thematic review

Thematic review series: Lipid Posttranslational Modifications

Geranylgeranylation of Rab GTPases

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Abstract Rab GTPases require special machinery for protein prenylation, which include Rab escort protein (REP) and Rab geranylgeranyl transferase (RGGT). The current model of Rab geranylgeranylation proposes that REP binds Rab and presents it to RGGT. After geranylgeranylation of Rab C-terminal cysteines, REP delivers the prenylated protein to membranes. The REP-like protein Rab GDP dissociation inhibitor (RabGDI) then recycles the prenylated Rab between the membrane and the cytosol. The recent solution of crystal structures of the Rab prenylation machinery has helped to refine this model and provided further insights. The hydrophobic prenyl binding pocket of RGGT and geranylgeranyl transferase type-I (GGT-I) differs from that of farnesyl transferase (FT). A bulky tryptophan residue in FT restricts the size of the pocket, whereas in RGGT and GGT-I, this position is occupied by smaller residues. A highly conserved phenylalanine in REP, which is absent in RabGDI, is critical for the formation of the REP:RGGT complex. Finally, a geranylgeranyl binding site conserved in REP and RabGDI has been identified within helical domain II. The postprenylation events, including the specific targeting of Rabs to target membranes and the requirement for single versus double geranylgeranylation by different Rabs, remain obscure and should be the subject of future studies.—Leung, K. F., R. Baron, and M. C. Seabra. **Geranylgeranylation of Rab GTPases. *J. Lipid Res.* 2006.** 47: **467–475.**

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Members of the small GTPase Ras superfamily perform important regulatory functions, from cell growth to cytoskeleton dynamics to membrane trafficking. With the exception of Ran, Ras-like proteins undergo cotranslational or posttranslational lipid modifications, which act as hydrophobic membrane anchors, interacting with the cytoplasmic leaflet of cellular membranes and/or participating in protein-protein interactions. The most common lipid modification affecting small GTPases is protein prenylation, which involves the covalent addition of either farnesyl (15 carbon) or geranylgeranyl (20 carbon) pyrophosphate to proteins via thioether linkages catalyzed by protein prenyl transferases (1). Prenylation of Ras, Rho/Rac, and Rab is absolutely critical for the proper function of the modified protein in cellular processes (reviewed in the other reviews in this series). The importance of protein prenylation first gained focus when it was found that oncogenic forms of Ras proteins required prenylation for their ability to transform cells (2, 3). Since then, the search for inhibitors of prenylation has been an active area of research [reviewed in this series (4)].

Three distinct protein prenyl transferases have been identified and can be classified into two main functional classes: the CAAX prenyl transferases, consisting of farnesyl transferase (FT) and geranylgeranyl transferase type I (GGT-I) [reviewed in this series by Lane and Beese (4a)], and the Rab geranylgeranyl transferase (RGGT, also known as GGT-II) (1). Substrates for the first class include CAAX-containing farnesylated proteins (Ras, nuclear lamins, and others) and geranylgeranylated proteins of the Rho/Rac families and others. Rab protein family members are exclusive substrates of RGGT.

THE RAB PROTEIN FAMILY: STRUCTURE/FUNCTION

The Rab proteins (ras genes from rat brain), comprising >60 proteins, form the largest family of the Ras superfamily of small GTPases and are important regulators of organelle biogenesis and vesicle transport (5). They are conserved throughout evolution, from yeast to mammals (6). Some Rab proteins are ubiquitously expressed, whereas others are expressed in a tissue-specific or developmentally regulated manner. For example, Rab1 is found in all cell types, whereas others, such as Rab27a, are found in melanocytes and secretory cell types (7). Analysis of the budding yeast *Saccharomyces cerevisiae* genome indicates that there are 11 Rab genes called Ypt (yeast protein involved in transport), some of which are redundant

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in their function (8). In mammals, >60 Rab proteins have been identified, which is not surprising considering the increase in complexity of trafficking pathways required to carry out diverse functions in a variety of different cell types. The evolutionary conservation of Rabs is highlighted by the fact that mouse Rab1a can compensate for the loss of Ypt1p in yeast (9).

Studies of Rab protein function suggest that they are important in vesicular membrane transport (10, 11). Eukaryotic cells possess an elaborate internal membrane system composed of different intracellular compartments, each serving a different function. These compartments are highly dynamic and communicate with each other. Each Rab protein has a specific intracellular localization and thus regulates a specific membrane trafficking step. However, transport between two membrane compartments may be governed by more than one Rab member; thus, some Rab proteins may exhibit redundancy in their roles.

