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 the Rab protein must bind REP, which presents the Rab to RGGT. Following geranylgeranylation of C-terminal cysteines in Rab, 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 have helped to refine this model and provide further insights. The hydrophobic prenyl binding site of RGGT is similar to that of geranylgeranyl transferase type-I (GGT-I) but differs from farnesyl transferase (FT). At the bottom of the hydrophobic isoprenyl-binding pocket, a bulky Trp residue in FT prevents binding of the longer geranylgeranyl pyrophosphate group whereas in GGT-I and RGGT this position is occupied by the smaller residues Thr and Ser, respectively. At the REP:RGGT interface, a highly conserved Phe in REP, which is absent in RabGDI, plays a critical role in the formation of the REP:RGGT complex and may explain the inability of RabGDI to complex with RGGT. A geranylgeranyl-binding site in REP and RabGDI has been identified within domain II. However, as most Rabs undergo double geranylgeranylation, it is still unclear how and where the second geranylgeranyl group is stabilised. The post-prenylation 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 object of future studies.

Supplementary key words
Rab • GTPases • prenyltransferases • REP • GDI • geranylgeranyl
Members of the small GTPase Ras superfamily perform important regulatory functions, from cell growth to cytoskeleton dynamics and membrane trafficking. With the exception of Ran, Ras-like proteins undergo co- or post-translational 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 catalysed by protein prenyltransferases (1). Prenylation of Ras, Rho/Rac and Rab is absolutely critical for the proper function of the modified protein in cellular processes (also 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 by (4)).

Three distinct protein prenyltransferases have been identified and can be classified into two main functional classes: the CAAX prenyltransferases, consisting of farnesyltransferase (FT) and geranylgeranyltransferase type I (GGT-I) (reviewed in this series by Beese et al (editor please add reference, if it is too complicated then it is OK to delete the ref)), and the Rab geranylgeranyltransferase (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). The Rab protein family are exclusive substrates of RGGT.

THE RAB PROTEIN FAMILY – STRUCTURE/FUNCTION:

The Rab proteins (ras genes from rat brain), comprising over 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, while others are expressed in a tissue-specific or developmentally-regulated manner. For example, Rab1 is found in all cell types while others, such as Rab27a, are found in melanocytes and secretory cell types (7). Analysis of the budding yeast S. cerevisiae genome indicates that there are 11 Rab genes called Ypt (yeast
protein involved in transport), some of which are redundant in their function (8). In mammals, over 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 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 localisation and thus regulates a specific membrane trafficking step. However, transport between two membrane compartments may be governed by more than one Rab member and 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\(^{2+}\), and have been previously referred to as G1-G3 and PM1-PM3 respectively (12). There are two regions which 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 loop4/\(\alpha2/\text{loop5}\) region. While the presence of a di-cysteine 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, which were conserved only in Rab proteins and thus discriminated them from other Ras-like proteins (Figure 1). The RabF1 region is located in the so-called effector domain (loop2/\(\beta2\)) in the switch I region. The remaining four regions, RabF2 (\(\beta3\)), RabF3 (loop4), RabF4 (\(\alpha2/\text{loop5}\)) and RabF5 (\(\beta4/\text{loop6}\)), all reside in and around the switch II region between \(\beta\)-sheets \(\beta3\) and \(\beta4\) (5). Since 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 protein (RabGDI) and Rab escort protein (REP), as these regulatory proteins are nucleotide-sensitive (e.g. associate better with the GDP form of Rab proteins) and recognise all Rabs (13, 14).
In addition, four Rab subfamily regions (RabSF) were defined as regions of high conservation within subfamilies: RabSF1 (β1), RabSF2 (α1/loop2), RabSF3 (α3/loop7) and RabSF4 (α5) (5). RabSF1, RabSF3 and RabSF4 corresponded to three regions previously referred to as Rab complementary-determining regions (CDRs) 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 which mediates binding to Rabphilin3a, while 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, but also to RabSF regions to confer specificity.

RAB GERANYLGERANYLATION – TWO ALTERNATIVE PATHWAYS

Like the CAAX prenyltransferases, RGGT is heterodimeric and consists of distinct α- and β-subunits. However, its mechanism of action is distinct from the other prenyltransferases. The enzyme was first isolated from rat brain cytosol and purified as a multi-component 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 Rab Geranylgeranyl Transferase (RGGT). Unlike the CAAX prenyl transferases, RGGT does not recognise short peptides containing the Rab C-terminal prenylation motif, nor does it recognise the Rab protein alone (17, 18). Instead, it binds Component A, now called Rab Escort Protein (REP), which is a Rab-binding protein (19).

Several details concerning the mechanisms of Rab protein prenylation are yet unclear. Biochemical assays have led to the proposal of two possible pathways (Figure 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 0.2µM (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 recognised by RGGT (Kd<1nM) 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) (Figure 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 ($K_d \sim 10$nM) (Figure 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 RGGT:GGpp:REP complex becomes rate-determining and is favoured, while at low concentrations the classical pathway is preferred.

