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Rab Family of GTPases

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Abstract

Rab proteins represent the largest branch of the Ras-like small GTPase superfamily and there are 66 Rab genes in the human genome. They alternate between GTP- and GDP-bound states, which are facilitated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), and function as molecular switches in regulation of intracellular membrane trafficking in all eukaryotic cells. Each Rab targets to an organelle and specify a transport step along exocytic, endocytic, and recycling pathways as well as the crosstalk between these pathways. Through interactions with multiple effectors temporally, a Rab can control membrane budding and formation of transport vesicles, vesicle movement along cytoskeleton, and membrane fusion at the target compartment. The large number of Rab proteins reflects the complexity of the intracellular transport system, which is essential for the localization and function of membrane and secretory proteins such as hormones, growth factors, and their membrane receptors. As such, Rab proteins have emerged as important regulators for signal transduction, cell growth, and differentiation. Altered Rab expression and/or activity have been implicated in diseases ranging from neurological disorders, diabetes to cancer.

Keywords

Rab; GTPase; GTP-binding protein; Membrane trafficking; Vesicular transport; GAP; GEF; Effector

1 Introduction

Rab GTPases play an important role in specifying transport pathways in the intracellular membrane trafficking system of all eukaryotes from the last eukaryotic common ancestor (LECA) to mammals. In the LECA, there are at least 20 prototype Rabs forming six groups, e.g., Rab1/Ypt1 and Rab8/Sec4 in group I, Rab5/Ypt51 in group II, Rab7/Ypt7 and Rab9/Ypt9 in group III, Rab11/Ypt31 and Rab4/Ypt4 in group IV, Rab6/Ypt6 in group V, and Rab28 in group VI [1, 2] (Fig. 1). Most of these ancient Rabs are conserved throughout evolution while some are lost in certain species. There is significant expansion of the Rab family in mammalian cells to accommodate increasing complexity of the intracellular trafficking system, with 66 Rab genes in the human genome. Historically Rab GTPases are best characterized in the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) and in

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mammalian cells [3], and evolutionarily older Rabs tend to be more highly and widely expressed and more intensively studied [4]. This volume focuses on the techniques used for biochemical and functional characterizations of these *S. cerevisiae* and mammalian Rabs.

The intracellular membrane trafficking system governs protein secretion during exocytosis and uptake of extracellular nutrients during endocytosis in eukaryotic cells. It is also a fundamental transport system for targeting newly synthesized enzymes to correct membrane compartments/organelles, e.g., the lysosomal hydrolases. As such, it is essential for cell physiology. Intracellular membrane trafficking is mediated by vesicular carriers from donor to acceptor compartments and Rab GTPases are involved in every facet of the vesicular transport process via temporal and spatial interactions with a series of effectors [5, 3, 6]. These effectors include cargo proteins to be packaged into the vesicles, motor proteins that facilitate the movement of vesicles along actin and microtubule cytoskeletons, and tethering factors that dock vesicles to target compartments for membrane fusion.

Each Rab specifically targets to a distinct membrane compartment [7], e.g., Rab2 to the transport vesicles between the endoplasmic reticulum (ER) and the Golgi, Rab5 to early endosomes, Rab7 to late endosomes, etc. (Fig. 2). This membrane targeting process requires posttranslational isoprenylation (geranylgeranylation) of the two Cys residues at or near the C-terminus of each Rab as well as a cognate guanine nucleotide exchange factor (GEF) on the target membrane [8] (Fig. 3). In addition, a GDP displacement factor (GDF) is shown to facilitate Rab membrane targeting to endosomes [9] (Fig. 3). Upon membrane association, the GEF catalyzes nucleotide exchange of GDP with GTP on the Rab [10, 11], and the activated GTP-bound Rab then interacts with effectors and is packaged into transport vesicles to mediate the formation and movement of vesicles and their fusion with the target compartment (Fig. 3). The Rab functional cycle is completed by GTP hydrolysis, which is catalyzed by GTPase-activating proteins (GAPs) [12], and recycling back to the donor compartment, which is mediated by the GDP-dissociation inhibitor (GDI) [13–15] (Fig. 3).