All members of the Ras superfamily have conserved regions that are involved in binding guanine nucleotide and phosphate/Mg^{2+}; these have been referred to previously as G1–G3 and PM1–PM3, respectively (12). There are two regions that undergo a significant conformational change upon GTP binding and hydrolysis: the switch I domain, which lies in the loop 2 region; and the switch II domain, which resides in the loop 4/α2/loop 5 region. Although the presence of a dicysteine prenylation motif at the C terminus is generally considered a good defining feature of a Rab protein, it is not absolute, as a few Rab proteins, such as Rab8 and Rab13, contain only a single cysteine motif. More recently, diagnostic feature distinguishing to distinguish Rabs from other Ras-like GTPases has been proposed based on sequence alignments of Rab proteins (5). Using this approach, five Rab family regions (RabF) were identified that were conserved only in Rab proteins, thus distinguishing them from other Ras-like proteins (**Fig. 1**). The RabF1 region is located in the so-called effector domain (loop 2/β2) in the switch I region. The remaining four regions, RabF2 (β3), RabF3 (loop 4), RabF4 (α2/loop 5), and RabF5 (β4/loop 6), all reside in and around the switch II region between β-sheets β3 and β4 (5). Because the RabF regions cluster around the two switch domains, which undergo changes in conformation on binding GDP or GTP, it has been suggested that these regions are involved in binding to general regulators of Rab function, such as Rab GDP dissociation inhibitor (RabGDI) protein and Rab escort protein (REP), as these regulatory proteins are nucleotide-sensitive (e.g., they associate better with the GDP form of Rab proteins) and recognize all Rabs (13, 14).

In addition, four Rab subfamily regions (RabSF) were defined as regions of high conservation within subfamilies: RabSF1 (β1), RabSF2 (α1/loop 2), RabSF3 (α3/loop 7), and RabSF4 (α5) (5). RabSF1, RabSF3, and RabSF4

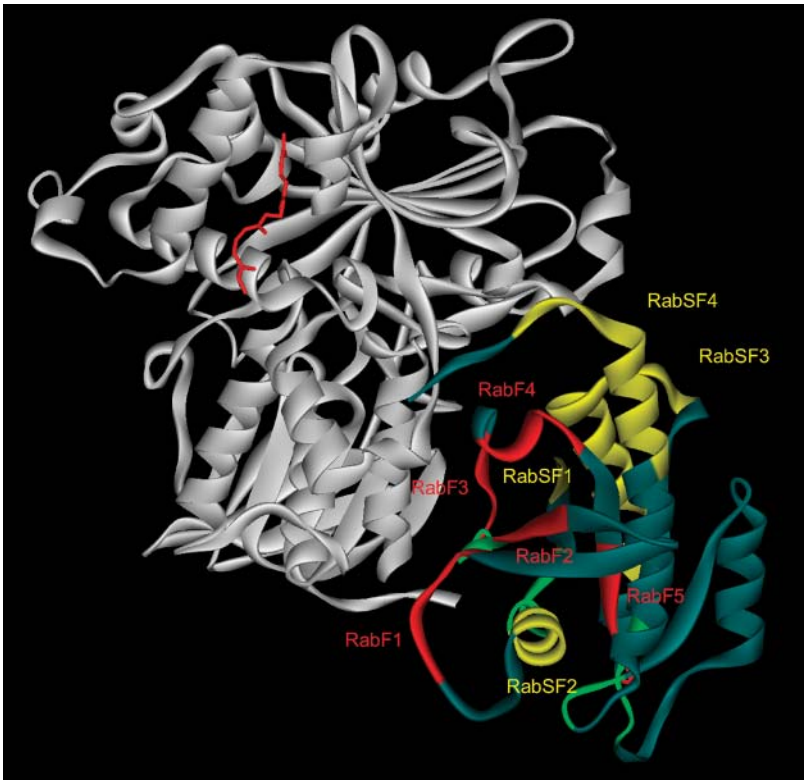

Fig. 1. Crystal structure of the Rab7:Rab escort protein-1 (Rab7:REP-1) complex showing regions conserved within the Rab family. Ribbon representation of REP-1 (white) bound to Rab7 (grayish blue). The Rab family regions (RabF) and Rab subfamily regions (RabSF) are highlighted in red and yellow, respectively. The guanine nucleotide binding regions are shown in green. Geranylgeranyl diphosphate (GGpp; brown) located in the prenyl binding pocket of REP-1 is shown in a ball-and-stick representation. All crystal structures were generated using Accelerys DS ViewerPro 5.0. The Protein Data Bank identifier for Rab7GG:REP-1 is 1VG0.

correspond to three regions previously referred to as Rab complementary-determining regions I, II, and III, based on the crystal structure of Rab3a complexed with its effector Rabphilin3a (15). Thus, RabSF1, RabSF3, and RabSF4 of Rab3a form a surface that mediates binding to Rabphilin3a, whereas RabSF2 forms another surface on the opposite face of Rab3a and could interact with other effectors. Based on these findings, it was proposed that effectors bind to RabF regions to discriminate between the nucleotide-bound states and to RabSF regions to confer specificity.

