

Chapter 1

Prenylation of RabGTPases, Their Delivery to Membranes, and Rab Recycling

Roger S. Goody, Yaowen Wu, and Aymelt Itzen

Abstract Rab proteins, in common with many other Ras superfamily members, need to be lipidated (in the case of Rab geranylgeranylated) at their C-terminus to be able to interact with membranes. Here, we review the basic mechanism of geranylgeranylation and then address the still unsolved question of how prenylated Rabs are delivered to specific membranes. The evidence reviewed leads to a model in which specifically localized guanine nucleotide exchange factors are an essential component of the targeting mechanism, and that additional stabilizing interactions are important in a number of cases, including effector interactions and positively charged residues in the C-terminus in at least one case examined. Finally, there is evidence that in some cases additional still unidentified factors are involved.

Keywords RabGTPases • Prenylation • Delivery • Localization • GEF • GDI • GDF

1.1 Introduction

Intracellular vesicular transport is an important and essential aspect of the functioning of eukaryotic cells. Transport between membrane-bound compartments occurs via vesicles or tubular structures in a highly complex and tightly regulated

R.S. Goody (✉)

Department of Physical Biochemistry, Max-Planck-Institute of Molecular Physiology,
Otto-Hahn-Str. 11, 44227 Dortmund, Germany
e-mail: roger.goody@mpi-dortmund.mpg.de

Y. Wu

Chemical Genomics Centre of the Max Planck Society, Otto-Hahn-Str. 15, 44227 Dortmund,
Germany

A. Itzen

Department Chemie, Center for Integrated Protein Science Munich, Technische Universität
München, Lichtenbergstr. 4, 85748 Garching, Germany

manner. While many different classes of proteins are involved in the overall process, a crucial regulatory role is played by members of the Rab family of small GTPases (Hutagalung and Novick 2011; Stenmark 2009). In order to fulfill their role, the 60 members (in humans) have to be modified by addition of geranylgeranyl groups (one, or in most cases two) to the C-terminus in a process referred to as prenylation. These prenyl groups endow the Rab proteins with a high affinity for membranes and allow them to play various roles, for example connecting vesicles to systems of motor-proteins or recognizing specific tethering factors that guide vesicles to a specific membrane or membrane compartment as a first step in the fusion process. While the principles involved in these events are quite well understood in a number of cases, one essential but unclarified property that Rab proteins must have in order to play the role envisaged for them is localization to a specific membrane or membrane domain. In this review, we will first discuss the events leading to prenylation, then discuss the principles involved in delivery of Rabs to membranes, and finally summarize the status of our knowledge on the targeting of Rab proteins to specific membranes.

1.2 Prenylation of Rab Proteins

Rab proteins differ from Ras and Rho proteins, which are also prenylated near their C-terminus, by not having a specific prenylation recognition sequence motif. Ras and Rho proteins have the so-called CaaX box at their very C-termini, with the nature of the X residue determining whether farnesylation (Ras proteins) or geranylgeranylation (Rho proteins) occurs. In Rab proteins, the C-terminal motifs are commonly CC, CXC, or CCXX, but in a few cases CXXX motifs occur (Rab8, Rab13, Rab18, Rab23, Rab38). The explanation for the lack of a specific prenylation motif is that specificity is in fact generated via an accessory protein, the Rab escort protein (REP), which interacts with all RabGTPases (but not other members of the Ras superfamily) and with Rab geranylgeranyl transferase (RabGGTase), presenting the Rab C-terminus to the active site of the prenylating enzyme. This leads to the curious situation of having a specific enzymatic modification of a group of substrates with similar overall structure but differently structured modification sites, with the added peculiarity that the modifying enzyme has little or no (measurable) affinity for its substrates. The latter point is put into perspective if the REP:Rab complexes are regarded as the substrates, and these do indeed have affinity and specificity for RabGGTase. In the presence of the substrate geranylgeranyl pyrophosphate, i.e., the lipid donor, the affinity of RabGGTase for REP:Rab complexes is in fact very high [K_d in the nM range (Thoma et al. 2001a, b)].

The Rab prenylation reaction has been investigated in detail in a number of publications, and many of the data obtained are summarized in Fig. 1.1 (Wu et al. 2009). The importance of the CIM (CBR interacting motif, where CBR means C-terminus binding region) region of Rab proteins was noticed in

