate [PI(4,5)P\(_2\)], a phosphoinositide that is normally found at the plasma membrane, is also a significant component for recruiting AP-2 during clathrin-mediated endocytosis (195, 495). Rab5 regulates CCV uncoating in two ways: 1) it induces displacement of the \( \mu_2 \) kinase (through the action of the Rab5 GAP GAPVD1) from AP-2 to prevent it from phosphorylating the \( \mu_2 \) subunit, and 2) it mediates PI(4,5)P\(_2\) turnover (374). Modulation of PI(4,5)P\(_2\) levels by Rab5 may occur through recruitment of effectors such as PI(3)P kinases or PI phosphatases (77, 379).

Another possible example is Ypt1-mediated regulation of traffic between the ER and Golgi that relies on COPII-coated vesicles. A subunit of the COPII coat, Sec23, has been shown to interact with Bet3, a subunit of the TRAPP complex that is a GEF for Ypt1 and tethers ER-derived vesicles to the Golgi prior to fusion (52). Ypt1, or Rab1, is required for ER-to-Golgi traffic and presumably recruits factors that facilitate uncoating of COPII vesicles in preparation for fusion (209, 243, 284, 321, 371).

**D. Rabs and Vesicle Tethering**

To ensure fidelity of transport, most membrane transport pathways require factors that “tether” the vesicles to the target membrane before they fuse. These tethering factors fall into two categories: long coiled-coil tethers or multiprotein complexes. Members of both categories of tethers are Rab effectors, and some also regulate the nucleotide-bound state of their associated Rabs (such as the TRAPP complex described above). Despite differences in structure and organization, all of these tethering factors ensure fidelity in transport as they regulate SNARE-mediated fusion of their respective vesicles to the target membrane.

1. Coiled-coil tethers

The Golgins are a family of coiled-coil tether proteins with members that include p115 (Uso1 in yeast), giantin, and GM130 (384). p115 was first discovered as a peripheral membrane protein required for an in vitro inter-Golgi transport assay (459). Sequence analysis suggested an evolutionary relationship of p115 with Usol, a protein previously defined as an essential factor in ER to Golgi transport in yeast (293, 359, 375, 376). Both Usol and p115 are homodimers that consist of a long coiled-coil tail that binds to factors such as the cis-Golgi-localized GM130 and the COPI vesicle factor giantin and a globular head that binds to Rab1 (6, 10, 284, 294, 384, 396a). GM130 associates with the cis-Golgi through its interaction with GRASP65, also an effector of Rab1, and this interaction regulates fusion of COPII vesicles with the cis-Golgi (26, 284). p115 has also been shown to bind to and regulate SNARE proteins; it can interact directly with both syntaxin 5 and Sly1 and binds to GM130 to disrupt its interaction with both Rab1 and syntaxin 5 (6, 105). Rab1 is the essential regulatory factor in this process that is recruited by its GEF, TRAPP I, to COPII-coated ER-derived vesicles (see above) to assemble its accessory factors (p115, GM130, etc.) that tether the incoming vesicles to the Golgi membrane for SNARE-mediated fusion. More recent data demonstrate that golgins containing a GRIP domain, such as golgin-97, GCC88, and GCC185, contain binding sites for multiple Rabs (177, 393). The GRIP domain mediates an interaction with the Arf-like protein Arl1 to participate in trans-Golgi recruitment of the golgin (312, 468), unlike the above golgins normally found at the cis-Golgi. These golgins would therefore potentially serve as scaffolds to recruit traffic from multiple Rab-regulated pathways to the correct side of the Golgi. Although the significant players in the process have been identified, defining how they interact at a molecular level to regulate ER-to-Golgi and intra-Golgi traffic still requires more work.

Another coiled-coil tether protein is early endosome antigen 1 (EEA1), an effector of Rab5 that is involved in
tethering and fusion of early endosomes (285, 388, 406). As a dimer, EEA1 is thought to bridge endosomes through its FYVE domain, an evolutionarily conserved phosphatidylinositol 3-phosphate [PI(3)P] binding motif, and through its interaction with the SNARE protein syntaxin 6 to mediate homotypic endosomal fusion (55, 56, 176, 233, 273, 387). Therefore, similar to Rab1, Rab5 interacts with coiled-coil tethers to connect membranes and specific SNARE proteins that mediate fusion in their respective pathways.