RAB GERANYLGERANYLATION: TWO ALTERNATIVE PATHWAYS

Like the CAAX prenyl transferases, RGGT is heterodimeric and consists of distinct α- and β-subunits. However, its mechanism of action is distinct from that of the other prenyl transferases. The enzyme was first isolated from rat brain cytosol and purified as a multicomponent enzyme (components A and B) that was able to attach geranylgeranyl groups onto Rab proteins (16, 17). Component B represents the catalytic component, now called RGGT. Unlike the CAAX prenyl transferases, RGGT does not recognize short peptides containing the Rab C-terminal prenylation motif, nor does it recognize the Rab protein alone (17, 18). Instead, it binds component A, now called REP, which is a Rab binding protein (19).

Several details concerning the mechanisms of Rab protein prenylation remain unclear. Biochemical assays have led to the proposal of two possible pathways (**Fig. 2**). The classical mechanism of Rab prenylation implicates first the association of an unprenylated Rab protein with REP (19). The equilibrium dissociation constant was measured to be 0.2 μM (although it varies between Rabs), and the interaction relies mostly on ionic bonds and does not involve the two C-terminal cysteine residues (18). The complex is then recognized by RGGT (K_d< 1 nM), which adds two geranylgeranyl moieties to the Rab protein without prior dissociation of REP (20, 21). After the transfer of the isoprenoids onto C-terminal cysteines, the ternary complex remains associated until the binding of a new geranylgeranyl diphosphate (GGpp) molecule, which stimulates the release of the Rab-GG:REP complex (20). REP is then believed to escort the prenylated Rab protein to its target membrane (22) (Fig. 2).

An alternative pathway for Rab prenylation was also proposed (23). Solid phase precipitation assays demonstrated that REP-1 and RGGT can form a tight complex in the presence of GGpp (K_d∼ 10 nM) (Fig. 2). This complex could associate with Rab protein, but 10 times slower than REP:Rab to RGGT:GGpp. It was proposed that in vivo the pathway followed should depend on the concentrations of the proteins involved. At high concentrations of RGGT, REP, Rab, and GGpp, the association of Rab with the RGGT:GGpp:REP complex becomes rate-determining

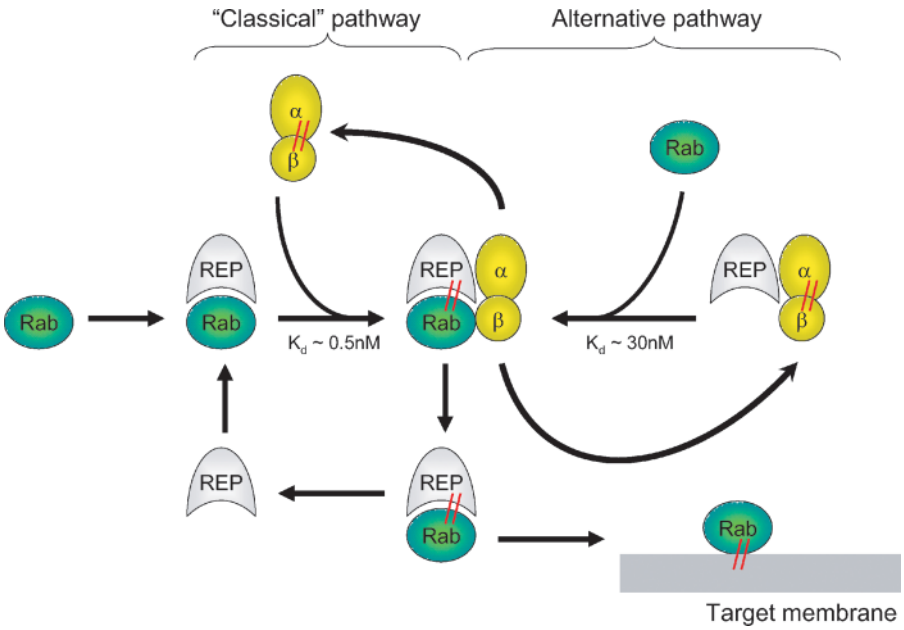

Fig. 2. Cartoon showing the two possible pathways for Rab protein prenylation. In the classical pathway, newly translated Rabs bind REP and the complex is recognized by GGpp-bound Rab geranylgeranyl transferase (RGGT). RGGT catalyzes the transfer of geranylgeranyl groups to C-terminal cysteines of the Rab protein. After prenylation, RGGT dissociates from REP, which remains bound to the prenylated Rab protein and delivers it to target membranes. REP is then released into the cytosol to take part in a new cycle of prenylation. In the alternative pathway, REP forms a complex with RGGT in the presence of GGpp under conditions in which these constituents are at higher concentrations relative to the Rab protein. The REP:RGGT:GGpp complex then binds newly translated Rab protein and the geranylgeranylation reaction takes place. RGGT dissociates as before, whereas REP escorts the prenylated Rab to membranes as in the classical pathway. K_d values of the Rab:REP:RGGT:GGpp complex for each pathway are indicated.

and is favored, whereas at low concentrations, the classical pathway is preferred.

RGGT

The heterodimeric enzyme, consisting of a 60 kDa α-subunit and a 38 kDa β-subunit, presents 30% homology with its counterparts FT and GGT-I (24). The yeast genes encoding the α- and β-subunits of RGGT were designated *BET4* and *BET2*, respectively (25, 26). Interestingly, a mutation termed *bet2-1* results in lower affinity of the enzyme for GGpp. This mutation can be suppressed by overexpression of *BTS1* (25), which encodes a GGpp synthase, suggesting that this enzyme is directly involved in GGpp accessibility by RGGT.