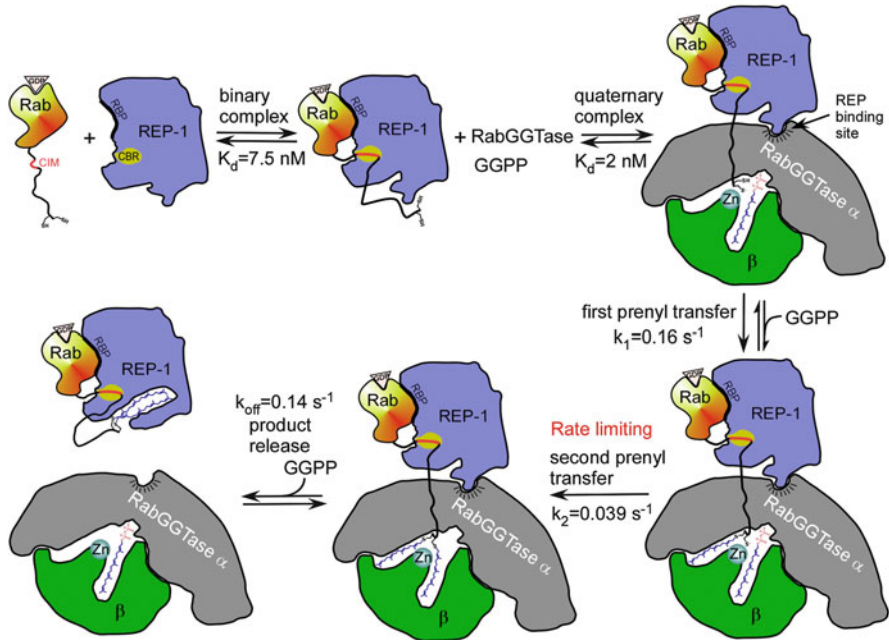


Fig. 1.1 The mechanism of Rab prenylation in the Rab:REP:RabGGTase complex. After an initial relatively weak association of the globular domain of Rab with REP, interaction of the CIM (C-terminal interaction motif) of Rab with the CBR (C-terminal binding region) of REP leads to tightening of this interaction. This is followed by a high-affinity interaction of the Rab:REP complex with RabGGTase and geranylgeranyl pyrophosphate (GGPP) to form the high-affinity quaternary complex. The first geranylgeranyl group is transferred to the most C-terminal cysteine of Rab with the help of a catalytic zinc ion. The second prenyl group is then transferred more slowly (in the case of Rab7) to the preceding cysteine, after which the prenylated Rab:REP complex is released under the influence of a further lipid substrate molecule. The constants given apply to Rab7 and REP-1. Figure modified from the publication of Wu et al. (2009)

structural investigations on a complex between prenylated Rab proteins and REP (Rak et al. 2004) or GDI (GDP dissociation inhibitor) (Rak et al. 2003). This short motif, consisting of a polar residue sandwiched between two hydrophobic residues, is essential for high-affinity binding to REP and for efficient prenylation. The positioning of the cysteine groups with respect to this motif is critical. In wild-type Rab7, there are 12 residues downstream of this motif before the first prenylatable cysteine, and this can be shortened by a further 3 residues without drastic effects on the prenylation reaction, but shortening to 7 residues leads to only residual activity and with 6 residues or less there is no detectable activity (Wu et al. 2009). Interestingly, it has recently been shown that the region between the CIM and the cysteines can be replaced by a non-peptidic structure and that cysteine can be replaced by a simple thiol group and still retain full prenylation

properties both *in vitro* and *in vivo* (Li et al. 2014). These results are discussed later in connection with the role of the hypervariable C-terminus in Rab localization.

The discussion so far has implicitly assumed that the Rab protein harbors a GDP molecule, since the GDP form is prenylated more efficiently than the GTP form (Seabra 1996). This is probably partially related to the fact that the GTP form of Rab proteins is bound a factor of ca. 10 more weakly than the GDP form to REP (Alexandrov et al. 1998). A conceptual problem that arises at this point is the fact that freshly synthesized Rabs in the cytoplasm will mainly have GTP bound, since the cellular concentration of GTP is ca. tenfold higher than that of GDP. The stronger binding of REP to the GDP form of Rabs will tend to compensate for the imbalance in the GTP/GDP concentrations, and taking the data available at their face value, this could lead to similar amounts of Rabs in the two different forms bound to REP (depending on the relative concentrations of REP and Rab molecules), and therefore to production of prenylated Rabs in both nucleotide-bound forms. Even if the rate of prenylation is slower for the GTP-bound form, this means that significant amounts of prenylated Rabs with bound GTP can be produced. As discussed in detail below, Rabs that associate with membranes in the GTP form are resistant to extraction by GDI, meaning that if association with a wrong membrane occurs initially, this will be essentially irreversible as long as GTP hydrolysis does not occur. This is an unlikely scenario, since production of activated (i.e., GTP-bound) Rabs at their appropriate membrane location needs to be strictly regulated. As described below, prenylated Rab:GTP is bound much more weakly than prenylated Rab:GDP to REP, increasing the probability of Rab release from its complex with GDI leading to membrane association. In this situation some additional factor that is responsible for targeting would be needed. Again, this is a theme that will arise in later discussions.

1.3 Delivery of Prenylated Rab Proteins to Membranes

An interesting question that has been touched upon but not examined extensively is whether direct delivery of Rabs from their complexes with REP to their specific membrane occurs, or whether they first locate to a default membrane, possibly the ER and/or Golgi. Rabs ending with CXC are carboxymethylated, as are some mono-prenylated Rabs (Leung et al. 2007), and the enzyme that performs this (isoprenylcysteine carboxymethylase, or Icmt) is ER localized. The authors conclude that Rabs with a CC motif, which are not carboxymethylated, are located directly to their target membrane, but that CXC Rabs first have to visit the ER, but evidence against initial localization to ER for all Rabs was not provided. Moreover, the ER and Golgi appear to be the default site of mistargeted Rab proteins arising from the loss of targeting elements on Rab proteins *per se* or the depletion of GEFs (Ali et al. 2004; Cabrera and Ungermann 2013; Li et al. 2014). However, if some are still in the GTP form, there would still be a problem for GDI to extract them

again to allow delivery to the correct target membrane, unless there is a generic RabGAP at the ER and Golgi that acts on them, for which there is no evidence.

After prenylation of Rab proteins in the complex between REP, Rab, and RabGGTase, a further incoming lipid substrate molecule (GGPP) is able to dissociate the RabGGTase and leave the Rab protein as a soluble complex with REP (Thoma et al. 2001b) (Fig. 1.1). This high-affinity complex [K_d in the nM range (Wu et al. 2007)] has to be disrupted to allow Rabs to be attached to membranes. A similar situation arises with recycling of Rabs by GDI, which has similar properties to REP in terms of binding and solubilizing prenylated Rab proteins. In both cases, there is a stable interaction that has to be overcome to allow insertion of the prenyl groups into the membrane.