2 Regulation of Rab GTPase Cycle

Like other small GTPases in the Ras superfamily, Rabs show high affinity for guanine nucleotides GTP and GDP (Kd in the nanomolar range) but weak intrinsic GTPase activity in GTP hydrolysis. As a result, both the GDP/GTP exchange reaction and the GTP hydrolysis reaction in a Rab GTPase cycle are accelerated by catalyzing protein factors such as GEFs and GAPs in the cell [12] (Fig. 3).

Rab GEFs show a general mechanism by displacing the switch I region, disrupting Mg^{2+} coordination, and stabilizing the nucleotide-free form of Rab proteins [12]. As such, the GEFs facilitate GDP dissociation and GTP loading on Rabs in the cell where GTP concentration is two orders of magnitude higher. However, the five families of Rab GEFs identified so far share no sequence and structural homology in the catalytic domain. The Vps9 domain-containing GEFs are specific for the Rab5 subfamily members on early endosomes [16] while the SAND1/Mon1-Ccz1 complex is a specific GEF for Rab7/Ypt7 on late endosomes [17, 18]. These endosomal GEFs promote endocytosis by activation of Rab5 and Rab7. For exocytosis, there are TRAPP complexes [19, 20] and Sec2/Rabin8 proteins

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[21] that are GEFs for Rab1/Ypt1 and Rab8/Sec4 to promote ER to Golgi transport and post-Golgi transport to the plasma membrane, respectively. In addition, the Ric1/Rgp1 complex is a GEF for Ypt6/Rab6 in the Golgi complex [22]. Finally, the DENN (differentially expressed normal vs. neoplastic) domain-containing GEFs [23] are specific for various Rabs that have no close yeast homologs, such as Rab3, Rab9, Rab10, Rab12, Rab27, Rab28, Rab35, and Rab39.

Rab GAPs, in contrast, contain a TBC (Tre-2/Bud2/Cdc16) domain for catalysis of GTP hydrolysis [12]. The TBC domain contains conserved catalytic motifs IxxDxxR and YxQ from which the Arg and Gln side chains insert into the GTP-binding site on the Rab to stabilize the transition state for GTP hydrolysis in a so-called dual finger mechanism [24].

The GEFs and GAPs are recruited to distinct organelles by proteins and lipids characteristic to each organelle to facilitate the establishment of functional Rab domains on the membrane. In a number of cases, a GEF is recruited to the membrane by an upstream Rab for activation of a downstream Rab, forming a Rab cascade (Fig. 4). For example, Sec2, a GEF for Sec4 in the budding yeast *S. cerevisiae*, is an effector of the upstream Rab Ypt32 and is recruited by Ypt32-GTP to post-Golgi vesicles for activation of Sec4 in exocytosis [25] (Fig. 4). Their mammalian homologs form a similar Rab cascade where Rab11-GTP recruits Rabin8 to secretory vesicles for activation of Rab8 to facilitate cilia biogenesis in mammalian cells [26] (Fig. 4). In addition, the Ric1/Rgp1 complex is recruited by Rab33B to the Golgi membrane to function as a GEF for Rab6 activation [27] (Fig. 4). Along the endocytic pathway, the Sand1/Mon1–Ccz1 complex is a GEF for Rab7 but an effector of the upstream Rab5 [18, 17]. As such, it is recruited by Rab5-GTP to the endosomal membrane for activation of Rab7 (Fig. 4). With the displacement of Rabex5, a Vps9 domain-containing Rab5 GEF, from the membrane, Rab5-GTP undergoes GTP hydrolysis and converts to Rab5-GDP that is removed from the membrane by GDI, leading to the conversion of early endosomes marked by Rab5 to late endosomes marked by Rab7. Interestingly, Rabex-5 itself can be recruited by another upstream Rab, Rab22, to early endosomes to establish a Rab22–Rabex-5–Rab5 cascade within the early endosomal network [28] (Fig. 4). In addition, within a late endosomal/premelanosomal network, there exists a Rab9–BLOC-3–Rab32/Rab38 cascade where Rab9-GTP recruits BLOC-3 to the membrane to function as a GEF for activation of Rab32/Rab38 [29, 30] (Fig. 4).

In contrast to the GEFs recruited by upstream Rabs, a GAP may be recruited by a downstream Rab to the membrane for inactivation of an upstream Rab to establish the boundary between the functional Rab domains. In the budding yeast, it is reported that a Ypt1 GAP, Gyp1, is recruited by a downstream Rab, Ypt32, to the membrane to inactivate and clear Ypt1 from the Ypt32 membrane domain [31]. In mammalian cells, Rab9 is shown to recruit the GAPs (RUTBC1 and RUTBC2) to late endosomes for inactivation of Rab32 and Rab36 on the membrane [32, 33].

