

RAB PROTEINS AS MEMBRANE ORGANIZERS

Marino Zerial and Heidi McBride*

Cellular organelles in the exocytic and endocytic pathways have a distinctive spatial distribution and communicate through an elaborate system of vesiculo-tubular transport. Rab proteins and their effectors coordinate consecutive stages of transport, such as vesicle formation, vesicle and organelle motility, and tethering of vesicles to their target compartment. These molecules are highly compartmentalized in organelle membranes, making them excellent candidates for determining transport specificity and organelle identity.

COGNATE SNARES
SNAREs on opposite membranes that are destined to form *trans*-SNARE complexes to mediate fusion.

NSF
Molecular chaperone involved in recycling SNAREs after one round of fusion.

EFFECTOR
A protein or protein complex that binds the GTPase directly and in a GTP-dependent manner and is required for the downstream function determined by that GTPase.

Max-Planck-Institute of Molecular Cell Biology and Genetics, c/o EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany.
*Current address: The University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Ontario, Canada, K1Y 4W7.
Correspondence to M.Z.
e-mail: Zerial@EMBL-Heidelberg.de

How do transport vesicles or tubular structures generated in one compartment encounter their target organelle and engage in membrane fusion? A typical transport reaction can be viewed as a four-step process that consists of formation of a vesicle or tubular intermediate, movement of the vesicle towards its target compartment, tethering/docking with the acceptor membrane and, ultimately, fusion of the lipid bilayers. The specificity of membrane tethering and fusion is critical to preserve organelle identity and the proper flow of cargo within the cell. The first specific event is the Rab-mediated tethering of an incoming vesicle to the correct target organelle. Following this, the specific topological pairing of COGNATE SNARES (soluble NSF attachment protein receptor, where NSF stands for *N*-ethylmaleimide-sensitive fusion protein) between the two bilayers (SNARE pins) ensures precision in the fusion event^{1–4} (see the review by Chen and Scheller on page 98 of this issue). SNAREs are enriched in certain organelles, which helps to identify the correct target and to limit nonspecific fusion events. However, during vesicular transport, SNAREs inevitably spread throughout many cellular compartments. Any given organelle will contain SNARE complexes that must remain inactive until they return to their specific place of function. An additional layer of regulation is therefore inevitable to ensure that *trans*-SNARE complexes fuse membranes only at the appropriate time and in the correct place.

In this review, we discuss how Rab GTPases and their EFFECTORS fit the criteria for a regulatory system that provides the complementary specificity to SNARE

complexes during membrane tethering and fusion. The discovery of molecular interactions between Rab effectors and components of the SNARE machinery provides a new understanding of how Rab proteins directly regulate SNARE function. We begin by summarizing the role of Rab proteins and the identification of some of their effectors. We then describe recent evidence indicating that Rab effectors are not randomly distributed on the organelle membrane but are clustered in distinct functional domains. The model emerging from these observations is that, rather than being mere regulators of SNARE protein complexes, Rab GTPases and their effectors are primary determinants of compartmental specificity in the organelles of eukaryotic cells.

Heterogeneity of Rab effectors

Rab proteins constitute the largest family of monomeric small GTPases. Eleven Rab (Ytp/Sec4p) proteins are expressed in the yeast *Saccharomyces cerevisiae* but there might be as many as 63 family members in humans (J. Schultz and P. Bork, personal communication) as estimated from expressed-sequence tags (ESTs) and the sequenced human genome. This increased complexity throughout evolution reflects a greater need for cell organization and intracellular transport in the different cell types of multicellular organisms. Numerous studies have established that Rab proteins are distributed to distinct intracellular compartments and regulate transport between organelles (reviewed in REF. 5, see FIG. 1 and TABLES 1,2) (see extra online material). The regulatory principle of Rab proteins, as for other GTPases, lies in

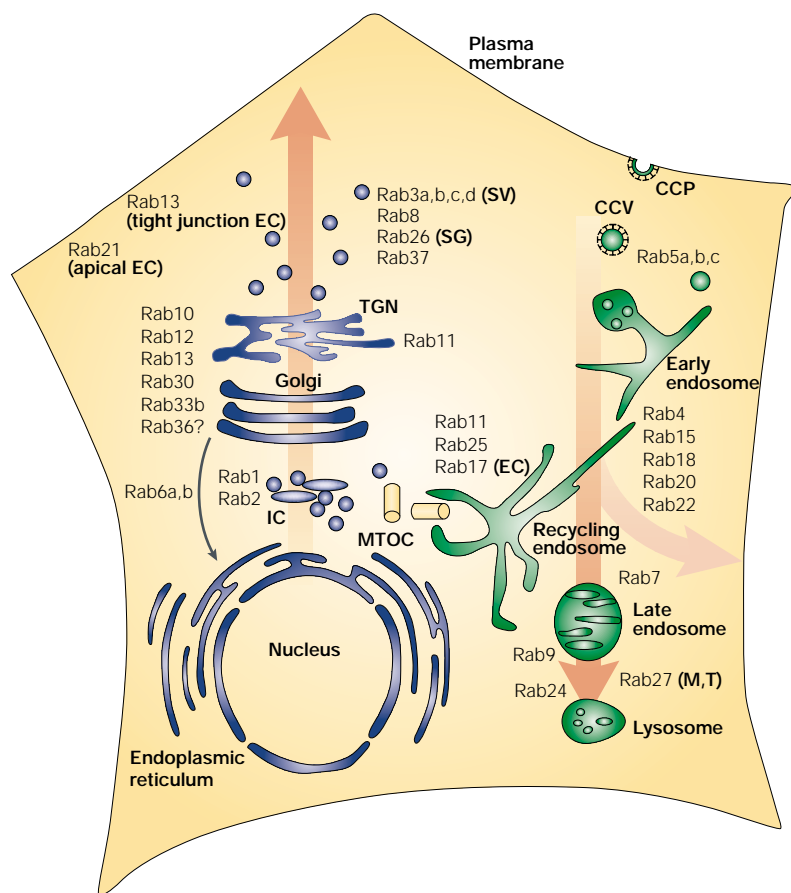


Figure 1 | Map of intracellular localization of Rab proteins. Summarizes the intracellular localization of Rab proteins in mammalian cells. Some proteins are cell- (for example, Rab3a in neurons) or tissue-specific (for example, Rab17 in epithelia) or show cell-type-specific localization (for example, Rab13 in tight junctions). (CCV, clathrin-coated vesicle; CCP, clathrin-coated pit; EC, epithelial cells; IC, ER–Golgi intermediate compartment; M, melanosomes; MTOC, microtubule-organizing centre; SG, secretory granules; SV, synaptic vesicles; T, T-cell granules; TGN, *trans*-Golgi network.)

their ability to function as molecular switches that oscillate between GTP- and GDP-bound conformations. The GTP-bound form is considered the ‘active’ form. However, with respect to the physiology of the regulated process, the most important feature is the ability of GTPases to cycle regularly between GTP- and GDP-bound states. This cycle imposes temporal and spatial regulation to membrane transport, with the Rab proteins acting like timers whose clocks are set depending on the (intrinsic and catalysed) rates of nucleotide exchange and hydrolysis. Their on/off regulatory function is restricted to the membrane compartments where they are localized.

Each transport step requires that the activated Rab proteins bind to soluble factors that act as ‘effectors’ to transduce the signal of the Rab GTPase in the transport mechanism. Many established or putative effector proteins and regulators have been identified and characterized (TABLES 1, 2). Given the structural conservation of Rab GTPases and SNAREs, one might expect that Rab effectors could also be grouped in a family of structurally conserved proteins. This does not seem to be the case.

EEA1

The antigen involved in a human autoimmune disease.

COPII VESICLES

Coated vesicles involved in transport from the endoplasmic reticulum to the Golgi.

The structural heterogeneity shown by Rab effectors implies that these are highly specialized molecules whose activities are exclusively tailored for individual organelles and transport systems. However, some Rab effectors do share structural features. For example, p115/Usolp, Rabaptin-5 and early endosome antigen 1 (EEA1), all contain predicted coiled-coil regions, and Rab3-interacting molecule (Rim1), EEA1 and Rabenosyn-5 contain zinc-fingers. A more comprehensive analysis of Rab effectors, taking into account their structural and functional properties, will be necessary to categorize these molecules.

Regulation of intracellular transport

It is well established that Rab proteins function in the tethering/docking of vesicles to their target compartment, leading to membrane fusion. However, Rab proteins have also been implicated in vesicle budding and, more recently, in the interaction of vesicles with cytoskeletal elements. The finding that Rab proteins have several functions suggests that all steps of vesicle transport could be coordinated by the same regulatory machinery.

Membrane tethering. Over the past few years, several studies have shown that membrane tethering is a conserved mechanism that depends on Rab effectors, rather than on SNARE complexes. Depending on the system, the recruitment of Rab and tethering effector proteins can be either symmetrical or asymmetrical between donor and acceptor membranes. In the yeast secretory pathway, tethering of endoplasmic reticulum (ER)-derived vesicles to the Golgi complex depends on the membrane recruitment of Usolp by Ypt1p but not on SNAREs⁶. Whereas no direct interaction between Usolp and the Rab protein could be detected in the latter study, the mammalian homologue of Usolp, p115 was recently shown to bind directly to Rab1 (REF 7). However, there are mechanistic differences between mammalian and yeast ER-to-Golgi transport. Whereas Rab1 recruits p115 onto COPII (coat protein 2) VESICLES already at the budding step, in yeast the requirement for Ypt1p might be only at the level of the target membrane⁸. Beside these proteins, a multi-protein complex called TRAPP (transport protein particle) also targets ER-derived vesicles to the Golgi apparatus⁹. The TRAPP complex accelerates nucleotide exchange on Ypt1p, probably in the Golgi¹⁰ where this GTPase is essential⁸. The precise function of the complex and its tethering role in relation with Ypt1p activation awaits further analysis.