2. Multisubunit tethers

In most cases, vesicle tethering is performed by multisubunit complexes. There are eight known complexes: TRAPP I and TRAPP II (ER-Golgi and intra-Golgi/endosome-late Golgi, respectively), the exocyst (Golgi-PM), the COG complex (endosome-Golgi, intra-Golgi), the Dsl complex (Golgier-ER), the HOPS complex (vacuole-vacuole and endosome-vacuole), the CORVET complex (endosome-Golgi), and the GARP/VFT complex (membrane protein recycling from endosome to Golgi). From recent structural data, an emerging theme is the structural similarity of several tethering complexes and their interface with components of the SNARE machinery as a mechanism of regulating fusion.

3. The TRAPP complexes

The TRAPP complexes are multisubunit tethers that regulate traffic at different parts of the Golgi (51). Unlike the tethers listed above, the TRAPP complexes are not recruited by a Rab but act as GEFs for Rab1, allowing it to interact with effectors to coordinate membrane traffic. In yeast, the TRAPP I complex functions in ER-to-Golgi traffic while the TRAPP II complex (that contains all TRAPP I subunits and an additional three subunits) regulates intra-Golgi and endosome-to-late Golgi traffic (53, 353). In mammalian cells, there appears to be only one TRAPP complex (271, 482). In addition to TRAPP I and TRAPP II, a recent discovery indicates there is a third TRAPP complex that is required for activating Ypt1 during autophagy in yeast (252). How does TRAPP act as a GEF and a tether? A recent discovery proved insightful: the TRAPP subunit Bet3 binds to the COP II subunit Sec23 (51, 482). Bet3 also has genetic interactions with Bet1, Sed5, and Sec22, all SNARE proteins that function in ER-to-Golgi traffic (347, 353). In studies of mammalian TRAPP, mBet3 is required for homotypic tethering of COP II-coated vesicles from vesiculotubular clusters, an intermediate compartment between the ER and Golgi (482). Following activation of Rab1/Ypt1, known effectors such as Uso1/p115 and giantin (see above) can tether these intermediate vesicles to the Golgi. TRAPP may perform its function in regulating intra-Golgi and endosome-to-late Golgi traffic through its interaction with the COP I coat (476).

4. The exocyst

The exocyst is an octameric complex that tethers secretory vesicles to the plasma membrane in preparation for fusion (286, 422). The vesicle-associated Rab Sec4 recruits the exocyst by interacting with one of its subunits, Sec15, as an effector protein (164). The exocyst is unique in that some of its subunits are also effectors of Rho proteins found on the plasma membrane. This arrangement presumably ensures efficient and accurate tethering to sites marked by these polarity determinants (165, 467, 489). Furthermore, the exocyst has both direct and indirect interactions with components of the SNARE machinery. The exocyst subunit Sec6 has been shown to interact with Sec9, a t-SNARE and SNAP25 homolog found at the plasma membrane (395), while Exo84 interacts with the SNARE regulatory protein SrO7 (491). Pull downs of the exocyst coprecipitate Sec1, a SM (Sec1/mUnc18) protein that binds to and promotes membrane fusion by assembled SNARE complexes (59, 161, 377, 465). It is unclear how exactly Sec4, Rho proteins, and SNAREs interact with the exocyst to control the fusion of secretory vesicles at the plasma membrane. However, some insight comes from recent crystal structures of Exo70, Exo84, and Sec6 from yeast, Sec15 from Drosophila, and mammalian Exo70 that reveal long, rod-shaped proteins composed of bundled α-helices (111, 281, 394, 469). These structures are consistent with quick-freeze/deep-etch micrographs of purified mammalian exocyst complexes that depict sets of “arms” ~10–30 nm long, consistent with the length of the Exo70 structure (190). Exocyst subunits, as rods, can potentially bundle together in a side-by-side fashion and perhaps in parallel to the two opposed vesicular and plasma membranes. This would bring together the SNARE proteins found on the opposing membranes, as well as SrO7 and Sec1 to regulate their assembly and function, and this process is controlled by the concurrent interactions of several exocyst subunits with Sec4 on vesicles and Rho proteins at the plasma membrane (286).

5. The COG complex

The conserved oligomeric Golgi (COG) complex is composed of eight subunits and regulates retrograde traffic within the Golgi as well as between the endosome and the Golgi (431). COG is an effector of Ypt1 and acts as a tether by interacting with the COPI coat, the SNARE protein Sed5, and the SM protein Sly1 (232, 300, 378, 415, 494). COG plays a role in recycling Golgi resident proteins, highlighted by the observation that mutations in subunits of the COG complex produce defects in glycosylation that lead to severe congenital disease phenotypes.
The crystal structure of the COG subunit COG4 revealed that a disease-causing mutation in the protein disrupts a COOH-terminal domain that is important for the role of COG complex in glycosylation (337). In addition to being structurally similar to the COG2 subunit, COG4 is remarkably similar in structure to Sec6 as well as the other solved structures of exocyst proteins (64). This observation is discussed further below.