**RAB GERANYLGERANYL TRANSFERASE**

The heterodimeric enzyme consisting of a 60kDa $\alpha$-subunit and a 38kDa $\beta$-subunit presents 30% homology with its counterparts FT and GGT-I (24). The yeast genes encoding the $\alpha$- and $\beta$-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 Å and revealed the presence of four distinct structural domains (27). The $\alpha$-subunit is composed of three domains: a helical domain, an immunoglobulin-like (Ig-like) domain and a leucine-rich repeat (LRR) domain. The helical domain is structurally very similar to the $\alpha$-subunit of FT despite only 22% sequence identity between FT$\alpha$ and the relevant region in RGGT$\alpha$. A major structural difference in RGGT is the presence of both the Ig-like domain and the LRR domain, absent in FT or 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 function of these unique regions remains unknown.

The $\beta$-subunit forms an $\alpha$-$\alpha$ barrel and contains a central cavity lined with hydrophobic residues very similar to the $\beta$-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 structure of GGT-I (30) helped define the isoprenoid specificity of each enzyme. In geranylgeranyl transferases, residue 49$\beta$ (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 the binding site (Figure 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 (Figure 3). These changes significantly enlarge the binding site to accommodate GGpp.

RGGT binds GGpp with an affinity of 8±4nM, while Fpp binds less strongly (Kd=60±8nM) and Gpp even less (Kd=330±40nM) (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 (HPS) in the gunmetal (gm) mouse (32). HPS is a rare autosomal recessive, genetic disease characterised by partial albinism, prolonged bleeding and platelet dysfunction (33). The gm mutation was identified as a Gly to Ala substitution in a splice acceptor site within the Rggta gene (32). While 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 suggests 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 hypothesised 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). Since 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. While no further studies have demonstrated phosphorylation of RGGT, future work should clarify its role in the regulation of RGGT activity.

While 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 analogue of the nitrogen-containing bisphosphonate, risedronate, was identified as a drug which inhibited not only prenylation of Rab proteins in osteoclasts and J774 macrophages in vitro and bone resorption in vivo. More recently, 3-PEPC, an analogue 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, though 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 homologue 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 as 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 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 (SCR). 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 form 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 while the opposing face is comprised 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 (CBR) 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 well as its structure, which was not visible in the crystal.

Since the Rab-binding platform (RBP) is a key functional element, it is highly conserved between REP-1 and RabGDI. Out 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 in order to promote their prenylation while 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 of 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 cannot explain why RabGDI binds preferentially 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) (Figure 1). This hydrophobic pocket appears to be blocked when REP-1 is in complex with RGGR. However, when REP is in complex with a mono-GG Rab protein, there is a change in the conformation of helix D resulting in opening of the binding site and allowing the accommodation of the GG moiety. The cavity is too small to accommodate two GG groups and 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 need first to be resolved to build a complete model of the interactions between Rab proteins and REP.

The mobile effector loop (MEL) is a short stretch in domain II which 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 MEL did not affect Rab binding but significantly reduced membrane association of RabGDI and prevented the extraction of Rabs by RabGDI (50). Therefore the MEL has been implicated in Rab recycling.

The C-terminal binding region (CBR) of REP-1 is composed of hydrophobic residues that form a cavity above the MEL. It has been proposed that the CBR 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 RGGR (see more details under REP:RGGR Complex). As discussed above, the issue of whether there are differences between REP and RabGDI in binding affinities towards unprenylated and prenylated Rabs remains to be
addressed experimentally, although this is often referred as a known fact. Under steady-state, endogenous RabGDI:Rab complexes can be isolated but not REP:Rab complexes. 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 whilst 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 2.7Å 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 implicated the LRR or Ig-like domains of RGGT to interact with the insert region REP, since these are unique sequences (27). This is in fact not the case, since this element faces the opposite direction to the interaction surface. The REP:RGGT interface is formed by helices 8, 10, 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 F279 of REP-1 plays a key role in mediating interaction with RGGT, since a F279A 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 stabilises the REP-1:RGGT complex (Figure 4). This phenylalanine is conserved throughout the REP family but is absent in the RabGDI family. The comparison between apo-RGGT and the REP-1:RGGT complex suggested that complex formation leads to a dramatic decrease in flexibility of RGGT helices 8 and 10. It was proposed that this change in conformation was to unblock a binding site for REP helix D, which would otherwise 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 V287 residue in REP-1 is replaced with a phenylalanine residue at this position in RabGDI (Figure 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 as to how the binding of phosphoisoprenoid was able to stimulate the interaction of RGGT and REP, since the distance between the REP:RGGT binding interface and the active site located on the RGGT β-subunit exceeded 30Å. The RGGT α-subunit residue Y107 was shown to be involved in phosphoisoprenoid-dependent interaction between REP and RGGT. Indeed, the RGGTy107A mutant lacks the ability to interact with REP-1 with high affinity ($K_d=10nM$) while the low-affinity ($K_d=2\mu M$) isoprenoid-independent binding was unaffected (49). Thus it was proposed that Y107α regulates a long-range conformational change that transduces phosphoisoprenoid-binding.