The crystal structure of RGGT has been solved to 2.0 angstrom (Å) and revealed the presence of four distinct structural domains (27). The α-subunit is composed of three domains: a helical domain, an Ig-like domain, and a leucine-rich repeat (LRR) domain. The helical domain is structurally very similar to the α-subunit of FT, despite only 22% sequence identity between FTα and the relevant region in RGGTα. A major structural difference in RGGT is the presence of both the Ig-like domain and the LRR domain, which are absent in FT and GGT-I. These domains are also absent from lower eukaryote versions of RGGT, suggesting that the LRR and Ig-like domains of the mammalian RGGT are not essential for the catalytic activity of the enzyme. The functions of these unique regions remain unknown.

The β-subunit forms an α-α barrel and contains a central cavity lined with hydrophobic residues very similar to the β-subunit of FT, which comprises the GGpp binding site. A positively charged cluster is located near the opening of the cavity. In the FT-Fpp structure, this cluster was shown to interact with the diphosphate head groups of Fpp (28, 29).

The recent elucidation of the structure of GGT-I (30) helped define the isoprenoid specificity of each enzyme. In geranylgeranyl transferases, residue 49β (in GGT-I) is always a small amino acid such as threonine, serine, valine, or alanine across many species, whereas in FT, it is always a tryptophan. This residue fills the space in FT where the larger isoprenoid GGpp fit within GGT-I and RGGT, thus constricting the length of the isoprenoid that fits in

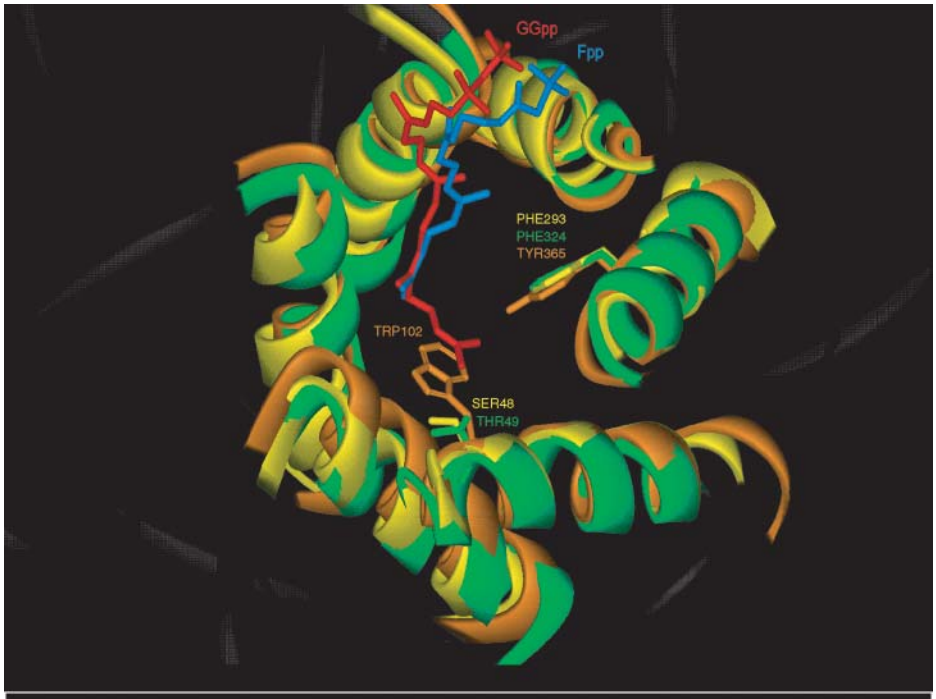

Fig. 3. The isoprenyl binding pocket of mammalian protein prenyl transferases. Superposition of the isoprenyl binding pocket of Zn^{2+}-depleted farnesyl transferase (FT; orange), geranylgeranyl transferase type-I (GGT-I; green), and RGGT (yellow). Fpp (blue), GGpp (red), and key amino acid side chains are shown in ball-and-stick representations. Trp102β of FT clashes with the fourth isoprene unit of GGpp and therefore sterically hinders GGpp from binding in the pocket. The smaller residues in GGT-I and RGGT (Thr49 and Ser48, respectively) accommodate the GGpp molecule. Sequence alignment of the β-subunits of human FT, GGT-I, and RGGT indicates the key residues Trp102β (orange), Thr49β (green), and Ser48β (yellow). The Protein Data Bank identifiers for FT, GGT-I, and RGGT are 1D8E, 1N4P, and 1LTX, respectively.

the binding site (**Fig. 3**). When the Trp residue was replaced by a Thr in FTβ, the resulting mutant preferably bound to GGpp without any significant alteration of CAAX sequence specificity (30). In RGGT, as in GGT-I, Ser48β and Leu99β replace the more bulky Trp102β and Tyr154β of FT at the bottom of the cavity (Fig. 3). These changes significantly enlarge the binding site to accommodate GGpp.

