CMLS, Cell. Mol. Life Sci. 62 (2005) 1657–1670
1420-682X/05/151657-14
DOI 10.1007/s00018-005-4486-8

CMLS Cellular and Molecular Life Sciences

Review

The structural and mechanistic basis for recycling of Rab proteins between membrane compartments

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Received 4 November 2004; received after revision 14 February 2005; accepted 31 March 2005
Online First 28 May 2005

Abstract. Rab proteins are members of the Ras superfamily of GTPases and are key regulators of intracellular vesicular transport. They undergo a cycle of GTPase activity, and this activity is interconnected to a cycle of reversible attachment to membranes. This cycle is mediated by geranylgeranylation of (usually) two C-terminal cysteines, which in turn is effected by Rab geranylgeranyltransferase in concert with REP (Rab escort protein). After delivery to their respective membranes, Rabs are activated by replacement of GDP by GTP, allowing interaction with a wide variety of effector molecules involved in vesicular transport, in particular with docking of transport vesicles to their specific target membranes. After completion of these events and GTP hydrolysis, Rabs are retrieved by GDI (GDP dissociation inhibitor) and delivered to their starting compartment. Here, the structural and mechanistic basis of events occurring in Rab delivery and cycling, and the differences between REP and GDI are discussed on the basis of recent advances in the field.

Key words. Posttranslational modification; Rab proteins; GDI; REP.

Introduction

The Rab proteins are members of the largest subgroup of the Ras superfamily of GTPases and are involved in multiple stages of intracellular vesicular transport. Like other members of the Ras superfamily, their cycle of GTPase activity is an essential feature of their mode of operation. This cycle is regulated by GTP/GDP exchange factors (GEFs), which accelerate the otherwise very slow rate of GDP dissociation, and by GTPase activating proteins (GAPs), which accelerate intrinsic GTPase activity. Replacement of GDP by GTP leads to an increase in affinity for other protein factors that are lumped together under the heading 'effectors', even though the purpose and outcome of such interactions varies widely. A large number of these interactions appear to be involved in essential steps of vesicular transport, including budding, transport, and tethering and docking of transport vesicles to target membranes. Like most (but not all) GTPases involved in signal transduction and regulation, the Rab proteins are conjugated to lipid moieties that allow their reversible attachment to membranes. For the Rab proteins, the reversibility of this association is of particular significance, since it allows recycling of proteins between different membranes upon completion of their functional cycle. This is summarized in figure 1, which shows the Rab cycle and illustrates how it is interconnected with the classical GTPase cycle of GDP/GTP exchange and GTP cleavage.

The aim of this review is to discuss the present state of knowledge on early events in the Rab activity cycle, including prenylation and delivery to membranes, and a mechanistically related late event, which is retrieval and

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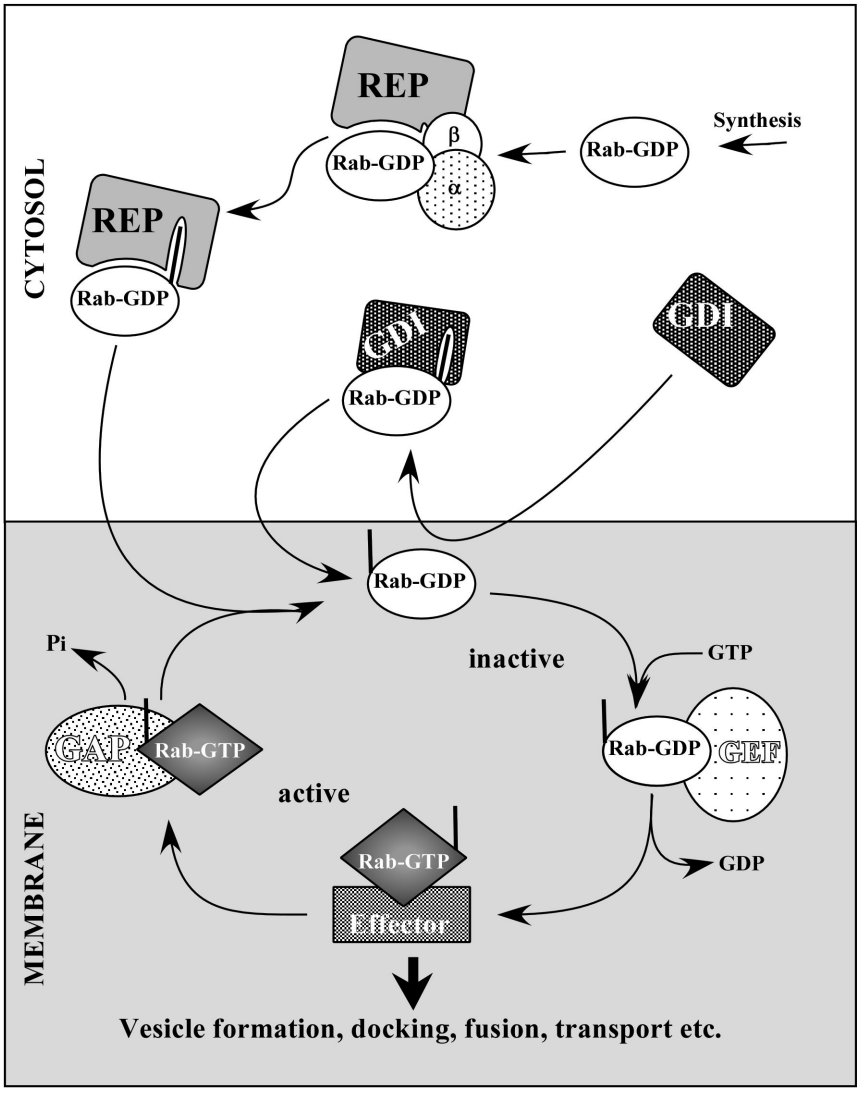

Figure 1. Rab prenylation, delivery to membranes and the GTPase cycle. The GDP form of Rab is recognized by REP (Rab escort protein) and presented to RabGGTase (α- and β-subunits), resulting in geranylgeranylation at one or two C-terminal cysteines. Prenylated Rab proteins are escorted to their specific membranes and anchored by insertion of the lipid residues. They are activated by the action of GEF (guanosine nucleotide exchange factor) molecules, which results in replacement of GDP by GTP. In their active GTP-bound form, Rabs can interact with effector molecules, examples of which are tethering factors which establish the initial specific contact between a transport vesicle and a target organelle membrane. After membrane fusion, a complex process in which many proteins, in particular the SNARE proteins, are involved [55], the slow intrinsic rate of GTP hydrolysis is accelerated by GAP (GTPase-activating protein) activity, after which the Rab molecule can be extracted from the membrane by GDI (GDP dissociation inhibitor), which returns the Rab molecule to its starting membrane. Rab proteins are also involved in other aspects of vesicular transport, including formation and transport of vesicles.

recycling of Rab proteins. We will place particular emphasis on structural and mechanistic evidence which has been obtained over the last few years.

Prenylation of Rab proteins

After their synthesis on cytosolic ribosomes, Rab proteins are recognized in their GDP-bound form by the Rab escort protein (REP), of which two isoforms (REP-1 and REP-2) are present in mammalian cells (fig. 1). In this complex, the Rab protein is presented to the prenylating enzyme Rab geranylgeranyltransferase (RabGGTase or GGTase II), which transfers two geranylgeranyl groups (for a few members of the over 60 mammalian Rabs only one geranylgeranyl group) to two cysteines at the C-terminus of the Rab molecule. The need for the REP molecule as an additional factor in prenylation is unique to the Rab family since other proteins (mainly GTPases) undergoing post-translational prenylation are modified directly by the prenylating enzyme (either farnesyl transferase or geranylgeranyl transferase type I) [1]. The latter enzymes

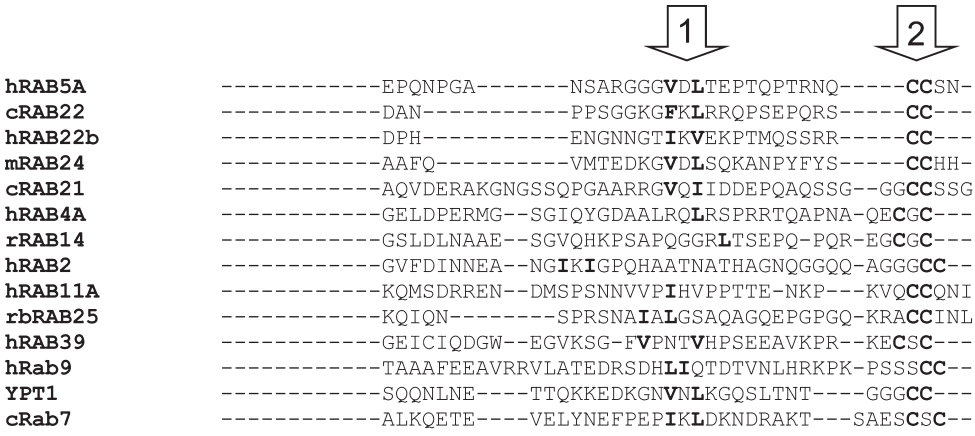

Figure 2. Examples of Rab C-termini. The large hydrophobic residues shown in bold typeface at the approximate position given by arrow 1 are thought to be important for interaction of the C-terminal region with REP and GDI. In the case of Rab39, it is difficult to assign this residue, since the general pattern involves either two large aliphatic residues two residues apart, or a single such residue in this region; but Rab39 harbors two large hydrophobic residues (valines) separated by three amino acids in this region. The prenylatable cysteines are in bold typeface underneath arrow 2.

