Rab GTPases, Directors of Vesicle Docking*

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Rab GTPases represent a large family of Ras-like enzymes that play key roles in the secretory and endocytic pathways. They are located on distinct membrane-bound compartments, and genetic experiments implicate Rab in the processes by which transport vesicles or membrane-bound compartments recognize their cognate fusion targets (see Refs. 1–4 for review). Because mutant forms of Rab proteins can block protein transport along a given route or actually change the sizes of entire organelles, Rab proteins obviously play key regulatory roles in membrane trafficking. This minireview will attempt to summarize our current view of what Rab proteins do.

Most Rabs are doubly geranylgeranylated at or near their C termini, which leads to their membrane association. The specificity of Rab localization is provided by structural determinants unique to each family member (5–8) that appear to be recognized by distinct sets of proteins on organelle surfaces (9–12). Like Ras, Rabs cycle between an active, GTP-bound form and an inactive, GDP-bound form. Transport vesicles carry Rab proteins with bound GTP; after membrane fusion, GTP hydrolysis converts them into their GDP-bound states. A cytosolic protein, termed GDI, retrieves prenylated, GDP-bound Rab proteins from their fusion targets and recycles them back to their membranes of origin. GDI delivers Rab to membranes with GDP bound; they are subsequently reactivated by Rab-specific, nucleotide exchange factors (13, 14). At steady state, the bulk of a given Rab is membrane-associated; however, between 10 and 50% can be detected in the cytosol.

**Rabs Initiate Vesicle Docking and Facilitate SNARE Complex Formation**

Proteins of the VAMP (v-SNARE) and syntaxin (t-SNARE) families are present on transport vesicles and their targets, respectively, and the pairing of cognate of v- and t-SNAREs is believed to provide the specificity of membrane fusion reactions (15). Genetic experiments in yeast first linked Rab function to SNARE proteins. Although the yeast ER-to-Golgi Rab, Ypt1p, is an essential gene product, a number of proteins that function as SNARE components in this transport reaction can compensate for the loss of Ypt1p when overexpressed (16, 17). Similarly, Brennwald et al. (18) found that a t-SNARE homolog could suppress an effector domain mutation in the secretory vesicle Rab, Sec4p. Overexpression of a SNARE could override the need for a protein that facilitates SNARE pairing by simple mass action. Subsequent work confirmed that Ypt1p is required for actual v-SNARE-t-SNARE complex formation (19, 20). The SNARE complexes analyzed by these workers contained the v- and t-SNAREs, Bos1p and Sed5p, but not the corresponding Rab protein. Thus, Rab proteins act to facilitate SNARE complex formation but are not core elements of such complexes.

If cognate v- and t-SNAREs could pair at all times, all of the organelles in the cytoplasm might become stuck together as part of a giant sandwich. For example, an ER transport vesicle v-SNARE, which resides in the ER, would bind tightly to a Golgi t-SNARE and thereby attach together two entire organelles. Similarly, a post-Golgi v-SNARE would bind the Golgi to a plasma membrane-localized t-SNARE. For this reason, sets of proteins ("SNARE protectors") must block SNARE accessibility.

Sec1p is the prototypic t-SNARE-protector. The mammalian homolog of yeast Sec1p (also named n-Sec1, munc18, or rbSec1) binds directly to the presynaptic plasma membrane t-SNARE, syntaxin-1A, with an apparent binding constant in the nanomolar range (21); moreover, Sec1p binding was shown to inhibit v-SNARE-t-SNARE association (21).

If t-SNAREs are protected and thus not available for v-SNARE interaction, an additional layer of specificity must be provided to help a vesicle or membrane bearing an active v-SNARE recognize its cognate t-SNARE. We propose that Rabs, in their GTP-bound conformations, recruit transport step-specific docking factors from the cytosol that facilitate v-SNARE-t-SNARE pairing (Fig. 1). Such docking factors would be predicted to catalyze the deprotection of t-SNAREs. If the initial Rab recruitment onto a nascent transport vesicle is coupled to (or quickly followed by) its conversion to Rab-GTP, only functional transport vesicles will recruit the docking factors. In this manner, docking will only take place between transport vesicles and their targets, rather than between entire organelles (22). In contrast, when homotypic fusion between two organelles is the goal, reaction rates would be modulated by the level of Rab-GTP (23).