Delivery of post-Golgi vesicles to the plasma membrane in yeast depends on Sec4p and the tethering factor that interacts with this Rab protein is also a multi-protein complex — the exocyst. The exocyst was identified by Novick and co-workers as a complex of seven proteins that are specifically required for exocytosis¹¹. An equivalent complex exists in mammals¹². One of its subunits, Sec3p, marks the sites of exocytosis on the plasma membrane in yeast¹³. The exocyst mediates vesicle targeting and, through the Sec15p subunit, interacts specifically and directly with Sec4p in a GTP-dependent manner¹⁴. These data raise several interesting questions. How

Table 1 | Rab proteins and their effectors

Rab	Rab function	Direct effector	Effector function	Rab specificity	Effector partners	Partner features
Rab1	• ER–Golgi transport	p115	• Tethering • Sequestering SNAREs into budded vesicles	Rab1–GTP	Giantin GM130	• Tethering of COPI-coated vesicles to Golgi
		PRA1	• Rab receptor (proposed)	Rab1 Rab3 Rab4b Rab5a Rab5c	VAMP2	• v-SNARE involved in bilayer fusion
Rab3	• Rab3a: synaptic vesicle and chromaffin granule secretion • Rab3b, c, d: regulated secretion	Rabphilin-3	• Potentiates fusion	Rab3–GTP	α -actinin Rabaptin-5	• Crosslinks actin filaments into bundles • Stimulated by Rabphilin-3 interactions • Also binds Rabaptin-5, an effector of Rab5 and Rab4
		RIM1 RIM2 Calmodulin	• Membrane fusion • Confers calcium sensitivity to protein interactions	Rab3–GTP Rab3	RIM–BP1 Many	• Contains fibronectin type III repeats and SH3 domains • Multiple functions
Rab4	• Localized to early/recycling endosomes • Role in sorting/recycling in early endosomes	Rabaptin-5, Rabaptin-5 β Rabaptin-4	• Activates Rab5 through complex with Rabex-5 • Implicated in protein sorting and recycling	Rab4–GTP Rab5–GTP	Rabex-5	• Nucleotide exchange factor
Rab5	• Ligand sequestration at plasma membrane • CCV–EE and EE–EE fusion • Endosome motility	Rabaptin-5 Rabaptin-5 β EEA1	• Stabilizes Rabex-5 recruitment • Tethering, core fusion component	Rab5–GTP Rab4–GTP Rab5–GTP	Rabex-5 Rabphilin-3 Syntaxin13 Syntaxin6 hVps34	• Nucleotide exchange factor • t-SNAREs essential for bilayer fusion • Class III PI(3)K catalytic subunit
		p150	• Class III PI(3)K regulatory subunit	Rab5–GTP		• Class I PI(3)K catalytic subunit
		p110 β	• Class I PI(3)K catalytic subunit	Rab5–GTP	p85- α	• Class I PI(3)K regulatory subunit
		Rabenosyn-5	• Required for CCV–EE and EE–EE fusion	Rab5–GTP Rab4–GTP	hVps45	• Regulates SNARE complex formation or disassembly
Rab6	• Retrograde Golgi–ER and intra-Golgi transport	Rabkinesin-6	• Vesicle motility • Cytokinesis	Rab6–GTP	Microtubules	
Rab8	• TGN–plasma membrane traffic (basolateral in epithelial cells)	Rab8IP	• Stress-activated protein kinase	Rab8–GTP		
Rab9	• Late endosome to Golgi	p40	• Stimulates fusion	Rab9–GTP		
Rab11	• Recycling through perinuclear recycling endosomes • Plasma membrane–Golgi traffic	Rab11BP	• Unclear	Rab11–GTP	mSec13	• Coat component of COPII vesicles
Rab13	• Involved in the formation of the tight junction	δ -PDE	• Extracts Rab13 from membrane	Rab13		
Rab33b	• Intra-Golgi transport	Rab33b-BP	• Probably regulates motility of Rab33 vesicles	Rab33b–GTP		
Ypt1p	• ER–Golgi	Uso1p	• Tethering of ER-derived vesicles	Recruitment regulated by Ypt1p		
		Yp1p–Yif1p complex	• Ypt GTPase binding to the Yp1p–Yif1p complex essential for vesicle docking and fusion	Ypt1p, Ypt31p Sec4p		• Integral membrane protein • Possible receptor and GDI-releasing factor
Sec4p	• Delivery of TGN-derived vesicles into the bud	Sec15p	• Tethering through interaction of vesicular Sec15p and Sec10p with target complex in the bud	Sec4p–GTP	Exocyst	• Marks the site for docking or fusion
Ypt51p/ Vps21p	• Golgi–endosome and plasma membrane–endosome transport	Vac1p	• TGN–Golgi transport	Ypt51p–GTP	Vps45p Pep12p	• Regulates SNARE complex formation or disassembly • Pep12p is a t-SNARE
Ypt7p	• Vacuole fusion	Vam2p– Vam6p	• Tethering and nucleotide exchange activity	Ypt7p–GTP	HOPS complex Vam3p SNARE complex δ -adaptin	• Links SNAREs with Ypt activation • Marks the site for tethering/fusion • AP3-vesicle formation at the Golgi
			• Budding of AP3 vesicles?			

(CCV, clathrin-coated vesicle; EE, early endosome; ER, endoplasmic reticulum; PDE, phosphodiesterase; PI(3)K, phosphoinositol-3-OH kinase; SH3, Src homology region 3 domain; TGN, *trans*-Golgi network; Vamp, vesicle-associated membrane protein.)

Table 2 | Regulatory proteins

Rab/Ypt	GAP	GEF
Rab1	?	Mss4
Rab3	Rab3-GAP	Mss4, possibly Rabin 3
Rab5	Tuberous sclerosis 2 (?) RN-Tre	Rabex5
Rab6	GAPcenA	?
Rab8	?	Mss4
Rab10	?	Mss4
Ypt1p	?	TRAPP [*] Dss4p
Ypt51p/Vps21p	Gyp1p, Gyp3p	Vps9p
Ypt6p	Gyp2p Gyp3p, Gyp4p, Gyp6p	Ric1p–Rigp1p
Ypt7p	Gyp4p, Gyp7p	HOPS [†]
Sec4p	Gyp1p, Gyp2p/Mdr1p, Gyp3p, Gyp4p/Msb4p	Sec2p Dss4p

^{*}TRAPP complex: Trs20p, Trs23p, Trs31p, Trs33p, Trs65p, Trs85p, Trs120p, Trs130p, Bet3p, Bet5p. [†]HOPS complex: Vps11p, Vps16p, Vps18p, Vps33p, Vps39p, Vps41p.

and where is assembly of this complex regulated? What is the role of each subunit and that of Sec4p in this process? What determines localization of Sec3p?

In the early ENDOCYTIC PATHWAY, **Rab5** regulates clathrin-coated-vesicle-mediated transport from the plasma membrane to the early endosomes as well as homotypic early endosome fusion^{15,16}. EEA1 is the Rab5 effector that mediates tethering/docking of early endosomes¹⁷. EEA1 is a largely coiled-coil protein that contains two zinc-fingers and two Rab5-binding domains at the amino and carboxyl termini¹⁸. Considering that Rab5 is also required on both donor and acceptor membranes for fusion to occur^{19,20}, EEA1 could bridge two membranes that bear Rab5 (see below).

A symmetrical requirement for a Rab protein in vesicle docking and fusion has also been determined for **Ypt7p** in yeast vacuole fusion^{21,22}. Ypt7p binds to and regulates the membrane localization of a multi-protein complex (**HOPS**, which stands for homotypic fusion and vacuole protein sorting; also referred to as Class C Vps protein complex²³) which includes the Vam2p/**Vps41p** and Vam6p/**Vps39p** proteins^{24,25}. As these proteins have been implicated in vacuolar membrane docking²⁶, HOPS seems to be the effector that mediates Ypt7p-dependent tethering. Consistent with the symmetrical requirement for Ypt7p, the HOPS complex is also needed on each vacuole partner undergoing homotypic fusion²⁶. Furthermore, HOPS stimulates nucleotide exchange on Ypt7p²⁷. So, as shown earlier for the Rabaptin-5–**Rabex-5** complex of Rab5 (REF. 28), HOPS couples nucleotide exchange on a Rab protein to effector recruitment and function.

Vesicle budding. More elusive is the role of Rab proteins in budding, for which there is contradictory evidence

depending on the experimental system. For example, *in vivo* studies have indicated a possible role for Rab1 in budding of vesicles from the ER²⁹ and for **Rab9** from endosomes directed to the *trans*-Golgi network (TGN)³⁰. More recently, however, Rab1 has been shown not to be essential for COPII vesicle formation *in vitro*, although it commits the vesicles to targeting and fusion⁷. In yeast, Ypt1p (Rab1) is also not needed for COPII vesicle formation but is exclusively required on target Golgi membranes^{8,31}.

A function in vesicle formation has also been attributed to Rab5. Rab5, which modulates the half-life of clathrin-coated pits (ccv) on the plasma membrane *in vivo*¹⁶, is required for vesicle formation *in vitro*³². Consistent with this finding, overexpression of RN-Tre, a newly identified Rab5 GAP (GTPase-activating protein), downregulates Rab5 and inhibits receptor internalization³³.

One component of the Ypt7p (Rab7)-tethering complex HOPS, Vam2p/Vps41p²⁴, has also been implicated in the budding of vesicles from the Golgi³⁴, although Ypt7p itself has not yet been directly involved in Golgi budding events²⁶.

Thus, depending on the specific transport event, Rab proteins might directly or indirectly influence vesicle budding. They could regulate the concentration and/or assembly of coat components or help to incorporate cargo molecules selectively into the nascent vesicles. Alternatively, their presence in an active state might function as a checkpoint that ensures the delivery of a vesicle to its appropriate target compartment.