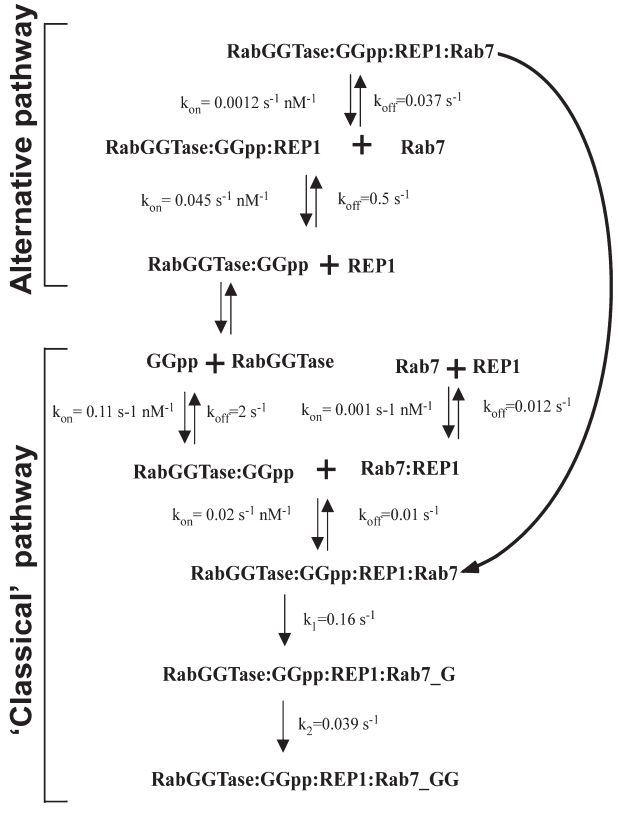

Figure 3. Rate constants in the system involving prenylation of Rab by RabGGTase. Two binary complexes ('binary' and 'ternary' refer only to the protein components) and three ternary complexes can be generated, the final product being doubly geranylgeranylated Rab complexed with Rep and RabGGTase. Not shown in the scheme is the effect of binding of lipid substrate to this complex, which results in a decrease in affinity of RabGGTase to the Rab:REP complex and frees the RabGGTase for further cycles of activity and releases the prenylated Rab as a soluble complex with REP.

recognize a specific C-terminal sequence motif (CAAX), whereas recognition in the case of RabGGTase is indirect, via REP, and there is no pronounced sequence specificity for the C-terminus (see fig. 2 for examples of Rab C-termini). Since prenylation is essential for Rab activity, it is not surprising that REP is essential, and loss or reduction of this activity results in lethal phenotypes or pathological conditions, as elaborated on later in this review [2, 3].

Generation of the ternary complex between a Rab protein, REP and RabGGTase is a complex process that has been investigated by kinetic and structural methods. The results of the kinetic studies are summarized in figure 3 [4–10]. Briefly, there are two routes to generate this complex. One of them, referred to as the 'classical' route, involves formation of the REP:Rab complex, followed by binding of the RabGGTase. REP has relatively low affinity for RabGGTase in the absence of Rab and of geranylgeranyl pyrophosphate, but both entities have a positive effect on this affinity. Thus, association of geranylgeranyl pyrophosphate with the active site of RabGGTase results in a K_d value of ca. 10 nM for the REP interaction with RabGGTase. Since Rab can now bind with high affinity to form the ternary complex, this order of binding represents an alternative route to formation of the ternary protein complex, at least in the presence of geranylgeranyl pyrophosphate. Although the physiological significance of the existence of two pathways is not understood, it appears that the 'alternative' pathway emerged relatively late in the evolution of eukaryotes, since it is absent in yeast cells [11]. However, the availability of two alternative binary protein complexes has provided a convenient starting point for investigation of the properties of the ternary complex (RabGGTase and Rab have no detectable affinity, so that a binary complex between these components cannot

be generated). The ability to generate stable binary complexes in the laboratory was of importance, since the ternary protein complex has so far proven refractory to structural analysis, although it can be generated and crystallized [12].

The first binary complex to be characterized structurally was that between REP-1 and RabGGTase in the presence of a lipid substrate analog that was resistant to hydrolysis and loss of the phosphate groups. The analog used was a stable phosphonate derivative of farnesylpyrophosphate,

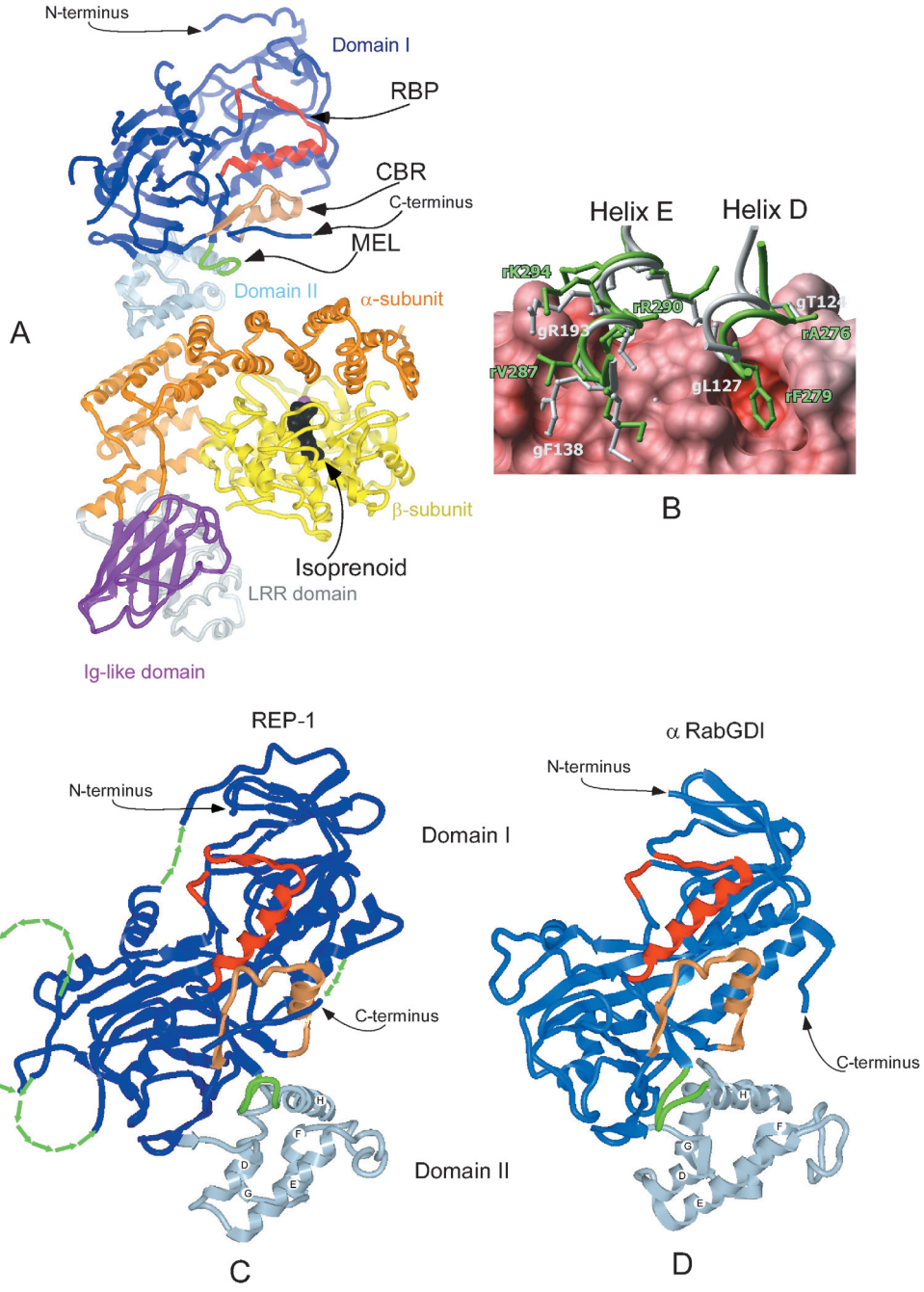

Figure 4. Structure of the complex between REP1 and RabGGTase and comparison of REP-1 and αGDI structures. (*A*) Overview of the REP-1:RabGGTase complex. Domain I of REP-1 is shown as a blue ribbon, while domain II is displayed in light blue, the Rab-binding platform in red, the C-terminal binding region (CBR) in pale orange and the mobile effector loop (MEL) in green. The partially obscured pink sphere adjacent to the isoprenoid group is the catalytic zinc ion. The α-helical region of the α-subunit of RabGGTase is in orange, while the β-subunit is shown in yellow, the Ig-like domain in magenta and the LRR (leucine-rich repeat) in gray. (*B*) Interface between REP-1 and RabGGTase. RabGGTase is shown in space-filling representation and the regions of REP-1 helices D and E involved in the interaction as a backbone worm model with side chains in ball and stick representation (green). The equivalent regions of GDI are shown in similar representation in gray. (*C*, *D*) Comparison of REP-1 (*C*) and αRabGDI (*D*). Color coding for REP-1 and GDI as in *A*.

