Protein prenylation is a type of lipid modification that affects about 0.5% of cellular proteins [1]. Prenylated proteins are covalently modified with either farnesyl or geranylgeranyl (GG) via thioether bonds to C-terminal cysteine residues (reviewed in [2]).

Three distinct protein prenyltransferases have been identified to date (reviewed in [3]), which can be classified into two functional classes: the CAAX prenyltransferases, farnesyltransferase and geranylgeranyltransferase type I (GGTase-I), and the Rab geranylgeranyltransferase or GGTase type II (Rab GGTase). CAAX prenyltransferases recognize a distinct motif at the C-terminus of intracellular protein substrates. The motif is designated CAAX, where C is cysteine, A is an aliphatic amino acid and X is any amino acid. The CAAX sequence is the primary determinant for transferase binding to a protein substrate. If the last amino acid in the CAAX sequence is a methionine or serine, the protein is a substrate of farnesyltransferase, whereas if it is a leucine it becomes a substrate for GGTase-I [4-8]. CAAX-containing proteins include members of the Ras and Rho/Rac family of low-molecular-mass GTPases, \( \gamma \)-subunits of G-proteins, nuclear lamins, G-protein-coupled receptor kinases and retinal GMP phosphodiesterase.

The third prenyltransferase, Rab GGTase, acts exclusively on Rab proteins [9,10] and is the focus of this paper. Rabs are digeranylgeranylated at cysteines near or at the C-terminus in different motifs such as CC, CXC or CCXX. In contrast with the CAAX prenyltransferases, Rab GGTase does not recognize exclusively the cysteine-rich motif at the C-terminus. Instead, it has an intrinsic low affinity for Rabs and requires the presence of another component, designated Rab escort protein (REP), for effective prenylation. A model for the molecular mechanisms involved in the geranylgeranylation of Rabs is detailed below.

Rab proteins
Rab GGTase modifies a subset of proteins, termed Rab in mammals and Ypt/Sec4 in yeast. Rabs belong to the Ras superfamily of small GTPases and act as regulators of exocytic and endocytic pathways. More than 30 different Rabs have been identified and each one has a charac-

Abbreviations used: GDI, GDP dissociation inhibitor; GG, geranylgeranyl; GGTase, geranylgeranyltransferase; REP, Rab escort protein.
teristic subcellular location. These observations led to the suggestion that subsets of Rabs regulate distinct membrane trafficking steps (reviewed in [11–13]). Rab action involves a complex cycle of events. First, Rabs associate with a specific transport vesicle in the inactive GDP-bound form. A GDP/GTP exchange factor catalyses the binding of GTP, which activates the Rab protein. GDP–Rab is required for the formation of the soluble N-ethylmaleimide-sensitive factor-attachment protein receptor complexes, which specify the correct targeting of the transport vesicle to the appropriate acceptor compartment [14]. After fusion of the vesicle with the acceptor compartment, GTP–Rab is inactivated by the hydrolysis of bound GTP catalysed by a Rab-specific GTPase-activating protein. In order to cycle back to the donor compartment, GDP–Rab is removed from the membrane by Rab GDP dissociation inhibitor (GDI) [15]. In response to subsequent signals, Rab GDI mediates the reassociation of GDP–Rab with the donor membrane and the cycle resumes. The participation of Rabs in this intermembrane cycle is dependent on their lipid modification with geranylgeranyl groups.

**REPs**

Geranylgeranylation of newly synthesized Rabs requires the formation of a stable 1:1 complex with REP (Figure 1), previously called component A of Rab GGTase [10,16,17]. Newly synthesized Rabs probably exist in a GTP-bound state, because the intracellular concentration of GTP is much higher than the concentration of GDP [18]. However, REP has a low affinity for GTP–Rab and it is as yet not clear how the initial interaction between REP and Rab forms. One possibility is that GTP hydrolysis must occur before REP binding. However, the rate of intrinsic hydrolysis is too low to allow rapid and efficient prenylation. In intact cells, Rab prenylation is efficient and at steady state no significant pool of unprenylated Rabs exist [19]. Therefore it is possible that newly synthesized GTP–Rabs are recognized by a Rab GTPase-activating protein that stimulates GTP hydrolysis and mediates REP and GDP–Rab binding. Another possibility is that newly synthesized Rabs never assume the GTP-bound conformation. REP and/or molecular chaperones could bind to Rab as the polypeptide chain is synthesized, helping nascent Rab to fold in the GDP-bound conformation and rendering the binary complex a substrate for geranylgeranylation.