Vesicle motility. The task of Rab proteins is not restricted to membrane budding and fusion. More recently, these GTPases have been shown to determine the distribution of cellular compartments by regulating the movement of vesicles and organelles along cytoskeletal filaments. A role for **Rab6** in microtubule-dependent transport has been inferred from the discovery that this GTPase interacts with a kinesin-like protein, **Rabkinesin-6** (REF. 35), which is important for cytokinesis³⁶. Rab5 regulates both the attachment of early endosomes to, and the motility along, microtubules³⁷. There are also functional connections between Rab proteins and motors of the actin cytoskeleton. Genetic interactions have been uncovered in yeast between Sec4p and the myosin heavy chain Myo2p, indicating a possible mechanism whereby vesicles are propelled by motor proteins along polarized actin cables towards the site of exocytosis^{38,39}. Another potential link between Rab and myosin proteins can be deduced from studies of a human disease, **Griscelli syndrome**. This is a rare, autosomal recessive disorder that is characterized by defective pigmentation of the skin and hair due to an aberrant accumulation of melanosomes in melanocytes. Mutations in two human genes have been associated with the disease, one in the **MYO5A** gene and the second in the **RAB27A** gene⁴⁰. Interestingly, the same proteins are lost in the mouse mutants *dilute* (**myosin-VA**) and *ashen* (**Rab27a**), which are defective in pigment granule transport^{41,42}. As Rab27a and myosin-VA

CCP
Area of the plasma membrane where receptors and the clathrin machinery are concentrated, preparing to form a vesicle.

function in the same pathway of melanosome transport in melanocytes, it will be interesting to see whether Rab27a and myosin-VA can directly interact.

Multiplicity of Rab5 effectors

The identification of interacting molecules has revealed an extraordinary complexity of the machinery downstream of Rab5. Using an affinity-chromatography procedure, it was possible to identify >20 polypeptides from bovine brain cytosol that interact directly or indirectly with the GTP-bound form of Rab5 (REF. 17). An important principle emerging from this analysis is that Rab5 effectors function in a cooperative fashion (BOX 1).

The first Rab5 effector identified and found to be essential for early endosome fusion was Rabaptin-5 (REF. 43). Rabaptin-5 forms a complex with another protein, Rabex-5, which catalyses nucleotide exchange on Rab5

(REF. 28). Upon activation of Rab5 by Rabex-5, the Rabaptin-5–Rabex-5 complex induces its own membrane recruitment through Rabaptin-5 (Lippe *et al.* manuscript in preparation). This positive-feedback loop counteracts GTP hydrolysis⁴⁴ and is thought to create a microenvironment that is enriched in active Rab5 on the membrane where other Rab5 effectors are recruited²⁸.

How does this local clustering of activated Rab5 proteins regulate the tethering machinery? The carboxy-terminal end of the tethering factor EEA1 contains two structural elements that are essential for targeting to the early endosome membrane⁴⁵. One is the **FYVE finger**, a zinc-finger that specifically binds to phosphatidylinositol-3-phosphate, PtdIns(3)P^{46,47}, and a Rab5-binding site located immediately upstream of the FYVE finger¹⁸. The discovery that phosphatidylinositol-3-OH kinases (PI(3)Ks) are Rab5 effectors⁴⁸ is

Box 1 | Rab5 effectors cluster in a Rab5 domain

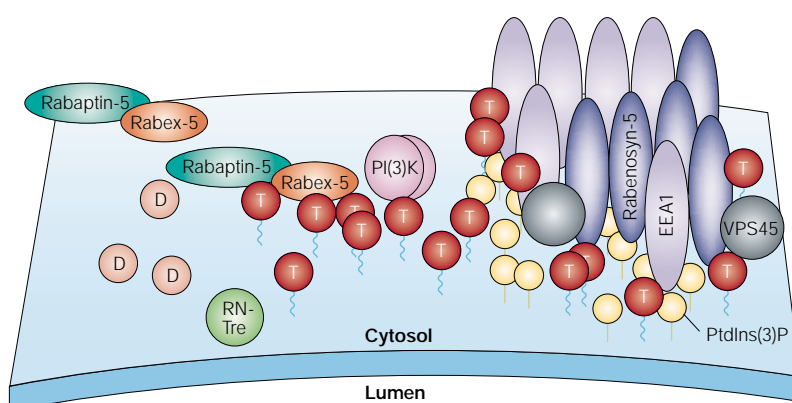
The complex network of Rab5 regulators and effectors involves positive feedback loops and, according to the model presented in the figure, is designed to generate a local amplification of active Rab5 and the clustered recruitment of Rab5 effectors on the early endosome membrane.

Rab5-GTP (T in the figure) is unstable on the early endosome where it undergoes continuous

cycles of GTP hydrolysis (Rab-GDP is shown as D in the figure), catalysed by RN-Tre³³ and nucleotide exchange⁴⁴. The first feedback loop is due to the Rabaptin-5–Rabex-5 complex that activates Rab5 through the nucleotide-exchange activity of Rabex-5 and gets recruited on the early endosome membrane through Rabaptin-5. In this case, the product of the reaction (Rab5-GTP) recruits the enzyme. A second feedback loop is due to the cooperativity between effectors. Active Rab5 interacts with the hVPS34-p150 phosphoinositol-3-OH kinase (PI(3)K), thus coupling phosphatidylinositol-3-phosphate (PtdIns(3)P) production to Rab5 localization. The concomitant presence of Rab5 and PtdIns(3)P allows the recruitment of the Rab5 FYVE effectors early endosome antigen 1 (EEA1) and Rabenosyn-5. It is also known that EEA1, Rabaptin-5 and Rabex-5 form high-molecular-weight oligomers on the early endosome membrane. Because the oligomers also incorporate the nucleotide-exchange factor for Rab5, Rabex-5, the effectors themselves feedback on the recruitment and clustering of Rab5 within a limited area of the early endosome.

But what determines the specific targeting of Rab5 to the early endosome to initiate a Rab domain? Candidates for Rab receptors and GDI-releasing factors have been found^{97–101} but, eventually, their localization also needs to be explained. Similarly, what determines the targeting of hVPS34/p150? Important factors for the generation of a Rab domain might be the cooperativity and the self-organization properties of its components described above. We have seen in fact that, through positive feedback loops, the localization of one component depends on the recruitment of the other. None of the individual components (Rab5 or PI(3)K) is sufficient to form a domain but it is the combinatorial use of all components that creates the specificity of that particular membrane environment. For example, EEA1 is absent from the plasma membrane and clathrin-coated vesicles^{20,102}, consistent with the idea that hVPS34 produces PtdIns(3)P on early endosomes^{48,55}. On the plasma membrane, therefore, Rab5 alone is not sufficient to recruit EEA1 and the other FYVE effectors, arguing that clusters of Rab5/hVPS34/PtdIns(3)P/EEA1/Rabenosyn-5 are present only on a subcompartment of the early endosome⁵⁷. In addition to these interactions, generation of a Rab domain will most probably require associations between Rab5 effectors and additional membrane proteins.

So the Rab5 machinery can be viewed as a typical modular system¹⁰³, in which specific biochemical interactions between Rab5 effectors and regulators as well as other endosomal proteins create spatial segregation. By regulating the assembly of a specific membrane domain, these molecules contribute to the compartmental specificity, robustness and dynamic properties of the early endosome.



another example of cooperativity between effector molecules. PI(3)Ks function in various cellular processes^{49,50}. Distinct types of PI(3)K have unique functions in signal transduction and mitogenesis, cytoskeletal organization and membrane transport⁵¹. Rab5 interacts directly with two types of PI(3)Ks. The first is **p85 α /p110 β** , a type I kinase that mainly phosphorylates PtdIns(4)P and phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂), generating PtdIns(3,4)P₂ and phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃) (REF. 49). The function of p85 α /p110 β with respect to Rab5 is not known, but, given that type I PI(3)Ks are established components of the signal-transduction machinery, the interaction between p85 α /p110 β and Rab5 might reveal new properties of Rab5 in response to intracellular signalling. The second PI(3)K is **hVPS34/p150**, the mammalian homologue of yeast Vps34p/Vps15p^{52,53}. This kinase preferentially phosphorylates phosphatidylinositol to PtdIns(3)P, which is necessary for the recruitment of FYVE finger proteins on the early endosome^{48,51,54}. Rab5 is therefore coupled to the generation of PtdIns(3)P by the recruitment of hVPS34/p150 (BOX 1), a result that is consistent with the enrichment of PtdIns(3)P on early endosomes⁵⁵. This mechanism is not limited to the membrane recruitment of EEA1. Rabenosyn-5 is another FYVE finger Rab5 effector that, similar to EEA1, is recruited in a Rab5- and PtdIns(3)P-dependent fashion to early endosomes, where it functions in docking and fusion⁵⁶. This mechanism could also indirectly regulate the recruitment of FYVE finger proteins other than Rab5 effectors to the early endosome.

Purified clathrin-coated vesicles (CCV) fail to recruit EEA1, despite the presence of Rab5 (REF. 20,57). EEA1 (and probably Rabenosyn-5) can be exclusively attached to early endosomes and this asymmetrical recruitment between transport vesicles and their target organelle directly correlates with the asymmetrical distribution of hVPS34 (REF. 48). Binding of EEA1 to activated Rab5 through its amino-terminal site¹⁸ is therefore not sufficient for membrane recruitment. However, a patch of EEA1 molecules⁵⁸ could provide several (low affinity) binding sites that are sufficient to tether an incoming CCV to the early endosome, thus providing directionality to the transport process.

So far, less than half of the Rab5-interacting proteins have been identified and several other molecules still need to be integrated in the Rab5 scheme. In addition, if other Rab GTPases interact with effector proteins with a complexity similar to that of Rab5, it means that the number of total Rab effectors is likely to increase considerably with the functional characterization of other Rab proteins.

Rab domains

The diversity of biochemical reactions that are regulated by Rab proteins raises the question of how these processes are coordinated. We propose that Rab5 and its effectors are not randomly recruited and distributed on the early endosome membrane but are spatially segregated in a defined membrane domain or Rab domain.

There is increasing evidence that membrane-bound molecules are not randomly distributed in the membrane bilayer but are enriched in membrane domains of varying lipid composition⁵⁹. However, the membrane arrangement that is regulated by Rab5 is very different from that of other membrane domains such as LIPID RAFTS, which primarily depend on the intercalation of sphingolipids with cholesterol⁶⁰. First, protein-lipid interactions are a central factor in the generation of the Rab5 domain. The localized synthesis of PtdIns(3)P not only allows the specific recruitment of FYVE effectors in conjunction with Rab5, but also contributes to their clustering. The dynamic properties of a Rab5 domain probably include a spatial and temporal control over PtdIns(3)P synthesis and turnover. As membrane flows through the early endosome, phosphoinositides other than PtdIns(3)P (for example, PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃)^{48,61}, could be either converted to PtdIns(3)P or excluded from the Rab5 domain. Conversely, the PtdIns(3)P that is generated in the Rab5 domain and not retained by Rab5 effectors could be used in other endosomal sub-compartments, undergo further modifications or be degraded^{55,62-64}. PtdIns(3)P is also enriched in the internal vesicles of multivesicular endosomes⁵⁵, suggesting that this phospholipid could also be generated at later stages of the endocytic pathway to function in combination with other Rab proteins.

