Controlling the location and activation of Rab GTPases

Miguel C Seabra[1] and Christina Wasmeier

The remarkable degree of specificity with which Rab GTPases recognise distinct subsets of intracellular membranes forms the basis of their ability to act as key cellular regulators, determining the recruitment of downstream effectors to the right membrane at the right time. The molecular mechanisms controlling Rab localisation, however, have proved tricky issues to address. It is becoming increasingly apparent that multiple factors contribute to the specificity of Rab localisation and the close coordination of membrane targeting with Rab activation. With important new insights into the mode of action of the general Rab regulators REP and RabGDI, as well as the demonstration that novel factors such as Yip3/Pra1 act as GDI displacement factors and that signals within Rab proteins contribute to targeting specificity, a better understanding of the concepts governing Rab recruitment and function is now beginning to emerge. The diversity of cellular processes regulated by Rab family members is made possible, not only by the wide range of effectors they recruit, but also by the different mechanisms regulating their own targeting and activation.

Addresses
Cell and Molecular Biology Section, Division of Biomedical Sciences, Faculty of Medicine, Imperial College London, London SW7 2AZ, UK
[1]e-mail: m.seabra@imperial.ac.uk

Current Opinion in Cell Biology 2004, **16**:451–457

This review comes from a themed issue on
Membranes and organelles
Edited by Judith Klumperman and Gillian Griffiths

Available online 20th June 2004

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2004.06.014

Abbreviations

ER	endoplasmic reticulum
GAP	GTPase-activating protein
GDF	GDI displacement factor
GEF	guanine nucleotide exchange factor
MEL	mobile effector loop
Pra1	prenylated Rab acceptor 1
RabGDI	Rab GDP dissociation inhibitor
REP	Rab escort protein
RGGT	Rab geranylgeranyl transferase
WPB	Weibel–Palade bodies
Yip3	Ypt-interacting protein 3

Introduction

Rab proteins, members of the Ras superfamily of small GTPases, control cellular events ranging from secretion and endocytosis to signal transduction and development. Their molecular mechanisms of action have been the focus of increasing attention since their initial discovery in the 1980s. Rab GTPases regulate vesicle budding, and vesicle tethering and fusion in vesicular transport [1]. Rabs also play important regulatory roles in motility by recruiting molecular motors to organelles and transport vesicles [2–6]. In addition, they coordinate intracellular signalling events with membrane traffic, a topic that is discussed in more detail elsewhere in this issue (see review by Miaczynska, Pelkmans and Zerial). Finally, Rabs have also been implicated in organising functionally distinct subdomains within a particular membrane and in defining organelle identity [1,7].

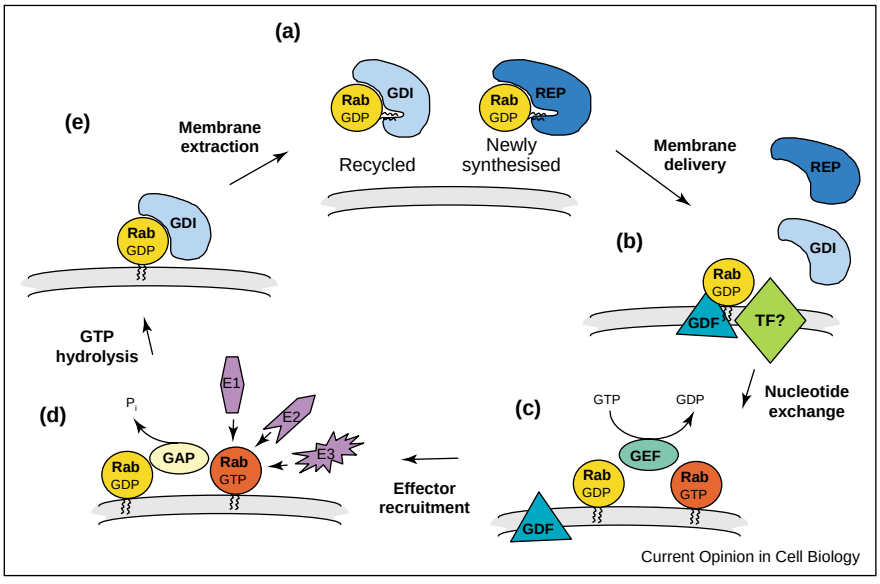
Reversible membrane association and the GTP/GDP switch

There are at least 63 different mammalian Rab proteins, many of which are restricted to specialised cell types [8,9]. A vital aspect of Rab function is the specific localisation of each Rab to a particular subcellular compartment, and its involvement in a specific transport step.