farnesyl-pCH_2p (FpCp). Although the hydrocarbon chain is shorter in the farnesyl than in the geranygeranyl group, relatively high binding affinity to RabGGTase is retained [5]. Crystallization of the REP-1:GGTAse:FpCp complex led to determination of its structure at a resolution of 2.7 Å [13]. As can be seen from figure 4A, RabGGTase interacts via its α-subunit with the lower domain (domain II) of REP-I, which is similar to the previously determined three-dimensional (3D) structure of αRabGDI [14]. As indicated in figure 1, RabGDI is the molecule responsible for retrieving Rab molecules from membranes after completion of their cycle of activity and for returning them to their starting membranes. REP and RabGDI molecules have stretches of high sequence homology, and partially shared properties, so that the similarity in structures is not surprising. More surprising is the fact that the binding interface in the RabGGTase:REP-1 complex is not formed by regions of the molecules which were believed to be the most likely candidates for the interaction. Since REP is significantly larger than GDI but unlike GDI is an interaction partner of RabGGTase, additional structural elements harboring the binding site for RabGGTase were expected. As can be seen in figure 4, this is not the case, and the additional sequences in REP in comparison with GDI are in fact largely disordered in this structure. Similarly, the interaction of RabGGTase with REP does not occur via either of the two domains of the α-subunit which were thought to be likely interaction sites. These are the leucine-rich repeat (LRR) domain and the immunoglobulin(Ig)-like domain, which are present in RabGGTase but not in the otherwise structurally homologous GGTase-I or farnesyl transferase [15, 16].

As mentioned above, the overall structure of REP-1 shows high similarity with that of mammalian αGDI [14] and yeast GDI [17]. In figure 4C and D, the arrangement of secondary structure elements at the interface with RabGGTase is seen to be very similar to that of GDI in the same region of domain II (helices D and E). The question therefore arises as to the reason for the inability of GDI to interact with RabGGTase. This is revealed by inspection of the detailed interactions of the side chains of REP-1 with RabGGTase (fig. 4B). Key residues in this interaction are F279 and R290. The latter residue had already been identified as essential for the interaction of the yeast homolog of REP and RabGGTase [9]. F279 of REP-1 anchors between helices 10 and 12 of RabGGTase α-subunit, and its mutation to alanine resulted in loss of ability of REP-1 to bind to RabGGTase and support prenylation of Rab proteins. Residues equivalent to F279 or R290 are not present in GDI, explaining the functional segregation of these two highly related molecules. Thus, discrimination between REP and GDI by RabGGTase occurs as a result of differences of local sequence rather than of overall 3D structure.

Although the Rab molecule is not present in the structure shown in figure 4, it was already clear from mutational analysis that the Rab binding site on REP is in the region referred to as the Rab-binding platform in figure 4A [16]. Positioning the Rab molecule in this area, making additional use of mutational analysis performed on Rab residues, shows that the flexible C-terminus of the Rab molecule would be long enough to extend into the active site of the RabGGTase, which is located in the β-subunit. This point will be discussed in more detail in a later section.

Interaction of REP and GDI with Rab proteins

As described above, one of the functions of REP is to present Rab proteins to RabGGTase in order to allow post-translational modification to occur. A second function is to sequester the prenyl groups attached to the C-terminus of Rab to form a soluble complex until completion of the third function, which is the delivery of the prenylated protein to its appropriate membrane. The task of retrieving Rab molecules from membranes after completion of their cycle is not performed by REP, although this was proposed several times, but by GDI [18, 19]. The structural similarity of REP and RabGDI suggests that their manner of interaction with Rabs should be similar. However, there are significant differences, perhaps the most important of which is the fact that REP binds with high affinity to both prenylated and unprenylated Rabs [20], whereas GDI interacts with prenylated Rabs with high affinity with a K_d in the nanomolar range, but 10^3–10^4 fold more weakly with unprenylated Rabs ([21] and B. Dursina and K. Alexandrov, unpublished data). This is related on the one hand, to the different roles of REP and GDI in the initial prenylation, but also, and less obviously, to the fact that REP need only deliver Rabs to membranes, whereas GDI must in addition be able to retrieve them. The background to these differences will become apparent when the interactions are reviewed in a later section.

Preparation of prenylated Rab proteins

One of the main problems in structural and mechanistic studies on protein-lipid conjugates is the preparation and isolation of adequate amounts of the post-translationally modified proteins. Obviously, direct expression in bacteria is not a viable approach to this problem, but even expression in eukaryotic cells presents problems in terms of yields and homogeneity of mature proteins. An additional difficulty arises from the insolubility of lipidated proteins. In the case of Rab proteins, two different approaches have been adopted to solve these problems. The simpler situation is presented by generation of complexes between the

geranylgeranylated RabGTPases and REP proteins. In this case, a stoichiometric complex between unmodified Rab and REP can be prepared, followed by in vitro prenylation by RabGGTase. The product of this reaction can then be isolated in soluble form due to the chaperoning nature of the REP molecule [22]. The situation is more difficult with Rab:GDI complexes, since GDI cannot support prenylation of Rab proteins by RabGGTase. The approach which was adopted to solving this problem was to use the method of expressed protein ligation [23], which has proven to be useful for generating C-terminally modified Rabs [17, 24–26]. To apply this approach to the construction of prenylated proteins, the yeast Rab GTPase Ypt1 lacking the two terminal cysteines was expressed in *Escherichia coli* as a C-terminal fusion with an intein and a chitin binding domain. The fusion protein was isolated by binding to chitin-agarose and cleaved at the junction with the intein domain by treatment with a high concentration of a thiol reagent, thus releasing Ypt1 with a reactive C-terminal thioester group. Incubation of this with a dicysteine peptide in which the C-terminal residue bore a geranylgeranyl group led to thioester exchange and rearrangement via an S → N acyl shift to generate full-length Ypt1 with a geranylgeranyl group on the terminal cysteine. This was carried out in detergent solution to render both the lipidated peptide and product soluble. After removal of the excess unligated peptide and detergent, the complex between prenylated Ypt1 and yeast GDI could be reconstituted in solution and isolated [17].