It has been suggested that the attachment of Rabs to membranes is catalyzed by a GDF (GDI displacement factor) that is able to disrupt the stable GDI:Rab complexes (Dirac-Svejstrup et al. 1997). A molecule that appears to have the desired properties is Pra-1 (Yip3 in yeast), an intrinsic membrane protein reportedly localized in the late Golgi and early endosomes with activity towards Rab9 and other endosome-associated Rabs (Rab5, Rab7) (Sivars et al. 2003). Although there is no convincing evidence for their mode of action, other members of the Yip family are Rab interacting proteins thought to be candidate GDFs. However, recent evidence has been presented indicating that the activities of these proteins are not required for correct localization of yeast Rabs (Cabrera and Ungermann 2013).

The lack of definitive evidence on the existence and properties of GDFs for Rabs was a reason for the enthusiastic reception of publications suggesting that one of the over 250 proteins injected by *Legionella pneumophila* into infected cells is a *bona fide* GDF as well as being a GEF towards host cell Rab1 (Ingmundson et al. 2007; Machner and Isberg 2007). While the GEF properties of DrrA/SidM have been amply confirmed and extensively characterized, it was also shown that the apparent GDF activity of this protein was actually a product of its GEF activity (Schoebel et al. 2009), and in fact all Rab GEFs will have this apparent activity towards their cognate Rabs. The source of this effect is the preference of GDI for Rab:GDP rather than for Rab:GTP. It was shown that replacement of GDP by GTP in prenylated Rabs leads to a loss of affinity of about three orders of magnitude towards both GDI and REP (Wu et al. 2010). Because of this, Rab:GDP that dissociates from its complex with GDI or REP spontaneously will undergo nucleotide exchange in the presence of a cognate GEF, thus preventing rebinding to GDI and possibly allowing membrane insertion. These considerations lead to the notion that the presence of a GEF at a specific membrane location might lead to trapping of its activated cognate Rab at the same location (Schoebel et al. 2009; Wu et al. 2010).

Since molecules with genuine GDF activity towards Rab:GDI complexes have so far proven elusive, with the exception of Yip3, the question of their necessity for the process arises. Since Rab:GDI and in particular Rab:REP complexes dissociate slowly, it appears that acceleration over the rate seen in vitro is likely to be required in vivo. One possibility that has not yet been considered is the role of membranes in this process, except in the very general sense that interaction with a membrane will make a thermodynamic contribution to the process by providing a trap, whose

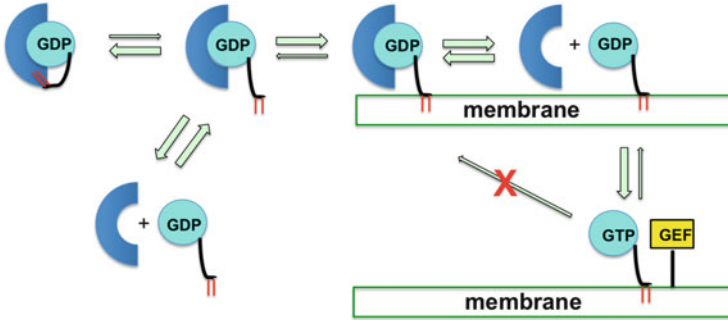


Fig. 1.2 Model for Rab delivery to membranes without a GDF. Assuming that there is a certain tendency for the lipid moieties at the C-terminus to dissociate from their binding site on Rab, this state might be temporarily trapped by the proximity of a membrane. If a cognate GEF is present at this membrane, membrane attachment will be made essentially irreversible because of generation of the GTP bound state of the Rab protein, which reduces the affinity to GDI dramatically

properties are enhanced by the influence of concerted or sequential GDP/GTP exchange on the Rab:GDI affinity.

A hint towards a possible active role of membranes is suggested by comparing the properties of Rab:GDI or Rho:GDI complexes with those of complexes of farnesylated Ras family proteins with PDE δ (Ismail et al. 2011). In this case, the interaction appears to be almost exclusively with the prenyl group, and the structure of farnesylated Rheb:GDP in complex with PDE δ complex confirms that there is little or no interaction between PDE δ and the body of the GTPase. In the case of Rab or Rho complexes with GDI, the main contribution to affinity and therefore specificity appears to come from the specific interaction of GDI with the GTPase domain. The idea therefore arises that whereas there is a relatively strong interaction of the farnesyl group with the lipid binding site of PDE δ , the interaction of geranylgeranyl groups with the lipid binding site of GDI or REP is weak, leading to a dynamic equilibrium between a state in which both the GTPase domain and lipid are bound to GDI, and a state in which the lipid group is transiently free (see Fig. 1.2). If this occurs in close proximity to a membrane, insertion of the lipid group could then lead to complete dissociation of Rab:GDP from GDI, which would again be reversible, unless a membrane-localized GEF can facilitate GDP replacement by GTP, thus preventing the reverse reaction (i.e., rebinding of Rab to GDI). These arguments are summarized in Fig. 1.2.

