Traffic Control: Rab GTPases and the Regulation of Interorganellar Transport

Michael J. Tuvim, Roberto Adachi, Simon Hoffenberg, and Burton F. Dickey

Membrane proteins, membrane lipids, and luminal contents are exchanged among the intracellular compartments of eukaryotic cells by vesicular transport. This process must be highly ordered to maintain cellular architecture in the face of rapid membrane turnover. The Ras-related Rab GTPases play multiple roles in regulating this traffic.

Viewed through a microscope, the architecture of a eukaryotic cell suggests the internal partitions of a complex building. Unlike inert walls, however, cellular membranes are highly dynamic structures. For example, the entire surface area of the plasma membrane turns over every hour in a typical cell. This turnover permits changes in the protein and lipid composition of cellular membranes and mediates transfer of soluble macromolecules among intracellular organelles. Multicellular organisms make extensive use of traffic to and from the plasma membrane to regulate systems physiology. For example, hormones are secreted by exocytosis in the afferent limb of endocrine signaling, glucose and water transporters are reversibly inserted into the plasma membrane in response to endocrine signaling, and adhesion molecules are translocated to the cell surface to mediate intercellular attachment.

How eukaryotic cells maintain their structure in the face of this heavy traffic is a classic problem of cellular biology that is rapidly yielding its secrets; the identities of numerous components of the molecular machines that mediate vesicular transport are known, high resolution structures of several components are available, and the molecular physiology of these machines is now coming into focus. Rab GTPases are ubiquitous components of vesicle trafficking machines, with different Rab proteins regulating traffic between different intracellular compartments (Fig. 1). Here we review the current understanding of the structure and function of Rab proteins in relation to other components of trafficking machines.

Discovery of Rab proteins

GTPases were among the first proteins implicated in vesicular transport processes when introduction of a hydrolysis-resistant GTP analog into the interior of permeabilized mast cells resulted in massive exocytosis (4). The precise molecular target of GTP in this experiment remains uncertain, since several GTPases are now known to participate in vesicle traffic (see below), but Rab family GTPases were definitively implicated in vesicle traffic in the budding yeast Saccharomyces cerevisiae when open reading frames that included consensus GTP-binding motifs were identified both serendipitously and by a directed genetic strategy to uncover trafficking proteins (6, 7). Mammalian counterparts of these yeast genes were soon identified and shown to be functional homologues capable of replacing the essential functions of Rab genes in yeast. The yeast genome is now known to encode 11 Rab GTPases, whereas mammalian genomes encode ~50. Each distinct step of intercompartmental traffic appears to be regulated by one or more Rab proteins (Fig. 1), with the greater subcellular complexity of higher eukaryotes being reflected in a larger complement of Rab proteins.

The conserved mechanism of vesicle traffic

Insights obtained from powerful genetic and biochemical approaches to functional analysis are providing a mechanistic description of vesicle traffic at the molecular level. For purposes of analysis, intercompartmental vesicular transport can be broken into several distinct stages (Fig. 2). Typically, transport vesicles are formed by localized deformation of the proteolipid membrane of a donor compartment induced by a protein coat (e.g., clathrin). Soluble cargo proteins and integral membrane proteins destined for export from the donor compartment are concentrated in the region of the nascent transport vesicle, along with membrane-bound and cytoplasmic components of the trafficking machinery. After budding of the fully formed transport vesicle, the protein coat dissociates and the vesicle travels to a target compartment, either by diffusion or by directed transport along cytoskeletal elements depending on the particular step. The transport vesicle initially becomes loosely tethered to the target membrane, then firmly docked, and finally fuses with the target membrane. Variations exist on this general scheme (for example, endocytic vesicles fuse homotypically to yield early endosomes, and regulated exocytic vesicles do not fuse until receipt of an external signal), but the underlying mechanism is widely conserved.