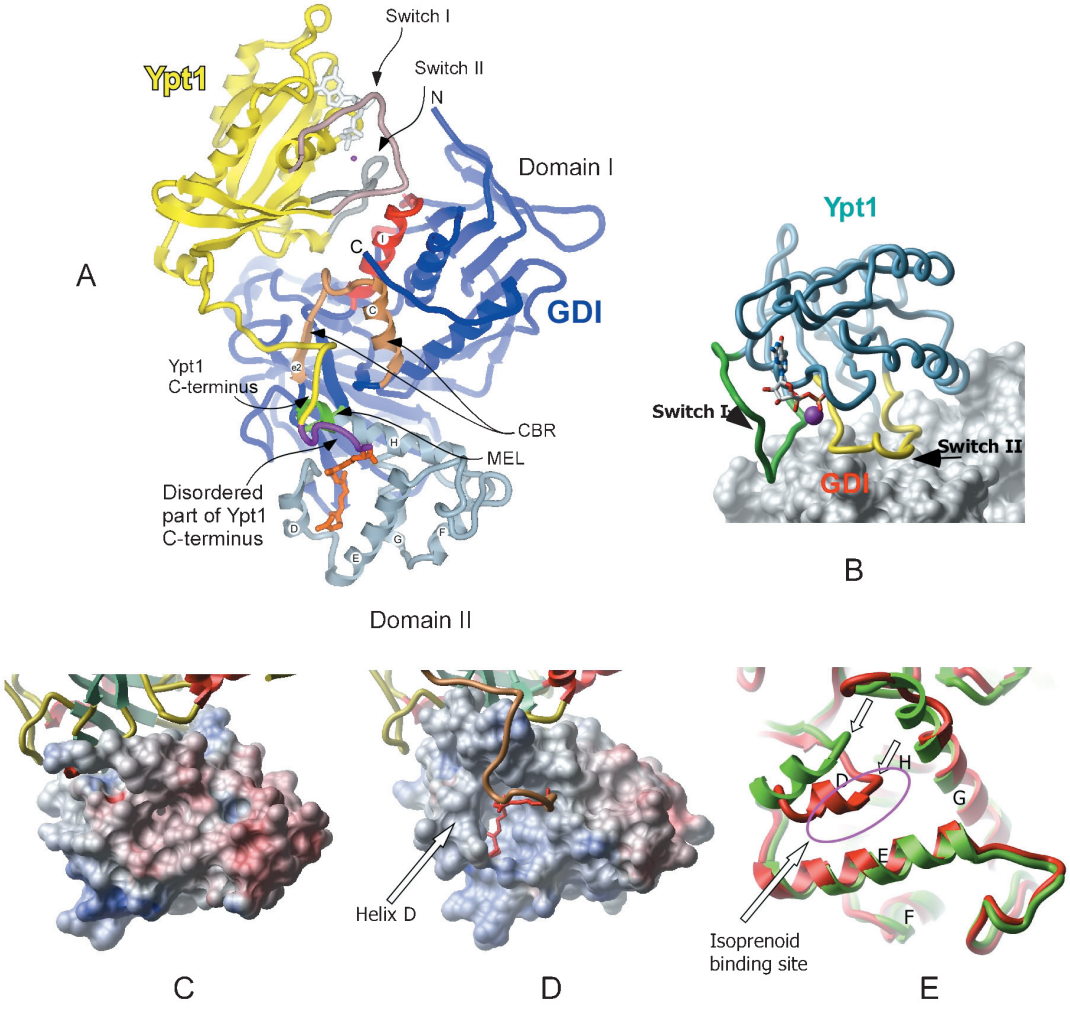

Figure 5. (*A*) Structure of the complex between monoprenylated Ypt1 and GDI. The Ypt1 molecule is shown mainly in yellow, with GDP in gray and Mg^{2+} in magenta. GDI is depicted in blue (domain I) and light blue (domain II) except for RBP (red), CBR (orange) and MEL (green). The disordered part of the Ypt1 C-terminus is shown in magenta. The geranylgeranyl group is green. (*B*) The ordered Switch I (green) and Switch II (yellow) regions of YPT1 on the surface of GDI (gray). (*C–E*) illustrate the structural changes occurring in domain II of GDI on binding the Rab geranylgeranyl group and C-terminus. (*C*) Domain II of αGDI in the absence of other bound proteins is displayed in surface representation and colored according to charge. (*D*) Domain II of GDI in the Ypt1:GDI complex displayed as in (*C, E*) Creation of the geranylgeranyl binding site by movement of helix D away from the core of domain II. The arrows show the positions of helix D in the closed (red) and open (green) states, respectively. The position of the lipid binding cavity is indicated by the magenta oval outline.

The 3D structure of the Ypt1:GDI complex

The complex prepared as described above was crystallized, and its structure was determined at 1.5 Å resolution [17]. A model of the structure is shown in figure 5A. The main sites of interaction of the globular GTPase domain of Ypt1 with GDI are consistent with conclusions drawn from mutational analysis of the binding interface [27, 28]. On the Rab side, the Switch I and, particularly, Switch II regions are heavily involved. These are regions occurring in all GTPases involved in signal transduction and regulation, and they are known to undergo structural changes on GTP hydrolysis. Switch I, referred to earlier as the effector loop because of mutational implication of this region in interaction with effector molecules, is generally disordered in the GDP-bound state but ordered in the GTP state. A similar situation pertains with Switch II. In the structure of Ypt1:RabGDI, Switch I and Switch II are completely traceable in electron density, despite the fact that GDP is bound to the Rab protein. This ordering effect is a consequence of the large number of interactions with GDI, particularly for Switch II (fig. 5B).

The structure of the Ypt1:GDI complex sheds light onto two related features of the interaction, namely the inhibition of GDP dissociation, and the more significant property of preferential binding of GDI to the GDP form of Rab molecules, which is an essential feature of the mode of action of Rab GDI [29, 30]. Inhibition of dissociation of GDP appears to be a consequence of ordering of Switch II and consequent stabilization of the water molecules which coordinate the essential Mg^{2+} ion, which in turn interacts with the β-phosphate of GDP. The basis for the preferential binding to the GDP form of Rabs should be derivable from a comparison of the structure of Ypt1 in the Ypt1:GDI complex with that of Ypt1 with a GTP analog bound. Since the structure of Ypt1 in the absence of a bound protein partner was not known, the structure of Ypt7 as a complex with the GTP-analog GppNHp was used [31]. This indicated that Switch II is stabilized in Ypt7:GppNHp in a completely different conformation than in Ypt1:GDI, so that the interactions shown in figure 5B would not be possible for the GTP-induced conformation of Switch II. A more direct comparison is possible in the case of the Rab7:REP-1 complex (see below), since the structure of Rab7 in the GTP-bound form could also be determined [32].

Of particular interest is the position of the C-terminus and the lipid residue in the Ypt1:GDI complex. In crystallographically determined structures of GTPases of the Ras superfamily in the apo form, the C-terminus is either removed by truncation, or is not seen due to disorder [33]. However, it is known that this is an important part of the structure, not only because it harbors the site or sites of prenylation, but because the sequence of this hypervariable region contains important targeting information [34]. In the complex structure described here, most of the C-terminus, with the exception of the last seven residues, is visible in electron density. The geranylgeranyl group is well defined and in a position which was unexpected based on previous evidence. Thus, model studies starting from the REP:RabGGTase structure suggested that the lipid binding site would be in domain I (upper domain) of the REP molecule, and by analogy of GDI [13]. This suggestion was supported by structural information on a complex between GDI and a geranylgeranyl cysteine derivative [35] that showed the lipidated amino acid to be bound in approximately the suggested position. It is unclear at present what the significance of this binding site is, but it seems highly likely that the domain II binding site identified in the Ypt1:GDI structure is in a correct binding position, regardless of a possible further significance of an additional binding site. It has been suggested recently that the putative domain I binding site might be an initial docking site at an early stage of extraction of Rabs from membrane [36], the final position being that seen in the Ypt1:GDI structure.

The structure of the Ypt1:GDI complex provides an unambiguous explanation of why the lipid binding sites of GDI and REP were so elusive despite the availability of high-resolution structures and a large number of genetic and biochemical studies. The lipid binding site is formed by helices D, E, H and F of domain II due to the outward movement of helix D (fig. 5E). This conformational change results in formation of a deep cavity penetrating the hydrophobic core of domain II that is occupied by the geranylgeranyl moiety in both the Ypt1:GDI and Rab7:REP-1 structures (see below). It is currently unclear where the second conjugated isoprenoid could be located, since the observed binding sites are not sufficiently large to accommodate both lipids. It is most likely that the second lipid is located on the surface of domain II of the GDI or REP molecules in the vicinity of the binding site identified here. The distance between the domain II binding site and the postulated site on domain I is too large to allow one lipid group to bind at one position and one in the other site.

The structure of the Rab7:REP-1 complex

Prenylation of the complex between Rab7 and REP-1 by RabGGTase followed by removal of the enzyme led to crystallization of the prenylated complex and determination of its structure [17, 37]. The overall structure (fig. 6A) is similar to that of Ypt:GDI. The C-terminus is less well defined than in the Ypt:GDI complex. Thus, density can only be traced until amino acid 193 (of 207), with the remaining residues being non-traceable in the electron density map. However, density corresponding to the lipid residue is seen in a position corresponding to

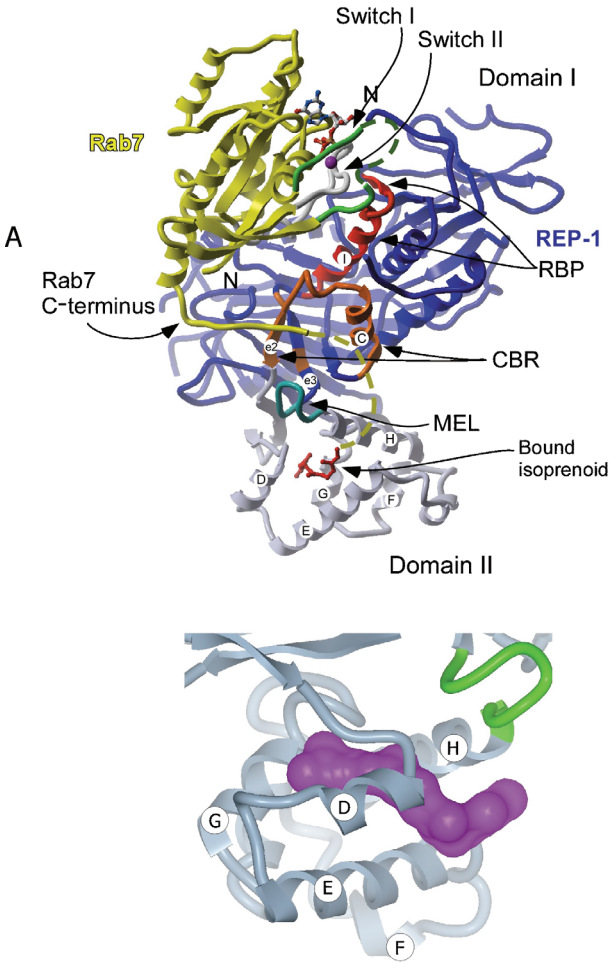

Figure 6. (*A*) Structure of the complex between monoprenylated Rab7 and REP-1. Color scheme as for the Ypt1:GDI complex. (*B*) The C-terminal geranylgeranyl group in its binding cavity on domain II of REP-1. The last 14 residues of the C-terminus cannot be seen in the electron-density map, but the prenyl group is visible (fig. 6B). Domain II is shown from the left side to illustrate the depth of isoprenoid penetration into the protein core. Due to this perspective, helix D appears be closer to helix E than it is.