Interestingly, a genuine GDF-type mechanism appears to operate in the case of interaction of farnesylated proteins with PDE δ (Chandra et al. 2012; Ismail et al. 2011). This protein binds farnesyl groups in a manner that is independent of the GTPase core domain of Ras-family members, as already discussed. It appears to act as a chaperone for farnesylated proteins in a similar manner to Rab and Rho GDIs, with the important exception that there is no dependence of the interaction on the nucleotide state of the GTPases. Intriguingly, PDE δ interacts with other

Ras-family GTPases, namely Arl2 and Arl3, in a GTP-dependent manner, and this interaction leads to active displacement of bound farnesyl groups by an allosteric competitive mechanism. This is therefore a genuine GDF-like mechanism and avoids the thermodynamic dead end that would be encountered with a GDF mechanism that involves a specific strong interaction with the chaperoned GTPase, as envisaged in the original Rab-GDF concept. In the PDE δ case, the interaction of the GDF (i.e., Arl2 or Arl3) is with the chaperone itself and is in fact reversible on hydrolysis of GTP at the active site of the Arl protein, resulting in loss of affinity to PDE δ . This process is therefore ultimately driven by GTP hydrolysis, although the directly relevant factor is the GTP/GDP concentration gradient, as for the hypothetical model for GEF-driven Rab localization. In both cases, the relative concentrations of GTPase:GTP and GTPase:GDP complexes will be regulated by the interplay of GEF and GAP activities in their various locations.

1.4 Testing the GEF Targeting Mechanism for Rab Proteins in Cells

If the arguments summarized above on the role of GEFs in Rab targeting are valid, it should be possible to manipulate the location of Rab proteins by controlling the localization of their cognate GEFs. This has been addressed by deliberate mislocalization of GEFs to the outer mitochondrial membrane in an acute fashion taking advantage of a mitochondrial binding domain (i.e., the *Listeria monocytogenes* ActA mitochondria-targeting sequence) fused to the FRB protein (FKB-rapamycin binding protein) (Blumer et al. 2013). Rab-GEFs fused to FKBP (FK506 binding protein) can then be recruited from their normal location to mitochondria on addition of a rapamycin analog, and in the three cases tested (Rab5/Rabex5, Rab1/DrrA, and Rab8/Rabin8), this is followed by relocation of the corresponding Rab proteins from their normal location to mitochondria. A similar approach in which the Rab32/Rab38 GEF BLOC-3 was mislocalized to mitochondria also resulted in the mislocalization of Rab32 (Gerondopoulos et al. 2012). This is strong evidence that GEFs can regulate the localization of their cognate Rabs. Obviously, this targeting mechanism can only function if Rab GEFs are localized to specific membranes. While known Rab GEFs are not integral membrane proteins, they appear to be recruited by a number of mechanisms to distinct membrane locations in practically all cases that have been sufficiently well investigated (Table 1.1) (Barr 2013; Blumer et al. 2013) excluding the atypical GEF Mss4 (Itzen et al. 2006; Wixler et al. 2011) (Dss4 in yeast) that is probably not a bona fide GEF but a type of chaperone for a number of nucleotide-free Rabs.

Table 1.1 Overview of Rab-GEFs, their sites of localization and their localization mechanism

GEF	Rab	Localization	Localizer(s)
TRAPP (Cai et al. 2007; Wang et al. 2000)	Rab1	ER-Golgi	Sec23 (CopII)
Ric1/Rgp1 (Pusapati et al. 2012)	Rab6	Golgi, cytosol	Rab33b:GTP
Sec2 (Ortiz et al. 2002; Walch-Solimena et al. 1997)	Sec4	Secretory vesicles	Ypt32:GTP, Sec15 (exocyst)
Rabin8 (Chiba et al. 2013; Hattula et al. 2002; Knodler et al. 2010)	Rab8	Cilia, cortical actin	Rab11:GTP, Sec15 (exocyst), Phosphatidylserine
Rabex5 (Horiuchi et al. 1997)	Rab5	Early endosomes	Rabaptin5, ubiquitinylation
DrrA/SidM (Brombacher et al. 2009; Machner and Isberg 2007; Murata et al. 2006)	Rab1	Legionella containing vacuole/plasma membrane	PI(4)P
Mon1/Ccz1 (Lachmann et al. 2012; Nordmann et al. 2010)	Ypt7/Rab7	Late endosomes	Vps21(Rab5):GTP
DENND1A (Allaire et al. 2010; Yoshimura et al. 2010)	Rab35	Clathrin coated pits, (plasma membrane)	AP2
DENND5A/5B (Miserey-Lenkei et al. 2007; Recacha et al. 2009; Yoshimura et al. 2010)	Rab39	Golgi	Rab6:GTP
DENND2 (Yoshimura et al. 2010)	Rab9	Actin filaments lysosomal trafficking	
MADD/Rab3Gep (Figueiredo et al. 2008; Yoshimura et al. 2010)	Rab27	Melanosomes	
Bloc-3 (Gerondopoulos et al. 2012)	Rab32/38	Melanosomes	
Crag (Xiong et al. 2012)	Rab11	Rhabdomeres	
FAM116 (Linford et al. 2012)	Rab14	Recycling endosomes, cytosol	
MTMR5/13 (Yoshimura et al. 2010)	Rab28		
DENND1C (Yoshimura et al. 2010)	Rab13		
DENND3 (Yoshimura et al. 2010)	Rab12		
DENND1B (Yoshimura et al. 2010)	Rab35		

In the case of recently discovered GEFs, this information is incomplete or absent

