Molecular control of Rab activity by GEFs, GAPs and GDI

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ABSTRACT

Rab proteins are the major regulators of vesicular trafficking in eukaryotic cells. Their activity can be tightly controlled within cells: Regulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), they switch between an active GTP-bound state and an inactive GDP-bound state, interacting with downstream effector proteins only in the active state. Additionally, they can bind to membranes via C-terminal prenylated cysteine residues and they can be solubilized and shuttled between membranes by chaperone-like molecules called GDP dissociation inhibitors (GDIs). In this review we give an overview of Rab proteins with a focus on the current understanding of their regulation by GEFs, GAPs and GDI.

Introduction

Rabs are the largest branch of the superfamily of small GTPases with more than 60 members in humans and 11 members in budding yeast.1,2 Since their discovery in the 1980s,3-5 their major role in regulation of vesicular trafficking has been well established and many regulating factors have been identified, some of which will be discussed in detail in the following. In their physiologic role, Rab proteins can attach reversibly to membranes and bind to the nucleotides guanosine-5’-di- (GDP) or -triphosphate (GTP).2

As a prerequisite for their function and to be able to localize to internal membranes within eukaryotic cells, Rab proteins need to become prenylated at C-terminal cysteine residues.6 In order for this to happen, the newly synthesized Rab protein binds to the Rab escort protein (REP, termed Mrs6 in yeast) and is only then prenylated by Rab geranylgeranyltransferase (RabGGTase or GGTTase-II, Fig. 1a).7-9 After prenylation, the Rab protein can be delivered to a target membrane, where it is activated by a guanine nucleotide exchange factor (GEF) and bound GDP is replaced by the approximately 10-fold more abundant GTP.1,10 It should be noted that GEFs catalyze nucleotide exchange in both directions and directionality is only a result of the higher concentration of GTP compared with GDP. In this active state, Rabs interact with a variety of different effector proteins which help to select cargo, form and bud vesicles from donor membranes, transport vesicles along cytoskeletal tracks and finally attach and fuse vesicles with the target membrane (reviewed in ref. 11). Finally, the Rab interacts with GTPase activating proteins (GAPs) and becomes inactivated by hydrolysis of GTP to GDP. It can then be extracted from the membrane by the GDP dissociation inhibitor (GDI), which solubilizes the inactive prenylated Rab protein to provide a pool of inactive Rab in the cytosol ready for the next round of vesicular transport12 (Fig. 1a).

The G-domain common to small GTPases and Rab proteins shows a globular fold with a 6-stranded β-sheet surrounded by 5 α-helices (Fig. 1b). The differences between the inactive and the active state of small GTPases were first resolved on a structural level in the late 1980s and the early 1990s,13,14 and are well understood today: Upon exchange of the nucleotide, the major changes of conformation take place within regions termed switch I and switch II, which adopt an ordered conformation in the triphosphate state and a less well defined conformation in the diphosphate state (reviewed in ref. 15 see Fig. 1b). The high affinity binding and the specific recognition and discrimination of guanine over other nucleotides takes place via interactions between the nucleotide and specific motifs termed G-motifs (G1-G5).16 The G1-motif (P-loop, GxxxxGK[S/T])...
Figure 1. Rab proteins as molecular switches. (a) After being synthesized, Rab proteins bind to the Rab escort protein (REP) and become prenylated by RabGGTase at C-terminal cysteine residues. The prenylated protein can be solubilized in the cytosol by the GDP dissociation inhibitor (GDI), which shields the hydrophobic geranylgeranyl groups from the hydrophilic environment (1). Guanine nucleotide exchange factors (GEFs) catalyze nucleotide exchange and help to recruit Rabs to certain membranes within the cell (2). In their GTP-bound active state, Rabs interact with effectors and regulate different steps in vesicular trafficking (budding, vesicular transport along the cytoskeleton, tethering and fusion with a target membrane; 3). Finally, inactivation takes place via interaction with GTPase activating proteins (GAPs) to yield the GDP-bound Rab (4). (b) Comparison of Rab proteins bound to GppNHp (left; Rab5 (1HUQ), Rab8 (4LHW), Rab9 (2O9B), Rab11 (2F9M), Sec 4 (1G17) and Ypt7 (1KY2)) and bound to GDP (right; Rab5 (3CLV), Rab8 (4LHV), Rab9 (1WMS), Rab11 (2F9L), Sec 4 (1G16) and Ypt7 (1KY3)). The nucleotide (sticks) and Mg\(^{2+}\) (green sphere), switch I (red), switch II (blue) and the P-loop (magenta) are highlighted (N: N-terminus, C: C-terminus). Note the switch regions that adopt an ordered and defined conformation in the active state, whereas more flexible and less well defined conformations can be seen in the inactive Rabs. (c) A sequence alignment of different representatives of Rab proteins from different Rab families (according to Klöpper et al.\(^{134}\)) is shown highlighting important features within the sequences. The P-loop (magenta), switch I (red), switch II (blue) and the P-loop (magenta) are highlighted. Additionally, the position of Rab-family (RabF, gray) and Rab-subfamily (RabSF, orange) motifs that play an important role in recognition of general Rab interacting proteins or Rab family specific interactions, respectively, are indicated. The secondary structure (\(\alpha\)-helices 1–5 and \(\beta\)-sheets 1–6) is shown below the aligned sequences.
is present in many nucleotide binding proteins and binds the β-phosphate and a Mg$^{2+}$-ion, the G2-motif (T, part of switch I) makes contacts with the γ-phosphate and the Mg$^{2+}$-ion, the G3-motif (DxxGQ at the beginning of switch II) contains the Gln-residue necessary for GTP-hydrolysis and the G4- (NKxD) and G5-motifs (TSAK) contain essential residues that form specific contacts with the guanine base to distinguish guanine from other nucleotides. \cite{15,19} Besides these G-motifs, Rab proteins also contain short stretches of amino acids that have been termed RabF and RabSF motifs \cite{1c}. These distinguish Rabs from other small GTPases and provide the specificity toward general Rab interaction partners such as REP/GDI (RabF motifs) or toward specific interaction partners that only bind a subset of or single Rabs, typically effector proteins (RabSF motifs). \cite{20,21}