Rabs act as molecular switches, cycling between 'inactive' GDP-bound and 'active' GTP-bound states [10]. Importantly, these changes in activity are coupled to reversible association with their target membranes. Whilst the GDP/GTP switch is a well-recognised molecular mechanism for controlling the activity of small GTPases, the membrane association/dissociation cycle, which could be described as a membrane in/out switch, is just as critical for the proper functioning of Rab proteins; to be in a truly activated state, a protein must be both GTP-bound and membrane-associated.

This cycle of events allows both spatial and temporal control of Rab activity and is coordinated by several factors. Geranylgeranylated Rabs are delivered to their target membranes by a Rab escort protein (REP) or a Rab GDP dissociation inhibitor (RabGDI), where they are activated by specific guanine nucleotide exchange factors (GEFs). Activated GTP-bound Rabs recruit a wide variety of downstream effector molecules to the membrane, which reflects the diverse functions Rabs play in membrane traffic. GTP hydrolysis catalysed by a GTPase-activating protein (GAP) returns Rabs to their inactive state. GDP-bound Rabs become susceptible to extraction from the membrane by RabGDI, with which they form stable cytosolic complexes representing a cytoplasmic reservoir of Rab proteins. Rabs appear to undergo multiple cycles of RabGDI-mediated delivery to, and extraction from, membranes (Figure 1). The mechanisms

Figure 1



Schematic representation of the Rab cycle showing membrane recruitment and activation. **(a)** GDP-bound Rab proteins form a cytosolic complex with RabGDI. **(b)** Membrane delivery and RabGDI displacement are mediated by a GDF, probably aided by unidentified targeting factors (TF), followed by **(c)** Rab activation through GEF-catalysed nucleotide exchange. **(d)** GTP-bound Rab recruits effector molecules to the membrane. **(e)** GAP-mediated GTP hydrolysis returns the Rab to its inactive state, resulting in re-extraction from the membrane by GDI.

underlying membrane association and its specificity remain rather mysterious, as do those underlying the control of the Rab cycle, and recent insights into these processes will be reviewed here.

Role of the general regulators REP and RabGDI

C-terminal modification of Rab proteins with at least one, and usually two, geranylgeranyl groups is essential for their association with membranes [11]. Newly synthesised Rabs require REP for recognition by Rab geranylgeranyl transferase (RGGT) [12]. REP is also thought to subsequently deliver the newly prenylated Rab to the target membrane, a role similar to that of RabGDI in the case of recycling Rabs [13,14]. REP and RabGDI proteins are closely related, both in sequence and structure, yet are functionally distinct [15•,16]. One important distinction is that RabGDI cannot assist in lipid modification of Rab proteins [13]. The recent elucidation of a crystal structure of the REP1:RGGT complex revealed that features observed in a small number of critical residues located at the interface of the two proteins are critical for their interaction. The absence from RabGDIs of a bulky hydrophobic phenylalanine side chain conserved in REPs, coupled with the presence of a conserved RabGDI-specific phenylalanine in the adjacent helix, may explain the inability of RabGDI to interact with RGGT [15•].

Both REP and RabGDI show a marked preference for GDP-bound Rabs [17,18]. Earlier studies had suggested an important role for the Rab switch regions (those regions showing the most significant conformational differences between the GTP- and the GDP-bound states) in the recognition of Rabs by REP and RabGDI [14,19,20]. A recently solved crystal structure of a complex between yeast RabGDI and Ypt1 (the yeast homologue of Rab1) reveals more precisely how RabGDI (and by inference REP) can discriminate between GDP- and GTP-bound Rabs [21••]. RabGDI contacts several residues within the highly conserved Rab switch I and II regions. The conformation of the switch regions in Rab–GTP [22–24] results in a poor fit with the Rab-binding site on RabGDI. In GDP-bound Rabs, these normally flexible loops adopt a more rigid conformation upon interaction with RabGDI. This results in a closed conformation, which may explain the inhibitory effect that RabGDI (and REP) have on GDP dissociation. Interestingly, the involvement of the switch regions in binding RabGDI has similarities with the way GEFs interact with other small GTPases to destabilise GDP binding [21••].