A large number of proteins collaborates to mediate traffic at each distinct step of interorganellar transport. In these complex machines, some components are essential mediators, whereas others regulate those core elements. There is now strong evidence that the soluble N-ethylmaleimide-sensitive factor (NSF) attachment receptor (SNARE) proteins form the essential fusion
mechanism; when reconstituted in artificial proteoliposomes, they are capable of mediating fusion (9), and disruption of their expression in model organisms results in profound trafficking defects (5, 14). SNAREs are membrane-associated proteins localized either on transport vesicles (v-SNAREs) or target membranes (t-SNAREs) that contain large, hydrophobic coiled-coil domains. The energetically favorable process of forming a parallel four-helix bundle with the coiled-coil domains of paired v-SNAREs and t-SNAREs appears to drive membrane fusion. The central role of SNARE proteins is highlighted by the fact that they are targets for multiple clostridial neurotoxins that block synaptic vesicle release (5). Other ubiquitous components of trafficking machines, such as the Sec1/Munc18 family, directly interact with and regulate the function of the core SNARE components. Still other proteins bring the core components together before fusion (see below) or separate them after fusion in an energy-dependent process (e.g., NSF and soluble NSF attachment proteins). Examination of the same step of traffic across species from yeast to humans reveals the participation of closely related orthologs, whereas comparison of different trafficking steps within a single species reveals the participation of more distantly related paralogs. Together, this structural conservation indicates the retention of a fundamental mechanism throughout the evolution of interorganellar traffic in eukaryotic organisms.

**Rab proteins in vesicle tethering**

Despite their discovery more than 15 years ago, the precise function of Rab proteins long remained elusive. It has been tempting to assume that different Rab proteins perform the same role at distinct steps of traffic, and the accumulation of transport vesicles in yeast with loss-of-function Rab mutations suggests that they function after vesicle budding (7). Recently, the use of cell-free assays that permit resolution of distinct substeps of vesicle traffic (Fig. 2) has enabled localization of the principal site of Rab action to the tethering step (6, 13). This step can be operationally defined as resistance of a cell-free trafficking assay to dilution or differential centrifugation but is distinguished from tight docking that is insensitive to clostridial neurotoxins and other proteases (3, 15). Tethering involves specific interactions among proteins in the transport vesicle, the target membrane, and the cytoplasm. Tethering complexes generally appear to be large, elongated multimeric assemblies, and several include phosphatidylinositol-binding proteins. Rab proteins have been shown to directly interact with tethering proteins and to promote their function (1, 3, 8, 15). After the initial tethering reaction, tight docking of the transport vesicle to the target membrane is mediated by the *trans* pairing of SNARE partners in the vesicle and target membrane, and Rab proteins promote *trans*-SNARE pairing through both tethering proteins and other effectors. Recent evidence indicates that the Rab and tethering proteins also transiently interact during vesicle budding to recruit SNAREs in *cis* onto a transport vesicle (1, 3, 15). Thus Rab proteins may act sequentially through the same tethering effectors both to load specific SNARE proteins onto transport vesicles during budding and then to pair them in *trans* during docking and fusion.

It is of interest that there is no apparent homology among tethering complexes from distinct steps of vesicle traffic (6). This stands in contrast to the homology among Rab family members, SNAREs, and Sec1 family proteins and suggests that tethering proteins play a major role in imparting specificity to trafficking reactions, in addition to their catalytic role.

The promotion of transport reactions by addition of purified Rab proteins in vitro or by overexpression of Rab pro-
Structural biology of Rab proteins and the stable docking of synaptic vesicles before fusion. Due to the high concentrations of SNARE proteins at synapses, these may not be representative of most steps of membrane traffic at the active zone (11). However, synaptic vesicle release has been shown to interact with cytoskeletal elements (dotted lines). In tethering, complexes of large, elongated proteins ( ) loosely attach the transport vesicle to the acceptor membrane under regulation by a Rab protein. In docking, v-SNAREs and target membrane SNAREs (t-SNAREs) form a parallel 4-helix bundle; in exocytosis, two of the helices are contributed by a t-SNARE of the SNAP-25 family, whereas in other steps of traffic all 3 t-SNARE helices are contributed by separate proteins. In fusion, completion of the energetically favorable SNARE-coiling process drives membrane fusion.