Here we present an overview of different regulatory factors of Rabs (GEFs, GAPs and GDI), with a focus on their discoveries and their molecular mechanisms of action and function within cells.

### Guanine nucleotide exchange factors (GEFs)

#### Families and numbers

Guanine nucleotide exchange factors play an important role in activating Rab proteins in a spatiotemporally controlled manner. \cite{23} In the past, a variety of different Rab GEFs have been identified (summarized in Table 1), including the Vps9 \cite{23} and DENN domain \cite{24} families of Rab GEFs as well as several other unrelated proteins. The low homology between Rab GEFs is probably the reason that for many Rabs, the corresponding GEFs are yet to be identified. \cite{25}

The first large family of Rab GEFs identified were Vps9-domain containing proteins (at least 9 members in humans) that are specific toward Rab5 family members and act in early endocytic trafficking. \cite{26,28} The second large family of RabGEFs, with 18 members in humans, are DENN domain GEFs. \cite{29} Compared to the Rab5-family specific Vps9 domains, this family has a broader substrate spectrum and DENN domain containing proteins act on several different Rab proteins (see Table 1). The domain architecture of these proteins shows 3 distinct regions, the upstream (uDENN), the central (DENN) and the downstream (dDENN) segments that are separated by linkers of different length within the primary sequence, \cite{30} but form a 2-domain closely packed tertiary structure with one longin domain (a domain found in several GEFs) \cite{31} and a C-terminal lobe. \cite{32} Besides these 2 major families of related Rab GEFs, several other proteins have been shown to possess GEF activity, including multi-subunit complexes such as the TRAPP I and II complexes (GEFs for Ypt1/Rab1 and Ypt31/32, respectively), \cite{33} the Mon1/Ccz1 complex (GEF for Rab7/Ypt7), \cite{34} Ric1-Rgp1 (GEF for Rab6/Ypt6) \cite{35} and BLOC-3 (GEF for Rab32 and Rab38). \cite{36} Interestingly, some of these contain longin-fold domains (Mon1/Ccz1, BLOC-3 and the TRAPP complex) \cite{31}, however they seem to fulfill different functions within these proteins compared with DENN domains. \cite{22} The related Sec2/GRAB/Rabin-8 GEFs that act on Rab8 or the yeast Rab8 homolog Sec4 all consist of a long parallel coiled-coil that catalyzes nucleotide exchange. \cite{7,38} Very recently, the *C. elegans* protein REI-1 and its human homolog SH3BP5 were found to possess GEF activity toward Rab11. \cite{39}

As a mechanism of fine-tuning their activity, several GEFs of small GTPases have been reported to be controlled by auto-inhibition and release of this autoinhibition by regulatory proteins. \cite{22} One such example is the Vps9 domain of Rabex-5, which shows basal activity toward Rab5, but is strongly activated in a positive feedback loop only upon interaction with a Rab5 effector protein, Rabaptin-5. \cite{5} Furthermore, several GEFs have been implicated in Rab cascades, where they are recruited by one Rab to activate another Rab protein acting further down the pathway. \cite{41} One example of this is the yeast Rab protein Ypt32p that recruits Sec2p, the GEF of downstream acting Sec4 (the yeast Rab8 homolog). \cite{42} Additionally it has been shown that GEFs (together with other factors) play a major role in recruitment of Rabs to certain sites within cells. \cite{43,44} Therefore, untangling the role of Rab-GEF networks including further interacting proteins and the identification of as yet unknown (possibly multi-subunit) GEFs will be a major challenge to fully understand their precise roles in the regulation of vesicular trafficking.