RabGDI plays a central role in the recycling of Rab proteins by mediating their reversible association with membranes [25]. This association requires the stabilisation of the highly hydrophobic geranylgeranyl groups in the RabGDI cytosolic complex. New structural information now sheds some light on how this might be achieved at the molecular level. Earlier studies had already defined two important domains within RabGDI: a Rab-binding platform and a region implicated in membrane interaction, termed the mobile effector loop (MEL) [16,26]. The important question of how RabGDI binds the lipid

groups, however, remained unanswered. Two crystal structures of RabGDI complexes have now been determined. The first study used a geranylgeranyl moiety linked to a single cysteine as a model binding partner [27•]. The geranylgeranyl ligand binds in a shallow hydrophobic groove on the surface of RabGDI and induces a conformational change in the MEL, which is proposed to result in the release of RabGDI:Rab complexes from the membrane. The second crystal structure of a yeast RabGDI:mono-prenylated Ypt1 complex, which was generated using the elegant approach of ligating a chemically synthesised lipidated peptide to a recombinant Rab, revealed a different location for the geranylgeranyl-binding site in RabGDI [21••]. The lipid is accommodated in a deep hydrophobic cavity within the core of the α-helical domain II of the protein. This cavity appears to result from conformational changes in RabGDI induced by interactions with the switch regions and the C terminus of the Rab protein, suggesting a biphasic model for the extraction of the first geranylgeranyl group from the membrane. The possible location of the second geranylgeranyl-binding site remains obscure. To resolve this issue, the crystallisation of a di-geranylgeranylated Rab in complex with RabGDI or REP will be required. REP shows higher affinity for the mono-geranylgeranylated reaction intermediate than for di-geranylgeranylated (or unmodified) Rab, indicating that the second geranylgeranyl group could be loosely bound to REP or RabGDI at the solvent interface [28]. Such a relatively unstable interaction may facilitate the transfer of the lipid anchor from RabGDI into the membrane bilayer.

Rab displacement from GDI and membrane delivery

As discussed above, it is important to understand in detail the process by which Rab dissociates from the soluble regulator (REP or RabGDI) and associates stably with the target membrane. Early experiments suggested that membrane translocation precedes nucleotide exchange, and that it was catalysed by a GDI displacement factor (GDF) [29–31]. Recently, Pfeffer and co-workers used an elegant biochemical approach to show that mammalian Ypt-interacting protein 3 (Yip3), also called prenylated Rab acceptor 1 (Pra1), acted as a GDF for Rab9 [32••]. Yip3 catalysed the dissociation of Rab9 from RabGDI *in vitro* and promoted the recruitment of Rab9 onto membranes. This activity displayed selectivity for endosomal Rabs (Rab9 and Rab5) versus early secretory pathway Rabs (Rab1 and Rab2) [32••].

Could other Yip family members similarly act as GDFs? Yips are small integral membrane proteins that, at least *in vitro*, bind promiscuously to prenylated Rab proteins [33,34] and perhaps other Ras-like GTPases [35]. Yip3/Pra1 appears to be present on endosomal membranes, but has also been reported to reside on the Golgi, so it will be important to examine its intracellular distribution in more detail in future studies [32••,36]. Also, the ability of Yip3/Pra1 to act on both Rab9 and Rab5 could point to a common delivery mechanism for different endosomal Rabs [32••]. However, the high degree of functional specialisation of membranes within the endocytic system would argue against such a model.

In vivo, additional specificity with respect to both Rab binding and subcellular localisation [36] could be achieved through the formation of Yip family heterodimers [37] or through interactions with other as-yet-unidentified factors. In addition, Yip proteins seem to have other roles in membrane trafficking, for example in mediating ER-to-Golgi transport, that appear to be independent of their interaction with Rabs [38–40]. Demonstrating a role for Yip3/Pra1 in recruiting Rabs to endosomal membranes *in vivo* will clearly be an important next step. The search for additional GDFs should also yield interesting results — will they turn out to be Yip family members, or could other unrelated types of proteins fulfil a similar role? The recent suggestion that a membrane-associated HSP90 chaperone complex binds RabGDI and participates in the extraction of Rab3 from synaptic membranes [41] highlights the existence of further factors with new potential roles in regulating Rab membrane delivery and retrieval, thus providing another impetus for future investigations in this area.

Role of exchange factors in Rab targeting

Early models for Rab membrane recruitment attributed specificity in targeting to RabGEFs, which were postulated to be integral membrane proteins [42,43]. Membrane association is accompanied by exchange of the bound GDP for GTP, activating the Rab and stabilising it on the membrane [44]. This activity seems to be distinguishable from that of the GDF [32••]. Since GEFs are specific for particular Rabs, they may make an important additional contribution to the fidelity of Rab targeting. However, the few RabGEFs identified to date are themselves peripheral membrane proteins requiring other factors for their targeting.

An interesting mechanism for the localisation of RabGEFs was suggested by the finding that activated Ypt31/Ypt32 proteins, small GTPases mediating vesicle budding from the Golgi, recruit a GEF (Sec2) required for the activation of Sec4, the Rab immediately downstream [45••]. A similar regulatory cascade linking two Rab proteins via an exchange factor was proposed for Ypt1 and Ypt31/Ypt32 [46]. By providing such functional links between sequential transport steps, Rab proteins may act as key coordinators of the entire pathway.