that of the lipid in Ypt1:GDI (fig. 6B), confirming the generic nature of this site. Since the structure of the unprenylated Rab7:REP-1 complex was also determined, and the structure of REP-1 in the absence of a Rab molecule is available from the REP-1:RabGGTase model, some details of the process leading to formation of this site can be derived. Inspection of domain II in these three structures reveals the presence of a hydrophobic tunnel, formed by helices D, E, G and H, of differing accessibility and form. Thus, in REP-1:RabGGTase it is practically closed, in unprenylated Rab7:REP-1 partially open and in prenylated Rab7:REP-1 fully open and occupied by the geranylgeranyl group. Apparently associated with these changes are changes in the relative disposition of domain II with respect to domain I. The conformational change occurring in domain II to create the lipid binding site has an additional consequence. It leads to disturbance of the residues involved in interaction with RabGGTase. In particular, the essential residue F279 is moved to a completely new position, the effect of which would be to destroy the binding interface and reduce the affinity to RabGGTase [32].

Interaction of the Rab C-terminal hypervariable domain with REP/GDI

In both the Ypt1:GDI and the Rab7:REP-1 complexes, the main interaction of the C-terminus, apart from the docking of the prenyl group into its binding site, is via two hydrophobic residues of the Rab molecule (V191 and L193 in Ypt1, I190 and L192 in Rab7; see fig. 2) with hydrophobic residues of the C-terminal binding domain (CBR). Inspection of other Rab sequences reveals that a motif consisting of a large aliphatic residue followed by a polar amino acid and a second large aliphatic residue is present in a many Rab hypervariable domains. In some cases, there are three large aliphatic residues, in others a single hydrophobic residue following a polar side chain. This motif is not positioned at constant distance from the prenylatable cysteine residues on the one hand or the start of the hypervariable domain on the other. Thus, comparing the long hypervariable sequence of Rab2a with the much shorter one of Rab9, there are 17 residues before the IKI motif, und 23 residues after this to the first prenylated cysteine in Rab2a, the corresponding numbers for Rab9 being 15 before VNL and 9 after it. Ypt1, which also has a relatively long hypervariable domain, has 21 residues before VNL and 11 after it (fig. 2). Thus, there appears to be length compensation in both regions. For the region after the hydrophobic motif, this fits in well with the observation that this is disordered in the Ypt1:GDI structure. The significance of the variation in the length of the hypervariable domain and the positioning of the hydrophobic binding motif within this domain is unclear. It is thought that insertion of the prenyl group of Rab proteins into specific membranes requires the assistance of at least one more protein which can also induce dissociation of GDI from Rab. Evidence for the existence of such factors was obtained as early as 10 years ago [38–40]. From this work, it was concluded that membrane-bound factors facilitate Rab recruitment, and the term ‘GDF’ (GDI displacement factor) was used to define their activity. Direct evidence has now been obtained that a small membrane protein called Yip3 (Pra1 in humans) does indeed show such activity towards Rab9 [41]. Yip3 is a member of the Yip family (Ypt-interacting proteins) first discovered in yeast to interact with several Rab proteins, and related proteins occur in mammals [42–45]. While the mechanism of action of these proteins remains unclear, they appear to interact with Rab proteins with a specificity which is not yet properly understood, although it is clear

that they show distinct organelle localization. Thus, although Yip3 interacts with a certain affinity with all yeast Rabs, it does not exhibit GDF activity towards all of them [41]. They interact weakly with GDI, and it has been suggested that the mechanism involves intermediate transfer of the prenyl group from GDI to the GDF [36, 41]. The assumption would then have to be made that this transfer reaction is specific, at least to a certain extent, and that in particular residues in the hypervariable domain are recognized to provide this specificity. In this respect it is of interest to note that a loop designated the mobile effector loop that has been identified in GDI/REP and appears to be essential for the generic ability of GDI/REP to deliver Rab proteins to membranes [46] is in direct contact with the Rab C-terminus in both the Ypt1:GDI and Rab7:REP-1 structures, being involved in the hydrophobic interaction with Rab C-terminus anchoring motif. Thus, elements required both for generic and specific aspects of membrane insertion are located physically close together and possibly form the most important point of interaction with GDFs or other proteins with receptor properties.

Mechanism of prenylation of Rab proteins

Advances in our understanding of this process have come from the results described so far, and are the basis for the following discussion. As already pointed out in the discussion of figure 3, the 'classical' mechanism for assembly of the prenylation-competent ternary complex involves initial interaction of Rab and REP molecules. This interaction has variable affinity, being very high for Rab7 and REP-1 (K_d = ca. 1 nM), lower for Rab1a and REP-1 (ca. 40 nM), and even lower for Rab27a and REP-1 (< 200 nM) [32]. The corresponding interactions with REP-2 are all weaker, but appear to retain the same relative order [32]. The possible significance of these varying affinities for the disease choroideremia, in which REP-1 activity is lost, will be discussed below.

After recruitment of the RabGGTase:GGpp complex in a high-affinity interaction (K_d = ca. 10 nM), prenylation can take place. This is a relatively slow reaction occurring first at the distal (final) cysteine in the Rab sequence ($t_{1/2}$ = ca. 5 s) and then at the proximal cysteine ($t_{1/2}$ = ca. 20 s). Using fluorescently labeled monoprenylated Rab proteins prepared by the expressed protein ligation method referred to above, it was possible to show that the second prenylation reaction occurs without dissociation of the monoprenylated intermediate [8, 25]. At this point in the mechanism, the high-affinity (K_d = ca. 1 nM) between RabGGTase and the binary Rab:REP complex presents an apparent problem, since the prenyl transferase must be released to allow transport to and membrane insertion of Rab proteins. This situation appears to be relieved by binding of a further lipid substrate molecule, since this binding was shown to reduce the affinity of RabGGTase by more than an order of magnitude [6]. At the structural level, we can envisage the following mechanism. Binding of lipid substrate to the doubly prenylated ternary complex can only occur if the lipid groups conjugated to Rab are displaced from their binding site on RabGGTase. After release, they must move towards their potential binding site on domain II of the REP molecule. However, from the structure of the REP:RabGGTase complex described above, it is highly likely that this site is in the 'closed' conformation. Generation of the binding pocket for the lipid moieties would involve destruction of the REP:RabGGTase interface, as described in connection with figure 4B, leading to dissociation of the RabGGTase. This provides a plausible explanation for the effect of lipid substrate binding to RabGGTase on the affinity to REP, although the sites of interaction of GGpp and REP are ca. 36 Å apart. Further mechanistic studies will be needed to confirm and refine this model.

The basis for the differential abilities of REP and GDI in extraction of Rab proteins from membranes

Knowledge of the characteristics of the interaction of Rab molecules with members of the REP/GDI family, and in particular of the structures described above, leads to the simple model for extraction of Rabs from membranes shown in figure 7. Initial interaction of the Rab binding platform of REP/GDI with the GTPase domain of the Rab molecule is followed by docking of the flexible C-terminus of Rab. This places domain II of the GDI molecule in close proximity to the membrane-anchored prenyl groups, finally leading to extraction of the prenyl groups from the membrane and docking to the binding site described above. We consider here the driving force for this sequence of events, and the differences between the relative efficiencies of REP and GDI for this process. Beginning with the latter point, REP appears to be much less efficient than GDI in Rab extraction. This difference in efficiency is in keeping with their biological roles, since REP is probably only involved in delivery of Rabs to membranes, whereas GDI, in addition to having this property, must also be able to extract them. These different properties can be explained by considering the affinities of REP/GDI for unmodified and prenylated forms of Rab, respectively. Although not all relevant quantitative data are available, it appears that whereas REP binds with high affinity to both unprenylated [20] and prenylated Rabs, enabling it to present the unprenylated form to RabGGTase, GDI binds only to prenylated Rabs with high affinity [21]. The large increase in affinity of GDI to Rab on docking of the C-terminus and the prenyl groups is the driving force for the extraction process. Expressed

in another manner, GDI is efficient in extracting Rabs from the membrane since there is a large difference in binding energy in the situation in which only the GTPase domain interacts with GDI and the situation in which the C-terminus and lipid moiety are also docked. The difference in binding energies provides the thermodynamic driving force for the extraction from the membrane. In contrast, most of the binding energy in the case of REP comes from the interaction with the GTPase domain, with only very little driving force for the extraction provided by the interaction of the C-terminus.