The possibility of manipulating vesicular transport for their own survival is exploited by several intracellular surviving pathogens. \cite{45} One example is the pathogenic bacterium *Legionella pneumophila* that provides a plethora of proteins for manipulation of vesicular transport and other physiologic processes. \cite{5} The protein DrrA (defect in Rab recruitment protein A) is among these and it was shown that Legionella injects this protein into infected host cells and uses its GEF activity to recruit and mislocalize Rab1 to the intracellular vacuole where Legionella resides. \cite{47,48} Similarly, the *Salmonella* protein SopE was reported to act as a GEF for and recruit Rab5 to the intracellular *Salmonella* containing phagosomes, thereby promoting fusion with endosomes. \cite{49}

#### Structures and mechanisms

The first structure of a Rab:GEF complex, published in 2006, was that of Rab8:Mss4, even though structural
| Rab   | GEFs                             | catalytic efficiency($M^{-1} s^{-1}$) | GAPs                             | catalytic efficiency($M^{-1} s^{-1}$) |
|-------|----------------------------------|--------------------------------------|----------------------------------|--------------------------------------|
| Rab1a | TRAPP 135,136                    |                                      | TBC1D20                          |                                      |
|       | DrnA (L. pneumophila) 67         |                                      | LepB (L. pneumophila) 69         |                                      |
|       |                                   |                                      | USP6 NL 66                       |                                      |
| Rab1b | TRAPP 135,136                    | 2.0 - 10^{48}                       | TBC1D20                          | 7.3 - 10^{70}                       |
|       | DrnA (L. pneumophila) 67         |                                      | LepB (L. pneumophila) 69         |                                      |
|       |                                   |                                      | USP6 NL 66                       |                                      |
| Rab2a |                                   |                                      | TBC1D 146/17/1BC1D4 / TBC1D 11 / | Three.6 - 10^{70}                    |
|       |                                   |                                      | TBC1D 125/1/USP6 NL 66           |                                      |
| Rab2b | MADD (Denn) 135,136              |                                      |                                  |                                      |
| Rab2c |                                   |                                      |                                  |                                      |
| Rab3a |                                   |                                      |                                  |                                      |
| Rab3b | MADD (Denn) 135,136              |                                      |                                  |                                      |
| Rab3c | MADD (Denn) 135,136              |                                      |                                  |                                      |
| Rab3d | MADD (Denn) 135,136              |                                      |                                  |                                      |
| Rab4a |                                   |                                      |                                  |                                      |
| Rab4b |                                   |                                      |                                  |                                      |
| Rab5a | Rab5-5 (Vps9) 135,136            | 2.3 - 10^{138}                      | TBC1D3 / RUBTC3 / USP6 NL 63    |                                      |
| Rab5b |                                   |                                      | TBC1D3 / RUBTC3 / USP6 NL 63    |                                      |
| Rab5c |                                   |                                      | TBC1D3 / RUBTC3 / USP6 NL 63    |                                      |
| Rab6a | Ric1-Rgp 135,136                 |                                      | TBC1D1                          |                                      |
| Rab6b | Ric1-Rgp 135,136                 |                                      | TBC1D1                          |                                      |
| Rab6c |                                   |                                      | TBC1D1                          |                                      |
| Rab7a | Mon1/Ccz1 135,136                |                                      | TBC1D2A / TBC1D5 / TBC1D 15 / |                                      |
| Rab7b | Mon1/Ccz1 135,136                |                                      | EVIS-L 66                       |                                      |
| Rab8a | Rab8-8                           | 2.6 - 10^{65}                       | TBC1D1                          | 5.3 - 10^{119}                      |
|       | GRAB 135,136                     |                                      | TBC1D4                          | 2.8 - 10^{119}                      |
|       | Moss 135,136                     |                                      |                                 |                                      |
| Rab8b |                                   |                                      |                                 |                                      |
| Rab9a | Denn2 135,136                    |                                      |                                 |                                      |
| Rab9b | Denn2 135,136                    |                                      |                                 |                                      |
| Rab10 | Denn4 135,136                    |                                      |                                 |                                      |
| Rab11a| SHBPS (REI-1) 19                 |                                      |                                 |                                      |
| Rab11b| SHBPS (REI-1) 19                 |                                      |                                 |                                      |
| Rab12 | Denn3 135,136                    |                                      |                                 |                                      |
| Rab13 | Denn1C 135,136                   |                                      |                                 |                                      |
| Rab14 | Denn6 135,136                    |                                      |                                 |                                      |
| Rab15 | Vps9 135,136                     |                                      |                                 |                                      |
| Rab17 |                                   |                                      |                                 |                                      |
| Rab18 |                                   |                                      |                                 |                                      |
| Rab19 |                                   |                                      |                                 |                                      |
| Rab20 |                                   |                                      |                                 |                                      |
| Rab21 | Rab5-5 (Vps9) 135,136            | 3.2 - 10^{138}                      | TBC1D 17                        |                                      |
| Rab22a| Rab5-5 (Vps9) 135,136            | 3.5 - 10^{138}                      | TBC1D10B / TBC1D 18             |                                      |
| Rab23 |                                   |                                      | EVIS-L 66                       |                                      |
| Rab24 |                                   |                                      |                                 |                                      |
| Rab25 |                                   |                                      |                                 |                                      |
| Rab26 |                                   |                                      |                                 |                                      |
| Rab27a| MADD (Denn) 135,136              |                                      | TBC1D10A / TBC1D 10B           |                                      |
| Rab27b| SBF1 (Denn) 135,136              |                                      |                                 |                                      |
| Rab28 |                                   |                                      |                                 |                                      |
| Rab29 |                                   |                                      |                                 |                                      |
| Rab30 |                                   |                                      |                                 |                                      |
| Rab31 |                                   |                                      |                                 |                                      |
| Rab32 | BLOC-3 36                        |                                      | TBC1D10B 66                     |                                      |
| Rab33a|                                   |                                      | TBC1D 25                        |                                      |
| Rab33b|                                   |                                      | TBC1D 25                        |                                      |
| Rab34 |                                   |                                      | TBC1D 18 / TBC1D 25            |                                      |
| Rab35 | Denn1A-C 135,136                 | 2.9 - 10^{132}                      | TBC1D 10A / TBC1D 10B / TBC1D 10C / |                                      |
|       |                                   |                                      | TBC1D 17 / EVIS 63             |                                      |
|       |                                   |                                      |                                 |                                      |
| Rab36 |                                   |                                      |                                 |                                      |
| Rab37 |                                   |                                      |                                 |                                      |
| Rab38 | BLOC-3 36                        |                                      | TBC1D10B 66                     |                                      |
| Rab39a| Denn5A 135,136                   | 2.8 - 10^{138}                      | TBC1D10B 66                     |                                      |
| Rab39b| Denn5A 135,136                   | 2.8 - 10^{138}                      | TBC1D10B 66                     |                                      |