A second important factor in the formation of a Rab5 domain is the effector cooperativity. The local generation of lipids and recruitment of individual effectors is not sufficient to maintain a membrane domain. In the absence of lateral interactions between the proteins within the domains, these lipid-protein complexes would rapidly diffuse throughout the plane of the membrane, filling the whole endocytic pathway. One possible mechanism to avoid this is protein oligomerization. On the membrane, EEA1, Rabaptin-5 and Rabex-5 form dynamic oligomeric complexes with NSF⁵⁸. However, oligomerization still might not be sufficient and a scaffold, such as the actin framework or one that is analogous to the spectrin/ankyrin system⁶⁵, or to the Golgi stacking machinery⁶⁶, might be needed to stabilize the local membrane composition of Rab effectors and arrange them in a precise architectural layout. Cytoskeletal tracks could also be connected with this scaffold to increase the efficiency of vesicle delivery to a Rab domain.

Last, the generation and maintenance of the Rab5 domain depends on energy. The hydrolysis of GTP regulates the kinetics and limits the extent of effector recruitment. Through PtdIns(3)P production, PI(3)K uses ATP to recruit and, more importantly, to cluster these molecules (BOX 1). The ATPase activity of NSF regulates the dynamics of the hetero-oligomers⁵⁸. The integration between GTPase and ATPase cycles therefore ensures a dynamic state between assembly and disassembly of oligomeric complexes of proteins and lipids and, consequently, confers a specific control on the size of that membrane domain.

Visualization of Rab domains
Morphological studies have lent support to the proposal

CCV
Coated vesicles involved in the endocytosis of receptors at the plasma membrane.

LIPID RAFTS
Lipids including cholesterol and sphingomyelin aggregated laterally to form membrane microdomains.

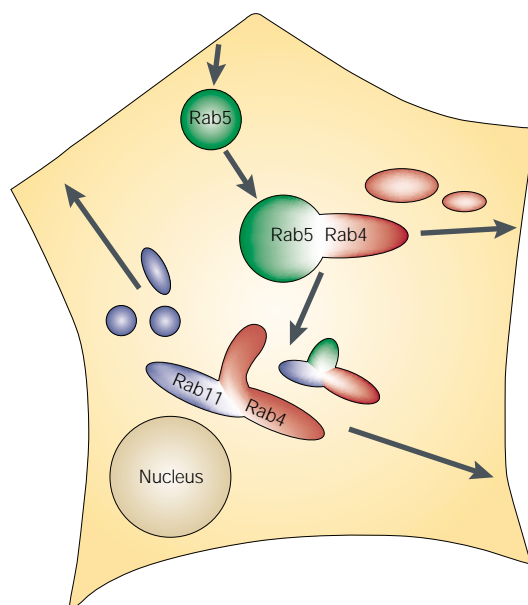


Figure 2 | Model of Rab domains on endosomes. Studies of Rab5, Rab4 and Rab11 tagged with green fluorescent protein (GFP) have shown that these Rab proteins are compartmentalized within the membrane of early endosomes⁶⁸. Cargo flows sequentially through these domains as indicated by the arrows. The Rab domains also have a specific distribution and different pharmacological properties⁶⁸. We propose that, similarly to the Rab5 effectors, Rab4 and Rab11 effectors are clustered in defined areas of the endosome membranes that are linked to each other through bifunctional Rab effectors.

GREEN FLUORESCENT PROTEIN
Autofluorescent protein originally identified in the jellyfish *Aequorea Victoria*.

RECYCLING ENDOSOME
About 90% of endocytosed receptors are recycled to the plasma membrane. At least part of this traffic occurs through recycling endosomes.

TRANSFERRIN
Protein involved in ferric ion uptake into the cell. The pathway followed by transferrin bound to its receptor defines the recycling pathway.

APICAL JUNCTIONAL COMPLEX
Desmosomes, adherens junctions and tight junctions make up the apical junctional complex.

NEURITE
Process extended by a nerve cell that can give rise to an axon or a dendrite.

CIS-SNARE COMPLEX
SNARE pairing occurring within the same membrane.

that Rab proteins and/or Rab effectors are clustered in defined membrane domains. Upon expression of the activated Rab5Q79L mutant of Rab5, Rab5 and EEA1 concentrate in brightly fluorescent spots on the enlarged endosomal membrane^{58,67}. Rab5, and probably its effectors, particularly accumulate at the interface between fusing vesicles and persist in a discrete spot for minutes after endosome fusion⁶⁷. Immunofluorescence and video microscopy studies conducted on cells that express Rab5, Rab4 and Rab11 tagged with GREEN FLUORESCENT PROTEIN (GFP) have also revealed compartmentalization of these three GTPases within the endosomal membrane⁶⁸. Three main populations of endosome could be distinguished: one that contains primarily Rab5, a second that contains both Rab5 and Rab4, and a third one that harbours Rab4 and Rab11. This distribution is consistent with biochemical and ultrastructural studies^{57,69,70}. The former two would correspond to early/sorting endosomes and the latter to perinuclear RECYCLING ENDOSOMES⁷¹. TRANSFERRIN internalized as endocytic tracer was found first to enter the Rab5 domain and then to move sequentially through Rab4- and Rab11-positive structures. Given the function of these Rab proteins^{16,72,73}, the Rab5 domain would be the gateway into the early endosome, whereas the Rab4 and Rab11 domains would contain the machinery that is necessary for sorting and recycling of transferrin to the cell surface. Endosomes can therefore be viewed as a

mosaic of Rab4, Rab5 and Rab11 (as well as other Rab) domains that dynamically interact but keep a relatively stable distribution over time (FIG. 2). These Rab domains could be connected through tethering molecules without intermixing. Molecules such as Rabaptin-5, which interacts with Rab5 and Rab4 through two distinct regions (REF. 74), might functionally and structurally link the Rab5 and Rab4 domains. Other molecules should then link other domains together (that is, Rab4 with Rab11). When combined with other types of lipid microdomains that have been shown to exist on endosomes^{75,76}, the multiplicity of membrane platforms could reach a high level of complexity.

This membrane compartmentalization is not limited to the Rab5 system, but has been observed for other Rab effectors that are involved in vesicle tethering. Docking sites could be inferred from the fluorescent morphology of discrete punctate sites around the yeast vacuole that contain at least two proteins required for vacuolar protein sorting and morphology, Vam2/Vps41p and Vam6/Vps39p⁷⁷. Striking fluorescent images of patching were also seen in budding yeast for Sec3p, a component of the exocyst, which is restricted to the bud site¹³. The exocyst localizes to APICAL JUNCTIONAL COMPLEXES in polarized mammalian cells⁷⁸, the apical pole of pancreatic acinar cells⁷⁹, and the tips of growing NEURITES, growth cones and synapse-assembly sites in developing neurons⁸⁰. Highly dynamic transport carriers bearing Rab6 and containing specific cargo molecules were observed to translocate from the Golgi to the ER⁸¹, supporting the idea that this retrograde transport pathway involves a subdomain of the Golgi complex. Thus, patches of Rab effectors have been observed within both endocytic and biosynthetic organelles, indicating that these molecules might generally be restricted spatially on the membrane of organelles.

Rabs link to SNAREs and motor proteins
A crucial factor in vesicular transport is the coordination between Rab-dependent membrane tethering, docking and SNARE-dependent membrane fusion. Upon successful tethering and priming, the SNAREs engage in *trans*-interactions between vesicle- and target-SNAREs. This interaction leads to closely apposed membranes, resulting in fusion. We propose that the selective incorporation of CIS-SNARE COMPLEXES within a Rab domain is a prerequisite for the cognate SNAREs to pair *in trans* upon tethering/docking (BOX 2). Molecular interactions with Rab effectors within the Rab domain might result in the selective enrichment of *cis*-SNARE complexes at their site of function.

Several recent studies have reported direct molecular links between Rab effector proteins and components of the SNARE machinery (BOX 2). Sec1p and related proteins, Vps33p and Vps45p, regulate SNARE pairing by sequestering syntaxin molecules^{82,83}. Interactions between Rab effectors and Sec1 family members have been observed both in mammalian cells⁵⁶ and in yeast^{25,84–86}. These interactions might function to retrieve the inhibitors from the SNAREs and might result in conformational changes that allow

Box 2 | Molecular interactions showing cooperativity between Rab and SNARE machineries

a | The Rab effectors Vac1p, Rabenosyn-5 and the HOPS complex can bind Sec1 homologues directly^{25,56,84–86}. The functional importance of this interaction can be seen as two distinct, but not mutually exclusive, possibilities. First (top), the Rab effector might remove Sec1 from the primed *cis*-SNARE complex to free the SNAREs for interactions *in trans*. Second (bottom), the Rab effector/Sec1 complex might stabilize *trans*-SNARE pairs after docking¹⁰⁴.

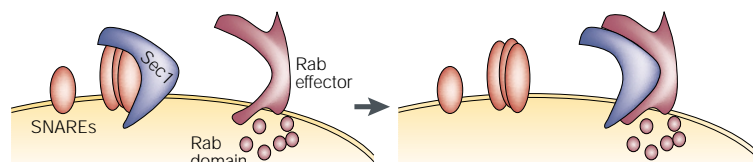
b | In two independent systems, the hydrolysis of ATP by *N*-ethyl-maleimide-sensitive fusion protein (NSF) and α -SNAP could regulate Rab effectors–SNARE interactions and oligomeric assembly of Rab effectors. The mechanistic consequences of these reactions are not clear but they might mediate the cyclical assembly/disassembly of protein complexes during membrane transport. In the early endosome, EEA1, Rabaptin-5 and Rabex-5 exist in oligomeric complexes with NSF⁵⁸. The presence of NSF within the oligomers could function either to locally prime SNAREs or to regulate interactions among Rab effectors and SNAREs, ultimately leading to *trans*-SNARE pairing. Sec18p (NSF homologue) activity might be required at several stages during vacuole fusion in yeast^{24–26}, in which hydrolysis of ATP by Sec18p releases the Ypt7p (Rab7 homologue)

HOPS effector complex from the Vam3p SNARE complex. HOPS can then bind and activate²⁷ Ypt7p leading to vacuole tethering (b, right). In addition, the Ypt7p effector complex can also bind to primed Vam3p, which might aid assembly of *trans*-SNARE complexes⁸⁷. Both data from mammalian and yeast systems therefore support the idea that vesicle tethering and SNARE priming are spatially and temporally coupled.