These properties of REP and GDI are commensurate with their known cell biological roles. Thus, the first role to be played by REP is to bind Rab proteins and present them to RabGGTase. In order to do this, REP binds Rab proteins with high affinity. The second role of REP is to confer solubility to the Rab molecule, which it can apparently do without strong fixation of the prenylated C-terminus. Finally, REP must release the Rab molecule to its specific membrane. In this role, the relatively weak affinity of the C-terminus for REP is advantageous, since it will not be costly in terms of energy. An unclarified aspect at this point is the fact that REP would still be bound with high affinity to the membrane-anchored Rab molecule. The recently identified GDF molecules that have the ability to displace GDI (discussed above) from Rab are candidates for the role of release factors for REP, if they have similar behaviour towards REP. Energetically, the problem would still remain that the GDF would have to undergo a strong interaction with either the REP/GDI or the Rab molecule (available evidence supports the latter) to disturb the Rab:REP interaction, leaving a similar energetic problem to be solved. While it is not clear what actually happens, one possibility is that at least part of the driving force for these events comes from subsequent GEF-catalyzed exchange of GTP for GDP, which reduces the affinity between Rab and REP/GDI. However, using purified complexes and membranes or in a permeabilized cell model, nucleotide exchange appears to occur after the release of REP or GDI from the membrane [38, 39, 47].

In contrast to REP, GDI does not have, and does not need, a high affinity for unprenylated Rabs, and this allows the large increase in affinity occurring via docking of the C-terminus needed for membrane extraction without the problem which would ensue for REP, which is that the overall affinity would then be so high that the Rab:REP complex would be so stable that a large energy input would be needed for the next round of membrane insertion.

These arguments are relevant to the question of why there are two similar molecules (REP and GDI) with partially overlapping functions. Thus, it might be conjectured that a single molecule with the combined properties of REP and GDI could suffice. In the light of the considerations elaborated here, this appears unlikely. Thus, a few modifications to GDI might well allow it to interact with RabGGTase in the correct manner, but the weak affinity of GDI for Rab would mean that a system consisting of modified GDI and RabGGTase would be inefficient in terms of the prenylation reaction. If further modifications of GDI were introduced to increase its affinity for the GTPase domain of Rab, the problem alluded to above (too high overall affinity between GDI and prenylated Rab) would pertain. If the docking affinity of the C-terminus of Rab were then decreased by mutations in

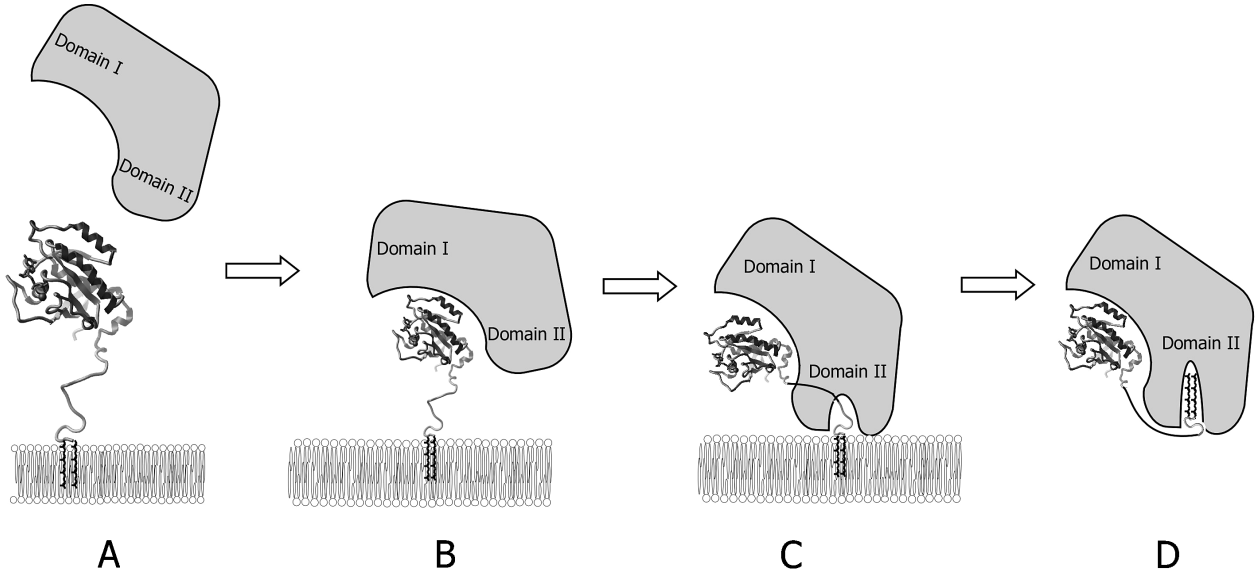

Figure 7. Simple model for the extraction of Rab from membranes by GDI. The model implies that the binding cavity for the geranylgeranyl group is at least partially formed by interaction of Rab with GDI even before interaction with the prenyl group, in keeping with partial opening of the binding site in a complex between REP-1 and C-terminally truncated Rab7 [32].

domain II of GDI, we would essentially have generated a REP molecule, and the ability of GDI to extract Rabs from membranes would be reduced or lost. Thus, it is not surprising that attempts to construct a molecule with the combined properties of REP and GDI have at best been only partially successful [48].

The discussion here has concentrated on general thermodynamic and structural aspects of the Rab extraction mechanism. Obviously, further studies will be needed to understand the mechanism more fully. What is missing at present is on the one hand more detailed knowledge of other proteins involved, including GDF molecules, and on the other hand information which should be available from properly designed time-resolved spectroscopic studies on individual steps, including the initial docking of GDI, interaction of C-terminal residues with the CBR, generation of the lipid binding site and finally extraction of the lipid from the membrane and insertion into its binding pocket. The influence of GDFs and GEFs will probably be seen to be of crucial importance for individual steps in the mechanism and for stabilization of the membrane-bound state of the Rab molecule.

Involvement of GDI and REP in diseases

Lack of or impaired GDI and REP activities are involved in a number of diseases [3]. The work discussed in this article is relevant to several such pathologic states, of which two will be discussed here. A mutation in αGDI (L92P) has been found to be associated with non-specific X-linked mental retardation [49]. The mutant protein was found to be less efficient than wild-type GDI in extracting Rab3A from membranes, and to thus reduce the pool of cytoplasmic Rab3A. Since Rab3 cycling is an essential aspect of neurotransmitter release, the possible connection to mental retardation is apparent. At the time the discovery was made, it seemed likely that the mutation had a direct effect on binding of the prenyl group by GDI. However, as can be seen by inspection of the structure of the Ypt1:GDI complex, this is not the case, since it is not near the lipid binding site. But it is highly likely to have a negative effect on the binding of the hydrophobic motif of the Rab hypervariable domain, since the residue is directly involved in this interaction [17]. It is conceivable that the mutation has a double effect, one being on the affinity of GDI to Rab, the other on the proper positioning of the Rab C-terminus for recognition by GDF or other putative receptor.