(Continued on next page)
analysis showed some peculiarities compared with other known GTPase:GEF structures and indicated a function of Mss4 as a chaperone rather than a GEF. In the following years, a variety of different GEFs in complex with their cognate Rabs were successfully crystallized and their structures solved (Sec4:Sec2, Rab21:Rabex-5, Ypt1p:TRAPP, Rab35:DennD1B, Rab8:GRAB/Rabin8 and Rab5:Rabex-5, Fig. 2a), allowing a structural view of intermediates of the nucleotide exchange reaction. Since the general mechanism of action of GEFs is the stabilization of the intermediate nucleotide-free state of small GTPases, GEFs bind these nucleotide-free GTases with high affinities. Accordingly, most structures of GTPase:GEF complexes have been obtained in the absence of nucleotides (Fig. 2a), with the exception of the recently published structures of Rab5:Vps9 and Rab8:Rabin8 that could be crystallized in the presence of different nucleotides.

The general exchange mechanism of all GEFs must follow a common route with a first low-affinity encounter complex with the nucleotide-bound GTPase and subsequent release of the nucleotide. In all cases, GEFs bind to the switch I/switch II and interswitch regions of the small GTPases, inducing structural rearrangements within these regions that are incompatible with high affinity nucleotide binding (Fig. 2a). The largest conformational changes (compared with the active GTP-bound GTases) generally take place in switch I, where interactions occur mostly between the GEF and the C-terminal half of switch I (where the γ-phosphate and Mg²⁺ binding G2-motif Thr is located) to pull the switch I into an open conformation. This movement also leads to displacement of a highly conserved aromatic Phe or Tyr residue in Rab proteins (Tyr33 in Rab1b) from an edge-to-face interaction with the guanine base, thus lowering the nucleotide affinity. In many cases, the residues surrounding this aromatic residue in switch I move approximately 10–30 Ångstrom away from their position in the GDP-bound GTPase and often adopt a disordered state. For this reason, amino acids within this region often cannot be traced in the electron density of Rab:GEF structures (Fig. 2a), similar to the situation in GDP-bound GTases. In contrast, switch II adopts an ordered conformation in Rab:GEF structures that is more similar to the GDP- than the GTP-bound state of Rabs and the overall conformational change of switch II shown in Fig. 2 is less dramatic than that of switch I. Since GEFs catalyze nucleotide exchange in both directions (i.e. from GDP- to GTP-bound state or vice versa), these results indicate that, depending on the educt of the GEF catalyzed reaction (i.e., Rab:GDP or Rab:GTP), the initial recognition of the GTPase by the GEF might be either by binding to switch I (GDP-bound state) or switch II (GTP-bound state).