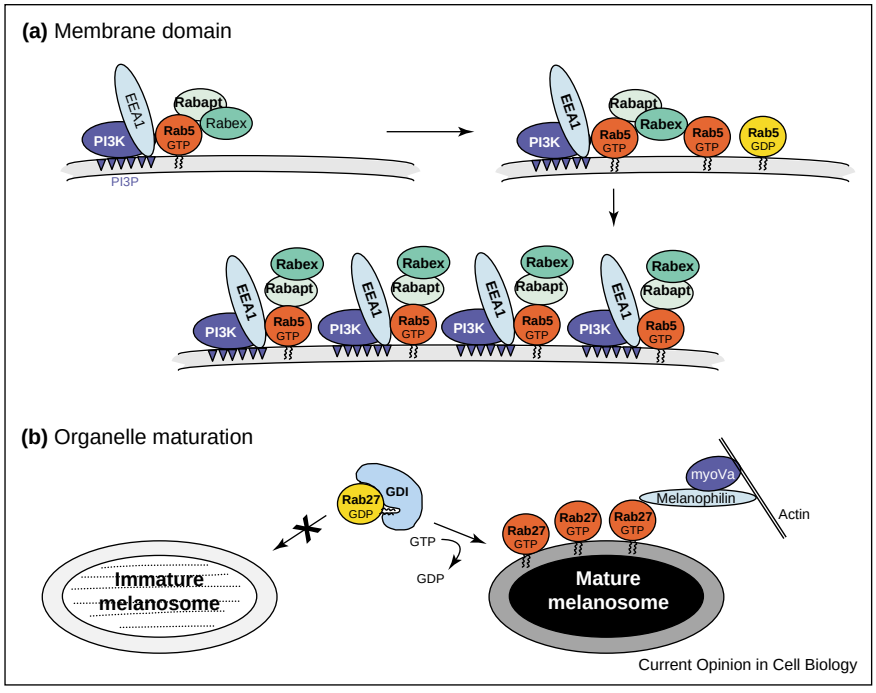
Rab targeting signals

The fundamental question of how specificity is imparted to the Rab membrane translocation event has remained

unanswered. Lipid modification of Rabs has long been thought of as simply providing a membrane anchor, but recent studies suggest that geranylgeranyl groups influence targeting specificity [47•,48•]. Unlike small GTPases of the Ras and Rho subfamilies, which bind membranes via a single lipid group, most Rabs contain two geranylgeranyl groups. Mutant mono-prenylated Ypt1 and Sec4 in yeast [47•], or Rab5 and Rab27 in mammalian cells [48•], are unable to localise to the correct subcellular compartment, and as a result are non-functional. Such proteins appear to undergo prenylation via the REP/RGGT system like wild-type Rabs, and are able to interact normally with RabGDI, but seem to be mistargeted to the ER [48•]. Initial association with the ER followed by further transport via the secretory pathway has been observed for Ras proteins [49,50], but is not seen with wild-type Rabs [48•]. This supports the idea that functional Rabs are delivered directly to their intracellular target membranes, an important issue considering the proposed role of Rabs as determinants of organelle identity. In contrast with yeast, several mammalian Rab proteins only possess a single geranylgeranyl group, suggesting that these Rabs use a different targeting mechanism. The physiological significance of this phenomenon is not clear. Interestingly, the human homologue of Yip1, Yip1A, binds di-prenylated Rabs such as Rab1 or Rab5, but not the mono-prenylated family members Rab8 or Rab13 [47•]. This finding again highlights the Yip family of proteins as potentially important mediators of Rab targeting and, indeed, loss of Yip1 disrupts the Golgi localisation of Ypt1 [47•].

Given that the majority of Rabs are di-geranylgeranylated, this modification cannot be sufficient for directing them to specific intracellular compartments. The C-terminal hypervariable region of a Rab protein has long been thought to contain its subcellular targeting signal [51]. However, a more recent re-examination of this question, using chimaeric proteins generated by exchanging domains between endosomal, Golgi and secretory-granule-associated Rabs, argues against a universal requirement for the divergent C termini in the localisation of these proteins (Ali, Wasmeier and Seabra, unpublished). Various elements within the conserved Rab family and subfamily regions [52], on the other hand, appear to be required for Rab targeting. Interestingly, the relative importance of each region varies between Rabs, which may reflect diverse mechanisms operating in the recruitment of different Rab family members (Ali, Seabra and Wasmeier, unpublished).