1.5 Do Additional Factors Contribute to Rab Targeting?

Although the evidence described is strong support for a decisive role of GEFs in Rab targeting, over the years other factors have been suggested to play an important part. The original hypothesis that the hypervariable C-terminal region alone acted as the sole determining factor (Chavrier et al. 1991) has been challenged and at least partially disproved, suggesting that multiple regions in Rabs contribute to membrane targeting (Ali et al. 2004). However, further testing of the role of the

C-terminus appeared appropriate, and recent experiments have addressed this question again (Li et al. 2014). Semisynthetic Rabs were prepared that contained C-termini in which much of the C-terminus was replaced by polyethylene glycol, except for a three amino acid motif known from earlier work to be essential for interaction with GDI and REP (CIM, or C-terminal interaction motif) (Rak et al. 2003, 2004; Wu et al. 2009). In some constructs, the unstructured region N-terminal to the CIM was replaced by a flexible Gly-Gly-Ser (GGS) repeat structure. In all constructs in which the appropriate lengths between the GTPase core domain and the CIM, as well as between the CIM and C-terminal cysteines, were maintained, the constructs could be geranylgeranylated both *in vitro* and *in vivo* (as judged by the evidence that membrane localization occurs). In the case of Rab1 and Rab5, such microinjected proteins localized in the same manner as wild-type constructs fused to fluorescent proteins, suggesting that here the exact structure of the C-terminus is not important. However, the targeting of C-terminally modified Rab7 and Rab35 is compromised in certain constructs, suggesting a role of parts of the C-terminus in correct localization. For Rab35, this is probably due to a requirement for a polybasic region near the C-terminus to interact with the highly negatively charged plasma membrane (Fig. 1.3). In Rab7, the sequence N-terminal to the CIM appears to be partially important, probably because of its known involvement in interaction with the effector RILP (Wu et al. 2005). This is reminiscent of evidence that effector binding is essential for the correct localization of Rab9 (Aivazian et al. 2006).

The notion that effector interactions are needed for localization of Rabs is not immediately attractive based on the accepted paradigm of Rab action, which is that recruitment to specific membranes in the active GTP-bound form is required to allow interaction with a cognate effector molecule, for example, to connect to transport systems or to engage tethering factors. If the interaction with one effector is required for localization, this interaction is presumably necessary for the continued residence of the Rab protein at its correct membrane localization. Effector interactions are typically not of very high affinity, and are rapidly reversible, so that it could be imagined that the initial localizing interaction might be replaced competitively by interaction with a different effector (that is not membrane localized, or at least not to the same membrane), but this would lead to destabilization of Rab in the membrane, which is probably not a desired consequence. Perhaps the essential equilibrium concepts behind this argument are too naïve. Thus, if the rate of spontaneous release of a Rab protein from the membrane is slow on the timescale of the events triggered by the second effector interaction, it is possible that the Rab molecule could fulfill its function before spontaneous or GAP/GDI-induced loss from the membrane, for example, after a fusion event.

A different situation can be envisaged with effectors that form heterotetrameric complexes with their cognate Rabs. Thus, it is possible that the dimeric structure of the Rab7 effector RILP is of importance in stabilizing the membrane-bound form of Rab7 (see Fig. 1.4). A complex with the stoichiometry of 2:2 (effector:Rab) will be stabilized in the membrane compared to Rab alone or to the situation in which there is only a 1:1 complex (unless there is an additional interaction of the effector with

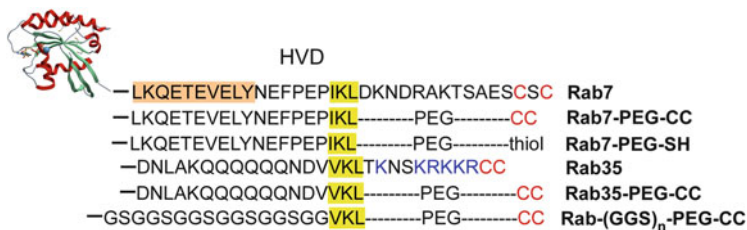


Fig. 1.3 Substitution of the Rab HVD with different C-terminal structures. The CIM is highlighted in yellow, the RILP interaction sequence of Rab7 in orange, while the basic residues in Rab35 are in blue and the prenylatable cysteines are in red

the membrane or another membrane component). This is because in the heterotetrameric complex there are four rather than two prenyl groups that interact with the membrane. It is conceivable that this is the reason for the importance of the Rab7 effector interaction for membrane stabilization, as described above. This is perhaps of more general significance, since several other Rab-effector interactions involve this dimeric type of interaction, including Rab5:Rabaptin5 (Zhu et al. 2004), Rab6:GCC185 (Burguete et al. 2008), Rab11:FIP2 (Jago et al. 2006), and Rab11:FIP3 (Eathiraj et al. 2006; Shiba et al. 2006). In the absence of additional interactions of the effector with the membrane or other membrane-bound components, this cannot be a targeting mechanism, but could be a mechanism to stabilize a Rab protein at a specific membrane after the initial targeting step, in which GDP/GTP exchange by a specifically localized GEF probably plays the most important role. Thus, a dimeric or better divalent effector would be able both to stabilize the membrane-bound form of the Rab protein and to interact with a further partner, for example, the dynein-dynactin complex in the case of Rab7 (Tan et al. 2011). It will be of interest to determine whether Rab effectors that also interact with the C-terminal region, but in a monovalent manner, also contribute to Rab targeting. Known examples are Rab3:Rabphilin (Ostermeier and Brunger 1999) and Rab27:melanophilin (Kukimoto-Niino et al. 2008).