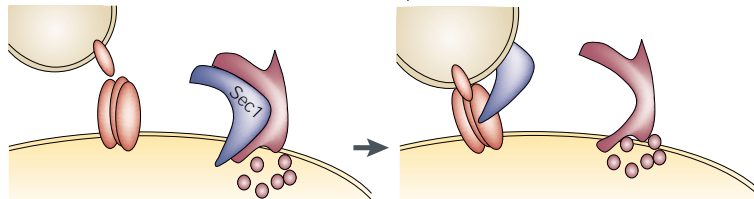
c | Multiple direct links have been established between Rab effectors and SNAREs. Complexes formed between EEA1 and two t-SNAREs, syntaxin13 and syntaxin6 (REFS 58,105) within the Rab5 domain might mediate fusion between endosomes, or between *trans*-Golgi-network-derived vesicles and endosomes, respectively. The Ypt7p effector proteins have also been found in complexes with the SNARE Vam3p complex^{24,25,87}. Through directly binding to syntaxin-5 complexes, the Rab1 effector p115 might ensure their incorporation into COPII vesicles⁷. The concentration of Rab effectors within a restricted membrane area would increase the apparent affinity and enhance the rate of Rab effectors–SNARE and SNARE–SNARE complex formation. In this way, only the correct SNAREs would be selectively incorporated within the Rab domain, at the target site for fusion (top). These interactions might also contribute to the steady-state localization of SNAREs, despite their regular cycle between organelles. In addition, effector–SNARE interactions could be functionally required for fusion^{58,87}, possibly for the architectural arrangement and bridging of *trans*-SNARE pairs (bottom).

a Sec1 homologues bind Rab effectors

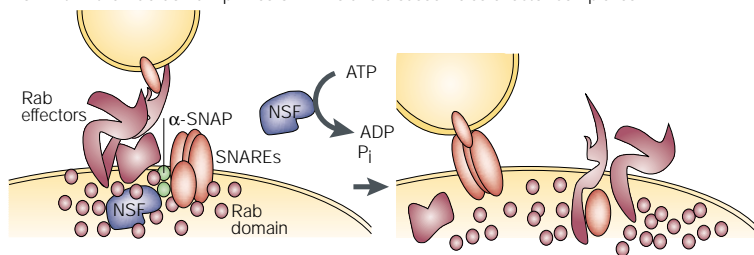
Rab effector removes negative regulation



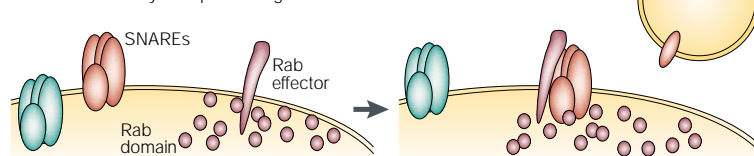
Rab effector delivers Sec1 to stabilize *trans*-SNARE pairs

**b Priming machinery and Rab effectors**

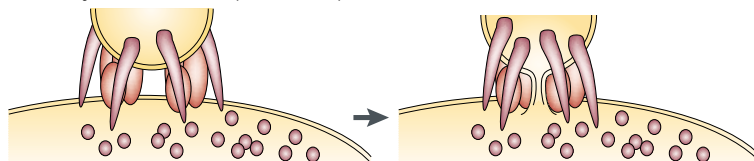
NSF within the Rab domain primes SNAREs and disassembles effector complexes

**c Rab effectors directly bind SNAREs**

Effectors selectively incorporate cognate SNAREs into Rab domains



Assembly of several *trans* pairs into a pore structure



SEC18P (Sec18p)
Saccharomyces cerevisiae
 homologue of NSF.

HAEMAGGLUTININ
 Spike protein of the influenza
 virus. HA is the best-
 understood fusion protein.

SNAREs to be primed or stabilized in a *trans*-paired conformation (BOX 2).

A second set of interactions has been observed between the Rab effectors and the SNARE priming machinery. For example, **Vac1p** could be precipitated together with **Pep12p** and SEC18P in the presence of a *sec18-1* mutant⁸⁴. The Rab5 effector proteins EEA1 and Rabaptin-5–Rabex-5 complex form oligomers on the endosome whose assembly is dependent on the ATPase activity of NSF⁵⁸. Interactions between the Ypt7p effector HOPS and SNAREs also depend on the ATPase activity of Sec18p (REF 24). Finally, in both systems there are specific links to the SNARE proteins themselves. EEA1 binds directly to **syntaxin 13** and this interaction is functionally required for endosome fusion⁵⁸. The Ypt7p effector proteins have also been found in complexes with the SNARE **Vam3p** complex^{24,25,87}.

The compartmentalization of Rab effectors and the SNARE machinery within specifically assembled domains explains how productive, fusion-competent SNARE pairing would occur only within the membrane environment that has been selected for vesicle tethering, despite the presence of a cycling population of SNAREs at any given time and despite promiscuous pairing. In this context, it is interesting to consider the mechanistic links between the tethering machinery and the activation of SNAREs. The transition from membrane tethering to fusion implies that a relatively long distance must be bridged after activation of the SNARE machinery within the Rab domain. Using electron microscopy, tethered vesicles (50–100 nm in diameter) have been observed ~75–150 nm away from the target⁸⁸. This is consistent with the length of the rod-like tethering factors. Ultrastructural analysis has shown that EEA1, for example, is associated with filamentous material that extends from the cytoplasmic surface of the endosome⁵⁷. The current hypothesis is that the tethered vesicle swings through the highly viscous cytosol, attached by its 'string', until it collides with the surface of the target. Once the vesicle lands, the activated SNAREs engage for close docking, which closes the gap between the two bilayers. This would suggest that the tethering factors can communicate across the ~100-nm distance the fact that a vesicle has been 'trapped' to signal the priming of SNAREs. An alternative possibility is that tethering factors might undergo a large conformational change upon binding a vesicle at the free end, which would reduce the distance gap and result in the vesicle being pulled into close proximity to its target. There are many proteins that undergo extensive conformational changes that are important for their function. For example, HAEMAGGLUTININ (HA) of the influenza virus drastically changes its shape upon pH reduction⁸⁹, the light-chain-(**calmodulin**)-binding domain of myosin swings to generate movement in response to conformational changes in the motor domain^{90,91}, and the flagellar protein spasmin extends from a tightly coiled spring to a long rod (a distance difference of microns) to propel the *Vorticella velogiines* protozoa⁹². Interestingly, calmodulin is required for endosome fusion⁹³ and EEA1 contains a putative **calmodulin-binding IQ motif**,

which might potentially function in such a conformational change.

Functional coordination is not restricted to vesicle tethering and fusion but is also important for vesicle motility. Strikingly, similarly to endosome membrane docking and fusion, Rab5-dependent endosome movement along microtubules depends on hVPS34 PI(3)K. So, Rab5 functionally links regulation of membrane transport, motility and intracellular distribution of early endosomes. The higher the levels of Rab5–GTP, the higher the recruitment of Rab5 effectors, including hVPS34, and the higher the movement, tethering and fusion activity of endosomes. This implies that a microtubule motor or a molecule that regulates its recruitment and/or activity might be a PtdIns(3)P-binding protein. Rab5 effectors implicated in microtubule-dependent early endosome motility have not yet been identified, although it is possible that a minus-ended kinesin might be involved³⁷. Rabkinesin-6 is a Rab6 effector³⁵ and a second kinesin-related protein, Rab33b-BP, binds to **Rab33b**⁹⁴. How these interactions result in vesicle motility remains to be determined.

Perspectives for membrane biology

We are beginning to understand the fundamental principles of membrane transport from one intracellular compartment to another. However, a more complex task will be to define an organelle in molecular terms, determine its boundaries, explain how its transport machinery is specifically localized and what controls its size and intracellular distribution. Understanding the mechanisms of membrane compartmentalization and organelle biogenesis will therefore be an important challenge for the future.

We have argued that the compartmentalization of Rab proteins and their effectors contributes to the formation of membrane domains and hence to the structural and functional properties of organelles. It will be important to find out whether this mode of function is limited to the early endosomal system or can be generalized to other Rab proteins of different organelles. Moreover, the idea of Rab domains needs to be integrated with other functional modules. For example, functional interactions of Rab proteins with Rho and ARF GTPases and their effectors^{95,96} could link vesicle formation and targeting with the role of the cytoskeleton in organelle structure and function. However, beside protein machineries, biological membranes contain different kinds of lipid microenvironments^{59,74,75}. It will be interesting to see how the physico-chemical properties of these membrane platforms participate in the membrane compartmentalization of organelles and act on the location and function of the transport machinery.

Morphological techniques will become increasingly important to assess the distribution and, most importantly, the dynamics of membrane compartments. In addition to studies *in vivo*, to elucidate the mechanisms that underlie the compartmentalization of biological membranes it will be necessary to develop biophysical methods (for example, artificial membrane systems) that recapitulate the clustering of molecules and their

TRANSCYTOSIS PATHWAYS

Transport of macromolecules across a cell, consisting of endocytosis of a macromolecule at one side of a monolayer and exocytosis at the other side.

APICAL

Plasma membrane surface of an epithelial cell that faces the lumen.

BASOLATERAL

Plasma membrane surface of an epithelial cell that adjoins underlying tissue.

behaviour *in vitro*. These systems should also help us understand the principles that govern the size of cellular organelles and their sub-compartments. Why are endosomes scattered in comparison with the Golgi complex? What determines the geometry of these compartments? It will be interesting to see, in relation to the regulation of homotypic endosome fusion by Rab5 (REF. 44), whether the timer function of Rab GTPases could have a general function in regulating organelle dynamics.