6. GARP/VFT

The GARP/VFT complex is composed of four subunits (Vps51/52/53/54) that function in the recycling of membrane proteins from late endosomes to Golgi (81). In yeast, the GARP complex is recruited by Ypt6/Rab6 to the late Golgi and also associates with Tlg1, a Golgi SNARE protein (392). Subunits of the GARP/VFT complex have regions of sequence similarity to subunits of the COG and exocyst tethering complexes and furthermore share the functional similarities of interacting with Rabs and SNARE proteins (464). The recent crystal structures of the COOH-terminal fragments of Vps53 and Vps54 confirm that these two subunits of the GARP complex are structurally similar to subunits of the exocyst and COG complexes (327, 444). The structure of Vps54 revealed that the mutation responsible for the wobbler mouse phenotype, which leads to spinal muscular atrophy and serves as an animal model for amyotrophic lateral sclerosis, destabilizes Vps54 and results in reduced levels of the protein and of the GARP complex (327). Functionally, the GARP/VFT complex overlaps with the retromer in the transport of cargo between endosomal compartments and the Golgi despite the different components and Rab regulation. A potential link may be Rab6 interacting protein 1, a protein that interacts with Rab6, Rab11, and the retromer (276, 457).

7. The Dsl complex: similarities in the structure of tethering complexes

The Dsl complex, composed of Dsl1, Tip20, and Sec39, regulates retrograde traffic from the Golgi to the ER. It does so by interacting with COPI-coated vesicles (Dsl1 interacts with the subunits of the COPI coat) originating from the Golgi and stabilizing the assembly of SNARE proteins required for this pathway (12, 13, 226, 443). No known Rab has been shown to participate in this process. However, the crystal structures of Dsl1 and Tip20 show both proteins to be structurally similar to COG4 and COG2 of the COG complex, Vps53 and Vps54 of the GARP complex, as well as subunits of the exocyst despite little, if any, sequence similarity (327, 337, 428, 444). All of these complexes interact directly with SNARE proteins: 1) Tip20 and Sec39 of the Dsl complex interact with the SNAREs Sec20 and Use1, respectively; 2) the COG complex interacts with multiple v- and t-SNAREs found at the Golgi; 3) GARP complex interacts with the SNARE Tlg1 at the Golgi and Vps53 and Vps54 interact with the SNARE syntaxin 6, syntaxin 16, and Vam4; and 4) the exocyst subunit Sec6 interacts with the SNAP-25 homolog Sec9 (222, 226, 328, 378, 392, 395, 415, 416, 428, 441, 442). How these common structural features contribute to the tethering process and SNARE function are undoubtedly a critical focus of future research.

8. HOPS and CORVET

The HOPS and CORVET complexes regulate traffic at the level of the endosome and the lysosome/vacuole and share certain subunits in common (369). The core of both complexes is composed of the class C Vps proteins (Vps11, Vps18, Vps16, and Vps33), first identified in yeast through the isolation of mutants that produce no identifiable vacuoles (22, 23, 342, 348, 349). The HOPS (homo-2

typic fusion and vacuole protein sorting) complex, in addition to the class C Vps proteins, also contains the subunits Vps39 and Vps41 that impart Ypt7 effector and GEF function to the HOPS complex. The HOPS complex and Ypt7 are required for efficient and accurate homotypic fusion of vacuolar membranes (79, 119, 325, 369, 402, 474). Vps41 interacts directly with Ypt7 to allow the HOPS complex to be a Ypt7 effector (41, 306). The HOPS complex is able to perform its tethering function through its interaction with phosphoinositides and the SNARE protein Vam7 found on vacuolar membranes (411). Furthermore, the class C protein Vps33 is a member of the SM family of proteins that regulates SNARE-mediated membrane fusion by binding to trans-SNARE complexes (169, 411a). The more recently discovered CORVET (class C core vacuole/endosome tethering) complex contains Vps3 and Vps8, instead of Vps39 and Vps41 found in the HOPS complex, and is an effector of Vps21, the yeast homolog of Rab5 (315). Both Vps3 and Vps8 are members of the Vps class D proteins, identified through the isolation of mutants with enlarged vacuoles, and are implicated in sorting of proteins to endosomes (71, 186). Vps21 is also a class D protein, and these data suggest that the CORVET complex is involved in recycling factors from late endosomal compartments marked by Rab7/Ypt7 (and interacting with HOPS) to those containing Vps21/Rab5. Thus the interchangeable nature of the HOPS and CORVET complexes facilitates regulation of traffic in both directions between endosomes and the vacuole/lysosome through their interaction with Rabs that define specific compartments in the pathway (81, 276, 392, 457, 464).