RGGT binds GGpp with an affinity of 8 ± 4 nM, whereas Fpp binds less strongly (K_d = 60 ± 8 nM) and Gpp even less (K_d = 330 ± 40 nM) (31). However, these differences in affinity are more significant when the Rab substrate is included. This may be explained by the fact that the reaction cycle progression requires the binding of fresh isoprenoid diphosphate to displace the product from the active site. Fpp was shown to be ineffective in displacing GGpp from the active site of GGT-I (30).

Mutations in RGGT have been shown to cause a disease similar to Hermansky-Pudlak syndrome in the *gunmetal* (*gm*) mouse (32). Hermansky-Pudlak syndrome is a rare autosomal recessive, genetic disease characterized by partial albinism, prolonged bleeding, and platelet dysfunction (33). The *gm* mutation was identified as a glycine-to-alanine substitution in a splice acceptor site within the *Rggta* gene (32). Although REP expression is unaffected, there is ~70% reduction in the expression of RGGT α-subunit, with a concomitant decrease in RGGT activity. Although the reduction in RGGT activity was observed in all tissues, defects in Rab prenylation are tissue-specific (32, 34, and our unpublished observations). One possible explanation for the tissue-specific phenotype is that concentrations of Rab proteins are considerably higher in platelets and melanocytes compared with other tissues (34). Alternatively, a subset of Rabs present lower affinity for the prenylation machinery and are selectively affected whenever the enzyme is limiting (32). Interestingly, Rab27 isoforms are highly expressed in tissues affected in *gm* mice, suggesting that hypoprenylation of Rab27 partly contributes to the *gm* phenotype.

REGULATION OF RGGT ACTIVITY

Very little is known about potential enzyme regulation. One possible mechanism involves an intramolecular interaction (27). The structural data suggest that the RGGTα N terminus is mobile. This region of the α-subunit binds to the β-subunit in an extended conformation by coordinating the zinc ion with residues from both α- and β-subunits, so it was hypothesized that this binding might act in an autoinhibitory manner to prevent the binding of short substrate peptides (27). This hypothesis remains to be tested.

There is also some evidence to suggest that RGGT activity may be regulated by phosphorylation. Stimulation of 3T3-L1 fibroblasts and adipocytes with insulin was shown to result in the concomitant phosphorylation of the RGGT α-subunit but not the β-subunit (35). This in turn led to a subtle increase in Rab3 and Rab4 prenylation. Therefore, it is possible that Rab prenylation can be influenced by environmental changes by responding to extracellular signals. Interestingly, insulin was also shown to induce the phosphorylation of the FT α-subunit, leading to increased farnesylation of Ras (36). Because FT and GGT-I share the same α-subunit, it was proposed that insulin-induced phosphorylation may also regulate GGT-I activity, although this has yet to be shown. Although no further studies have demonstrated phosphorylation of RGGT, future work should clarify its role in the regulation of RGGT activity.

Although many specific inhibitors have been found to inhibit FT and GGT-I activity, including FTI-277 and GGTI-298 (4), a limited number of specific inhibitors for RGGT have been identified. For example, the monoterpene perillyl alcohol has been shown to inhibit RGGT, but it also inhibits GGT-I. The first specific inhibitor of RGGT was a phosphonocarboxylate called NE10790 or 3-PEHPC (37). This analog of the nitrogen-containing bisphosphonate risedronate was identified as a drug that inhibited not only the prenylation of Rab proteins in osteoclasts and J774 macrophages in vitro but also bone resorption in vivo. More recently, 3-PEPC, an analog of 3-PEHPC, was also demonstrated to inhibit the activity of RGGT (38). The availability of such inhibitors specific for RGGT may help in our understanding of RGGT regulation and the mechanism of Rab prenylation.

THE REP/RabGDI PROTEIN FAMILY

Two REP isoforms are known in mammals: REP-1 and REP-2, both of which are ubiquitously expressed, although their expression levels vary in different tissues (39). In yeast, only a single essential gene has been identified, encoding the MRS6/MSI4 gene product (40). The REP proteins are structurally related to RabGDI, such that they have been grouped to form the REP/RabGDI superfamily.

REP-1 was shown to be the product of the choroideremia (CHM) gene, which maps to human locus Xq21 (41). CHM is an X-linked progressive retinal degenerative disease affecting photoreceptors, retinal pigment epithelium, and choroid (42). The autosomal homolog REP-2 (also known as CHML) can functionally replace REP-1, as in vitro assays suggest that it behaves as a REP in the geranylgeranylation of Rabs (43). The substrate specificity between REPs may differ, because REP-2 may have lower affinity for Rab3 and Rab27, although the molecular basis for this effect remains unresolved (43–45). Mutation of REP1 in CHM leads to a selective defect in the prenylation of some Rabs, including Rab27a (46). These defects may trigger the degenerative process in CHM.