In contrast to the switch regions, the conformational change of the P-loop is usually less dramatic and changes in the P-loop are mostly secondary effects due to a collapse of this loop into the nucleotide-binding pocket in the absence of interactions with negatively charged phosphates. In fact, PO₄³⁻ or SO₄²⁻ ions were found to be bound at a position resembling the β-phosphate of the nucleotides in some structures (e.g. Rab1b:DrrA and Rab8:Rabin8) and seem to stabilize the P-loop in a conformation similar to the
nucleotide-bound form. In this respect, comparison of Sec2:Sec4 structures with (pdb 2EQB) and without (pdb 2OCY) a bound PO$_4^{2-}$ ion nicely illustrate this effect with a collapsed conformation of the P-loop only in the PO$_4^{2-}$-free structure (Fig. 2). Furthermore, a similar collapsed state of the P-loop can also be observed in the related P-loop NTPase myosin in the absence of both an exchange factor and a bound nucleotide (Fig. 2b). However, in some GEF:Rab complexes (Ypt1:TRAPP, Rab21:Rabex-5), a negatively charged residue from the GEF is projected into the vicinity of the highly conserved P-loop lysine, thus substituting for the missing negative charge.

In addition to binding to the switch and interswitch regions and opening of the nucleotide binding pocket, binding of the GEF in many cases involves projection of residues toward and steric hindrance of Mg$^{2+}$ binding (e.g., Sec2, Vps9). As well as electrostatic repulsion between acidic residues of GEFs or the Rab itself pointing toward the phosphate groups (e.g., Vps9, TRAPP). These effects additionally lower the nucleotide affinity and thereby further accelerate nucleotide dissociation from the open nucleotide binding pocket.

**GTPase activating proteins (GAPs)**

**Families and numbers**

Small GTPases have a low intrinsic nucleotide hydrolysis activity with the half-life of the active state being of the
order of 30 min to several hours. Therefore, to be turned off in a physiologically meaningful timeframe, additional proteins termed GTPase activating proteins need to bind to the small GTPases and assist in hydrolysis. Whereas Rab GEFs are highly diverse in primary sequence and structure, Rab GAPs consist of one major family, the TBC- (Tre-2/Bub2/Cdc16) domain GAPs with more than 40 different members present in humans. They were first described in yeast in the early 1990s where they are called Gyps ("GAP for Ypt proteins"). Only one GAP in humans is known that does not contain this conserved TBC domain: The Rab3GAP complex, consisting of 2 different proteins and acts on members of the Rab3 family.

As can be seen in Table 1, for many Rabs, no corresponding GAP has been identified. However, many GAPs have been found to be promiscuous toward Rabs and possibly evolved low specificity to ensure that different active Rabs that reach a certain destination/organelle in a cell are inactivated by the GAP(s) present at this site. Additionally, the boundaries between different Rabs are regulated by cascades similar to the case of GEFs described above. Thus, besides recruiting the downstream acting GEF Sec2, Ypt32 has also been described to recruit Gyp1, the GAP acting on Ypt1, as well as Gyp6, the GAP for Ypt6, thus helping to establish a sharp transition between active Ypt1/Ypt6 and Ypt32 on intracellular membranes.

Similar to DrrA as a GEF, Legionella pneumophila also harbors a Rab GAP in its arsenal of translocated proteins for the manipulation of host vesicular transport, the GAP protein LepB. LepB also binds to the Legionella containing vacuole and appears to inactivate Rab1 (and possibly other Rab proteins) to allow their removal at a later stage in the reproduction cycle.

**Structures and mechanisms**

The first structure of a TBC domain GAP was that of Gyp1 in 2000, followed by the structure of the complex between Gyp1 and Rab33b in 2006. In this paper, Lambergh and colleagues made the surprising discovery that, in contrast to other known GAPS of small GTPases, TBC-domain GAPs use a slightly different mechanism for catalysis. Whereas other GAPS were shown to provide an Arg finger to assist hydrolysis together with the Gln within the G3-motif of the small GTPases, Gyp1 provides an Arg and an additional Gln-finger in trans that substitutes the G3-motif Gln that is used by many other GTPases in their GAP activated mechanism. TBC-domain GAPs were thus shown to act via a dual-finger mechanism (Fig. 3a).