FIGURE 2. Substeps of vesicle traffic. In budding, a nascent vesicle forms on a donor membrane and includes trafficking proteins such as Rab GTPases (●) and vesicle-associated SNAREs (v-SNARES; —) in addition to cargo molecules. In transport, the budded vesicle travels through the cytoplasm by diffusion or directed transport, and in some cases Rab effector proteins (○) have been shown to interact with cytoskeletal elements (dotted lines). In tethering, complexes of large, elongated proteins ( ) loosely attach the transport vesicle to the acceptor membrane under regulation by a Rab protein. In docking, v-SNAREs and target membrane SNAREs (t-SNAREs) form a parallel 4-helix bundle; in exocytosis, two of the helices are contributed by a t-SNARE of the SNAP-25 family, whereas in other steps of traffic all 3 t-SNARE helices are contributed by separate proteins. In fusion, completion of the energetically favorable SNARE-coiling process drives membrane fusion.

Proteins in vivo indicate that Rab action is limiting for at least some steps of transport (2, 8). Most of the eleven Rab proteins of Saccharomyces are essential, with the nonessential Rabs mediating nonessential steps of traffic (e.g., transport to the vacuole, equivalent to the mammalian lysosome) or with more than one closely related Rab protein acting redundantly in the same step of traffic (7). The only mammalian Rab gene deletion reported to date (Rab3A) has a mild defect in neurotransmission, and deletion of the Caenorhabditis elegans homolog resulted only in a small behavioral change and moderate decrease in the number of synaptic vesicles docked at the active zone (11). However, synaptic vesicle release may not be representative of most steps of membrane traffic due to the high concentrations of SNARE proteins at synapses and the stable docking of synaptic vesicles before fusion.

Structure of Rab proteins

The mammalian Rab family comprises ~50 members that can be identified in a database search of the human genome. The primary structure of these 24- to 25-kDa proteins contains several well known guanine nucleotide-binding motifs shared with other GTPases such as elongation factor-Tu, Ras, and trimeric G proteins (Fig. 3). In addition, several other short sequence motifs are shared exclusively among Rab proteins (10, 12). Amino acid identity among Rab family members ranges from 35 to >80%; for comparison, identity between Rab proteins and Ras, a small GTPase that regulates growth, is only ~30%. Rab proteins with >75% sequence identity are typically assigned the same number, followed by a unique letter (e.g., Rab5A, B, or C). Such closely related Rab proteins may be considered functional isoforms because they localize to the same intracellular organelles and show evidence of at least some functional redundancy. Rab proteins have been highly conserved through evolution, with 55-75% identity between orthologs from yeast and mammals compared with 40-50% identity between SNARE orthologs.

Most Rab proteins are tightly associated with membranes through the posttranslational addition of two geranylgeranyl (20-carbon polyisoprenoid) groups to two cysteines near the COOH terminus, although a few Rab proteins have only one geranylgeranyl group. Rab3A with its COOH terminus deleted has been crystallized both alone and in complex with a large domain of one of its effector proteins, Rabphilin-3 (5). The tertiary structure closely resembles that of other GTPases, with a central barrel composed of a six-stranded β-sheet surrounded by α-helices (Fig. 3). Extensive analysis of other GTPases has defined two regions, termed switches I and II, that are located near the phosphate region of the bound guanine nucleotide and undergo dramatic conformational change on nucleotide exchange. These regions are involved in protein-protein contact and account for the nucleotide dependency of most GTPase interactions. In addition, a third region of protein-protein contact formed by three loops was identified in the Rab3A/ Rabphilin-3 crystal structure and termed the complementarity-determining region (CDR). The residues that comprise the CDR pocket are conserved among Rab isoforms (e.g., RabA–D) but differ among distantly related Rabs. Conversely, multiple Rab3 effectors share a motif (SGAWFF) that interacts with the Rab3 CDR, but this motif is not present in effectors of other Rab proteins.

Cycles within cycles: membrane association and GTP hydrolysis

Vesicle traffic is a quantal process, with repeated rounds of transport maintaining a steady flow of membrane into and out of subcellular compartments. Some of the proteins mediating this traffic, including Rabs, are physically translocated from donor to target compartment (Fig. 4). These must then recycle back to begin another duty cycle. Despite their tight membrane association mediated by COOH terminal prenylation, Rab proteins are recycled through the cytoplasm. This occurs in complex with a chaperone, named guanine nucleotide dissociation inhibitor (GDI), that both extracts GDP-bound Rab proteins from target membranes and retards dissociation of the bound GDP (5, 13). When a Rab protein complexed with

