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Rab GTPase localization and Rab cascades in Golgi transport

Suzanne R. Pfeffer¹

Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305-5307, U.S.A.

Abstract

Rab GTPases are master regulators of membrane traffic. By binding to distinct sets of effector proteins, Rabs catalyse the formation of function-specifying membrane microdomains. They are delivered to membranes by a protein named GDI (guanine-nucleotide-dissociation inhibitor) and are stabilized there after nucleotide exchange by effector binding. In the present mini-review, I discuss what we know about how Rab GTPases are delivered to the correct membrane-bound compartments and how Rab GTPase cascades order Rabs within the secretory and endocytic pathways. Finally, I describe how Rab cascades may establish the distinct compartments of the Golgi complex to permit ordered processing, sorting and secretion of secretory cargoes.

Keywords

guanine-nucleotide-dissociation inhibitor displacement factor (GDF); guanine-nucleotide-dissociation inhibitor (GDI); Golgi complex; GTPase-activating protein (GAP); guanine-nucleotide-exchange factor (GEF); Rab GTPase

How are Rab GTPases delivered to membranes? Rabs exist in the cytoplasm as 1:1 complexes with GDIs (guanine-nucleotide-dissociation inhibitors) [1,2]. For most Rab proteins, the cytosolic pool represents approximately half of the total Rab proteins; the remainder is found in the cytoplasm [3]. Rab–GDI complexes contain all of the information needed to be loaded on to specific membrane-bound compartments [4,5]. Multiple components contribute to Rab localization and these components cooperate to enable cells to establish the function-specifying membrane microdomains that comprise distinct membrane-bound compartments [1,2].

Level one targeting: GDIs compared with GEFs (guanine-nucleotide-exchange factors)

Rab-specific GDIs and GEFs work in concert to contribute to Rab targeting (Figure 1). Rab-specific GDI has the marvellous ability to bind GDP-bearing prenylated Rabs and remove them from membranes. Thus, if a Rab is delivered to the incorrect compartment, GDI corrects the mistargeting by extracting the Rab to permit delivery to the correct membrane domain. If a Rab-specific GEF is localized near a Rab, it will convert the Rab-GDP into Rab-GTP and thereby subvert GDI extraction. The prediction is that the anchoring of a GEF

¹pfefferstanford.edu.

to an intracellular compartment will lead to the accumulation of a Rab at that site. This result would require expression of the GEF at a level higher than the cognate GAP (GTPase-activating protein).

Level two targeting: effector binding

Rab-specific GAPs counteract the action of Rab-specific GEFs and reverse the process of trapping Rabs on membranes. Many GAPs are found in the cytosol, suggesting that they have continual access to Rabs on membranes [6,7]. Thus an additional layer of interactions is needed to stabilize Rabs at a particular membrane compartment. We have shown that interaction with some, but not all, effectors can stabilize Rabs on membranes. We have referred to these as 'key' effector interactions [3]. The Rab9 effector TIP47 (47 kDa tail-interacting protein), binds to Rab9 in part by recognizing the Rab9 hypervariable domain. We generated a chimaeric Rab protein comprising the globular portion of Rab5 attached to the Rab9 hypervariable domain [3]. This Rab5/9 protein bound the Rab5 effectors EEA1 (early endosome antigen 1) and Rabaptin 5. Rab5/9 also bound to TIP47. In cells, the Rab5 effectors EEA1 and Rabaptin 5 are more abundant than TIP47. At steady state, Rab5/9 localized to early endosomes; upon expression of exogenous TIP47, the Rab5/9 protein moved to late endosomes. These experiments demonstrate the importance of effector interactions for Rab localization.

Can GEFs suffice to drive Rab localization?

Some have argued that GEFs are key to Rab localization and they are surely important contributors (Figure 1). However, if this were true, why would changing the cellular concentration of an effector protein move Rab5/9 from early endosomes to late endosomes? Presumably, the chimaeric Rab5/9 protein is activated by a Rab5-specific GEF. One possibility is that the Rab5/9 chimaera is converted into the GTP-bound form on early endosomes; cytoplasmic TIP47 could then have been recruited to the membrane surface of early endosomes and remained bound to the Rab5/9 protein as it transitioned to late endosomes, where additional Rab9 effectors (as well as a Rab9 GEF) would be localized. Whatever the mechanism, the effector relocates the Rab protein. Indeed, expression of a point mutant that is unable to bind Rab9 failed to relocate Rab5/9 [3].