RabGDI was originally isolated from bovine brain as a protein that inhibited the dissociation of GDP from Rab3a (13). RabGDI and REP family members all share regions of high homology known as sequence conserved regions (SCRs). Sequence alignment of the RabGDI and REP sequences reveal five SCRs located at the N-terminal and central portions of the molecule. The crystal structures of RabGDIα (47) and in complex with prenylated Ypt1 (48) have been solved. They reveal that RabGDIα is composed

of two main structural units, a large multisheet domain I and a small α-helical domain II. Domain I is composed of SCR1A, SCR1B, and SCR3B, which fold back together to form a compact structure at the apex of the molecule to create the Rab binding platform (RBP). The less conserved SCR2 and SCR3A constitute domain II. The SCRs form a conserved face on one side of the molecule, whereas the opposing face is composed of less conserved regions.

Two crystal structures of REP-1 in complex with either RGGT or Rab7 have been described (44, 49). As with RabGDI, REP-1 consists of two domains: a large cylindrical domain I made up of four β-sheets and six α-helices, and a smaller domain II composed of five α-helices. The largest region of sequence conservation with RabGDI, SCR2, covers helices D and E of domain II and the C-terminal binding region on domain I of REP-1. The most significant difference between REP-1 and RabGDI is in domain I, where REP contains a large insertion between SCR1 and SCR2. Unfortunately, the function of this insert remains unknown, as does its structure, which was not visible in the crystal.

Because the RBP is a key functional element, it is highly conserved between REP-1 and RabGDI. Of 32 residues of REP-1 involved in the formation of the Rab complex interface, 15 are invariant between RabGDI and REP-1, 11 are conserved, and 6 are unique for the REP-1:Rab complex (44). The contacts between the RBP and Rab7 are nearly identical in the prenylated and unprenylated complexes, suggesting that the association of REP-1 with the geranylgeranyl moiety does not alter the RBP. REP-1 needs to bind unprenylated proteins to promote their prenylation, whereas this property is not required for RabGDI function. In fact, it is widely thought that RabGDI cannot bind unprenylated Rabs effectively. However, comparison of structural data from Rab7:REP-1 (44) and Ypt1:yGdi1p (48) complexes indicates that they are in fact very similar in terms of hydrophobic and hydrophilic interactions between REP/GDI and Rab proteins; therefore, such data cannot explain why RabGDI preferentially binds prenylated proteins.

The crystal structure of RabGDI in complex with prenylated Ypt1p revealed that the geranylgeranyl moiety is accommodated in a hydrophobic lipid binding site in domain II (48). A similar binding pocket was observed in REP (44) (Fig. 1). This hydrophobic pocket appears to be blocked when REP-1 is in complex with RGGT. However, when REP is in complex with a mono-GG Rab protein, there is a change in the conformation of helix D, resulting in an opening of the binding site and allowing the accommodation of the GG moiety. The cavity is too small to accommodate two GG groups, so it was postulated that the second lipid moiety lies in an adjacent groove located outside the cavity. However, the structures of apo-REP-1 and di-GG-Rab:REP-1 first need to be resolved to build a complete model of the interactions between Rab proteins and REP.

The mobile effector loop is a short stretch in domain II that is thought to be another conserved functional element, although it adopts a different conformation in REP-1 than in RabGDI (49). Mutations in the RabGDI mobile effector loop did not affect Rab binding but significantly reduced the membrane association of RabGDI and prevented the extraction of Rabs by RabGDI (50). Therefore, the mobile effector loop has been implicated in Rab recycling.

The C-terminal binding region of REP-1 is composed of hydrophobic residues that form a cavity above the mobile effector loop. It has been proposed that the C-terminal binding region interacts with the distal part of the C terminus of Rab proteins. More importantly, it was demonstrated that a motif in the HVR of Rab proteins, similar to the IKL sequence of Rab7, consisting of a polar residue flanked by hydrophobic residues, is important for efficient Rab prenylation.

Despite the structural similarities, REP and RabGDI have unique functions. The best understood difference involves the inability of RabGDI to bind RGGT (for more details, see below). As discussed above, the issue of whether there are differences between REP and RabGDI in binding affinities toward unprenylated and prenylated Rabs remains to be addressed experimentally, although this is often referred to as a known fact. Under steady-state conditions, endogenous RabGDI:Rab complexes can be isolated but REP:Rab complexes cannot. This may reflect the fact that RabGDI is more abundantly expressed than REP and/or functional differences. The most likely hypothesis at present is that REP works at the initial prenylation/membrane association event, whereas RabGDI works at a later stage in recycling Rabs on/off membranes (22).