**Rab-recruited Vesicle Docking Proteins**

Three macromolecular complexes have been identified that are likely to serve as docking factors: the Exocyst (24, 25), the Rabaptin-5 complex (14, 26), and another large complex required for ER-to-Golgi transport in yeast (27). According to our model, such complexes would be recruited onto membranes by a Rab-GTP to help link or direct vesicles to their targets.

The Exocyst is a large, 19.5 S particle complex that contains Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, and Exo70p and is required for exocytosis. A spectacular finding is the fact that this large "Exocyst" complex is localized to the tips of yeast buds, the site of exocytosis in *Saccharomyces cerevisiae*. Sec3p appears to localize the Exocyst to the plasma membrane target (28). In contrast, the corresponding t-SNAREs are distributed uniformly throughout the plasma membrane (18). It seems likely, then, that the Exocyst functions to deliver transport vesicles to the docking site on the plasma membrane for the ultimate SNARE assembly reaction.

How does a vesicle find the complex? Genetic and biochemical experiments suggest a link between the Sec4p Rab and Exocyst subunits. The data imply that Rabs on a transport vesicle may bind to the Exocyst to position the vesicle for
subsequent fusion. Either the docking complex or the Rab (20) could then free the t-SNARE, allowing for subsequent fusion. In this process, the Exocyst would function as a true “docking” machine.

Another docking complex is the Rabaptin-5 complex that interacts with Rab5-GTP in preference to Rab5-GDP (26). Rabaptin-5 is contained within a complex comprised of several subunits, including a nucleotide exchange factor (Rabex-5) that can activate Rab5 (14). Interestingly, Rabex-5 activates Rab5, which would further promote Rab5-Rabaptin association. Presumably, while the Rab5-GTP-Rabaptin-5 complex exists, fusion is promoted. Rab5-bound Rabaptin will be in equilibrium with an unbound form. One could imagine that upon Rabaptin-5 release, Rab5-GTP would be instantly hydrolyzed (23). Subsequent Rabex-5 interaction would restore activity to the endosome-associated Rab5. Thus, the Rabaptin-5 complex contains self-regulatory elements that have the capacity to modulate the extent to which this complex is likely to facilitate SNARE pairing in endosome fusion reactions.

Some but Not All Rabs Act as Docking Timers

Expression of a mutant Rab5 that is significantly slowed in its GTP hydrolysis rate triggers the formation of oversized endosomes (29). Because endosome fusion still takes place, these experiments showed elegantly that GTP hydrolysis is not required for nor directly coupled to the membrane fusion event. Even more striking was the recent demonstration that Rab5 hydrolyzes GTP in a continuous cycle that is completely uncoupled to membrane fusion (23). In this study, the authors engineered a Rab5 mutant protein that prefers xanthosine triphosphate to GTP; this mutant enabled them to monitor Rab5-specific nucleotide hydrolysis during an endosome fusion reaction. Zerial and co-workers (23) concluded that the rate of nucleotide hydrolysis by Rab5 is rate-determining for membrane docking and fusion reactions. They proposed that cells could regulate the extent of fusion reactions by regulating the steady state level of Rab5-GTP via nucleotide exchange rate-enhancing factors and GTPase-activating proteins. The data showing that Rab5 acts as a timer for endosome fusion are rigorous and convincing. In homotypic endosome fusion reactions, they proposed that their primary role is to recruit cytosolic docking factors that are needed for membrane docking and fusion.