Figure 2



Different models for Rab recruitment to intracellular membranes. **(a)** Model depicting the recruitment of several Rab5 effectors that may act in concert to generate a membrane domain. Activated Rab5 recruits PtdIns(3)K, leading to the generation of PtdIns(3)P in the endosomal membrane. The presence of the lipid then allows the binding of EEA1. GTP–Rab5 also interacts with the Rabaptin/Rabex complex which, through the GEF activity of Rabex, results in the activation of additional Rab5 molecules. This in turn is followed by recruitment of further effectors, leading to the formation of a functionally distinct membrane subdomain. **(b)** Rab27 as a potential sensor of organelle maturation. Rab27 is not present on early-stage melanosomes. Maturation-dependent changes within the organelle membrane result in a melanosome competent for the recruitment of Rab27. Once bound, Rab27 interacts with its effector melanophilin, linking it to myosinVa (myoVa) and the actin cytoskeleton, to position the mature melanosome at the cell periphery for transfer to keratinocytes.

Rabs and organelle identity

The precise targeting of Rab proteins to distinct subsets of membranes is crucial since it determines the localisation of downstream effectors. However, the characteristics of a particular compartment can also control effector binding. For example, Rab5 recruits the phosphatidylinositol 3-kinase hVps34 to early endosomes but not to clathrin-coated vesicles, resulting in the generation of PI(3)P only on a subset of Rab5-positive membranes, which in turn governs the binding of lipid-dependent effectors such as EEA1 to endosomes [53]. The novel Rab5 effector proteins APPL1/2, which are implicated in linking endocytosis to signal transduction into the nucleus, now define an additional, previously undetected, subcompartment of the early endosome [54].

While certain Rab5 effectors appear to define particular membrane subcompartments, others are recruited in a cooperative manner. Moreover, the formation of complexes with regulatory factors, such as Rabaptin-5 and the GEF Rabex-5, amplifies recruitment and activation of the GTPase in a positive feedback loop [53] (Figure 2a). In this way, Rab5, Rab4 and Rab11 in early endosomes, and Rab7 and Rab9 in late endosomes, have been suggested to act as membrane organisers that form functionally distinct subdomains within a single compartment.

A potential novel aspect of Rab recruitment has recently been suggested for Rab27 [55••]. Rab27 is present on a wide range of lysosome-related and other secretory organelles, and is involved in the intracellular transport of melanosomes in melanocytes, as well as in the regulation of secretion from endocrine and exocrine cells, cytotoxic T-lymphocytes and platelets [6]. Rab27 was also recruited to Weibel–Palade bodies (WPBs), the specialised lysosome-related organelles of endothelial cells. Intriguingly, two different populations of WPBs could be distinguished in these cells: older organelles that were positive for Rab27, and newly synthesised ones that did not contain the GTPase. This raises the possibility that Rab27 could act as a sensor for the maturation stage of WPBs, perhaps marking mature organelles for exocytosis. The molecular basis of such selective maturation-dependent recruitment is still obscure. Surprisingly, Rab27 is also recruited to the WPB-like organelles induced upon expression of von Willebrand factor, the lumenal content of WPBs, in non-endothelial cells [55••]. Such cells are not expected to possess any other specialised targeting factors that may be present in endothelial cells, so that this observation could indicate a key role for maturation-dependent changes in membrane lipids (and proteins) in the recruitment of Rab proteins.