Barr and co-workers have shown that the DENND2 [DENN (differentially expressed in normal and neoplastic cells)/MADD (mitogen-activated protein kinase-activating death domain) domain-containing 2] proteins function as Rab9 GEFs in cells [8]. It is noteworthy that these proteins are entirely cytosolic and would therefore have the capacity to activate Rab9 wherever it is targeted. It will be very important to determine the fraction of Rab-specific GEFs and GAPs that are membrane-associated. If a significant proportion of these enzymes reside in the cytosol, they will not have the spatial capacity to drive specific membrane targeting.

Additional targeting factors: GDFs (GDI displacement factors)

My laboratory was the first to report an affinity for the interaction between Rab9 and GDI [9]. Because binding was very tight (<20 nM), we reasoned that membranes may contain a

factor that facilitates dissociation of Rab–GDI complexes. Subsequent work by Roger Goody and his co-workers have confirmed very tight binding for prenylated Rabs bound to GDI, of the order of 1–5 nM for Rab7, for example [10,11].

We have reconstituted the delivery of Rab9 on to membranes using pure complexes of doubly geranylgeranylated Rab9 bound to GDI [4]. We showed that membranes contained a protease-sensitive factor that led to the delivery of the Rab on to the membrane surface [4,12,13]. We went on to show that a protein named PRA1 (prenylated Rab acceptor protein 1) (orYip3), purified from the membranes of bacteria expressing it, could catalyse the release of Rab9 from GDI, with the product being a detergent micelle containing prenylated Rab9-GTP, as well as free GDI [14]. We named this activity GDF [13].

How might a GDF act? From the work of Goody and co-workers, we know that prenylated Rabs bind to GDI via their switch regions that display their GDP-bound status [10,15]. The hypervariable domain of the Rab is presented on the surface of GDI; the prenyl groups tuck into the bottom of the GDI protein. Prenylated Rabs bind GDI much more tightly than non-prenylated Rabs. Thus, if GDF were to extract the prenyl groups from the Rab–GDI complex, the Rab would be released. GDF can be considered a reaction catalyst if the process occurs on a membrane surface: the Rab released would be trapped on a membrane. GDI could re-extract the protein or the Rab could undergo rapid nucleotide exchange. A GDF would not be a conventional catalyst in the sense that GDI can re-extract the product. However, it would certainly be an ‘effective’ catalyst if Rab conversion into the GTP-bound form occurred quickly in relation to the GDI extraction process.

Rab cascades in localization

A very important discovery by Peter Novick and co-workers was the finding that Rab proteins template the localization of subsequent acting Rab proteins [16]. Thus Ypt32p recruits the GEF for the later acting Sec4p GTPase. Moreover, Ypt32p recruits the GAP for the previous acting Ypt1p GTPase [17]. This suggests that GEFs and GAPs are responsible for the vectorial nature of the secretory pathway and can template its organization. A similar cascade was reported by Zerial and co-workers to explain the conversion of early endosomes into late endosomes: Rab5 recruits the GEF for Rab7 protein [18]. Rab cascades have the capacity to keep Rab domains distinct and remove a Rab from a domain in which it does not belong [19].

Other examples of Rab cascades will help to establish the links between Rab GTPases of yet unknown function. My laboratory recently identified two proteins that bind Rab9A but serve as GAPs for other Rab GTPases: RUTBC1 is a GAP for Rab32 [20] and RUTBC2 is a GAP for Rab36 [7]. In cells and cell extracts, RUTBC1 influences the ability of Rab32 to bind its effector protein, Varp. For RUTBC2, a small amount of the protein is membrane-associated where it co-localizes with Rab36, and expression of wild-type RUTBC2, but not the catalytically inactive RUTBC2 R829A mutant, decreases the amount of membrane-associated Rab36 protein. These data are consistent with a physiological role for RUTBC1 in regulating Rab32 and RUTBC2 regulating Rab36. Since both of these GAP enzymes bind but cannot act on Rab9A, we conclude that Rab32 and Rab36 act upstream of Rab9A and

cells have devised a mechanism to segregate these Rab proteins on membrane surfaces. Rab36 is in brain, lung and melanocytes; Rab32 is more ubiquitously expressed. Additional work is required to reveal their physiological roles.