In contrast, the Legionella pneumophila GAP LepB uses a single Arg-finger comparable to other GAPS of small GTPases in conjunction with the cis-Gln provided by the Rab protein (Fig. 3b). The general role of the Gln (whether provided by the GAP or the Rab itself) is to align a water molecule for an in-line attack of the γ-phosphate, whereas the Arg finger (generally provided by the GAP) neutralizes developing negative charges during the hydrolysis reaction. Since TBC-domain GAPs are not dependent on the Gln provided by the small GTPase, they have also been shown to still be able to catalyze GTP-hydrolysis in Rab proteins carrying a mutation often considered to stabilize GTPases in the active state (e.g., the G3-Gln mutated to Leu or Ala), thus possibly leading to misinterpretations obtained with Rabs carrying these mutations in *in vivo* experiments. On the other hand, some TBC-domain GAPs lack the Arg, the Gln or both and might thus be either inactive or act via a different mechanism.

**Prenylation of Rab proteins and membrane binding**

Besides cycling between an active and an inactive state regulated by GEFs and GAPs, Rab proteins also cycle between a cytosolic and a membrane bound form and these cycles are tightly interconnected. As a necessary requirement for the reversible membrane attachment, Rab proteins need to become irreversibly prenylated at C-terminal cysteine residues.

Prenylation as a posttranslational modification was first discovered in the late 1970s, but it took another decade until C-terminal prenylation was also shown for Rabs and Rabs. Prenylation is catalyzed by 3 different enzymes using either farnesylpyrophosphate (FPP) or geranylgeranylprophosphate (GGPP) for modification of target proteins. Farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I) both recognize C-terminal CaaX-motifs (C - Cys, a - aliphatic amino acid, X - any amino acid) and attach a single farnesyl- or geranylgeranyl-group to the cysteine via a thioether linkage. In contrast, geranylgeranyltransferase II (GGTase II, also termed RabGGTase) recognizes C-terminal cysteine residues within different motifs (e.g., CC, CXC, see Fig. 1) and usually modifies 2 cysteine residues at the C-terminus of Rab proteins. Rab proteins ending on CXC, but not CC, are then further processed by carboxymethylation.

**Prenylation machinery and REP**

After ribosomal synthesis, Rab proteins first need to bind to the Rab escort protein (REP), which only then presents the Rab proteins to RabGGTase for
The double prenylation observed in most Rab proteins proceeds without intermediate release of the mono-prenylated Rab protein. After binding of a new molecule of GGPP to the active site of the RabGGTase, the doubly prenylated Rab in complex with REP dissociates from RabGGTase and can deliver the Rab protein to a target membrane.

The X-ray structures of mono-prenylated Rab7 in complex with REP have been solved (Fig. 4a). REP, which exists in 2 different isoforms in humans and one isoform (termed Mrs6) in yeast, consists of 2 different domains. Whereas domain I recognizes and binds the globular G-domain and the extended C-terminus of the Rab protein via regions referred to as the Rab binding platform (RBP) and the C-terminus binding region (CBR), respectively, the prenyl-groups bind between helices D and E of domain II (Fig. 4). These 2 helices within domain II of REP additionally form the binding platform for the heterodimeric RabGGTase which consists of one large (60kDa) α-subunit and a smaller (38kDa) β-subunit (termed Bet4 and Bet2, respectively, in yeast). The large subunit consists of 3 domains, an immunoglobulin- (Ig-) like domain, a leucine-rich repeat- (LRR-) domain and a solely α-helical domain which provides the binding interface with REP. The interaction is formed by the α-helices 8, 10 and 12 within this domain interacting with helices D and E of REP-1 (Fig. 4a and c). The active site, containing a Zn$^{2+}$-ion for catalysis of prenylation, is located within the β-subunit.

Based on the available structures of Rab7:REP and REP:RabGGTase, modeling of the ternary complex was also possible which in turn allowed proposal of a model of the catalytic mechanism: Whereas the Rab-protein is bound to domain I of REP distal from the RabGGTase, the flexible C-terminus can extend toward and bind within the active site of the β-subunit of RabGGTase to become prenylated. As discussed before, binding of

![Figure 3. Dual- and single-finger mechanism used by RabGAPs. The structures of Gyp1:Rab33 (a) and LepB:Rab1 (b) are shown (left; Rab: gray cartoon with the switch I and II in red and blue, respectively and the P-loop in magenta (the switch regions and the P-loop are shown as ribbon), the GAP is shown as rainbow colored cartoon) and a zoom into the active site (right; residues provided by the GAP are colored in green, residues of the Rab protein in gray). Both structures were obtained in the presence of GDP and BeF$_3$ or AlF$_3$ as transition state mimetics of the hydrolysis reaction. (a) Gyp1 uses a dual-finger mechanism to catalyze GTP-hydrolysis and provides both Arg$_{343}$ and Gln$_{378}$ (green) in trans. The G3-motif Gln$_{92}$ (gray) of Rab33 is repositioned by interactions with backbone atoms of Gyp1 and does not participate in catalysis. (b) In contrast to TBC-domain GAPs, the *Legionella pneumophila* GAP LepB uses a single-finger mechanism and provides only one residue (Arg$_{444}$, green). Gln$_{67}$ of Rab1b remains in its usual conformation to position the attacking water for the in-line attack of the γ-phosphate.