A different X-linked disease, choroideremia, leads to progressive loss of sight due to chorioretinal degradation, an affect arising from loss of function of REP-1 [50]. It appears that REP-2 can take over the prenylation and escort functions for most Rabs for most purposes, but Rab27A, which is expressed in the cell types affected in the disease (the retinal pigment epithelium and the

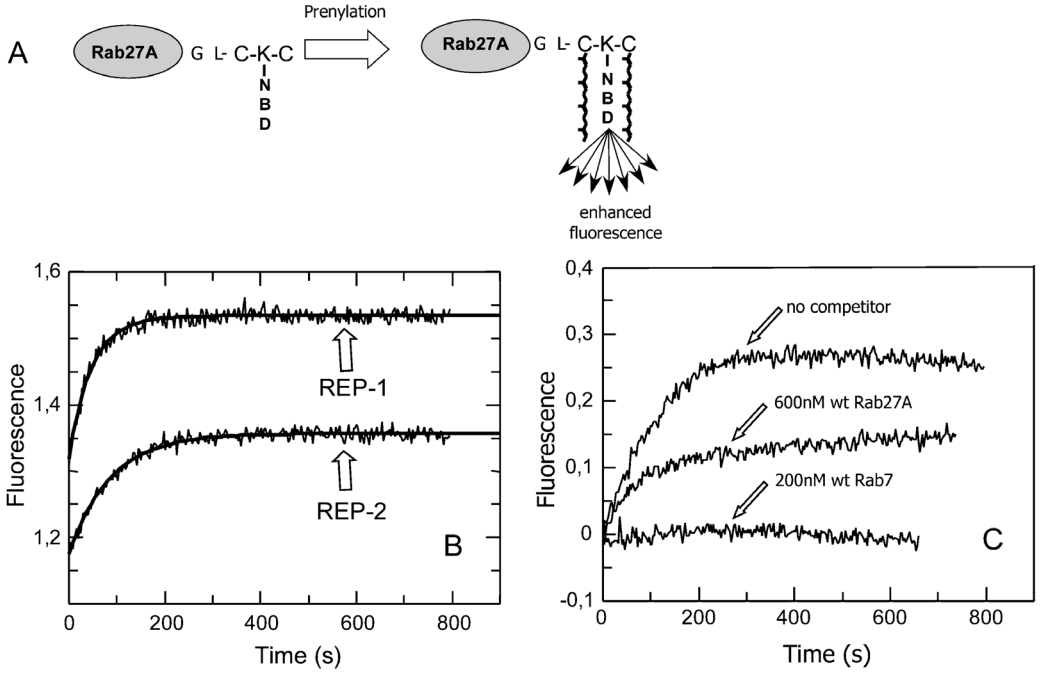

Figure 8. (*A*) A Rab27 derivative with a fluorescent label sensitive to prenylation. (*B*) Prenylation of Rab27-CK(NBD)C by RabGGTase in the presence of REP-1 or REP-2. The fitted rate constants are 0.02 and 0.01 s^{-1}, repectively. (*C*) Prenylation of 200 nM Rab27-CK(NBD)C in the presence of REP-1 (top curve); inhibition of prenylation of the same concentration of labeled Rab27 by 600 nM of unlabeled Rab27 (middle curve) and by 200 nM Rab7 (lower curve).

choroid), appears to accumulate in unprenylated form [51]. This was assumed to be due to the specific inability of REP-2 to support Rab27A prenylation at the required level in these cells. This question has now been reexamined, making use of specifically labeled Rab molecules [32]. Application of expressed protein ligation allowed incorporation of a fluorescent label (NBD) between the two prenylatable cysteines in Rab 27a (fig. 8A). On prenylation, the fluorescence of this group increased significantly, so that the fluorescence signal could be used as a continuous monitor of the prenylation state. As shown in fig. 8B, geranylgeranylation of Rab27A-CK (NBD)C in the presence of REP-2 was a factor of ca. 2 slower than in the presence of REP-1. While this appears to confirm the interpretation that REP-2 is deficient in supporting Rab27a prenylation, subsequent experiments showed that this effect also applied to other Rabs, so that this cannot be an explanation for specific Rab27a underprenylation. A clue to the probable explanation arose from experiments on the affinity of the interaction of different Rab proteins with REP-1 and REP-2. Here it was found that REP-2 bound several Rabs tested a factor of ca. 5-fold less strongly than REP-1 [32]. More significantly, Rab27A was found to have a generally very low affinity to both REP-1 and REP-2. This means that if Rabs compete for binding to REP, then limitation of REP activity will affect the weakly bound Rabs most. This situation is simulated in figure 8C, where it is shown that 200 nM Rab7 dramatically inhibits the prenylation of the same concentration of Rab27a. The suggestion was therefore made that loss of REP-1 activity in choroideremia reduces the total REP activity in the cell, and that this will affect weakly binding Rabs such as Rab27A more profoundly than others, leading to specific deficits in cells for which Rab27a plays an essential role. While this does not lead directly to specific approaches to the therapy of choroideremia, it suggests that gene therapy with the aim of replacing the missing REP-1 activity is not the only possible line of attack. Thus, if, as seems likely, the problem arises because of the low overall REP activity, measures to increase REP-2 levels, for example by increasing the expression level of REP-2, stabilizing the REP-2 message or reducing the rate of its degradation, might be successful.

Summary

Progress over the last several years has led to a partial understanding of the mechanisms of reversible association and dissociation of Rab proteins with membranes. However, the fundamental problem of the mechanism of targeting still remains. Thus, despite the fact that a potential GDF has been identified, there are still no data on how the factor recognizes a complex between a Rab protein and GDI or REP, and how transfer of the lipid moiety to the membrane occurs. The mechanisms of these processes must be unraveled, and even then assuming that specificity of Rab targeting is found to be dependent on specific receptors, the presence of these receptors in specific membranes has to be explained. It seems likely that it is the interplay of several aspects which is important, including GDFs, GEFs and more indirect cascade type mechanisms, including complex interactions such as those characterized for Rab5, Rabaptin and Rabex-5 [52]. There is also evidence suggesting that the lumenal content of an organelle might be involved in recruitment of Rabs on the cytoplasmic side of the membrane [53]. Clearly, the final answer to the question of targeting will be complex and is likely to be connected with the integrated nature of specific membranes or specific domains rather than being dictated by a single protein with receptor qualities [54]. These relationships remain to be established initially at the level of knowing which molecules interact with which other molecules, but finally at the level of resolution already achieved for the interactions discussed in this review.

1 CaseyP. J. and Seabra M. C. (1996) Protein prenyltransferases. J. Biol. Chem. **271:** 5289–5292
2 Jiang Y. and Ferro-Novick S. (1994) Identification of yeast component A: reconstitution of the geranylgeranyltransferase that modifies Ypt1p and Sec4p. Proc. Natl. Acad. Sci. USA **91:** 4377–4381
3 Seabra M. C., Mules E. H. and Hume A. N. (2002) Rab GTPases, intracellular traffic and disease. Trends Mol. Med. **8:** 23–30
4 Anant J. S., Desnoyers L., Machius M., Demeler B., Hansen J. C., Westover K. D. et al. (1998) Mechanism of Rab geranylgeranylation: formation of the catalytic ternary complex. Biochemistry **37:** 12559–12568
5 Thoma N. H., Iakovenko A., Owen D., Scheidig A. S., Waldmann H., Goody R. S. et al. (2000) Phosphoisoprenoid binding specificity of geranylgeranyltransferase type II. Biochemistry **39:** 12043–12052
6 Thoma N. H., Iakovenko A., Kalinin A., Waldmann H., Goody R. S. and Alexandrov K. (2001) Allosteric regulation of substrate binding and product release in geranylgeranyltransferase type II. Biochemistry **40:** 268–274
7 Thoma N. H., Iakovenko A., Goody R. S. and Alexandrov K. (2001) Phosphoisoprenoids modulate association of Rab geranylgeranyltransferase with REP-1. J. Biol. Chem. **276:** 48637–48643
8 Thoma N. H., Niculae A., Goody R. S. and Alexandrov K. (2001) Double prenylation by RabGGTase can proceed without dissociation of the mono-prenylated intermediate. J. Biol. Chem. **276:** 48631–48636
9 Alory C. and Balch W. E. (2000) Molecular basis for Rab prenylation. J. Cell Biol. **150:** 89–103
10 Shen F. and Seabra M. C. (1996) Mechanism of digeranylgeranylation of Rab proteins. Formation of a complex between monogeranylgeranyl-Rab and Rab escort protein. J. Biol. Chem. **271:** 3692–3698
11 Dursina B., Thoma N. H., Sidorovitch V., Niculae A., Iakovenko A., Rak A. et al. (2002) Interaction of yeast Rab geranylgeranyl transferase with its protein and lipid substrates. Biochemistry **41:** 6805–6816
12 Rak A., Niculae A., Kalinin A., Thoma N. H., Sidorovitch V., Goody R. S. et al. (2002) In vitro assembly, purification and crystallization of the rab geranylgeranyl transferase:substrate complex. Protein Expr. Purif. **25:** 23–30

13 Pylypenko O., Rak A., Reents R., Niculae A., Sidorovitch V., Cioaca M. D. et al. (2003) Structure of rab escort protein-1 in complex with rab geranylgeranyltransferase. Mol.Cell **11:** 483–494

14 Schalk I., Zeng K., Wu S. K., Stura E. A., Matteson J., Huang M. et al. (1996) Structure and mutational analysis of Rab GDP-dissociation inhibitor. Nature **381:** 42–48

15 Zhang H., Seabra M. C. and Deisenhofer J. (2000) Crystal structure of Rab geranylgeranyltransferase at 2.0 A resolution. Structure Fold. Des **8:** 241–251

16 Alory C. and Balch W. E. (2001) Organization of the Rab-GDI/CHM superfamily: the functional basis for choroideremia disease. Traffic **2:** 532–543