Rab cascades at the Golgi: a cisternal progenitor model

It seems clear that we will discover many more examples of Rab-specific GEFs and GAPs that bind one Rab and act on another. Thus it is entirely reasonable to presume that Rab cascades establish the order of compartments in the secretory pathway, completely analogous to the way that they template the endocytic pathway. The existence of Rab cascades on the Golgi and the capacity of all membrane-bound compartments to undergo homotypic fusion have led to a new model for how protein transport may take place in the Golgi complex [21].

Highlights of the new 'cisternal progenitor model' are summarized in this section. The Golgi comprises a series of flattened cisterna that link to one another to form a ribbon. During mitosis, the ribbon is fragmented and the Golgi breaks down into much smaller units that distribute to daughter cells during cytokinesis [22]. The reassembly of these Golgi fragments relies upon the p97 ATPase and its p47 adaptor subunit. Golgi membranes can undergo fusion during interphase; this process is mediated by p97 in conjunction with a p37 adaptor protein. In cells depleted of p37, the Golgi fragments into small vesicles [23]. This argues strongly that the Golgi undergoes fusion during interphase and the product of these events is an intact Golgi ribbon. Fission reactions can also occur; these are microtubule-dependent and rely on a connection between membranes and motor proteins. The importance of microtubules in Golgi structure maintenance has long been a known addition of nocodazole to cells disrupts the Golgi ribbon and the ministacks of Golgi complexes are found throughout the cytoplasm. Simple washout of nocodazole leads to reformation of the Golgi ribbon [24].

The fusion of Golgi compartments is mediated by a set of SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor) proteins that drive Golgi reassembly after mitosis. These SNAREs can be thought of as homotypic fusion SNAREs. (Homotypic fusion refers to the coalescence of two molecularly similar compartments; this is in contrast with heterotypic fusion of molecularly distinct compartments such as a transport vesicle fusing with a distinct target membrane.) GS15 (Golgi SNARE 15 kDa) is important for p37/p97-mediated cisterna formation [23] and is localized throughout the Golgi stack. GS15 acts with Syntaxin 5, Gos28 and Ykt6 proteins to mediate intra-Golgi transport [25]. Unlike other Golgi SNAREs, these are located at the Golgi rims and also in the middle of the stack where they can mediate local fusion events. The importance of homotypic fusion, especially for the transport of large cargoes that cannot be accommodated in regular transport vesicles, may be testable in cells in which the localization of GS15 or Syntaxin 5 are altered artificially.

If one overlays a Rab cascade on a membrane-bound compartment that is capable of undergoing homotypic fusion and fission, one can imagine a scenario where forward transport is driven by increasing concentrations of Rab proteins with their associated

SNAREs and tethers. Overlay on this a Rab cascade in which RabA can recruit the GEF for RabB. A RabA domain could begin to form an adjacent RabB domain (Figure 2). This domain could separate from the RabA domain by fission as part of the steady-state fission and fusion that would be taking place in the Golgi, and it could segregate from the RabA domain by RabB recruitment of a RabA-specific GAP (Figure 2). Under physiological conditions in which secretion is activated, it may be important for the Golgi to expand to accommodate the increased volume of cargo. This could involve increased production of RabB and RabC domains to build a larger Golgi. Under these conditions, one might detect cisternal expansion at the edges of the stack. A RabB domain present there would have the capability of fusing with another RabB domain. This would lead to connections between cisternae at different levels of the stack that would then be resolved by fission events.

This cisternal progenitor model involves stable compartments that form sequentially and retain their stacked nature. In this model, homotypic fusion would represent the predominant form of fusion used for cargo transport through the Golgi. It is important to note that two stacks are not needed to accomplish such transport: because even ministack cisternae are discontinuous, such a fusion event could occur between cisternae within a ministack. In addition, this class of fusion may explain the tubules that have been detected in some labs to connect Golgi cisternae [21].