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another GGPP leads to displacement of the already prenylated C-terminus from the active site which then migrates toward and binds to helices D and E of domain II of REP. This in turn leads to a conformational change that interferes with the interaction of Phe279 (α-helix D) and Arg290 (α-helix E) with RabGGTase (Fig. 4a and c), so that the Rab:REP complex finally dissociates from RabGGTase and the prenylated Rab can be delivered to a target membrane.87

GDI function and structures

To be recycled after one round of vesicular trafficking, Rab proteins need to be extracted from membranes and solubilized in the cytosol. The protein responsible for this was originally identified as a Rab3 interacting protein that inhibited dissociation of GDP and was therefore named GDP dissociation inhibitor (GDI).91 In contrast to REP, GDI only binds prenylated and inactive GDP-
bound Rab proteins with high affinity,\textsuperscript{91,92} thus ensuring extraction of the Rab proteins only after their trafficking cycle is complete and they have been inactivated by a GAP. Whereas GDI displays similar affinities for mono- and di-prenylated Rabs ($K_D = 1.5$ nM and 5.2 nM, respectively), but does not bind to unprenylated Rab, REP has a higher affinity for the mono-prenylated form ($K_D = 61$ pM) compared with the unprenylated or diprenylated Rab ($K_D = 1$ nM and 1.3 nM, respectively).\textsuperscript{92,93} These observations provide an explanation for the thermodynamic driving force of extraction of Rab proteins from membranes by GDI, but not REP: Whereas GDI binds only weakly to the G-domain of a Rab, the affinity increases approximately 3 orders of magnitude upon additional binding of the prenylated C-terminus. On the other hand, REP binds with similar affinities to the prenylated and unprenylated Rab, thus allowing binding and presentation of the unprenylated Rab to RabGGTase.\textsuperscript{92}

The first structure of GDI was solved in 1996, displaying 2 separate domains termed domain I and II.\textsuperscript{94} A first structure of GDI in complex with a prenylated cysteine\textsuperscript{95} initially suggested the lipid binding site to be located in domain I. However some years later, the structures of mono-\textsuperscript{96} and di-prenylated\textsuperscript{97} Rab proteins bound to GDI showed that the lipid binding site was actually located in domain II, whereas the globular G-domain and the extended C-terminus both bind to domain I, with an overall fold, binding regions and relative orientation of Rab and GDI similar to the situation in the Rab:REP complex (see comparison in Fig. 4a and b).

**Targeting and retrieval of Rab proteins to and from intracellular membranes**

One major issue that still remains to be fully resolved is the mechanism of specific targeting of Rab proteins to certain membranes within a cell. Initially, the hypervariable C-terminus was thought to contain the information encoded in the amino acid sequence for targeting to certain membranes,\textsuperscript{98,99} but it was later shown that this does not apply to all Rabs and that sequences important for the intracellular localization are distributed throughout the primary sequence of Rabs.\textsuperscript{100} Further work implied membrane localized GDI dissociation factors (GDFs) that are able to specifically dissociate Rab:GDI complexes and thereby contribute to specific localization.\textsuperscript{101,102} However, Pra1 and the yeast homolog Yip3 remain the only proteins with proven GDF activities that have been identified. Since GDIs bind with high affinity to Rabs in their inactive, but not their active state,\textsuperscript{103} a role of specific membrane targeting was also suggested for GEFs, since they can catalyze activation and therefore stabilize Rabs at a membrane.\textsuperscript{43,48} A present consensus view is that targeting of Rabs to certain membranes is most probably a process involving many different factors, including the C-terminus of Rabs, further putative GDFs, GEFs, GAPs and effectors.\textsuperscript{44} On the other hand, it is currently not clear whether extraction of Rabs by GDI is actively regulated and possibly accelerated beyond the presumably slow spontaneous dissociation rate of Rabs from membranes. The precise mechanisms of delivery and extraction of Rabs to and from intracellular membranes and additional factors that might catalyze these processes and contribute to spatial specificity therefore need to be established in future research.