Conclusions

Taken together, a picture is beginning to emerge of how the mechanisms underlying the function of individual Rab GTPases may have been fine-tuned to serve the diverse requirements of different membrane trafficking steps. The early endosome is a highly dynamic organelle that needs to maintain its identity in the face of continuous exchange of membrane with various other compartments. A mosaic of functionally distinct subdomains defined by sets of Rab proteins and their effectors, stabilised through cooperative recruitment, is ideally suited to that purpose. Organelles destined for regulated secretion, on the other hand, generally undergo a process of maturation. This needs to be monitored by the cellular machinery, and may require the association of a Rab protein such as Rab27 with a membrane initially devoid of Rab27 (Figure 2b). It would not come as a surprise to find that different Rabs achieve their specific subcellular localisation at the appropriate point in time through distinct targeting mechanisms.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zerial M, McBride H: **Rab proteins as membrane organizers**. *Nat Rev Mol Cell Biol* 2001, **2**:107-117.
2. Nielsen E, Severin F, Backer JM, Hyman AA, Zerial M: **Rab5 regulates motility of early endosomes on microtubules**. *Nat Cell Biol* 1999, **1**:376-382.
3. Jordens I, Fernandez-Borja M, Marsman M, Dusseljee S, Janssen L, Calafat J, Janssen H, Wubbolts R, Neefjes J: **The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein–dynactin motors**. *Curr Biol* 2001, **11**:1680-1685.
4. Echard A, Jollivet F, Martinez O, Lacapere JJ, Rousselet A, Janoueix-Lerosey I, Goud B: **Interaction of a Golgi-associated kinesin-like protein with Rab6**. *Science* 1998, **279**:580-585.
5. Hammer JA III, Wu XS: **Rabs grab motors: defining the connections between Rab GTPases and motor proteins**. *Curr Opin Cell Biol* 2002, **14**:69-75.
6. Seabra MC, Coudrier E: **Rab GTPases and myosin motors in organelle motility**. *Traffic* 2004, **5**:393-399.
7. Pfeffer S: **Membrane domains in the secretory and endocytic pathways**. *Cell* 2003, **112**:507-517.
8. Pereira-Leal JB, Seabra MC: **Evolution of the Rab family of small GTP-binding proteins**. *J Mol Biol* 2001, **313**:889-901.
9. Bock JB, Matern HT, Peden AA, Scheller RH: **A genomic perspective on membrane compartment organization**. *Nature* 2001, **409**:839-841.
10. Takai Y, Sasaki T, Matozaki T: **Small GTP-binding proteins**. *Physiol Rev* 2001, **81**:153-208.
11. Seabra MC: **Biochemistry of Rab geranylgeranyl transferase**. In *Lipid Modifications of Proteins*. Edited by Tamanoi FA and Sigman DS: Academic Press; 2000:131-154.
12. Andres DA, Seabra MC, Brown MS, Armstrong SA, Smeland TE, Cremers FP, Goldstein JL: **cDNA cloning of component A of Rab geranylgeranyl transferase and demonstration of its role as a Rab escort protein**. *Cell* 1993, **73**:1091-1099.
13. Alexandrov K, Horiuchi H, Steele-Mortimer O, Seabra MC, Zerial M: **Rab escort protein-1 is a multifunctional protein that accompanies newly prenylated rab proteins to their target membranes**. *EMBO J* 1994, **13**:5262-5273.
14. Wilson AL, Erdman RA, Maltese WA: **Association of Rab1B with GDP-dissociation inhibitor (GDI) is required for recycling but**

not initial membrane targeting of the Rab protein. *J Biol Chem* 1996, **271**:10932-10940.

15. Pylypenko O, Rak A, Reents R, Niculae A, Sidorovitch V,
• Cioaca MD, Bessolitsyna E, Thoma NH, Waldmann H, Schlichting I *et al.*: **Structure of Rab escort protein-1 in complex with Rab geranylgeranyltransferase**. *Mol Cell* 2003, **11**:483-494.
Analysis of a REP-1/RGGT complex reveals the interface between the two molecules, and provides a structural explanation for why REP can mediate lipid modification by RGGT whereas the closely related RabGDI cannot.

16. Alory C, Balch WE: **Organization of the Rab-GDI/CHM superfamily: the functional basis for choroideremia disease**. *Traffic* 2001, **2**:532-543.

17. Sasaki T, Kikuchi A, Araki S, Hata Y, Isomura M, Kuroda S, Takai Y: **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* 1990, **265**:2333-2337.

18. Seabra MC: **Nucleotide dependence of Rab geranylgeranylation. Rab escort protein interacts preferentially with GDP-bound Rab**. *J Biol Chem* 1996, **271**:14398-14404.

19. Overmeyer JH, Wilson AL, Erdman RA, Maltese WA: **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* 1998, **9**:223-235.

20. Pereira-Leal JB, Strom M, Godfrey RF, Seabra MC: **Structural determinants of Rab and Rab escort protein interaction: Rab family motifs define a conserved binding surface**. *Biochem Biophys Res Commun* 2003, **301**:92-97.

21. Rak A, Pylypenko O, Durek T, Watzke A, Kushnir S, Brunsveld L,
•• Waldmann H, Goody RS, Alexandrov K: **Structure of Rab GDP-dissociation inhibitor in complex with prenylated YPT1 GTPase**. *Science* 2003, **302**:646-650.
This study, in solving the technically challenging problem of determining the structure of a RabGDI/prenyl-Rab complex, provides several key insights into RabGDI function. It shows how RabGDI may inhibit GDP release and discriminate between GTP- and GDP-bound Rabs. Most importantly, through revealing a binding pocket for the prenyl group in RabGDI, it suggests a mechanism for the extraction of the Rab hydrophobic anchor from the membrane by RabGDI (see also [27•]).