E. Rabs and Membrane Fusion

In addition to recruiting tethers that ensure the proper association of cargo and target membranes, Rab proteins also regulate the SNARE-dependent fusion of
transport and target membranes. Rabs can either interact directly with SNARE proteins or with proteins that regulate SNARE function, such as SM or Lgl proteins, to perform this regulatory function.

1. Sec4 and Sro7

The Rab Sec4 is a yeast homolog of Rab8 and regulates the final stage of the secretory pathway in yeast. A recently discovered effector of Sec4 is Sro7, a member of the lethal giant larvae (lgl) family of proteins that interacts with Sec9 and regulates SNARE function (159). Several mutations that disrupt the secretory pathway can be rescued by overexpression of Sec4, and this mechanism of rescue requires the function of Sro7 (159, 465).

2. Rab5 interacts with rabenosyn-5 and EEA1

Rab5 is found on early endosomes and plays a critical role in targeting endosomal traffic towards lysosomes through the function of its numerous effectors. EEA1 and rabenosyn-5 are Rab5 effectors that interact with the SNARE protein syntaxin-6 and the SM protein VPS45, respectively (296, 387). Both EEA1 and rabenosyn-5 also possess a FYVE domain that binds to the phosphoinositide PI(3)P, which is normally found on early endosomal membranes (103, 233, 296). PI(3)P is enriched on early endosomal membranes through the action of the PI 3-OH kinase Vps34 and PI(4)- and PI(5)-phosphatases, all of them being effectors of Rab5 (77, 379). Recruitment of effectors using this dual mechanism is physiologically important because in the absence of Vps34 function, recruitment of both EEA1 and rabenosyn-5 is prevented and fusion of early endosomes is blocked (273, 274, 296, 388).

VI. Rab CASCADES: TRANSITIONING FROM ONE Rab TO ANOTHER

As membrane flows from one organelle to another, it must transition through different Rab-defined compartments. To what extent the compartment defines the Rab, or vice versa, has been an open question, which has been framed primarily by studies of specific pathways, the Rab proteins that are involved, and how they are each activated and inactivated to generate a programmed transition from one Rab to the next. How does this process occur? What mechanisms ensure the directionality of the switch and that the compartment is ready to receive the next Rab and its set of effectors? In several specific cases, recent evidence supports a maturation model whereby the compartment transitions from an upstream Rab to a downstream Rab by recruiting as effectors the GAP and GEF for the upstream and downstream Rabs, respectively (Fig. 4). The countercurrent cascades of GAPs and GEFs not only ensure that the appropriate downstream Rab is recruited but that the upstream Rab is concomitantly inactivated to delineate one compartment from another.

A. A Transition From Ypt31/32 to Sec4

Ypt31/32, two yeast paralogs homologous to Rab11, are found on late Golgi compartments destined to generate the secretory vesicles, marked with Sec4 (homolog of Rab8), that will go on to fuse with the plasma membrane (30, 154, 208, 356). To initiate this Rab switch, Ypt31/32, in its GTP-bound state, recruits the Sec4 GEF, Sec2. By activating Sec4, Sec2 promotes the stable association of Sec4 with secretory vesicles that ensures their delivery to and fusion with the plasma membrane (304). In this manner, Sec4 is assured of its involvement in the correct pathway because its association with secretory vesicles is dependent on the Rab directly upstream of it. Furthermore, Sec2 also associates with Sec15, a component of the exocyst and an effector of Sec4 as an additional mechanism to recruit Sec4 to secretory vesicles (164, 269, 357). A similar mechanism is in play for Rabex5, the GEF for Rab5. Rabex5 interacts with Rabaptin5, an effector of Rab5, to ensure proper spatiotemporal activation of Rab5 (188).

A somewhat related example of an effector playing a role in targeting a Rab to a specific membrane involves the interaction of Rab9 with its effector TIP47. Several chimeras of Rab1 or Rab5 with the hypervariable region of Rab9 (that interacts with TIP47) can be relocated from the Golgi (normal Rab1 localization) or the early endosome (normal Rab5 localization) to the late endosome (normal Rab9 localization) upon overexpression of TIP47 (1). This result indicates the importance of the Rab-effec-
tor interaction in determining the proper localization of the Rab of interest.