The combination of a GEF and a GAP recruited in such a fashion by upstream and downstream Rabs can effectively sharpen the boundary of Rab membrane domains and facilitate the transition from early to late compartments during intracellular transport [34]. It may also generate ultrasensitivity and “all-or-none” switch-like behavior in the Rab activity

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[35, 36]. In addition to the Rabs, other protein and lipid factors in the membrane are also known to regulate the recruitment and activity of Rab GEFs, which is exemplified by the regulation of Sec2/Rabin8 by phosphatidylinositol-4-phosphate (PI4P) [37] and phosphorylation [38].

3 Rab Functions in Vesicular Transport

Once activated and GTP bound, Rabs can temporally and spatially interact with multiple effectors to facilitate the selection of cargoes into vesicles, vesicle movement on actin and microtubule cables, and tethering of vesicles to target compartment for membrane fusion.

Rabs can interact with the cytoplasmic domains of transmembrane proteins/receptors to facilitate their packaging into transport vesicles. Rab5 and Rab21 on the early endocytic pathway directly bind to the α subunit of β1 integrins and promote their endocytosis and recycling to remodel the cell surface for migration and cytokinesis [39, 40]. In addition, Rab5 also directly interacts with angiotensin II Type 1A receptor ($AT_{1A}R$) to facilitate its endocytic trafficking [41]. On the exocytic pathway, Rab3b is shown to bind directly to polymeric IgA receptor (pIgR) to modulate its transcytosis in polarized epithelial cells [42]. Furthermore, some Rabs are involved in packaging of cargo proteins into transport vesicles through interactions with adaptor proteins. In this regard, Rab5 is shown to concentrate transferrin receptor into coated pits for endocytosis [43], while Rab9 facilitates the recruitment of the cargo protein (mannose-6-phosphate receptor) into late-endosome derived transport vesicles via its effector TIP47 [44].

Rabs are also known to interact with actin and microtubule motor proteins such as myosins, kinesins, and dyneins to facilitate the movement of transport vesicles on the actin and microtubule cytoskeleton. Class V myosins are actin motors that consist of an N-terminal actin-binding motor domain and a C-terminal cargo-binding globular tail domain (GTD), which can bind to a number of Rabs on post-Golgi secretory vesicles or recycling endosomes and get recruited to these exocytic compartments [45]. These exocytic and recycling Rabs are more closely related in evolution and belong to groups I, IV, and V, including Rab3, Rab6, Rab8/Sec4, Rab10, Rab11, Rab14, Rab25, and Rab39 [1, 46, 2, 45]. In addition, Rab27 indirectly recruits myosin V to melanosomes via a linker protein Slac2/melanophilin [47, 48]. The Rab-myosin V interaction links transport vesicles to actin and facilitate their movement toward the cell surface. These Rabs are also known for recruitment of microtubule-based kinesin and dynein motors, especially Rab6 that directly binds to both kinesin (KIF20A) and dynein (DYNLRB1 and dynactin) [49–51]. Rab14 also directly binds to a kinesin, kinesin-3 (KIF16B) [52]. Some of the Rabs interact with kinesins and dyneins indirectly via linker proteins, e.g., Rab11 proteins can recruit kinesin-1, kinesin-2, dynein LIC1, and dynein LIC2 via Rab11 effectors FIP3 and FIP5 [53–56]. Another interesting example is the endocytic Rab5 that binds and activates one of its effectors hVps34, a PI 3 kinase, and its product PI3P on the membrane in turn recruits the kinesin KIF16B [57]. This plus-end microtubule motor may play a role in the peripheral distribution of Rab5-positive early endosomes, suggesting the necessity of Rab5 removal for transition to late endosomes and movement toward perinuclear region.