22. Dumas JJ, Zhu Z, Connolly JL, Lambright DG: **Structural basis of activation and GTP hydrolysis in Rab proteins**. *Structure Fold Des* 1999, **7**:413-423.

23. Esters H, Alexandrov K, Constantinescu AT, Goody RS, Scheidig AJ: **High-resolution crystal structure of *S. cerevisiae* Ypt51(δC15)-GppNHp, a small GTP-binding protein involved in regulation of endocytosis**. *J Mol Biol* 2000, **298**:111-121.

24. Ostermeier C, Brunger AT: **Structural basis of Rab effector specificity: crystal structure of the small G protein Rab3A complexed with the effector domain of rabphilin-3A**. *Cell* 1999, **96**:363-374.

25. Araki S, Kikuchi A, Hata Y, Isomura M, Takai Y: **Regulation of reversible binding of smg p25A, a ras p21-like GTP-binding protein, to synaptic plasma membranes and vesicles by its specific regulatory protein, GDP dissociation inhibitor**. *J Biol Chem* 1990, **265**:13007-13015.

26. Luan P, Heine A, Zeng K, Moyer B, Greasely SE, Kuhn P, Balch WE, Wilson IA: **A new functional domain of guanine nucleotide dissociation inhibitor (α-GDI) involved in Rab recycling**. *Traffic* 2000, **1**:270-281.

27. An Y, Shao Y, Alory C, Matteson J, Sakisaka T, Chen W, Gibbs RA,
• Wilson IA, Balch WE: **Geranylgeranyl switching regulates GDI-Rab GTPase recycling**. *Structure* 2003, **11**:347-357.
The crystal structure of RabGDI in complex with a geranylgeranyl ligand shows the lipid bound in a shallow hydrophobic groove that contains residues involved in membrane extraction. A conformational shift upon lipid binding is proposed to lead to release of the RabGDI–Rab complex from the membrane (see also [21••]).

28. Shen F, Seabra MC: **Mechanism of digeranylgeranylation of Rab proteins. Formation of a complex between monogeranylgeranyl-Rab and Rab escort protein**. *J Biol Chem* 1996, **271**:3692-3698.

29. Soldati T, Shapiro AD, Svejstrup AB, Pfeffer SR: **Membrane targeting of the small GTPase Rab9 is accompanied by nucleotide exchange**. *Nature* 1994, **369**:76-78.

30. Dirac-Svejstrup AB, Sumizawa T, Pfeffer SR: **Identification of a GDI displacement factor that releases endosomal Rab GTPases from Rab-GDI**. *EMBO J* 1997, **16**:465-472.

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

32. Sivars U, Aivazian D, Pfeffer SR: **Yip3 catalyses the**
•• **dissociation of endosomal Rab-GDI complexes**. *Nature* 2003, **425**:856-859.
An essential role for GDFs in recruiting Rabs to their target membranes had long been postulated. For the first time, the molecular identity of such a factor is described here. Yip3 shows GDF activity in biochemical studies, and preferentially acts on endosomal Rabs, suggesting that it could contribute to the specificity of Rab membrane recruitment. This raises the question of whether other Yip family proteins might fulfil similar roles for other Rab GTPases.

33. Calero M, Collins RN: ***Saccharomyces cerevisiae* Pra1p/Yip3p interacts with Yip1p and Rab proteins**. *Biochem Biophys Res Commun* 2002, **290**:676-681.

34. Martincic I, Peralta ME, Ngsee JK: **Isolation and characterization of a dual prenylated Rab and VAMP2 receptor**. *J Biol Chem* 1997, **272**:26991-26998.

35. Figueroa C, Taylor J, Vojtek AB: **Prenylated Rab acceptor protein is a receptor for prenylated small GTPases**. *J Biol Chem* 2001, **276**:28219-28225.

36. Abdul-Ghani M, Gougeon PY, Prosser DC, Da-Silva LF, Ngsee JK: **PRA isoforms are targeted to distinct membrane compartments**. *J Biol Chem* 2001, **276**:6225-6233.

37. Calero M, Winand NJ, Collins RN: **Identification of the novel proteins Yip4p and Yip5p as Rab GTPase interacting factors**. *FEBS Lett* 2002, **515**:89-98.

38. Heidtman M, Chen CZ, Collins RN, Barlowe C: **A role for Yip1p in COPII vesicle biogenesis**. *J Cell Biol* 2003, **163**:57-69.

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

40. Barrowman J, Wang W, Zhang Y, Ferro-Novick S: **The Yip1p.Yif1p complex is required for the fusion competence of endoplasmic-reticulum-derived vesicles**. *J Biol Chem* 2003, **278**:19878-19884.