The disclosure of new protein sequences from the different genome projects provides an extraordinary number of novel tools with which to explore the organization of biological membranes. We will also learn a great deal from the generation and expansion of protein families throughout evolution. How has the basic transport machinery of yeast evolved from this simple eukaryote to multicellular organisms? Physiological processes typical of tissues and organs, such as neurotransmitter release, insulin-regulated traffic, recycling and transcytosis, require cell-type specific modifications of the intracellular pathways for the transport of particular cargo molecules. How is transport regulated in polarized epithelial cells and neurons? Different Rab domains containing different sets of Rab effectors could govern

protein sorting and transport in the secretory, endocytic/recycling and TRANSCYTOTIC PATHWAYS of polarized cells. Efforts in this direction are expected to provide clues into, for example, what makes APICAL and BASOLATERAL early endosomes functionally distinct in polarized epithelial cells. How is their spatial distribution controlled? It will be fascinating to see how the basic molecular principles that account for membrane compartmentalization and organelle polarity can contribute, at larger scale, to an integrated understanding of cell polarity and complex tissues in multicellular organisms.

Links

DATABASE LINKS **p115** | **Rabaptin-5** | **EEA1** | **Rabenosyn-5** | **Ypt1p** | **Rab1** | **TRAPP** | **Sec4p** | **Exocyst** | **Rab5** | **Ypt7p** | **HOPS** | **Vps41p** | **Vps39p** | **Rabex-5** | **Rab9** | **Rab6** | **Rabkinesin-6** | **Griscelli syndrome** | **MYO5A** | **RAB27A** | **myosin-VA** | **Rab27a** | **FYVE finger** | **p85 α** | **p110 β** | **hVPS34** | **p150** | **Rab4** | **Rab11** | **Sec1p** | **Vps33p** | **Vps45p** | **Vac1p** | **Pep12p** | **syntaxin 13** | **Vam3p** | **calmodulin** | **calmodulin-binding IQ motif** | **Rab33b** | **syntaxin 6**
FURTHER INFORMATION **Zerial lab home page** | **University of Ottawa Heart Institute**

- Rothman, J. E. Mechanisms of intracellular protein transport. *Nature* **372**, 55–63 (1994).
- Weber, T. *et al.* SNAREpins: minimal machinery for membrane fusion. *Cell* **92**, 759–772 (1998).
- McNew, J. A. *et al.* Compartmental specificity of cellular membrane fusion encoded in SNARE proteins. *Nature* **407**, 153–159 (2000).
Using the reconstituted liposome fusion assay, Rothman and colleagues systematically tested liposomes containing each of the yeast v-SNAREs for fusion with three potential t-SNARE complexes. The cognate SNARE pairs showed a high degree of selectivity, an important tenant of the SNARE hypothesis.
- Parlati, F. *et al.* Topological restriction of SNARE-dependent membrane fusion. *Nature* **407**, 194–198 (2000).
- Novick, P. & Zerial, M. The diversity of Rab proteins in vesicle transport. *Curr. Opin. Cell Biol.* **9**, 496–504 (1997).
- Cao, X., Ballew, N. & Barlowe, C. Initial docking of ER-derived vesicles requires Usa1p and Ypt1p but is independent of SNARE proteins. *EMBO J.* **17**, 2156–2165 (1998).
- Allan, B. B., Moyer, B. D. & Balch, W. E. Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. *Science* **289**, 444–448 (2000).
Shows that the previously identified tethering factor, p115, is a Rab1 effector and directly interacts with the SNARE machinery. The functional importance of an interaction between a Rab effector and the cis-SNARE complex during vesicle budding highlights the multiple roles of Rab effectors.
- Cao, X. & Barlowe, C. Asymmetric requirements for a Rab GTPase and SNARE proteins in fusion of COPII vesicles with acceptor membranes. *J. Cell Biol.* **149**, 55–66 (2000).
- Sacher, M. *et al.* TRAPP, a highly conserved novel complex on the cis-Golgi that mediates vesicle docking and fusion. *EMBO J.* **17**, 2494–2503 (1998).
- Wang, W., Sacher, M. & Ferro-Novick, S. TRAPP stimulates guanine nucleotide exchange on Ypt1p. *J. Cell Biol.* **151**, 289–296 (2000).
- TerBush, D. R., Maurice, T., Roth, D. & Novick, P. The exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO J.* **15**, 6483–6494 (1996).
- Kee, Y. *et al.* Subunit structure of the mammalian exocyst complex. *Proc. Natl Acad. Sci. USA* **94**, 14438–14443 (1997).
- Finger, F. P., Hughes, T. E. & Novick, P. Sec3p is a spatial landmark for polarized secretion in budding yeast. *Cell* **92**, 559–571 (1998).
Exocytosis in yeast occurs in a polarized fashion. This study shows that Sec3p localizes to the site of polarized exocytosis and is required for the targeting of secretory vesicles to the bud. This is in line with the concept that Rab effectors specify where the vesicles should tether on their target compartment, allowing trans-SNARE complex formation to mediate fusion.
- Guo, W., Roth, D., Walch-Solimena, C. & Novick, P. The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *EMBO J.* **18**, 1071–1080 (1999).
This work established that Sec15p, a subunit of the exocyst, is a Sec4p effector protein. It supports the idea that Rab proteins regulate the function of multimeric protein complexes important in the initial recognition of the docking site for an incoming vesicle.
- Govel, J. -P., Chavrier, P., Zerial, M. & Gruenberg, J. Rab5 controls early endosome fusion *in vitro*. *Cell* **64**, 915–925 (1991).
- Bucci, C. *et al.* The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell* **70**, 715–728 (1992).
- Chistoforidis, S., McBride, H. M., Burgoyne, R., D. & Zerial, M. The Rab5 effector EEA1 is a core component of endosome docking. *Nature* **397**, 621–625 (1999).
The identification of over 20 proteins that bind specifically to activated Rab5 opened up the idea that a much more complex protein machinery could be downstream of a Rab protein and have a wider regulatory role than previously imagined. Second, this paper shows that EEA1 alone can tether early endosomes and allow the SNARE machinery to mediate fusion.
- Simonsen, A. *et al.* EEA1 links phosphatidylinositol 3-kinase function to Rab5 regulation of endosome fusion. *Nature* **394**, 494–498 (1998).
- Barbieri, M. A. *et al.* Evidence for a symmetrical requirement for Rab5-GTP in *in vitro* endosome–endosome fusion. *J. Biol. Chem.* **273**, 25850–25855 (1998).
- Rubino, M., Miaczynska, M., Lippe, R. & Zerial, M. Selective membrane recruitment of EEA1 suggests a role in directional transport of clathrin-coated vesicles to early endosomes. *J. Biol. Chem.* **275**, 3745–3748 (2000).
- Haas, A., Scheglmann, D., Lazar, T., Gallwitz, D. & Wickner, W. The GTPase Ypt7p of *Saccharomyces cerevisiae* is required on both partner vacuoles for the homotypic fusion step of vacuole inheritance. *EMBO J.* **14**, 5258–5270 (1995).
- Mayer, A. & Wickner, W. Docking of yeast vacuoles is catalyzed by the Ras-like GTPase Ypt7p after symmetric priming by Sec18p (NSF). *J. Cell Biol.* **136**, 307–317 (1997).
- Rieder, S. E. & Emr, S. D. A novel RING finger protein complex essential for a late step in protein transport to the yeast vacuole. *Mol. Biol. Cell* **8**, 2307–2327 (1997).
- Price, A., Seals, D., Wickner, W. & Ungermann, C. The docking stage of yeast vacuole fusion requires the transfer of proteins from a cis-SNARE complex to a Rab/Ypt protein. *J. Cell Biol.* **148**, 1231–1238 (2000).
These experiments have shown that the HOPS complex is an effector of Ypt7p in vacuole tethering. Furthermore, this work, together with References 57 and 86 provided the first demonstrations of the dynamics of the interactions between Rab effector and SNARE machineries.
- Seals, D. F., Eitzen, G., Margolis, N., Wickner, W. T. & Price, A. A Ypt/Rab effector complex containing the Sec1 homolog Vps33p is required for homotypic vacuole fusion. *Proc. Natl Acad. Sci. USA* **97**, 9402–9407 (2000).
- Price, A., Wickner, W. & Ungermann, C. Proteins needed for vesicle budding from the Golgi complex are also required for the docking step of homotypic vacuole fusion. *J. Cell Biol.* **148**, 1223–1230 (2000).
- Wurmser, A. E., Sato, T. K. & Emr, S. D. New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. *J. Cell Biol.* **151**, 551–562 (2000).
- Horiuchi, H. *et al.* A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell* **90**, 1149–1159 (1997).
- Nuoffer, C., Davidson, H. W., Matteson, J., Meinkoth, J. & Balch, W. E. A GDP-bound form of rab1 inhibits protein export from the endoplasmic reticulum and transport between Golgi compartments. *J. Cell Biol.* **125**, 225–237 (1994).
- Riederer, M. A., Soldati, T., Shapiro, A. D., Lin, J. & Pfeffer, S. Lysosome biogenesis requires Rab9 function and receptor recycling from endosomes to the trans Golgi network. *J. Cell Biol.* **125**, 573–582 (1994).
- Barlowe, C. *et al.* COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. *Cell* **77**, 895–907 (1994).
- McLauchlan, H. *et al.* A novel role for Rab5-GDI in ligand sequestration into clathrin-coated pits. *Curr. Biol.* **8**, 34–45 (1998).
- Lanzetti, L. *et al.* The Eps8 protein coordinates EGF receptor signalling through Rac and trafficking through Rab5. *Nature* **408**, 374–377 (2000).
- Rehling, P., Darsow, T., Katzmann, D. J. & Emr, S. D. Formation of AP-3 transport intermediates requires Vps41 function. *Nature Cell Biol.* **1**, 346–353 (1999).
- Echard, A. *et al.* Interaction of a Golgi-associated kinesin-like protein with Rab6. *Science* **279**, 580–585 (1998).
A yeast two-hybrid search for effectors identified a kinesin as a Rab6-interacting protein. In line with the

concept that Rab proteins can have different functions, this result suggests that a Rab protein might be functionally connected with the microtubule-dependent motility of vesicles and organelles.