In the case of targeting of Rab27a to melanosomes, it has been shown that impaired effector binding does not affect membrane localization, whereas the nonredundant GEF activity of Rab3GEP/MADD is essential (Tarafder et al. 2011). However, mutants of Rab27a were found that were substrates for the GEF activity of Rab3GEP in vitro but were not correctly localized, suggesting that an additional factor or activity (i.e., not just GEF activity) is required for targeting. However, it is not clear what the effect of these mutations on Rab27 cycling is. In a similar vein, it was recently shown that the Ypt7 GEF Mon1:Ccz1 is required for correct localization of wild-type Ypt7, but not for a Ypt7 mutant that showed facilitated (i.e., GEF-independent) nucleotide exchange (Cabrera and Ungermann 2013). This again suggests that an additional factor or factors are required for targeting and even suggests that the exact site of the exchange reaction is not important.

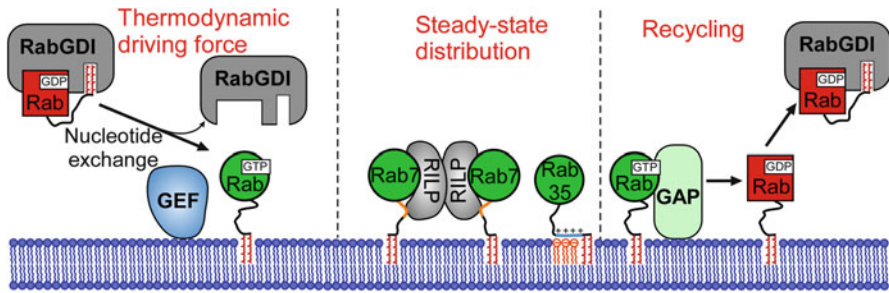


Fig. 1.4 Overview of important factors and principles in Rab targeting. GEF-mediated nucleotide exchange appears to be the major factor for Initial insertion of Rab proteins into membranes. Further interactions are probably of importance in determining the steady-state distribution between membrane and GDI. Note that univalent interaction with an effector will not increase stability unless there is an additional membrane-anchoring interaction of the effector (direct or indirect). A bivalent effector interaction of the type depicted could lead to stabilization of the Rab-membrane interaction, but the targeting step would still be dependent on GEFs. [Figure modified from Li et al. (2014)]

1.6 Conclusion

The work described briefly here leads to the conclusion that GEFs are major determinants in Rab targeting, but numerous observations suggest that a model in which GEFs are the sole determining factor is not correct. In some situations, there is evidence that effector interactions are important, while other observations demand another, additional targeting factor or principle. As a working model, we suggest that GTP/GDP exchange at a specific location plays a crucial role in targeting, but that additional stabilization of the membrane-bound state occurs in at least some cases, possibly including formation of a heterotetrameric complex containing two effector and two Rab molecules. In general, it appears to be the interplay of GEFs, GAPs, effectors, and possibly still unrecognized principles that determines localization in a highly dynamic and complex process.

References

- Aivazian D, Serrano RL, Pfeffer S (2006) TIP47 is a key effector for Rab9 localization. *J Cell Biol* 173:917–926
- Alexandrov K, Simon I, Iakovenko A, Holz B, Goody RS, Scheidig AJ (1998) Moderate discrimination of REP-1 between Rab7-GDP and Rab7-GTP arises from a difference of an order of magnitude in dissociation rates. *FEBS Lett* 425:460–464
- Ali BR, Wasmeier C, Lamoreux L, Strom M, Seabra MC (2004) Multiple regions contribute to membrane targeting of Rab GTPases. *J Cell Sci* 117:6401–6412
- Allaire PD, Marat AL, Dall'Armi C, Di Paolo G, McPherson PS, Ritter B (2010) The connectin DENN domain: a GEF for Rab35 mediating cargo-specific exit from early endosomes. *Mol Cell* 37:370–382