The structural requirements for REP–GDP–Rab interaction have not been elucidated. The preference for GDP–Rab implies that the interaction involves a nucleotide-sensitive region of Rab. Assuming that Rab and Ras have similar three-dimensional structures, there are two regions of Rab that change significantly upon binding guanine nucleotide: the loop2/beta2 region, also called the effector domain, and the loop4/alpha2 region [18]. Either one or both Rab regions could be involved in the interaction with REP. Three other sequences in Rab have been implicated in REP binding: the N-terminus including a conserved lysine residue, the loop3/beta3 region and the C-terminus [3]. All of these regions are juxtaposed on one side of the molecule and may thus contribute to REP binding.

Two REPs, REP-1 and REP-2, sharing 75% identity, have been identified in mammals. In the

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**Figure 1**

Model for cyclic action of REP

Step 1: REP binds newly synthesized GDP-bound Rab. Step 2: REP:Rab complex interacts with Rab GGTase heterodimer which catalyses the transfer of two GG groups to Rab. Step 3: REP:diGG Rab complex interacts with a receptor at the donor membrane from which nascent transport vesicles form. Step 4: diGG Rab dissociates from REP and inserts into the membrane via its GG groups. Free REP is recycled to the cytoplasm, and membrane-bound diGG Rab is activated by nucleotide exchange (GTP for GDP).
rat, both REPs are ubiquitously expressed as judged by immunoblot analysis of many tissues using an antibody that recognizes both proteins (Figure 2). The proportions of REP-1 and REP-2 vary from tissue to tissue. Brain contains mostly REP-1 while heart contains mostly REP-2. This analysis does not exclude the possibility that individual cell types within a given tissue may express only one of the other REP. REPs appear to be functionally redundant with respect to the prenylation of most Rabs with one notable exception [19,20]. The Rab27 protein is more efficiently prenylated by REP-1 than by REP-2. In choroideraemia cells, where REP-1 is missing, there is a deficiency of prenylation of Rab27 while other Rabs are appropriately prenylated [18]. This finding suggests that the retinal degeneration in choroideraemia may in part be due to Rab27 dysfunction.

REP proteins are homologous to Rab GDI, the function of which in Rab recycling is outlined above. REP and Rab GDI share structural homology in three regions, termed SCR1, 2 and 3 [21], and may thus have similar three-dimensional structures. Functionally, REPs and Rab GDIs also share many similarities. In semipermeabilized cells, both REP and Rab GDI can deliver Rab5 to endosomal membranes when added to the cells in a complex with prenylated Rab5. Both REP and Rab GDI are able to extract Rab5 from membranes when added to cells in the free form [22]. REP and Rab GDI both also serve as GDIs that preferentially bind to GDP–Rabs [22]. The major difference between REP and Rab GDI is the ability of REP to recognize both unprenylated and prenylated Rabs and to assist in the prenylation reaction. Rab GDI cannot assist in the prenylation reaction [22].

**Rab GGTase**

Rab GGTase (previously called component B of Rab GGTase) non-covalently binds the lipid substrate GGPP, and acts as a prenyl diphosphate carrier, as was demonstrated for the CAAX prenyltransferases [3,23,24]. However, unlike the CAAX prenyltransferases, Rab GGTase does not bind the protein substrate but instead recognizes the REP-GDP-Rab complex (Figure 1) [3,17]. Once the transient tetramer REP–GDP–Rab–GGTase complex is formed, two GG groups are transferred from the GGTase binding pocket to cysteine residues at or near the C-terminus of Rab [25]. Cysteines are geranylgeranylated with the formation of a thioether bond between the cysteine sulphur atom and the GG group [26]. The precise chemical mechanism of catalysis is not known.

Unlike the CAAX prenyltransferases, Rab prenylation involves the addition of two GG groups. The ability of a mutant Rab (Rabl-CS), in which one of the acceptor cysteines has been mutated to a serine, to accept one GG group suggests that the reaction proceeds with two independent additions of the prenyl group [17]. After prenylation of Rab1a-CS, the monoGG-Rab remains complexed to REP and Rab GGTase dissociates from the complex. In the prenylation of wild-type Rab1a (Rab1a-CC), it is unclear whether Rab GGTase dissociates from the REP–Rab complex after the first GG addition. The product of the reaction is the REP–diGG-Rab complex (Figure 1).

The stoichiometry of the REP–Rab complex before prenylation is 1:1. Rab geranylgeranylation triggers a change in the stoichiometry of the
REP–Rab interaction to 2:2 (or 2:1) [17]. This change may reflect a rearrangement of the REP–Rab interaction induced by the GG groups. The GG moieties could bind a hydrophobic pocket present in either Rab or REP or created by the interaction of both proteins.