36. Hill, E., Clarke, M. & Barr, F. A. The Rab6-binding kinesin, Rab6-KIFL, is required for cytokinesis. *EMBO J.* **19**, 5711–5719 (2000).
37. Nielsen, E., Severin, F., Backer, J. M., Hyman, A. A. & Zerial, M. Rab5 regulates motility of early endosomes on microtubules. *Nature Cell Biol.* **1**, 376–382 (1999).
- A role of Rab5 in the attachment and motility of early endosomes along microtubules was uncovered using *in vitro* assays. These data together with the findings of Reference 35, strongly support an additional role of Rab proteins in organelle movement along the cytoskeleton.**
38. Pruyne, D. W., Schott, D. H. & Bretscher, A. Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. *J. Cell Biol.* **143**, 1931–1945 (1998).
39. Schott, D., Ho, J., Pruyne, D. & Bretscher, A. The COOH-terminal domain of Myo2p, a yeast myosin V, has a direct role in secretory vesicle targeting. *J. Cell Biol.* **147**, 791–808 (1999).
40. Menasche, G. *et al.* Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. *Nature Genet.* **25**, 173–176 (2000).
41. Mercer, J. A., Seperack, P. K., Strobel, M. C., Copeland, N. G. & Jenkins, N. A. Novel myosin heavy chain encoded by murine dilute coat colour locus. *Nature* **349**, 709–713 (1991); erratum **352**, 547 (1992).
42. Wilson, S. M. *et al.* A mutation in Rab27a causes the vesicle transport defects observed in ashen mice. *Proc. Natl Acad. Sci. USA* **97**, 7933–7938 (2000).
43. Stenmark, H., Vitale, G., Ullrich, O. & Zerial, M. Rabaptin-5 is a direct effector of the small GTPase Rab5 in endocytic membrane fusion. *Cell* **83**, 423–432 (1995).
44. Rybin, V. *et al.* GTPase activity of Rab5 acts as a timer for endocytic membrane fusion. *Nature* **383**, 266–269 (1996).
45. Stenmark, H., Aasland, R., Toh, B. H. & D'Arrigo, A. Endosomal localization of the autoantigen EEA1 is mediated by a zinc-binding FYVE finger. *J. Biol. Chem.* **271**, 24048–24054 (1996).
46. Stenmark, H. & Aasland, R. FYVE-finger proteins — effectors of an inositol lipid. *J. Cell Sci.* **112**, 4175–4183 (1999).
47. Lawe, D. C., Patki, V., Heller-Harrison, R., Lambright, D. & Corvera, S. The FYVE domain of early endosome antigen 1 is required for both phosphatidylinositol 3-phosphate and Rab5 binding. Critical role of this dual interaction for endosomal localization. *J. Biol. Chem.* **275**, 3699–3705 (2000).
48. Christoforidis, S. *et al.* Phosphatidylinositol 3-OH kinases are Rab5 effectors. *Nature Cell Biol.* **1**, 249–252 (1999).
- The identification of two distinct lipid kinases as effectors of a Rab protein linked the Rab field to signal transduction and strengthened the idea that Rabs regulate the assembly of specialized lipid and protein microdomains.**
49. Vanhaesebroeck, B., Leeyers, S. J., Panayotou, G. & Waterfield, M. D. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem. Sci.* **22**, 267–272 (1997).
50. Rameh, L. E. & Cantley, L. C. The role of phosphoinositide 3-kinase lipid products in cell function. *J. Biol. Chem.* **274**, 8347–8350 (1999).
51. Siddhanta, U., McIlroy, J., Shah, A., Zhang, Y. & Backer, J. M. Distinct roles for the p110 α and hVPS34 phosphatidylinositol 3'-kinases in vesicular trafficking, regulation of the actin cytoskeleton, and mitogenesis. *J. Cell Biol.* **143**, 1647–1659 (1998).
52. Schu, P. V. *et al.* Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science* **260**, 88–91 (1993).
- First demonstration of a functional connection between PI(3)K and membrane transport.**
53. Volinia, S. *et al.* A human phosphatidylinositol 3-kinase complex related to the yeast Vps34p-Vps15p protein sorting system. *EMBO J.* **14**, 3339–3348 (1995).
54. Burd, C. G. & Emr, S. D. Phosphatidylinositol(3)-phosphate signaling mediated by specific binding to RING FYVE domains. *Mol. Cell* **2**, 157–162 (1998).
55. Gilliooty, D. J. *et al.* Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. *EMBO J.* **19**, 4577–4588 (2000).
56. Nielsen, E. *et al.* Rabenosyn-5, a novel Rab5 effector, is complexed with hVPS45 and recruited to endosomes through a FYVE finger domain. *J. Cell Biol.* **151**, 601–612 (2000).
57. Wilson, J. M. *et al.* EEA1, a tethering protein of the early sorting endosome, shows a polarized distribution in hippocampal neurons, epithelial cells, and fibroblasts. *Mol. Biol. Cell* **11**, 2657–2671 (2000).
- References 57, 68–70 support the idea that Rab proteins and their effectors are compartmentalized in early endosomes and show a non-random distribution.**
58. McBride, H. M. *et al.* Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. *Cell* **98**, 377–386 (1999).
59. Mukherjee, S. & Maxfield, F. R. Role of membrane organization and membrane domains in endocytic lipid trafficking. *Traffic* **1**, 203–211 (2000).
60. Simons, K. & Ikonen, E. Functional rafts in cell membranes. *Nature* **387**, 569–572 (1997).
61. Domin, J., Gaidarov, I., Smith, M. E., Keen, J. H. & Waterfield, M. D. The class II phosphoinositide 3-kinase PI3K-C2 α is concentrated in the *trans*-Golgi network and present in clathrin-coated vesicles. *J. Biol. Chem.* **275**, 11943–11950 (2000).
62. Wurmser, A. E. & Emr, S. D. Phosphoinositide signaling and turnover: PtdIns(3)P, a regulator of membrane traffic, is transported to the vacuole and degraded by a process that requires luminal vacuolar hydrolase activities. *EMBO J.* **17**, 4930–4942 (1998).
63. Gary, J. D., Wurmser, A. E., Bonangelino, C. J., Weisman, L. S. & Emr, S. D. Fab1p is essential for PtdIns(3)P 5-kinase activity and the maintenance of vacuolar size and membrane homeostasis. *J. Cell Biol.* **143**, 65–79 (1998).
64. Blondeau, F. *et al.* Myotubularin, a phosphatase deficient in myotubular myopathy, acts on phosphatidylinositol 3-kinase and phosphatidylinositol 3-phosphate pathway. *Hum. Mol. Genet.* **9**, 2223–2229 (2000).
65. De Matteis, M. A. & Morrow, J. S. The role of ankyrin and spectrin in membrane transport and domain formation. *Curr. Opin. Cell Biol.* **10**, 542–549 (1998).
66. Shorter, J. *et al.* GRASP55, a second mammalian GRASP protein involved in the stacking of Golgi cisternae in a cell-free system. *EMBO J.* **18**, 4949–4960 (1999).
67. Roberts, R. L. *et al.* Endosome fusion in living cells overexpressing GFP-rab5. *J. Cell Sci.* **112**, 3667–3675 (1999).
68. Sonnichsen, B., De Renzis, S., Nielsen, E., Rietdorf, J. & Zerial, M. Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. *J. Cell Biol.* **149**, 901–914 (2000).
69. Sheff, D. R., Daro, E. A., Hull, M. & Mellman, I. The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions. *J. Cell Biol.* **145**, 123–139 (1999).
70. Trischler, M., Stoorvogel, W. & Ullrich, O. Biochemical analysis of distinct Rab5- and Rab11-positive endosomes along the transferrin pathway. *J. Cell Sci.* **112**, 4773–4783 (1999).
71. Gruenberg, J. & Maxfield, F. R. Membrane transport in the endocytic pathway. *Curr. Opin. Cell Biol.* **7**, 552–563 (1995).
72. van der Sluijs, P. *et al.* The small GTP-binding protein rab4 controls an early sorting event on the endocytic pathway. *Cell* **70**, 729–740 (1992).
73. Ullrich, O., Reinsch, S., Urbé, S., Zerial, M. & Parton, R. G. Rab11 regulates recycling through the pericentriolar recycling endosome. *J. Cell Biol.* **135**, 913–924 (1996).
74. Vitale, G. *et al.* Distinct Rab-binding domains mediate the interaction of Rabaptin-5 with GTP-bound Rab4 and Rab5. *EMBO J.* **17**, 1941–1951 (1998).
75. Simons, K. & Gruenberg, J. Jamming the endosomal system: lipid rafts and lysosomal storage diseases. *Trends Cell Biol.* **10**, 459–462 (2000).
76. Burd, C. G., Babst, M. & Emr, S. D. Novel pathways, membrane coats and PI kinase regulation in yeast lysosomal trafficking. *Semin. Cell Dev. Biol.* **9**, 527–533 (1998).
77. Nakamura, N., Hirata, A., Ohsumi, Y. & Wada, Y. Vam2/Vps41p and Vam6/Vps39p are components of a protein complex on the vacuolar membranes and involved in the vacuolar assembly in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **272**, 11344–11349 (1997).
78. Grindstaff, K. K. *et al.* Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* **93**, 731–740 (1998).
79. Shin, D. M., Zhao, X. S., Zeng, W., Mozhayeva, M. & Muallem, S. The mammalian Sec6/8 complex interacts with Ca²⁺ signaling complexes and regulates their activity. *J. Cell Biol.* **150**, 1101–1112 (2000).
80. Hazuka, C. D. *et al.* The sec6/8 complex is located at neurite outgrowth and axonal synapse-assembly domains. *J. Neurosci.* **19**, 1324–1334 (1999).
81. White, J. *et al.* Rab6 coordinates a novel Golgi to ER retrograde transport pathway in live cells. *J. Cell Biol.* **147**, 743–760 (1999).
82. Pevsner, J. *et al.* Specificity and regulation of a synaptic vesicle docking complex. *Neuron* **13**, 353–361 (1994).
83. Yang, B., Steegmaier, M., Gonzalez, L. C., Jr & Scheller, R. H. nSec1 binds a closed conformation of syntaxin1A. *J. Cell Biol.* **148**, 247–252 (2000).
84. Burd, C. G., Peterson, M., Cowles, C. R. & Emr, S. D. A novel Sec18p/NSF-dependent complex required for Golgi-to-endosome transport in yeast. *Mol. Biol. Cell* **8**, 1089–1104 (1997).
85. Peterson, M. R., Burd, C. G. & Emr, S. D. Vac1p coordinates Rab and phosphatidylinositol 3-kinase signaling in Vps45p-dependent vesicle docking/fusion at the endosome. *Curr. Biol.* **9**, 159–162 (1999).
86. Tall, G. G., Hama, H., DeWald, D. B. & Horadzovsky, B. F. The phosphatidylinositol 3-phosphate binding protein Vac1p interacts with a Rab GTPase and a Sec1p homologue to facilitate vesicle-mediated vacuolar protein sorting. *Mol. Biol. Cell* **10**, 1873–1889 (1999).
87. Sato, T. K., Rehling, P., Peterson, M. R. & Emr, S. D. Class C Vps protein complex regulates vacuolar SNARE pairing and is required for vesicle docking/fusion. *Mol. Cell* **6**, 661–671 (2000).
88. Orci, L., Perrelet, A. & Rothman, J. E. Vesicles on strings: morphological evidence for processive transport within the Golgi stack. *Proc. Natl Acad. Sci. USA* **95**, 2279–2283 (1998).
89. Wiley, D. C. & Skehel, J. J. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annu. Rev. Biochem.* **56**, 365–394 (1987).
90. Uyeda, T. Q., Abramson, P. D. & Spudich, J. A. The neck region of the myosin motor domain acts as a lever arm to generate movement. *Proc. Natl Acad. Sci. USA* **93**, 4459–4464 (1996).
91. Howard, J. & Spudich, J. A. Is the lever arm of myosin a molecular elastic element? *Proc. Natl Acad. Sci. USA* **93**, 4462–4464 (1996).
92. Mahadevan, L. & Matsudaira, P. Motility powered by supramolecular springs and ratchets. *Science* **288**, 95–100 (2000).
93. Colombo, M. I., Beron, W. & Stahl, P. D. Calmodulin regulates endosome fusion. *J. Biol. Chem.* **272**, 7707–7712 (1997).
94. Koda, T., Zheng, J. Y. & Ishibe, M. Rab33B regulates intra-Golgi transport and utilizes a novel kinesin as an effector. *39th Annual Meeting of the American Society for Cell Biology Abstract no. 1244, 214a* (Washington, D. C., 1999).
95. Imamura, H. *et al.* Rho and Rab small G proteins coordinately reorganize stress fibers and focal adhesions in MDCK cells. *Mol. Biol. Cell* **9**, 2561–2575 (1998).
96. Chavrier, P. & Goud, B. The role of ARF and Rab GTPases in membrane transport. *Curr. Opin. Cell Biol.* **11**, 466–475 (1999).
97. Dirac-Svejstrup, A. B., Sumizawa, T. & Pfeffer, S. R. Identification of a GDI displacement factor that releases endosomal Rab GTPases from Rab-GDI. *EMBO J.* **16**, 465–472 (1997).
98. Ayad, N., Hull, M. & Mellman, I. Mitotic phosphorylation of rab4 prevents binding to a specific receptor on endosome membranes. *EMBO J.* **16**, 4497–4507 (1997).
99. Bucci, C., Chiariello, M., Lattaro, D., Maiorano, M. & Bruni, C. B. Interaction cloning and characterization of the cDNA encoding the human prenylated rab acceptor (PRA1). *Biochem. Biophys. Res. Commun.* **258**, 657–662 (1999).
100. Matern, H. *et al.* A novel Golgi membrane protein is part of a GTPase-binding protein complex involved in vesicle targeting. *EMBO J.* **19**, 4485–4492 (2000).
101. Hoffenberg, S. *et al.* A novel membrane-anchored Rab5 interacting protein required for homotypic endosome fusion. *J. Biol. Chem.* **275**, 24661–24669 (2000).
102. Mu, F. T. *et al.* EEA1, an early endosome-associated protein. EEA1 is a conserved α -helical peripheral membrane protein flanked by cysteine 'fingers' and contains a calmodulin-binding IQ motif. *J. Biol. Chem.* **270**, 13503–13511 (1995).
103. Hartwell, L. H., Hopfield, J. J., Leibler, S. & Murray, A. W. From molecular to modular cell biology. *Nature* **402**, C47–C52 (1999).
104. Carr, C. M., Grote, E., Munson, M., Hughson, F. M. & Novick, P. J. Sec1p binds to SNARE complexes and concentrates at sites of secretion. *J. Cell Biol.* **146**, 333–344 (1999).
105. Simonsen, A., Gaullier, J. M., D'Arrigo, A. & Stenmark, H. The Rab5 effector EEA1 interacts directly with syntaxin-6. *J. Biol. Chem.* **274**, 28857–28860 (1999).