**PTMs and their role in regulation**

Besides prenylation of the C-terminus, several other posttranslational modifications (PTMs) at different positions within the primary sequence of Rabs have been reported, including for example phosphorylation and sero-tonylation.\textsuperscript{104-109} p34cdc2 kinase for example phosphorylates Ser$_{204}$ within the C-terminus of Rab4 close to the prenylatable cysteines.\textsuperscript{105} Parkinson’s disease kinase LRRK2 phosphorylates several Rabs including Rab10 at position Thr$_{73}$ within switch II and a yet to be identified kinase phosphorylates Rab8 at Ser$_{111}$ within the RabSF3 motif and close to the switch II region in the tertiary structure.\textsuperscript{108} The latter was also shown to negatively influence the activation of Rab8 by its cognate GEF Rabin8.\textsuperscript{108} In a very recent study, TGF-$\beta$ activated kinase 1 mediated phosphorylation of Rab1a at Thr$_{75}$ in switch II was shown to disrupt interaction with GDI\textsuperscript{110} Serotonin (the attachment of serotonin via an amide bond to the catalytic glutamine side chain by transglutaminases) on the other hand has been shown to cause constitutive activation of the modified GTPase.\textsuperscript{106} Additionally, not only Rabs can be posttranslationally modified, but also their regulatory proteins including for example GEFs, GAPs and GDIs.\textsuperscript{111-115}

Again, much has been learned from the pathogenic bacterium *Legionella pneumophila* concerning the potential of PTMs for manipulation of Rabs. The Legionella proteins DrsA/SidD\textsuperscript{116-119} and AnkX/Lem3\textsuperscript{120-122} were shown to reversibly modify a Tyr and a Ser/Thr residue (Tyr$_{77}$ and Ser$_{76}$ in Rab1b within switch II) with AMP or phosphocholine, respectively, having an impact on the interaction of these Rab proteins with effectors, GAPs, GEFs and GDI. The potential role of PTMs was therefore discussed as means of modulating Rab interactions, including an effective
displacement of Rab-GDI complexes and stabilizing Rabs at a certain membrane.\textsuperscript{114,123}

Even though several examples have been published, a universal regulatory role or the impact of the different PTMs on Rab regulated membrane trafficking is still uncertain for many PTMs and will be subject to further interesting investigations in the future. However, the positions of the posttranslational modification within functionally important regions of Rab proteins indicate that regulatory roles are highly probable.

**Genetic disorders involving regulatory proteins**

Besides being targeted by pathogenic bacteria, Rabs and associated regulatory proteins are also mutated in several genetic disorders.\textsuperscript{45,124} Mutations of the GAPs Rab3GAP or TBC1D20 for instance are known to cause the severe Warburg Micro syndrome with neurologic and ocular disorders as well as microgenitalia.\textsuperscript{125,126} Another mutation in Rab3GAP causes cataracts, mental retardation and hypogonadism in Martsolf syndrome.\textsuperscript{127} The GEF BLOC-3 is mutated in Hermansky-Pudlak syndrome, causing albinism and bleeding disorders.\textsuperscript{36} A very recent discovery shows that the product of the C9orf72 gene, which harbors expanded GGGCCC repeats in non-coding regions in many cases of amyotrophic lateral sclerosis (ALS) leading to underexpression of the gene product, is a GEF for Rab8 and Rab39. These Rabs are involved in autophagy, which is defective in ALS.\textsuperscript{128,129}

Many other mutations are known or can be assumed to effect prenylation, delivery and/or retrieval of Rabs from membranes. For instance, Hermansky-Pudlak syndrome is also caused by a mutation of RabGGTase presumably causing deficient prenylation.\textsuperscript{130} Several mutations within the gene coding for REP-1 cause Choroideremia, an X-chromosomal disease causing degeneration of the retina and resulting night blindness as well as loss of peripheral vision,\textsuperscript{131,132} and another X-chromosomal mutation within the gene for GDI-1 is known to cause X-linked non-specific mental retardation.\textsuperscript{133} Together, these severe impacts on human physiology illustrate the importance of regulated membrane cycling of Rab proteins.

**Outlook**

Even though further GEFs and GAPs still will (and need to be) identified, many have been found and biochemically and structurally characterized in vitro. Similarly, the structures of GDI and REP in complex with Rabs have been reported as discussed above. Together, these insights give a rough scheme of the regulation of vesicular transport and factors involved. However, comparatively little is still known about the situation in vivo, especially regarding the localization and the specificity of these regulatory proteins toward Rabs as well as regarding their spatiotemporal regulation by additional proteins and posttranslational modifications. Our knowledge of the underlying protein interaction networks therefore still needs to be expanded to finally understand the fine details of the different Rab regulated vesicular transport processes in physiological and pathological states.

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