The cisternal progenitor model provides a simple molecular model for how one might build a Golgi complex *de novo*. The model involves a Golgi comprising three distinct compartments: a Rab GTPase would organize each compartment. That Rab would template the recruitment of a subsequent acting Rab that would organize its own SNAREs and tethering proteins. Rabs would organize the glycosyltransferases that define the residents of each Golgi compartment.

A satisfying aspect of the model is that it incorporates many of the previously discrepant experimental observations reported in yeast, plant and human cells. It explains why Golgi compartments appear to mature in live-cell video micrographs and why the kinetics of Golgi export may not match that predicted by a Golgi maturation model. Compartments have the capacity to 'grow' the next compartment in a templated Rab-dependent fashion, but they also fuse homotypically with one another. Thus each cisterna can serve as a progenitor of the next. Glycosyltransferases are mostly excluded from the rims where cargo is present, and would be predicted to be retained in a given compartment organized by Rab GTPases. Moreover, any enzymes seen at the rims may be the product of an intercisternal fusion event.

Future work will define the Rabs that may template the creation of the mammalian Golgi complex and their cognate GEFs and GAPs that create function-specifying membrane microdomains that generate the polarized Golgi complex that drives cargo processing and sorting to multiple intracellular compartments.

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Abbreviations used

EEA1	early endosome antigen 1
GAP	GTPase-activating protein
GDI	guanine-nucleotide-dissociation inhibitor
GDF	GDI displacement factor
GEF	guanine-nucleotide-exchange factor
SNARE	soluble <i>N</i> -ethylmaleimide-sensitive fusion protein-attachment protein receptor
GS15	Golgi SNARE 15 kDa
TIP47	47 kDa tail-interacting protein

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Figure 1. A model for Rab recruitment on to membranes

GDP-bound Rabs are recruited to membranes from cytosolic complexes with GDI through the catalytic action of a GDF. GEF catalyses nucleotide exchange. Interaction of the Rab-GTP with an effector that has been recruited to the same membrane by a Rab (option A) or by an effector binding partner (option B) will stabilize both the Rab and the effector on the membrane. If not bound to the effector, a GAP can activate the Rab to hydrolyse its GTP to GDP, thus allowing for potential membrane extraction of the Rab by GDI. Thus the Rab GTPase is dependent on its effectors, and vice versa, for stable interaction with the membrane. The effector binding partner need not be a protein and can be particular phospholipids, such as phosphoinositides, that are specific to a given compartment. © 2006 Rockefeller University Press. Reproduced from Aivazian, D., Serrano, R.L. and Pfeffer, S. (2006) TIP47 is a key effector for Rab9 localization. *J. Cell Biol.* **173**, 917–926 with permission.

A reversible lateral fission and fusion

B RabB-directed homotypic fusion

C RabB-directed removal of RabA

D Loss of stacking in cells depleted of p37

Figure 2. A cisternal progenitor model for transport through the Golgi

Transport through the Golgi and Golgi stack creation in a cisternal progenitor model. **(A)** Premise 1 states that Golgi stacks undergo continuous reversible fission and fusion. Consider a stably stacked Golgi where each cisterna is marked by a different Rab protein. The stack can grow if a Rab cascade builds sequential domains that can fuse with like domains (e.g. RabB regions with other RabB regions). RabA will create an adjacent RabB domain that may segregate by fission within the stack. The RabB bleb would fuse with the stable RabB cisterna, thereby growing. **(B)** This process can include cargo. Alternatively (or

simultaneously), vesicles may carry cargo from a RabA compartment to a RabB compartment by ‘heterotypic fusion’. Importantly, the RabA compartment is stable and is the progenitor of the RabB compartment. **(C)** The RabB compartment has the capacity to remove RabA for redelivery to the *cis*-Golgi. **(D)** The Golgi vesiculates in cells lacking the homotypic fusion factor p37 [23]. Reproduced from Pfeffer, S.R. (2010) How the Golgi works: a cisternal progenitor model. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19614–19618 with permission. © 2010 National Academy of Sciences U.S.A.

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