B. From Rab5 to Rab7

Endocytic cargo is initially found in Rab5-containing early endosomal compartments that can undergo maturation to become Rab7-containing late endosomal compartments targeted for lysosomes (340). In a Rab GEF cascade similar to that described above, among the effectors of Rab5 is the HOPS complex (potentially through an interaction with the HOPS subunit Vps41), which contains as one of its subunits the Vps39 protein, a GEF for Rab7 (50, 339, 474). Additionally, the HOPS complex is also an effector of Rab7 (369). Thus Rab5-mediated recruitment of the HOPS complex in turn promotes the association of Rab7 with this membrane thereby initiating maturation towards the lysosome/vacuole. This process of Rab conversion has been visualized in mammalian cells and appears to progress in several steps: 1) a dynamically fluctuating association of Rab5 with early endosomes, 2) an association of Rab5 with progressively fewer and larger endosomal compartments (that form through homotypic fusion) that move from the cell periphery towards the cell center, 3) a transient overlap with Rab7 that is dependent on the HOPS complex, and 4) a rapid conversion to a Rab7 compartment destined for the lysosome. These data suggest a maturation model whereby each transport compartment gains the necessary factors to move forward along the pathway while losing those that define the previous compartment (340). Additional support for the maturation model comes from elegant studies of the Golgi in Saccharomyces cerevisiae. Both studies show specific Golgi cisternae transitioning from being marked with an early Golgi marker to a late Golgi marker at a rate consistent with that seen for cargo transitioning through the secretory pathway (251, 266).

In the early endocytic pathway, the early endosome serves as a hub for traffic directed in several different directions through the action of various Rabs. Rab5 can recruit the HOPS complex that mediates a conversion to a Rab7-positive compartment and directs traffic towards the lysosome/vacuole. Another Rab5 effector, rabenosyn-5, has a binding site for and is an effector of Rab4 that is involved in targeting proteins to the Rab11-positive recycling endosome (97). Overexpression of rabenosyn-5 leads to prolonged overlap of Rab5 and Rab4 and shows how a divalent effector can influence Rab conversion and target traffic appropriately from a compartment that serves multiple pathways.

C. A Rab GAP Cascade

The GEF cascades above describe how a Rab conversion can be initiated. However, to avoid an extended period of overlap of Rab domains within a compartment, it is also important to inactivate the upstream Rab once the downstream Rab has been recruited and activated. GAPs are the primary players in this process. For example, the GAP for Rab5 (RabGAP-5) has been shown to play a role in regulating endosomal traffic; either overexpression or loss of RabGAP-5 in HeLa cells blocked trafficking of substrates from early endosomes to the lysosome (167). Therefore, to counterbalance the activating GEF cascade, recent evidence supports a countercurrent GAP cascade whereby the downstream Rab recruits the GAP that inactivates the upstream Rab. In yeast, compartments marked with Ypt1 at the early Golgi mature to contain Ypt32 at the late Golgi. A key step in this process is the recruitment of Gyp1, the GAP for Ypt1, by Ypt32 to inactivate Ypt1 and promote its removal from membranes (341). Loss of Gyp1, which is normally found at the Golgi as an effector of Ypt32, results in the prolonged overlap of Ypt1 and Ypt32 in a Golgi compartment. Ypt1 had previously been shown to recruit the GEF for Ypt32, but the identity of this GEF remains unclear (451).

VII. Rabs AND CANCER

The role of Ras, Ral, and Rho GTPases in oncogenesis is well-documented. However, several Rabs have also been implicated in the progression of multiple cancers (75) as membrane traffic plays a significant role in cancer biology, primarily in the loss of cell polarity and in the metastatic transformation of tumor cells (282). This includes the upregulation of Rab5 in malignant and metastatic human lung cell adenocarcinoma, Rab1 in tongue squamous cell carcinomas, and Rab3 in cancers of the nervous system (447). Rab5 is an appropriate target due to its role in receptor-mediated endocytosis. Modulating Rab5 function can significantly alter signaling from growth factors to promote tumorigenesis, and both up- and downregulation of Rab5a is associated with cancer in different tissues (90, 139, 241). The best characterized example of a Rab implicated in cancer is Rab25, a Rab closely related to Rab11 that regulates apical endocytosis and transcytosis in epithelial cells (61, 151, 453). Rab25 is upregulated in certain ovarian and breast cancers due to amplification of a chromosomal region containing the Rab25 gene. The resulting overexpression of Rab25 is associated with more aggressive forms of the associated cancer and a lower patient survival rate (73). Recent studies have demonstrated an interaction between Rab25 and the β1-integrin subunit, and this interaction is required for promoting invasiveness of tumor cells into a three-dimensional extracellular environment (63). Rab25 appears to retain a pool of αvβ1-integrin heterodimers at the tip of the invasive pseudopod to facilitate efficient integrin recycling and maintain a stable association of the
pseudopod with the extracellular environment. Therefore, Rab25 does not play a role in tumor initiation but facilitates its progression by allowing it to be invasive.