THE REP:RGGT COMPLEX

Recent studies described the crystal structure of REP-1 complexed with RGGT to 2.7 Å resolution and provided more insight into the interaction between the two proteins as well as why RGGT can interact with REP but not with RabGDI (49). A previous suggestion suggested that the LRR or Ig-like domain of RGGT interacts with the insert region of REP, because these are unique sequences (27). This is in fact not the case, because this element faces the opposite direction to the interaction surface. The REP:RGGT interface is formed by helices 8, 10, and 12 of the RGGT α-subunit and helices D and E in domain II of REP-1 (49). Despite the high affinity with which REP-1 can bind RGGT, the contact area is surprisingly small. An important finding was that residue Phe279 of REP-1 plays a key role in mediating the interaction with RGGT, because an Phe279Ala substitution abolishes binding to RGGT (49). This residue protrudes deeply into the cavity formed by α-helices 8 and 10 of RGGT and by establishing hydrophobic interactions stabilizes the REP-1:RGGT complex (**Fig. 4**). This phenylalanine is conserved throughout the REP family but is absent in the RabGDI family. Comparison between apo-RGGT and the REP-1:RGGT complex suggested that complex formation leads to a dramatic decrease in the flexibility of RGGT helices 8 and 10. It was proposed that this change in conformation unblocked a binding site for REP helix D, which would otherwise

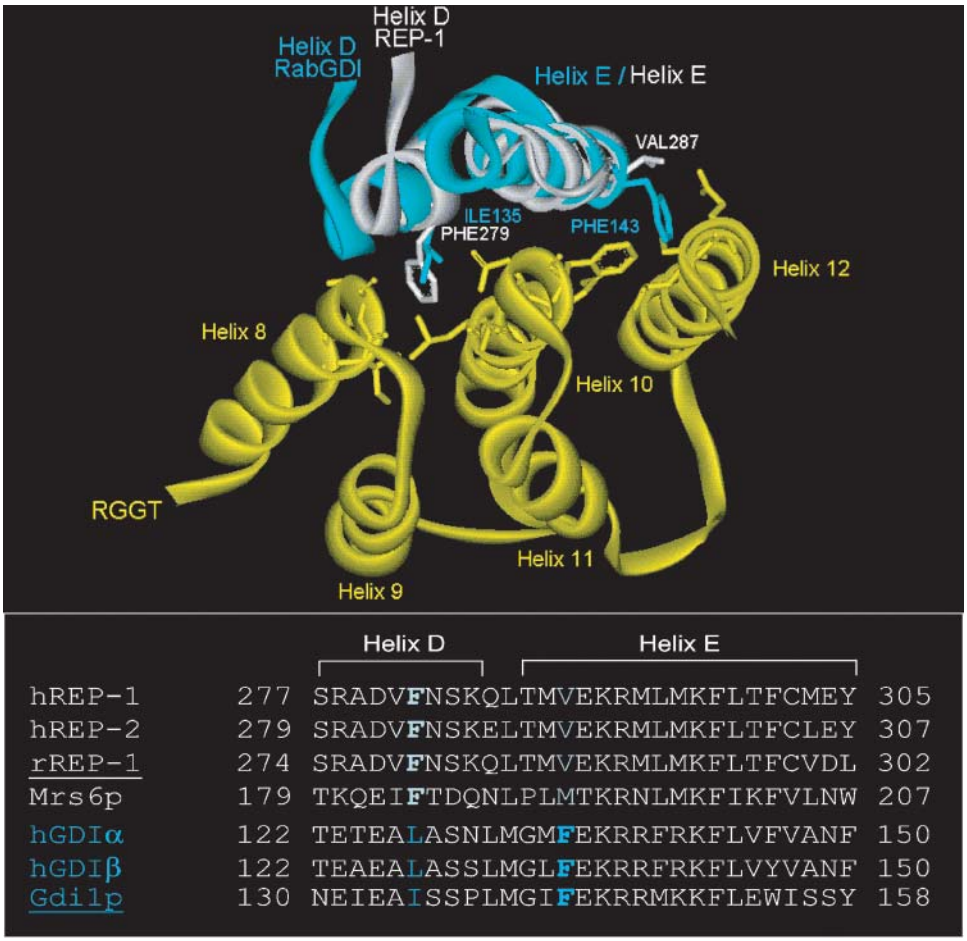

Fig. 4. Comparison of REP-1 versus Rab GDP dissociation inhibitor (RabGDI) for RGGT association. Helices D and E of yeast Gdi1p (turquoise) were superimposed onto the corresponding helices of rat REP-1 (white). Phe279 in helix D of REP-1 anchors between helices 8 and 10 of RGGT (yellow), but the smaller Ile135 residue of Gdi1p is unable to do so. In helix E, Val287 of rREP-1 is occupied in the same position by Phe143 in Gdi1p, which clashes with residues in the REP binding site located on RGGT helices 10 and 12. The amino acid side chains of the aforementioned residues are shown in ball-and-stick representations. Sequence alignment of the mammalian and yeast isoforms of REP and GDI is shown at bottom. The phenylalanine residues (white) in helix D are conserved among the REP family but are not present in GDI, whereas in helix E, the phenylalanine corresponding to residue 143 of Gdi1p (turquoise) is conserved among GDI proteins but not in REPs. The Protein Data Bank identifiers for Gdi1p and rREP-1 with RGGT are 1URV and 1LTX, respectively.

be hindered by RGGT helix 8, allowing the formation of a high-affinity complex. Comparison of RabGDI with REP-1 reveals another important difference. In helix E, the conserved Val287 residue in REP-1 is replaced with a phenylalanine residue at this position in RabGDI (Fig. 4). This phenylalanine is highly conserved in the RabGDI family but is absent in the REP family. It was suggested that the bulky aromatic side chain would clash with residues in the REP-1 binding site of RGGT. Thus, despite sharing structurally conserved domains, REP and RabGDI can be functionally segregated through a small number of amino acid substitutions.