Membrane delivery of prenylated Rab proteins

After prenylation, the REP–diGG-Rab complex dissociates from Rab GGTase and diGG-Rab translocates into specific organelar membranes, determined by each specific Rab (Figure 1). Free REP is recycled for another round of prenylation by binding a nascent Rab. We speculate that a Rab receptor promotes the dissociation of the REP–Rab complex (Figure 1) [16]. The Rab receptor remains unidentified and therefore it is not clear whether this putative protein is present in the cytoplasm or is membrane-associated. Rab GDI is an obvious candidate for the Rab receptor. Rab GDI interacts with prenylated GDP–Rabs and is involved in membrane delivery of prenylated Rabs. In coupled translation–prenylation reactions in vitro, Rab GDI can serve as the acceptor for prenylated Rabs [27]. However, in in vitro prenylation reactions using purified components, Rab GDI is a very weak acceptor of prenylated Rabs (L. Desnoyers, J. S. Anant and M. C. Seabra, unpublished work). In semi-permeabilized cells, introduction of the REP–diGG-Rab complex leads to the correct targeting and translocation of the diGG-Rab to the membrane, in the absence of cytosolic factors. We currently favour the hypothesis that the REP–diGG-Rab complex is competent for membrane delivery of prenylated Rabs (Figure 1), and that REPS act in the delivery of newly synthesized prenylated Rabs whereas Rab GDI acts in the recycling of Rabs for their action in multiple rounds of vesicular transport.

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Prenylation-dependent interaction of Rab proteins with GDP dissociation inhibitors

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Introduction

Prenylation is one of the most common lipid modifications of proteins in mammalian cells [1]. Studies using model peptides have confirmed that the increased hydrophobicity conferred on proteins by this post-translational modification can be sufficient to promote insertion into lipid bilayers [2], supporting the long-held view of prenylation as a mechanism for anchoring proteins to membranes [3]. In recent years it has become clear that protein-bound prenyl groups may play additional roles as mediators of protein–protein interactions [4]. One of the best examples of this type of interaction is the association between Rab proteins and a class of soluble regulatory proteins termed Rab GDP dissociation inhibitors (GDIs) [5].

Role of Rab proteins and Rab GDIs in vesicular transport

In mammalian cells, the family of low-molecular-mass (21–25 kDa) GTP-binding proteins encoded by the rab genes consists of at least 30 members [6]. All Rab proteins show some degree of homology to yeast GTP-binding proteins, Ypt1 and Sec4, which play essential roles in the vectorial transport of secretory proteins from the endoplasmic reticulum to the Golgi apparatus (Ypt1) and from the Golgi apparatus to the plasma membrane (Sec4) [7,8]. Individual Rab proteins are localized in discrete intracellular membranes and organelles, including the endoplasmic reticulum (Rab1A, Rab1B, Rab2), Golgi stack (Rab6, Rab10, Rab11), early endosomes (Rab4, Rab5), late endosomes (Rab7, Rab9) and synaptic vesicles (Rab3) [9]. It is now generally accepted that, like their counterparts in yeast, Rab proteins serve to regulate or direct vesicular transport within specific segments of the exocytic or endocytic pathways in mammalian cells. The precise role of Rab proteins in the vesicular transport machinery has not yet been defined at the molecular level. However, considerable evidence suggests that the mechanism entails the cycling of Rab proteins on and off donor and acceptor membranes, in conjunction with changes in their guanine nucleotide state (GTP = on, GDP = off) [10]. Post-translational modification of Rab proteins by one or two 20-carbon geranylgeranyl groups plays an important role in this process, since it is required for their membrane association [11,12] and binding to cytosolic GDIs [13].

A current hypothetical model of Rab function is depicted in Figure 1. The model emphasizes the key role played by GDI in the cycling of Rab proteins on and off intracellular membranes. The Rab cycle may be envisioned as beginning with the inactive GDP form of Rab residing in the cytosol in a 1:1 complex with a GDI. The GDP cycle may be envisioned as beginning with the inactive GDP form of Rab residing in the cytosol in a 1:1 complex with a GDI. The GDI serves three roles: (i) it binds to the prenylated GDP form of Rab [14] and slows the rate of GDP dissociation [15,16]; (ii) it masks the hydrophobic geranylgeranyl groups on the Rab protein, thus keeping the protein in solution [17]; (iii) it participates in the delivery of Rab–GDP to the membrane of the budding vesicle in the donor compartment. The last event has been studied in considerable detail in cell-free systems and perforated cells, where it has been demonstrated that preformed complexes between GDI

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