- Barr FA (2013) Review series: Rab GTPases and membrane identity: causal or inconsequential? *J Cell Biol* 202:191–199
- Blumer J, Rey J, Dehmelt L, Mazel T, Wu YW, Bastiaens P, Goody RS, Itzen A (2013) RabGEFs are a major determinant for specific Rab membrane targeting. *J Cell Biol* 200:287–300
- Brombacher E, Urwyler S, Ragaz C, Weber SS, Kami K, Overduin M, Hilbi H (2009) Rab1 guanine nucleotide exchange factor SidM is a major phosphatidylinositol 4-phosphate-binding effector protein of *Legionella pneumophila*. *J Biol Chem* 284:4846–4856
- Burguete AS, Fenn TD, Brunger AT, Pfeffer SR (2008) Rab and Arl GTPase family members cooperate in the localization of the golgin GCC185. *Cell* 132:286–298
- Cabrera M, Ungermann C (2013) Guanine nucleotide exchange factors (GEFs) have a critical but not exclusive role in organelle localization of Rab GTPases. *J Biol Chem* 288:28704–28712
- Cai H, Yu S, Menon S, Cai Y, Lazarova D, Fu C, Reinisch K, Hay JC, Ferro-Novick S (2007) TRAPPI tethers COPII vesicles by binding the coat subunit Sec23. *Nature* 445:941–944
- Chandra A, Grecco HE, Pisupati V, Perera D, Cassidy L, Skoulidis F, Ismail SA, Hedberg C, Hanzal-Bayer M, Venkitaraman AR, Wittinghofer A, Bastiaens PI (2012) The GDI-like solubilizing factor PDEdelta sustains the spatial organization and signalling of Ras family proteins. *Nat Cell Biol* 14:148–158
- Chavrier P, Gorvel JP, Stelzer E, Simons K, Gruenberg J, Zerial M (1991) Hypervariable C-terminal domain of rab proteins acts as a targeting signal. *Nature* 353:769–772
- Chiba S, Amagai Y, Homma Y, Fukuda M, Mizuno K (2013) NDR2-mediated Rabin8 phosphorylation is crucial for ciliogenesis by switching binding specificity from phosphatidylserine to Sec15. *EMBO J* 32:874–885
- Dirac-Svejstrup AB, Sumizawa T, Pfeffer SR (1997) Identification of a GDI displacement factor that releases endosomal Rab GTPases from Rab-GDI. *EMBO J* 16:465–472
- Eathiraj S, Mishra A, Prekeris R, Lambright DG (2006) Structural basis for Rab11-mediated recruitment of FIP3 to recycling endosomes. *J Mol Biol* 364:121–135
- Figueiredo AC, Wasmeier C, Tarafder AK, Ramalho JS, Baron RA, Seabra MC (2008) Rab3GEP is the non-redundant guanine nucleotide exchange factor for Rab27a in melanocytes. *J Biol Chem* 283:23209–23216
- Gerondopoulos A, Langemeyer L, Liang JR, Linford A, Barr FA (2012) BLOC-3 mutated in Hermansky-Pudlak syndrome is a Rab32/38 guanine nucleotide exchange factor. *Curr Biol* 22:2135–2139
- Hattula K, Furuholm J, Arffman A, Peranen J (2002) A Rab8-specific GDP/GTP exchange factor is involved in actin remodeling and polarized membrane transport. *Mol Biol Cell* 13:3268–3280
- Horiuchi H, Lippe R, McBride HM, Rubino M, Woodman P, Stenmark H, Rybin V, Wilm M, Ashman K, Mann M, Zerial M (1997) A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell* 90:1149–1159
- Hutagalung AH, Novick PJ (2011) Role of Rab GTPases in membrane traffic and cell physiology. *Physiol Rev* 91:119–149
- Ingmundson A, Delprato A, Lambright DG, Roy CR (2007) *Legionella pneumophila* proteins that regulate Rab1 membrane cycling. *Nature* 450:365–369
- Ismail SA, Chen YX, Rusinova A, Chandra A, Bierbaum M, Gremer L, Triola G, Waldmann H, Bastiaens PI, Wittinghofer A (2011) Arl2-GTP and Arl3-GTP regulate a GDI-like transport system for farnesylated cargo. *Nat Chem Biol* 7:942–949
- Itzen A, Pylypenko O, Goody RS, Alexandrov K, Rak A (2006) Nucleotide exchange via local protein unfolding-structure of Rab8 in complex with MSS4. *EMBO J* 25:1445–1455
- Jagoe WN, Lindsay AJ, Read RJ, McCoy AJ, McCaffrey MW, Khan AR (2006) Crystal structure of rab11 in complex with rab11 family interacting protein 2. *Structure* 14:1273–1283
- Knodler A, Feng S, Zhang J, Zhang X, Das A, Peranen J, Guo W (2010) Coordination of Rab8 and Rab11 in primary ciliogenesis. *Proc Natl Acad Sci USA* 107:6346–6351

- Kukimoto-Niino M, Sakamoto A, Kanno E, Hanawa-Suetsugu K, Terada T, Shirouzu M, Fukuda M, Yokoyama S (2008) Structural basis for the exclusive specificity of Slac2-a/melanophilin for the Rab27 GTPases. *Structure* 16:1478–1490
- Lachmann J, Barr FA, Ungermann C (2012) The Msb3/Gyp3 GAP controls the activity of the Rab GTPases Vps21 and Ypt7 at endosomes and vacuoles. *Mol Biol Cell* 23:2516–2526
- Leung KF, Baron R, Ali BR, Magee AI, Seabra MC (2007) Rab GTPases containing a CAAX motif are processed post-geranylgeranylation by proteolysis and methylation. *J Biol Chem* 282:1487–1497
- Li F, Yi L, Zhao L, Itzen A, Goody RS, Wu YW (2014) The role of the hypervariable C-terminal domain in Rab GTPases membrane targeting. *Proc Natl Acad Sci USA* 111:2572–2577
- Linford A, Yoshimura S, Nunes Bastos R, Langemeyer L, Gerondopoulos A, Rigden DJ, Barr FA (2012) Rab14 and its exchange factor FAM116 link endocytic recycling and adherens junction stability in migrating cells. *Dev Cell* 22:952–966
- Machner MP, Isberg RR (2007) A bifunctional bacterial protein links GDI displacement to Rab1 activation. *Science* 318:974–977
- Miserey-Lenkei S, Waharte F, Boulet A, Cuif MH, Tenza D, El Marjou A, Raposo G, Salamero J, Heliot L, Goud B, Monier S (2007) Rab6-interacting protein 1 links Rab6 and Rab11 function. *Traffic* 8:1385–1403
- Murata T, Delprato A, Ingmundson A, Toomre DK, Lambright DG, Roy CR (2006) The *Legionella pneumophila* effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nat Cell Biol* 8:971–977
- Nordmann M, Cabrera M, Perz A, Brocker C, Ostrowicz C, Engelbrecht-Vandre S, Ungermann C (2010) The Mon1-Ccz1 complex is the GEF of the late endosomal Rab7 homolog Ypt7. *Curr Biol* 20:1654–1659
- Ortiz D, Medkova M, Walch-Solimena C, Novick P (2002) Ypt32 recruits the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles; evidence for a Rab cascade in yeast. *J Cell Biol* 157:1005–1015
- Ostermeier C, Brunger AT (1999) Structural basis of Rab effector specificity: crystal structure of the small G protein Rab3A complexed with the effector domain of rabphilin-3A. *Cell* 96:363–374
- Pusapati GV, Luchetti G, Pfeffer SR (2012) Ric1-Rgp1 complex is a guanine nucleotide exchange factor for the late Golgi Rab6A GTPase and an effector of the medial Golgi Rab33B GTPase. *J Biol Chem* 287:42129–42137
- Rak A, Pylypenko O, Durek T, Watzke A, Kushnir S, Brunsveld L, Waldmann H, Goody RS, Alexandrov K (2003) Structure of Rab GDP-dissociation inhibitor in complex with prenylated YPT1 GTPase. *Science* 302:646–650
- Rak A, Pylypenko O, Niculae A, Pyatkov K, Goody RS, Alexandrov K (2004) Structure of the Rab7: REP-1 complex: insights into the mechanism of rab prenylation and choroideremia disease. *Cell* 117:749–760
- Recacha R, Boulet A, Jollivet F, Monier S, Houdusse A, Goud B, Khan AR (2009) Structural basis for recruitment of Rab6-interacting protein 1 to Golgi via a RUN domain. *Structure* 17:21–30
- Schoebel S, Oesterlin LK, Blankenfeldt W, Goody RS, Itzen A (2009) RabGDI displacement by DrrA from *Legionella* is a consequence of its guanine nucleotide exchange activity. *Mol Cell* 36:1060–1072
- Seabra MC (1996) Nucleotide dependence of Rab geranylgeranylation. Rab escort protein interacts preferentially with GDP-bound Rab. *J Biol Chem* 271:14398–14404
- Shiba T, Koga H, Shin HW, Kawasaki M, Kato R, Nakayama K, Wakatsuki S (2006) Structural basis for Rab11-dependent membrane recruitment of a family of Rab11-interacting protein 3 (FIP3)/Arfophilin-1. *Proc Natl Acad Sci USA* 103:15416–15421
- Sivars U, Aivazian D, Pfeffer SR (2003) Yip3 catalyses the dissociation of endosomal Rab-GDI complexes. *Nature* 425:856–859
- Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. *Nat Rev Mol Cell Biol* 10:513–525