17 Rak A., Pylypenko O., Durek T., Watzke A., Kushnir S., Brunsveld L. et al. (2003) Structure of Rab GDP-dissociation inhibitor in complex with prenylated YPT1 GTPase. Science **302:** 646–650

18 Andres D. A., Seabra M. C., Brown M. S., Armstrong S. A., Smeland T. E., Cremers F. P. et al. (1993) cDNA cloning of component A of Rab geranylgeranyl transferase and demonstration of its role as a Rab escort protein. Cell **73:** 1091–1099

19 Sanford J. C., Yu, J., Pan J. Y. and Wessling-Resnick M. (1995) GDP dissociation inhibitor serves as a cytosolic acceptor for newly synthesized and prenylated Rab5. J. Biol. Chem. **270:** 26904–26909

20 Alexandrov K., Simon I., Yurchenko V., Iakovenko A., Rostkova E., Scheidig A. J. et al. (1999) Characterization of the ternary complex between Rab7, REP-1 and Rab geranylgeranyl transferase. Eur. J. Biochem. **265:** 160–170

21 Shapiro A. D. and Pfeffer S. R. (1995) Quantitative analysis of the interactions between prenyl Rab9, GDP dissociation inhibitor-alpha, and guanine nucleotides. J. Biol. Chem. **270:** 11085–11090

22 Kalinin A., Thoma N. H., Iakovenko A., Heinemann I., Rostkova E., Constantinescu A. T. et al. (2001) Expression of mammalian geranylgeranyltransferase type-II in *Escherichia coli* and its application for in vitro prenylation of Rab proteins. Protein Expr.Purif. **22:** 84–91

23 Muir T. W., Sondhi D. and Cole P. A. (1998) Expressed protein ligation: a general method for protein engineering. Proc. Natl. Acad. Sci. USA **95:** 6705–6710

24 Iakovenko A., Rostkova E., Merzlyak E., Hillebrand A. M., Thoma N. H., Goody R. S. et al. (2000) Semi-synthetic Rab proteins as tools for studying intermolecular interactions. FEBS Lett. **468:** 155–158

25 Alexandrov K., Heinemann I., Durek T., Sidorovitch V., Goody R. S. and Waldmann H. (2002) Intein-mediated synthesis of geranylgeranylated Rab7 protein in vitro. J. Am. Chem. Soc. **124:** 5648–5649

26 Durek T., Goody R. S. and Alexandrov K. (2004) In vitro semi-synthesis and applications of C-terminally modified rab proteins. Methods Mol. Biol. **283:** 233–244

27 Wilson A. L. and Maltese W. A. (1993) Isoprenylation of Rab1B is impaired by mutations in its effector domain. J. Biol. Chem. **268:** 14561–14564

28 Overmeyer J. H., Wilson A. L., Erdman R. A. and Maltese W. A. (1998) The putative 'switch 2' domain of the Ras-related GTPase, Rab1B, plays an essential role in the interaction with Rab escort protein. Mol. Biol. Cell **9:** 223–235

29 Sasaki T., Kikuchi A., Araki S., Hata Y., Isomura M., Kuroda S. et al. (1990) Purification and characterization from bovine brain cytosol of a protein that inhibits the dissociation of GDP from and the subsequent binding of GTP to smg p25A, a ras p21-like GTP-binding protein. J. Biol. Chem. **265:** 2333–2337

30 Sasaki T., Kato M., and Takai Y. (1993) Consequences of weak interaction of rho GDI with the GTP-bound forms of rho p21 and rac p21. J. Biol. Chem. **268:** 23959–23963

31 Constantinescu A. T., Rak A., Alexandrov K., Esters H., Goody R. S. and Scheidig A. J. (2002) Rab-subfamily-specific regions of Ypt7p are atructurally different from other RabGTPases. Structure (Camb.) **10:** 569–579

32 Rak A., Pylypenko O., Niculae A., Pyatkov K., Goody R. S. and Alexandrov K. (2004) Structure of the Rab7:REP-1 complex; insights into the mechanism of Rab prenylation and choroideremia disease. Cell **117:** 749–760

33 Neu M., Brachvogel V., Oschkinat H., Zerial M. and Metcalf P. (1997) Rab7: NMR and kinetics analysis of intact and C-terminal truncated constructs. Proteins **27:** 204–209

34 Chavrier P., Gorvel J. P., Stelzer E., Simons K., Gruenberg J. and Zerial M. (1991) Hypervariable C-terminal domain of rab proteins acts as a targeting signal. Nature **353:** 769–772

35 An Y., Shao Y., Alory C., Matteson J., Sakisaka T., Chen W. et al. (2003) Geranylgeranyl switching regulates GDI-Rab GTPase recycling. Structure (Camb.) **11:** 347–357

36 Pfeffer S. R. and Aivazian D. (2004) Targeting Rab GTPases to distinct membrane compartments. Nat. Rev. Mol. Cell. Biol. **5:** 886–896

37 Rak A., Reents R., Pylypenko O., Niculae A., Sidorovitch V., Thoma N. H. et al. (2001) Crystallization and preliminary X-ray diffraction analysis of the Rab escort protein-1 in complex with Rab geranylgeranyltransferase. J. Struct. Biol. **136:** 158–161

38 Soldati T., Shapiro A. D., Svejstrup A. B. and Pfeffer S. R. (1994) Membrane targeting of the small GTPase Rab9 is accompanied by nucleotide exchange. Nature **369:** 76–78

39 Ullrich O., Horiuchi H., Bucci C. and Zerial M. (1994) Membrane association of Rab5 mediated by GDP-dissociation inhibitor and accompanied by GDP/GTP exchange. Nature **368:** 157–160

40 Dirac-Svejstrup A. B., Sumizawa T. and Pfeffer S. R. (1997) Identification of a GDI displacement factor that releases endosomal Rab GTPases from Rab-GDI. EMBO J. **16:** 465–472

41 Sivars U., Aivazian D. and Pfeffer S. R. (2003) Yip3 catalyses the dissociation of endosomal Rab-GDI complexes. Nature **425:** 856–859

42 Yang X., Matern H. T. and Gallwitz D. (1998) Specific binding to a novel and essential Golgi membrane protein (Yip1p) functionally links the transport GTPases Ypt1p and Ypt31p. EMBO J. **17:** 4954–4963

43 Calero M. and Collins R. N. (2002) *Saccharomyces cerevisiae* Pra1p/Yip3p interacts with Yip1p and Rab proteins. Biochem. Biophys. Res. Commun. **290:** 676–681

44 Calero M., Winand N. J. and Collins R. N. (2002) Identification of the novel proteins Yip4p and Yip5p as Rab GTPase interacting factors. FEBS Lett. **515:** 89–98

45 Matern H., Yang X., Andrulis E., Sternglanz R., Trepte H. H., and Gallwitz D. (2000) A novel Golgi membrane protein is part of a GTPase-binding protein complex involved in vesicle targeting. EMBO J. **19:** 4485–4492

46 Luan P., Heine A., Zeng K., Moyer B., Greasely S. E., Kuhn P. et al. (2000) A new functional domain of guanine nucleotide dissociation inhibitor (alpha-GDI) involved in Rab recycling. Traffic **1:** 270–281

47 Alexandrov K., Horiuchi H., Steele-Mortimer O., Seabra M. C., and Zerial M. (1994) Rab escort protein-1 is a multifunctional protein that accompanies newly prenylated rab proteins to their target membranes. EMBO J. **13:** 5262–5273

48 Alory C. and Balch W. E. (2003) Molecular evolution of the Rab-escort-protein/guanine-nucleotide-dissociation-inhibitor superfamily. Mol. Biol. Cell **14:** 3857–3867

49 D'Adamo P., Menegon A., Lo N. C., Grasso M., Gulisano M., Tamanini F. et al. (1998) Mutations in GDI1 are responsible for X-linked non-specific mental retardation. Nat. Genet. **19:** 134–139

50 Seabra M. C. (1996) New insights into the pathogenesis of choroideremia: a tale of two REPs. Ophthalmic Genet. **17:** 43–46

51 Seabra M. C., Ho Y. K. and Anant J. S. (1995) Deficient geranylgeranylation of Ram/Rab27 in choroideremia. J. Biol. Chem. **270:** 24420–24427
52 Miaczynska M. and Zerial M. (2002) Mosaic organization of the endocytic pathway. Exp. Cell Res. **272:** 8–14
53 Seabra M. C. and Wasmeier C. (2004) Controlling the location and activation of Rab GTPases. Curr. Opin. Cell Biol. **16:** 451–457
54 Hannah M. J., Hume A. N., Arribas M., Williams R., Hewlett L. J., Seabra M. C. et al. (2003) Weibel-Palade bodies recruit Rab27 by a content-driven, maturation-dependent mechanism that is independent of cell type. J. Cell Sci. **116:** 3939–3948
55 Jahn R., Lang T. and Sudhof T. C. (2003) Membrane fusion. Cell **112:** 519–533.