VIII. Rab8 AND NEUROLOGICAL DISEASE

Recent discoveries implicate Rab8 in several prevalent neurological diseases. Neurons may be more sensitive to perturbations in membrane traffic because of their unique polarized structure and function. Specialized functions of Rab8 are critically important for synaptic function (Rab3), neurite growth and remodeling (Rab11 and Rab13), and general nervous system development (Rab23) (211). The section below highlights the connection between Parkinson’s disease and Rab1, Huntington’s disease with Rab8 in post-Golgi trafficking, and neuropathies due to activating mutations of Rab7.

A. Parkinson’s Disease

Parkinson’s disease (PD) is the most prevalent neurological disorder of movement due primarily to loss of dopaminergic nerve cells in the substantia nigra (126). The gene encoding the α-synuclein protein, when mutated, causes an autosomally dominant inherited form of PD with several identified missense point mutations (141). α-Synuclein (αsyn) is a major constituent of Lewy bodies, an intracellular protein aggregate found in neurons that are the hallmark pathological feature of PD, one of several neurological diseases commonly referred to as synucleinopathies (399, 400). The connection between Rab8 and Parkinson’s disease first came from studies of one of the αsyn point mutations, A30P, expressed in a transgenic mouse model. Rab3a, Rab5, and Rab8 interacted with αsyn in brain extracts from mutant mice and not those containing the wild-type control αsyn (93). In addition to point mutations in αsyn, additional copies of the gene can also lead to PD (196, 390). When overexpressed in yeast, α-synuclein disrupts ER-to-Golgi transport, and this phenotype can be rescued by overexpression of Ypt1, the yeast Rab1 homolog (82). Animal models of PD and mammalian dopaminergic cells also showed reduced α-synuclein-induced toxicity when overexpressing Rab1. Further studies implicated Rab3 and Rab8, suggesting that α-synuclein may affect several membrane traffic pathways (149).

B. Huntington’s Disease

Huntington’s disease (HD) is an autosomal inherited neurological disorder caused by expansion of a trinucleotide repeat in the gene encoding huntingtin (htt) protein (150). The resulting mutation produces an NH2-terminal polyglutamine repeat, and the length of the expansion, and subsequently the polyQ repeat, correlates inversely with age of onset (146, 185). It is unclear how the polyQ repeat produces a disease state, but htt is normally associated with membranes and plays a role in membrane traffic (109, 446). A recent study shows that mutant htt disrupts clathrin-dependent post-Golgi traffic targeted for lysosomes (100). Mutant htt prevents the association of Rab8 with optineurin at the Golgi and results in reduced AP-1- and clathrin-mediated traffic to lysosomes. Htt interacts with the optineurin protein and FIP-2 that are both effectors of Rab8 at the Golgi (121, 175, 355). Rab8 and FIP-2 recruit htt to the Golgi, and the interaction of optineurin with myosin VI is important for maintaining Golgi structure (355). However, it is not known what role htt normally plays in its association with Rab8, FIP-2, and optineurin at the Golgi. In addition to its interaction with Rab8, based on studies of a mouse model for HD, Htt may interact with a GEF for Rab11 (238, 239). Membrane fractions from mutant mouse brains did not catalyze nucleotide exchange on Rab11, and a Rab11 dominant-negative mutant expressed in normal adult brains led to neurodegeneration similar to the HD mutant mouse model. Very recent data show delayed recycling of transferrin to the plasma membrane and impaired Rab11-dependent vesicle formation from recycling endosomes in fibroblasts from Huntington patients compared with healthy individuals (240). Both Rab8 and Rab11 localize to the recycling endosome (RE) and target proteins for the plasma membrane, although it is unclear how the two Rabs are differentiated in function (15, 16, 429, 487). It will be interesting to see how the interplay between Rab8, Rab11, and htt become altered during the onset of HD and how that contributes to the pathophysiology of the disease.