FIGURE 3. The Rab protein family. Rab proteins share a motif (SGAWFF) that interacts with the Rab3 CDR, but this motif is not present in effectors of other Rab proteins.
GDI encounters its cognate donor compartment, the dissociation of GDI is catalyzed by a GDI displacement factor. The membrane-associated Rab protein with GDP bound can then interact with a guanine nucleotide dissociation stimulator (GDS) that promotes dissociation of GDP (13). Since the intracellular concentration of GTP is higher than GDP, the empty Rab protein generally binds GTP and thereby becomes resistant to extraction by GDI. There is evidence that association of a GTP-bound Rab protein is a precondition of vesicle budding, followed by recruitment of a tethering protein that in turn recruits SNAREs and other trafficking proteins (1, 3, 13). This appears to be followed by GTP hydrolysis since the Rab protein does not remain associated with the tethering protein and SNAREs on the transport vesicle.

The transport vesicle with Rab protein on its surface travels to the target compartment, where tethering and tight docking take place. Additional rounds of nucleotide exchange and hydrolysis occur at the target membrane, and then following fusion the GDP-bound Rab protein is extracted by GDI for recycling back to the donor membrane. The precise role of the GTPase cycle in membrane fusion was long confusing because of the participation of multiple GTPases in vesicle transport processes (e.g., ADP ribosylation factors, trimeric G proteins, dynamin), confounding interpretation of experiments that used metabolically stable nucleotide analogs to lock protein conformation. However, recent experiments using Rab proteins preloaded with nucleotide analogs, and mutant Rab proteins with defects in their GTPase cycle or switched nucleotide specificity, have provided clarification (2, 8). GTP hydrolysis is not tightly coupled to transport (i.e., fusion is not strictly conditional on GTP hydrolysis, nor is hydrolysis strictly conditional on fusion); rather, GTP-bound Rab proteins appear to act as accelerators, promoting the rate of traffic. The rate of the hydrolysis reaction is itself regulated by GTPase activating proteins (GAPs), and effector proteins can act to retard the rate of GTP hydrolysis (e.g., Rabphilin-3 and Rab3) (5, 13). In the case of Rab5, there is evidence of dynamic regulation of the nucleotide-bound state of the protein, with both GDS and GAP activities present in a large Rab5-interactive protein complex at the target membrane (8). Presumably, this mechanism integrates information about overall traffic into and out of a compartment to fine tune the rate of docking and fusion.

Proteins that interact with individual Rabbs

Besides tethering factors and the proteins that regulate Rab GTPase cycles, additional proteins of highly varied function have been found to interact directly with individual Rab proteins (5, 13). This suggests that, in addition to their central effector functions of recruiting SNAREs and promoting vesicle tethering, Rab proteins also regulate functions specific to individual steps of transport. For example, a kinesin homolog interacts with Rab6, suggesting a role in cytoskeleton-mediated vesicle transport from the trans-Golgi; α-actinin interacts with...
the Rab3 effector, Rabphilin-3, suggesting a role in rearrangement of the cortical actin cytoskeleton related to exocytosis; and a serine/threonine kinase interacts with Rab8, suggesting cross-talk with signal transduction pathways. When these diverse effector interactions are added to ubiquitous interactions with tethering complexes and GTPase cycle regulatory factors, the extraordinary complexity of the switching functions performed by these small proteins becomes apparent.

**Concluding remarks**

Rab proteins are ubiquitous regulators of vesicle traffic whose significance is indicated by their evolutionary conservation and the severe transport defects that can result from their mutation. Increasing evidence implicates them in regulation of the tethering of transport vesicles to cognate target membranes and in the assembly of SNARE complexes both during transport vesicle loading and target membrane docking. The Rab GTPase cycle is loosely coupled to these biochemical processes, such that GTP-bound Rab proteins act to accelerate vesicular transport. Of interest, there is great variation among the Rab-regulated tethering complexes that have been studied to date, suggesting that these complexes impart specificity to intercompartmental transport reactions. Future work will primarily be directed toward elucidating the distinct effector interactions of individual Rab proteins that confer additional unique functions on individual steps of transport.

**References**

8. McBride HM, Rybin V, Murphy C, Giner A, Teasdale R, and Zerial M. Oligomeric complexes link Rab5 effectors with NSF and drive