- Tan SC, Scherer J, Vallee RB (2011) Recruitment of dynein to late endosomes and lysosomes through light intermediate chains. *Mol Biol Cell* 22:467–477
- Tarafder AK, Wasmeier C, Figueiredo AC, Booth AE, Orihara A, Ramalho JS, Hume AN, Seabra MC (2011) Rab27a targeting to melanosomes requires nucleotide exchange but not effector binding. *Traffic* 12:1056–1066
- Thoma NH, Iakovenko A, Goody RS, Alexandrov K (2001a) Phosphoisoprenoids modulate association of Rab geranylgeranyltransferase with REP-1. *J Biol Chem* 276:48637–48643
- Thoma NH, Iakovenko A, Kalinin A, Waldmann H, Goody RS, Alexandrov K (2001b) Allosteric regulation of substrate binding and product release in geranylgeranyltransferase type II. *Biochemistry* 40:268–274
- Walch-Solimena C, Collins RN, Novick PJ (1997) Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles. *J Cell Biol* 137:1495–1509
- Wang W, Sacher M, Ferro-Novick S (2000) TRAPP stimulates guanine nucleotide exchange on Ypt1p. *J Cell Biol* 151:289–296
- Wixler V, Wixler L, Altenfeld A, Ludwig S, Goody RS, Itzen A (2011) Identification and characterisation of novel Mss4-binding Rab GTPases. *Biol Chem* 392:239–248
- Wu M, Wang T, Loh E, Hong W, Song H (2005) Structural basis for recruitment of RILP by small GTPase Rab7. *EMBO J* 24:1491–1501
- Wu YW, Tan KT, Waldmann H, Goody RS, Alexandrov K (2007) Interaction analysis of prenylated Rab GTPase with Rab escort protein and GDP dissociation inhibitor explains the need for both regulators. *Proc Natl Acad Sci USA* 104:12294–12299
- Wu YW, Goody RS, Abagyan R, Alexandrov K (2009) Structure of the disordered C terminus of Rab7 GTPase induced by binding to the Rab geranylgeranyl transferase catalytic complex reveals the mechanism of Rab prenylation. *J Biol Chem* 284:13185–13192
- Wu YW, Oesterlin LK, Tan KT, Waldmann H, Alexandrov K, Goody RS (2010) Membrane targeting mechanism of Rab GTPases elucidated by semisynthetic protein probes. *Nat Chem Biol* 6:534–540
- Xiong B, Bayat V, Jaiswal M, Zhang K, Sandoval H, Charng WL, Li T, David G, Duraine L, Lin YQ, Neely GG, Yamamoto S, Bellen HJ (2012) Crag is a GEF for Rab11 required for rhodopsin trafficking and maintenance of adult photoreceptor cells. *PLoS Biol* 10:e1001438
- Yoshimura S, Gerondopoulos A, Linford A, Rigden DJ, Barr FA (2010) Family-wide characterization of the DENN domain Rab GDP-GTP exchange factors. *J Cell Biol* 191:367–381
- Zhu G, Zhai P, Liu J, Terzyan S, Li G, Zhang XC (2004) Structural basis of Rab5-Rabaptin5 interaction in endocytosis. *Nat Struct Mol Biol* 11:975–983