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Another important Rab function in vesicular transport is to tether transport vesicles to target compartments for membrane fusion. In this regard, Rab5/Vps21 is shown to tether vesicles directly via Rab–Rab interaction *in trans* [58]. However, the tethering function is more commonly performed by Rab effectors including long coiled-coil homodimers and large multi-subunit complexes. The former may be exemplified by the Rab5 effectors EEA1/Vac1 and Rabenosyn-5 [59–61] and the Rab1/Ypt1 effectors p115/Uso1 [62, 63], while the latter include the Sec4/Rab8 effector exocyst [64, 65], the Rab5/Vps21 effector CORVET (class C core vacuole/endosome tethering) complex [66], the Rab7/Ypt7 effector HOPS (homotypic fusion and vacuole protein sorting) complex [67], and the Rab1/Ypt1 effectors TRAPPI and TRAPPII complexes [68, 69]. These tethering factors are recruited by the Rabs to mediate vesicle docking and often interact with the SM (Sec1–Munc18) proteins to facilitate the assembly of SNARE complexes for membrane fusion. For example, Rabenosyn-5 contains an N-terminal FYVE domain for binding to PI3P on endosomes and a C-terminal Rab5-binding domain for tethering Rab5-positive vesicles. Furthermore, Rabenosyn-5 interacts with hVps45, a SM protein, to facilitate SNARE-mediated membrane fusion [59].

4 Other Rab Functions

The large number of effectors for each Rab, e.g., more than 20 for Rab5 [70], suggests that Rabs may have additional functions beyond intracellular membrane trafficking. Indeed, Rabs play important roles in signal transduction and autophagy. Some of the Rab5 effectors are signaling molecules such as APPL1 and APPL2, which are recruited to early endosomes by Rab5 [71, 72] and in turn recruit Akt and modulate its phosphorylation specificity for GSK-3β rather than TSC2 [73]. This Rab5-mediated APPL signaling on endosomes is essential for cell survival and development in zebrafish [73]. Another Rab5 effector is Vps34 [74], a class III PI 3-kinase that produces PI3P on early endosomes and promotes autophagosome formation during autophagy [75–77]. Vps34 is also an effector for the late endosome-associated Rab7 [78] and may play a similar role in autophagy on late endosomes. In addition, the exocytic Rab1/Ypt1 is also known for its essential role in the formation of preautophagosomal structure (PAS) via TRAPPIII complex during the initiation of autophagy [79–81].

5 Rabs and Disease

The fundamental function of Rabs and membrane trafficking in cell physiology is reflected by various diseases due to mutations or altered expression of Rab genes. Mutations in five of the 66 human Rab genes (Rab7, Rab23, Rab27, Rab38, and Rab39b) are known to cause genetic disorders. Among them, Rab7 is ubiquitously expressed in all tissues while the other four Rabs are expressed only in certain cell types and tissues. Importantly, they are also different in the nature of mutations.

Four gain-of-function mutations in Rab7 are linked to Charcot–Marie–Tooth Type 2B (CMT2B) disease [82–84], which is a form of hereditary motor and sensory neuropathy with symptoms of distal sensory loss and muscle weakness, leading to toe ulcers, infections, and ultimately amputation [85]. These gain-of-function mutations enhance Rab7 activity by increasing the nucleotide exchange reaction independent of GEFs [86, 87]. It is worth noting

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that enhanced Rab7 activity affects mainly peripheral neurons and CMT2B is a neurological disease, despite the ubiquitous expression of Rab7 in all tissues.

Mutations in the other four Rabs that lead to autosomal recessive disorders are all loss-of-function mutations. Mutations in Rab23 are linked to Carpenter syndrome [88], which is a neurological disorder of craniosynostosis and limb malformation. Rab23 is highly expressed in the brain and neurons [89] and is localized on the plasma membrane and early endosomes [90] involved in sorting and function of signaling molecules in Sonic Hedgehog signal transduction [91]. The *open brain* (*opb*) gene that inhibits the Sonic Hedgehog signaling in mice is mapped to Rab23 and the *opb* mouse model recapitulates some of the neurological defects of Carpenter syndrome [91]. Mutations in Rab27 are linked to Griscelli syndrome, which is an immunological disorder with excessive T lymphocyte and macrophage activation called hemophagocytic syndrome as well as defects in skin pigmentation [92, 93]. Rab27 is expressed in highly secretory cells such as cytotoxic T lymphocytes (CTL) [94] and melanocytes [95, 96] and localized to secretory granules and melanosomes in these cell types. Inactivation of Rab27 by the mutations blocks the transport and function of the secretory granules and melanosomes and contributes to the hemophagocytic syndrome and partial albinism in Griscelli patients [93]. This phenotype can be recapitulated by a Rab27 mutation in *ashen* mice [97]. Rab38 is one of multiple genes linked to Hermansky–Pudlak syndrome caused by defective melanocytes and platelets [98]. *Chocolate* mice [99] and *Fawn-hooded and Tester-Moriyama* rats [100] are animal models for Hermansky–Pudlak syndrome and they contain inactivating mutations in the Rab38 gene. A cell biology study suggests that Rab38 is essential for the biogenesis of melanosomes [101]. Finally, Rab39b is specifically expressed in the brain and neurons and mutations in Rab39b are associated with one form of X-linked mental retardation (XLMR) [102].