A model of the sequence of events occurring 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 F279 (helix D) and R290 (helix E) are disrupted, leading to a decrease in affinity of REP for RGGT.

FUNCTIONAL CONSEQUENCES OF RAB PRENYLATION: MONO VERSUS DI-GERANYLGERANYLATION

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 mono-cysteine Rabs possess a CXXX motif, and in some cases a canonical CAAX motif similar to members of the Ras and Rho family proteins. 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). While the presence of a single GG group is sufficient to target mono-cysteine Rab proteins, two GG moieties are required for the faithful targeting of di-cysteine Rabs. Recent studies have shown that when the C-terminus of Rab proteins normally containing a di-cysteine motif, such as Rab5a and Rab27a, was replaced
with a mono-cysteine motif, such as CSLG or CVLL, the mutants were mistargeted to the ER/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 mis-localised 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 di-geranylgeranylation.

The reason why some Rabs are mono-prenylated and some are di-prenylated 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 di-prenylated and not mono-cysteine Rabs, suggesting that different factors may be involved in membrane recruitment (55).

Post-prenylation processing is another factor that may assist membrane recruitment of Rabs. Rab proteins with a CXC motif, but not a CC motif, are carboxyl methylated on the C-terminal prenylcysteine (56). Our unpublished data suggest that mono-cysteine Rabs undergo post-prenylation processing, ie, 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 as absence of methylation does not affect the localisation of Rab proteins ((57) and our unpublished data). The post-prenylation processing enzymes, Rce1 and Lcmt, are localised in the ER (58, 59), raising the possibility that mono-cysteine Rab proteins and CXC Rabs must transiently interact with the ER following 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.
ACKNOWLEDGEMENTS

We thank members of the lab for many insightful discussions. K.F.L and R.B. are supported by Wellcome Trust.
REFERENCES


FIGURE LEGENDS

Figure 1: Crystal structure of the Rab7:REP-1 complex – regions conserved within the Rab family. Ribbon representation of REP-1 (white) bound to Rab7 (greyish blue). The RabF regions and RabSF regions are highlighted in red and yellow respectively. The guanine nucleotide-binding regions are in green. GGpp (brown) located in the prenyl-binding pocket of REP-1 is shown in ball and stick representation. All crystal structures were generated using Accelrys DS ViewerPro 5.0. The PDB ID of Rab7GG:REP-1 is 1VG0.

Figure 2: A cartoon showing the two possible pathways for Rab protein prenylation. In the classical pathway, newly translated Rabs bind REP and the complex is recognised by GGpp-bound RGGT. RGGT catalyses the transfer of geranylgeranyl groups to C-terminal cysteines of the Rab protein. Following 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 where these constituents are in higher concentration 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 while REP escorts the prenylated Rab to membranes as in the classical pathway. The $K_d$ values of the Rab:REP:RGGT:GGpp complex for each pathway are indicated.

Figure 3: The isoprenyl binding pocket of mammalian protein prenyltransferases. Superposition of the isoprenyl binding pocket of Zn$^{2+}$-depleted FT (orange), GGT-I (green) and RGGT (yellow). Fpp (blue), GGpp (red) and key amino acid side chains are in ball and stick representation. Trp102$^\beta$ 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 $\beta$-subunits of human FT, GGT-I and RGGT indicating the key residues, Trp102$^\beta$ (orange), Thr49$^\beta$ (green) and Ser48$^\beta$ (yellow). The PDB ID of FT, GGT-I and RGGT are 1D8E, 1N4P and 1LTX, respectively.
Figure 4: Comparison of REP-1 versus 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 represented in ball and stick. Sequence alignment of mammalian and yeast isoforms of REP and GDI is shown in the inset. The phenylalanine residues (white) in helix D are conserved among the REP family but are not present in GDI, while in helix E, the phenylalanine corresponding to residue 143 of Gdi1p (turquoise) are conserved among GDI proteins but not in REPs. The PDB ID of Gdi1p and rREP-1 with RGGT are 1URV and 1LTX, respectively.
"Classical" pathway

Alternative pathway

Rab

REP

K_d ~ 0.5nM

K_d ~ 30nM

Target membrane

FIGURE 2
**FIGURE 3**

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<td>GGT-β 38 ERYSSLETSRLTIAFFALSGLDMLDS 63</td>
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<tr>
<td>RGGTβ</td>
<td>GGT-β 37 YEYCMSEYLRMSGIYWGLTVMDLMGQ 62</td>
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FIGURE 4