Acknowledgments

We thank members of the Zerial lab, R. Lippe, M. Miaczynska and S. De Renzis, as well as our colleagues K. Simons, J. Gruenberg, G. Griffiths, J. Howard for their helpful comments and critical reading of the manuscript. We are grateful to I. Kastner for superb secretarial help.



Marino Zerial did his biology studies in Triest (Italy) where he worked on lysosomal storage disorders. He conducted postdoctoral work in Paris with Giorgio Bernardi (1983; Institut Jacques Monod) and in Heidelberg at the EMBL with Henrik Garoff (1985). From 1989, he was an EMBL staff scientist with Kai Simons. Then, in 1991, he became a group leader and, in 1998, a Director in the new Max Planck Institute MPI-CGB in Dresden. He is interested in the mechanisms of endocytosis and cell polarity.

Heidi McBride began her career in 1991 as a graduate student working in the field of mitochondrial biogenesis in Gordon Shore's laboratory at McGill University in Montreal, Quebec. In 1996, she moved to the EMBL in Heidelberg to complete her training as a cell biologist working with Marino Zerial. In 2000, Heidi returned home as Assistant Professor at the University of Ottawa Heart Institute in Canada.

RAB1

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5861>

RAB3

<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab3a%20or%20rab3b%20or%20rab3d%20not%20interacting&ORG=Hs>

RAB4

<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab4%20or%20rab4b&ORG=Hs>

RAB5

<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab5a%20or%20rab5b%20or%20rab5c&ORG=Hs>

RAB6

<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab6%20or%20rab6b%20not%20interacting%20not%20similar%20not%20activating&ORG=Hs>

RAB8

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=4218>

RAB9

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=9367>

RAB11

<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab11a%20or%20rab11b&ORG=Hs>

RAB13

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5872>

Rab33b

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19338>

p115

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=8615>

PRAl

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=10567>

rabphilin3

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19894>

calmodulin

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=801>

rabaptin 5

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=9135>

EEA1

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=8411>

p110 β

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5291>

VAMP2

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=6844>

α -actinin

http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=actn*&ORG=Hs

Rabex 5

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=27342>

syntaxin 13

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=23673>

syntaxin 6

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=10228>

hVps34

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5289>

p150

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=30849>

p85 α

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5290>

rabenosyn-5

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=64145>

rabkinesin-6

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19348>

RAB8IP

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5871>

p40

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=10244>

Rab11bp

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=54521>

VPS45

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=11311>

Sec13r

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=20332>

MSS4

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5877>

RAB3GAP

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=22930>

Tuberous sclerosis 2

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=7249>

EPS8

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=2059>

δ -PDE

<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5147>

YEAST GENES

Sec4

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=sec4>

Exocyst

<http://genome-www4.stanford.edu/cgi-bin/SGD/GO/go.pl?goid=145>

Sec15

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=sec15>

Vps45

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps45>

Pep12p

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=pep12>

Vam3p

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vam3>

Vac1p

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=Nucleotide&list_uids=173156&dopt=GenBank

Vps39p

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps39>

Vps41p

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps41>

Yip1p

<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=yip1>

Yif1
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=yif1>

Ypt51p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=ypt51>

Ypt6p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=ypt6>

Ypt7p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=ypt7>

Ypt1
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=ypt1>

Dss4p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=dss4>

Gyp1
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=gyp1>

Gyp2
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=gyp2>

Gyp3
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=gyp3>

Gyp6
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=gyp6>

Vps9p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps9>

Sec2p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=sec2>

Gyp7p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=gyp7>

TRAPP intermediate page

Bet3
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=bet3>

Bet5
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=bet5>

Trs20
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs20>

Trs23
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs23>

Trs 31
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs31>

Trs33
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs33>

Trs65
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs65>

Trs85
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs85>

Trs120
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs120>

Trs130
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=trs130>

HOPS complex intermediate page

Vps11p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps11>

Vps16p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps16>

Vps18p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps18>

Vps33p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps33>

Vps39p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps39>

Vps41p
<http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=vps41>

RAB13
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5872>

Rab21
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19333>

Rab6a,b
<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab6%20or%20rab6b%20not%20interacting%20not%20similar%20not%20activating&ORG=Hs>

Rab10
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=10890>

Rab12
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19328>

Rab30
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=27314>

Rab33b
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19338>

Rab36
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=9609>

RAB6a,b
<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab6%20or%20rab6b%20not%20interacting%20not%20similar%20not%20activating&ORG=Hs>

RAB1
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5861>

Rab2
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5862>

RAB3
<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab3a%20or%20rab3b%20or%20rab3d%20not%20interacting&ORG=Hs>

RAB8
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=4218>

Rab26
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=25837>

Rab37
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=58222>

RAB11
<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab11a%20or%20rab11b&ORG=Hs>

Rab17
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19329>

RAB9
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=9367>

Rab24
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19336>

RAB5
<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab5a%20or%20rab5b%20or%20rab5c&ORG=Hs>

RAB4
<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=rab4%20or%20rab4b&ORG=Hs>

Rab25
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=53868>

Rab18
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=22931>

Rab20
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19332>

Rab22
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19334>

RAB27A
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5873>

Rab33b
<http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=19338>

Zerial Lab home page
<http://www.mpi-cbg.de/content.php3?lang=en&aktID=zerial>
University of Ottawa Heart Institute
<http://www.ottawaheart.ca/hcwelcome.htm>