C. Carpenter Syndrome and Rab23, Charcot-Marie-Tooth Disease and Rab7

Carpenter syndrome is an autosomal recessive disorder with symptoms that include skull abnormalities, polydactyly, brachydactyly (shortness of fingers and toes), obesity, congenital heart disease, and mental retardation (183). Mutations in Rab23 have been identified as the causative agent of the disease, and surprisingly, the associated phenotypes differ quite dramatically from the mouse Rab23 open brain (opb) mutant that is embryonically lethal (117, 166, 211). Rab23 acts as a negative regulator of sonic hedgehog (shh) signaling during dorsal-ventral axis formation of the neural tube. By activating Rab23, dorsal neural cells can prevent shh signaling that is required for ventral cells of the spinal cord (117). Rab23 signaling through shh is more than likely the cause of symptoms seen in Carpenter syndrome as mutations in
shh signaling components also produce phenotypes such as polydactyly and brachydactyly (211). However, Rab23 was first cloned as a Rab predominantly expressed in the mouse brain (302), and although there are potential similarities, Carpenter syndrome phenotypes are more pleiotropic than those seen for opb mice (211). The Rab23 mutations that cause Carpenter syndrome may have uncovered novel signaling pathways involving shh that will require further attention to characterize these connections.

Rab7 is a critical regulatory component that directs traffic in the endosomal pathway to the lysosome (44, 66). Point mutations in Rab7 lead to Charcot-Marie-Tooth disease type 2B, an inherited motor and sensory neurological disorder characterized primarily by distal muscle weakness and atrophy (24, 189, 270, 447). Biochemical analysis indicates that Rab7 carrying any of the identified point mutations is preferentially GTP bound and has a slower rate of GTP hydrolysis (96, 401). Therefore, Rab7 in a prolonged “on” state may be the cause of the disease. It is interesting to note that mutations in an endocytosis-related gene, dynamin 2, that impair clathrin-mediated endocytosis also produce Charcot-Marie-Tooth disease phenotypes (33, 122, 497).

IX. MICROORGANISMS, Rabs, AND DISEASE

The above examples show how Rab-regulated pathways can be perturbed to cause disease. In a related manner, Rabs and their effectors have become targets for infectious microorganisms that have developed mechanisms to evade host defenses by hiding and replicating in an intracellular environment. To avoid the host cell degradation machinery and obtain nutrients and building blocks to multiply, such organisms manipulate several different Rabs to their advantage. The majority of intracellular pathogens hijack Rabs involved in the endocytic pathway, while the causative agent of Legionnaire’s disease uses a bifunctional protein to capture Rab1.

A. Salmonella enterica and Chlamydia pneumonia

Salmonella enterica and Serovar typhimurium, the cause of gastroenteritis commonly referred to as salmonellosis, are initially taken up by epithelial cells that line the gut. They reside in Salmonella-containing vacuoles (SCVs) in the cell that transition from a Rab5- to a Rab7-containing compartment (21, 173, 288, 403, 404). Rab7 effectors position the compartment at a perinuclear location close to the Golgi (39, 181). Acidification of the compartment causes release of Salmonella virulence factors that act to block the compartment from fusing with the lysosome, anchor the SCV to the Golgi, and recruit traffic from the Golgi (21, 43, 320, 403). For example, the Salmonella SopB protein, a PI phosphatase, recruits sorting nexin 1 (Snx1) to the SCV membrane for retrotranslocation of mannose-6-phosphate receptors from its membrane (46, 298). Mannose-6-phosphate receptors are integral membrane proteins that sort acid hydrolases from the Golgi to the vacuole/lysosome and are then recycled back to the Golgi through the action of the retromer complex (36, 37). The SopB protein, therefore, prevents maturation by enhancing recycling of unwanted lysosomal proteins from the SCV. The Salmonella Pip2B and SifA interact with the host SKIP (SifA and kinesin interacting protein) to prevent kinesin-powered movement of SCVs away from their perinuclear localization (39, 181, 205). The SCVs accumulate a variety of Rab proteins on their membranes but not those indicative of phagosomes undergoing a normal maturation process towards lysosomes (43). It is unclear how SCVs bypass this process. The SCVs also extend membranous fingers called Salmonella-induced filaments, or Sifs, that hijack traffic between endosomes and the Golgi through the recruitment of Rab9 by SKIP (205).