Other Rab Functions

Several lines of evidence suggest a link between the Rab-mediated process of vesicle docking and the actin- and microtubule-based cytoskeletons (cf. Refs. 13 and 33). An unexpected link was identified for Rab6, which in its GTP-bound conformation interacts with a novel, kinesin-like motor protein named Rabkinesin-6 (34). Rabkinesin-6 is localized to the Golgi complex and may be part of a larger docking complex that uses the microtubule-based cytoskeleton to direct vesicle trafficking. Rabphilin, a Rab3A effector, interacts with the actin-bundling protein, α-actinin, in the absence of Rab3A-GTP (35). This may provide a means for Rab3A-GTP to modulate the organization of the local actin-based cytoskeleton in relation to events preceding synaptic vesicle exocytosis.

A Role for Rabs in Vesicle Formation

Current models for Rab function come in large part from the phenotype of sec4 mutants strains that accumulate secretory vesicles at the non-permissive temperature. This provides strong evidence that Rabs are needed for vesicle docking. Nevertheless, there is accumulating evidence that Rabs must be present in a particular conformation on nascent, budding transport vesicles to permit those vesicles to form (3). This does not necessarily mean that Rab function in vesicle budding per se; rather, it is possible that vesicle formation is regulated such that budding only occurs if the vesicle contains everything it needs for docking and fusion.

Indirect clues for a requirement for Rabs in vesicle budding came from two studies looking at Rab mutants that bind GDP>GTP. Nuoffer et al. (36) found that ER-to-Golgi export was inhibited in the presence of this class of Rab1a mutant. Similar results were obtained by Riederer et al. (37) studying the role of Rab9 in mannose-6-phosphate receptor trafficking. A Rab9 mutant that bound GDP>GTP did not lead to the
accumulation of mannose 6-phosphate receptors in transport vesicles; rather these receptors traveled along other transport routes. In both of these sets of experiments, the mutant protein could have blocked vesicle budding indirectly by sequestering another component required for a downstream process.

Very recently, Smythe and co-workers (38) showed directly that Rab5 is required for ligand sequestration into clathrin-coated pits. Given the well established role of Rab5 in the fusion of endocytic vesicles with endosomes, these data support the proposal that the recruitment of essential components of the targeting and fusion machinery is coupled to the formation of functional transport vesicles.

If Rab5 are needed for vesicle formation, why would vesicles accumulate in a sec4 mutant strain? Perhaps the budding machinery can sense the presence of a Rab in its GTP-bound conformation. Thus, certain mutant Rab alleles could be incorporated into a vesicle budding machine but not able to function in docking, although other mutant alleles could yield a Rab that blocked vesicle formation as well. Two new yeast Ypt (Rab) proteins have redundant functions in TGN export; when YPT31 is deleted, cells accumulate aberrant Golgi structures in strains bearing a conditional allele (39, 40). This phenotype could be explained if this ypt31 allele cannot be recognized by the vesicle budding machinery.

Conclusion

In summary, cells have gone to a lot of trouble to produce at least 30 Rab GTAPases, most with distinct localizations and unique roles in the secretory and endocytic pathways. Many of these proteins are essential for viability in yeast; if they aren’t, their functions are usually covered by redundant Rab GTAPases. Thus, Rab’s are key regulators of membrane trafficking reactions.

We have proposed that Rab functions in vesicle docking but must be present in nascent transport vesicles in their active conformations to permit vesicle budding. Have we stretched the data to fit all the results within a single unified model? We do not think so, but we may be incorrect. We know of no single membrane trafficking step that requires a distinct Rab for budding versus fusion processes. It is true that Rab effectors represent a diverse set of proteins, but homotypic fusion and directed vesicle targeting may require different modes of regulation.

Each of the Rab GTAPases is likely to interact with both specific and general regulatory and effector proteins, and new Rab-modulating enzymes are being discovered all the time (e.g. Ref. 41). Although a large body of evidence suggests that Rab facilitates vesicle docking reactions, they clearly do so within the context of the cytoskeleton, and they may interact with multiple partners to be incorporated into the right vesicle and facilitate docking, which may include vesicle translocation to the target. A major future challenge will be to discover how they facilitate vesicle delivery and how they catalyze the formation of correct SNARE pairs.

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