In addition to mutations, many Rabs show altered expression level or activity in such diseases as cancer, Alzheimer's disease, and diabetes. It appears a common theme that a Rab may be up-regulated in certain types of cancers but down-regulated in other types of cancers [4]. For example, Rab25 is known to promote α5β1 integrin recycling in epithelial cells [103] and overexpression of Rab25 is associated with aggressiveness of ovarian and breast cancers [104], suggesting a role for Rab25 in cancer cell invasion and metastasis. However, Rab25 is down-regulated in colon cancer with poor patient prognosis [105], suggesting a tumor suppressor function. Indeed, Rab25 deficiency in mouse models of colon cancer promotes colonic tumor growth [105]. The reconciliation of this apparent conflict over Rab25 function in promoting or blocking tumor growth in different cancers may involve the CLIC3 protein, which is necessary for Rab25-mediated integrin recycling [106]. It is suggested that Rab25 may sort integrins to lysosomes for degradation in cell types that don't express CLIC3, acting like a tumor suppressor [106]. The opposite may be true in cell types with high levels of CLIC3 where Rab25 can promote integrin recycling and cell migration and invasion [106]. Another example is Rab31, which is overexpressed in breast cancer, brain cancer, skin cancer, and several other types of cancers but is down-regulated in leukemia, lung cancer, and colon cancer [4].

Endocytic Rabs such as Rab5 and Rab7 are overexpressed in hippocampal neurons of Alzheimer's patients [107] and the enhanced endocytic activity is suggested to promote the

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proteolytic processing of amyloid precursor protein (APP) in endosomes [108], which may lead to increased production and accumulation of amyloid-β peptide (Aβ) in the brain, a hallmark of Alzheimer's disease. Recycling Rabs such as Rab10 and Rab14 are activated by insulin signal transduction to promote the translocation of glucose transporter 4 (GLUT4) from intracellular vesicles to the plasma membrane of adipocytes for glucose uptake and metabolism [109]. Rab10 and Rab14 are kept in the inactive GDP-bound state by AS160, a GAP for both Rabs [110]. Upon insulin stimulation, AS160 is inactivated by phosphorylation [111, 112] and consequently Rab10 and Rab14 can be activated by GTP loading to promote docking and fusion of GLUT4-containing vesicles with the plasma membrane [109]. Malfunction of the Rab10- and Rab14-mediated GLUT4 translocation processes is implicated in type II diabetes.