41. Sakisaka T, Meerlo T, Matteson J, Plutner H, Balch WE: **Rab-alphaGDI activity is regulated by a Hsp90 chaperone complex**. *Embo J* 2002, **21**:6125-6135.

42. Goud B, Salminen A, Walworth NC, Novick PJ: **A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast**. *Cell* 1988, **53**:753-768.

43. Bourne HR: **Do GTPases direct membrane traffic in secretion?** *Cell* 1988, **53**:669-671.

44. Stenmark H, Valencia A, Martinez O, Ullrich O, Goud B, Zerial M: **Distinct structural elements of rab5 define its functional specificity**. *EMBO J* 1994, **13**:575-583.

45. Ortiz D, Medkova M, Walch-Solimena C, Novick P: **Ypt32 recruits**
•• **the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles evidence for a Rab cascade in yeast**. *J Cell Biol* 2002, **157**:1005-1015.
The demonstration that the localisation of Sec2, the exchange factor for Sec4, is regulated by Ypt31/32 suggests the intriguing possibility that the recruitment and activation of Rab proteins may be controlled via regulatory factors that are effectors of an upstream Rab within the same pathway. In such a model, a series of functionally linked Rabs could be recruited sequentially to a membrane without the need for specific integral membrane proteins. (See also [45••].)

46. Wang W, Ferro-Novick S: **A Ypt32p exchange factor is a putative effector of Ypt1p**. *Mol Biol Cell* 2002, **13**:3336-3343.

47. • Calero M, Chen CZ, Zhu W, Winand N, Havas KA, Gilbert PM, Burd CG, Collins RN: **Dual prenylation is required for rab protein localization and function**. *Mol Biol Cell* 2003, **14**:1852-1867.
This paper shows that the appropriate lipid modification, besides providing a membrane anchor, also influences correct subcellular targeting of yeast Rabs, and hence their functional activity. The finding that Yip1 binds di-geranylgeranylated but not mono-prenylated Rabs makes this protein a candidate component of the targeting machinery. (See also [47•].)

48. • Gomes AQ, Ali BR, Ramalho JS, Godfrey RF, Barral DC, Hume AN, Seabra MC: **Membrane targeting of rab GTPases is influenced by the prenylation motif**. *Mol Biol Cell* 2003, **14**:1882-1899.
This paper demonstrates that the mammalian Rab GTPases Rab5 and Rab27 require di-geranylgeranylation for their correct subcellular localisation and function. Mono-prenylated mutants were shown to interact normally with general Rab regulators, which further argues for a role of the lipid modification specifically in determining recruitment to the appropriate target membranes. (See also [46].)

49. Choy E, Chiu VK, Silletti J, Feoktistov M, Morimoto T, Michaelson D, Ivanov IE, Philips MR: **Endomembrane trafficking of ras: the CAAX motif targets proteins to the ER and Golgi**. *Cell* 1999, **98**:69-80.

50. Apolloni A, Prior IA, Lindsay M, Parton RG, Hancock JF: **H-ras but not K-ras traffics to the plasma membrane through the exocytic pathway**. *Mol Cell Biol* 2000, **20**:2475-2487.

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

52. Pereira-Leal JB, Seabra MC: **The mammalian Rab family of small GTPases: definition of family and subfamily sequence motifs suggests a mechanism for functional specificity in the Ras superfamily**. *J Mol Biol* 2000, **301**:1077-1087.

53. Miaczynska M, Zerial M: **Mosaic organization of the endocytic pathway**. *Exp Cell Res* 2002, **272**:8-14.

54. Miaczynska M, Christoforidis S, Giner A, Shevchenko A, Uttenweiler-Joseph S, Habermann B, Wilm M, Parton RG, Zerial M: **APPL proteins link Rab5 to nuclear signal transduction via an endosomal compartment**. *Cell* 2004, **116**:445-456.

55. •• Hannah MJ, Hume AN, Arribas M, Williams R, Hewlett LJ, Seabra MC, Cutler DF: **Weibel-Palade bodies recruit Rab27 by a content-driven, maturation-dependent mechanism that is independent of cell type**. *J Cell Sci* 2003, **116**:3939-3948.
Analysis of the recruitment of Rab27a to WPBs and WPB-like organelles reveals two intriguing novel phenomena. Firstly, Rab27a appears to be able to distinguish between newly synthesized and older organelles in endothelial cells, suggesting that it could serve as a cellular organelle maturation sensor. Secondly, expression of lumenal content in non-specialized cells seems to be sufficient to generate a novel compartment that is capable of recruiting Rab27a to the cytoplasmic side of the membrane, via an as-yet-uncharacterised mechanism.