The REP:RGGT crystal structure also raised an intriguing question regarding how the binding of phosphoisoprenoid was able to stimulate the interaction of RGGT and REP, because the distance between the REP:RGGT binding interface and the active site located on the RGGT β-subunit exceeded 30 Å. The RGGT α-subunit residue Tyr107 was shown to be involved in phosphoisoprenoid-dependent interaction between REP and RGGT. Indeed, the RGGTαY107A mutant lacks the ability to interact with REP-1 with high affinity (K_d = 10 nM), whereas low-affinity (K_d = 2 μM) isoprenoid-independent binding was unaffected (49). Thus, it was proposed that Tyr107α regulates a long-range conformational change that transduces phosphoisoprenoid binding.

A model of the sequence of events that occurs during the prenylation reaction has thus begun to emerge. Upon prenylation, the newly conjugated GGpp molecule moves from RGGT to the REP-1 hydrophobic cavity. By invading this hydrophobic core, helices D and E of REP-1 are displaced and the RGGT interaction with residues Phe279 (helix D) and Arg290 (helix E) are disrupted, leading to a decrease in the affinity of REP for RGGT.

FUNCTIONAL CONSEQUENCES OF RAB PRENYLATION: MONOGERANYLGERANYLATION VERSUS DIGERANYLGERANYLATION

The majority of Rab proteins contain two cysteine residues, such as CC or CXC at the C terminus, and undergo two geranylgeranylation reactions, probably via consecutive independent steps (21, 51). This double prenylation makes the Rab proteins considerably more hydrophobic than other prenylated proteins, which may be the reason why REP is required to chaperone Rab proteins during and after prenylation. Intriguingly, a subset of Rab proteins possess only one C-terminal cysteine residue, usually within a CXXX motif, and hence are only modified by a single GG group (52). Interestingly, these monocysteine Rabs possess a CXXX motif and in some cases a canonic CAAX motif similar to members of the Ras and Rho families. Rab8a possesses a CVLL motif, which is potentially a substrate of GGT-I, although it is preferentially modified by RGGT (53 and our unpublished observations). Although the presence of a single GG group is sufficient to target monocysteine Rab proteins, two GG moieties are required for the faithful targeting of dicysteine Rabs. Recent studies have shown that when the C terminus of Rab proteins normally containing a dicysteine motif, such as Rab5a and Rab27a, was replaced with a monocysteine motif, such as CSLG or CVLL, the mutants were mistargeted to the endoplasmic reticulum/Golgi region (54). Furthermore, Rab27a-CVLL was unable to rescue the function of wild-type Rab27a in Rab27a$^{-/-}$ cells (54). These findings indicate that the prenylation status is important for the correct targeting and function of Rab proteins. Similar studies in yeast have demonstrated that Ypt1p and Sec4p mutants with one prenylatable cysteine were similarly mislocalized and were unable to support growth when the mutant Rab represented the sole copy in the cell (55). This is consistent with the studies in mammalian cells and further demonstrates the importance of digeranylgeranylation.

The reason why some Rabs are monoprenylated and some are diprenylated is not clear, but recent studies suggest that they may be targeted by different routes. The integral membrane protein Yip1p, which has been implicated in Rab recruitment to membranes, interacts preferentially with diprenylated and not monocysteine Rabs, suggesting that different factors may be involved in membrane recruitment (55).

Postprenylation processing is another factor that may assist the membrane recruitment of Rabs. Rab proteins with a CXC motif, but not a CC motif, are carboxylmethylated on the C-terminal prenylcysteine (56). Our unpublished data suggest that monocysteine Rabs undergo postprenylation processing (i.e., proteolysis of the AAX tripeptide and carboxyl methylation), as observed in CAAX-containing Ras family proteins. However, the contribution of carboxyl methylation in Rab targeting is unclear, because the absence of methylation does not affect the localization of Rab proteins (57 and our unpublished data). The postprenylation processing enzymes Rce1 and Icmt are localized in the endoplasmic reticulum (58, 59), raising the possibility that monocysteine Rab proteins and CXC Rabs must transiently interact with the endoplasmic reticulum after prenylation, before delivery to their target organelle. Rab proteins with a CC motif do not undergo methylation and therefore are likely to be delivered directly to the target membrane.

In summary, recent biochemical and structural studies have led to an incremental advance in our understanding of Rab geranylgeranylation. Nevertheless, much remains unknown, in particular the molecular mechanisms underlying the exquisitely specific targeting of Rabs to their target intracellular membranes.

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