Chlamydia does not take advantage of the endosomal/lysosomal pathway but, like Salmonella, releases proteins to avoid being directed to the lysosome. Once inside the cell in a structure known as an inclusion, it releases effector proteins termed “integral inclusion membrane” (Inc) proteins that prevent recruitment of Rab5, Rab7, and Rab9 and recruit exocytic and Golgi-bound traffic marked by Rabs such as Rab4, Rab11, and Rab1 (320, 351, 352, 434). A key component is the Chlamydia Inc protein Cpn0585 that has similar features to Golgin proteins and interacts with Rab1, Rab10 and Rab11 (86).

B. Legionella pneumophila

Although Legionella disrupt the endosomal/lysosomal pathway, a recent discovery places it between the ER and Golgi. The SidM/DrrA protein from Legionella pneumophila, the cause of the pneumonia known as Legionnaire’s disease, is a bifunctional protein that was first characterized as both a GDF and a GEF for Rab1 (256, 257, 287). The co-crystal structure of SidM/DrrA with Rab1 indicated that the GDF activity is mediated by the region of SidM/DrrA that mediates GEF activity on Rab1. The high affinity of SidM/DrrA for GDP-bound Rab1 may account for the GDF activity demonstrated by SidM/DrrA (412). The NH2-terminal domain of SidM/DrrA mediates adenosine monophosphorylation (AMPylation) of the switch II region of Rab1, and GTP-bound Rab1 is the preferred substrate for SidM/DrrA-mediated AMPylation (289). The AMPylation activity of SidM/DrrA causes cytotoxicity in mammalian cells and reduces the interaction of Rab1 with the host effector protein MICAL-3 but not the bacterially encoded effector LdA (289). Through the

**X. CONCLUSIONS**

The volume of information describing Rab function in membrane traffic has grown dramatically in recent years. In addition to identifying the many Rab proteins, defining their subcellular localizations, and isolating their regulators and effectors, we are beginning to understand how Rabs communicate with each other to specify where their respective territories begin and end. Although we have provided a few examples of how one Rab domain might transition to another, it is presently unclear if these mechanisms are universally applicable to all Rab-regulated pathways. If not, how do these other Rabs determine the pathways that they regulate? The mechanism of Rab conversion, described above, relies on their associated GAPs and GEFs. However, do GDFs also play a role in this process? Does each pathway have a specific GDF, or are they shared among sets of pathways? How is this sharing regulated? We may have identified the major factors that regulate Rab function, but establishing how they are coordinated to achieve a common goal will require further analysis.

While several Rabs have been very intensively studied, a large fraction of the Rab proteins expressed in mammalian cells have not, and relatively little is known regarding their function and regulation. A recent study indicated that 42 Rab GTPases are expressed in COS7 cells, with the abundant Rabs being those that regulate endocytosis, secretion, and traffic to, from, and within the Golgi (295). Are these uncharacterized Rabs simply redundant with the better-known members of their branch of the Rab family or have they acquired unique functions? Do these Rabs serve tissue-specific roles? Will the same mechanisms act to control their function? How do they interact with the other Rabs found inside the cell? To understand the forces underlying the dramatic expansion of the Rab family during evolution, we must begin by describing the function of each Rab in greater detail. Knockouts and knockdowns of the less-studied Rabs, both singly and in a combinatorial fashion, will help to reveal the common and unique functions of each Rab. In vitro assays using donor and target membranes and all identified factors are now a realistic goal for many Rabs. In addition to describing Rab function at a molecular level, assays such as these can be used to identify and analyze novel factors that affect the pathway of interest.

Rabs are involved in the pathogenesis of a wide range of diseases but exactly what role they play in some of these disorders is still unclear. Analyzing the role of Rabs in the pathogenesis of Parkinson’s or Huntington’s disease provides a unique angle to approach the study of these diseases. Recent discoveries of the interaction of Rab35 with the actin bundling protein fascin to regulate intracellular actin assembly (488) or the function of Rab23 in brain and chondrocyte (477) development highlight the diverse roles of Rab proteins. Their involvement in signaling pathways outside of their stereotypical role in membrane traffic only magnifies our need to investigate in greater detail how Rabs work.

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**REFERENCES**


Rab GTPases in Membrane Traffic and Disease


328. Pfeffer S. 329. Pereira-Leal J, Seabra M.


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410. Sönnichsen B, Lowe M, Levine T, Jämsä E, Dirac-Svejstrup B, Sorkin A. Endosomal GTPases IN MEMBRANE TRAFFIC AND DISEASE 147


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