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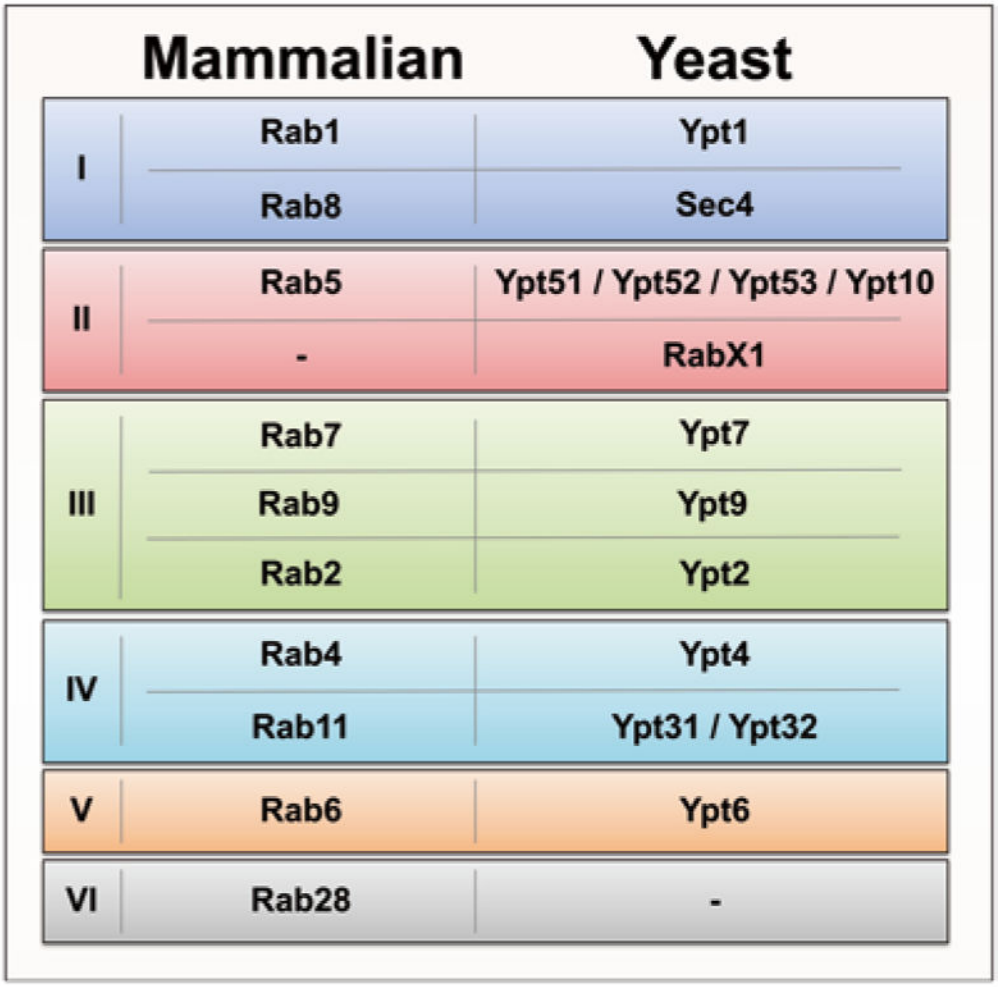

Fig. 1.

The mammalian and yeast Rab/Ypt homologs. Data from Diekmann et al. and Klöpper et al. [1, 2] reveal six major groups of Rab GTPases from the LECA to mammals. Shown are only the mammalian Rabs with yeast Rab/Ypt homologs. RabX1 is not present in mammalian cells, while there are no members of group VI, including Rab28, found in yeast

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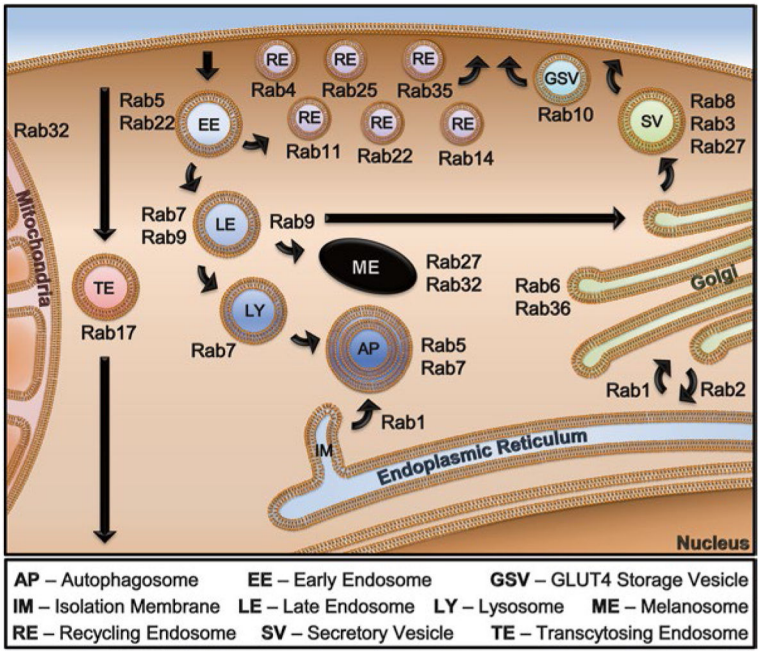

Fig. 2.

Rabs throughout the mammalian cell. Rabs are found in virtually every membranous compartment in eukaryotic cells. Above is a schematic representation of intracellular localization of the Rabs from Fig. 1 and discussed in this volume of MiMB

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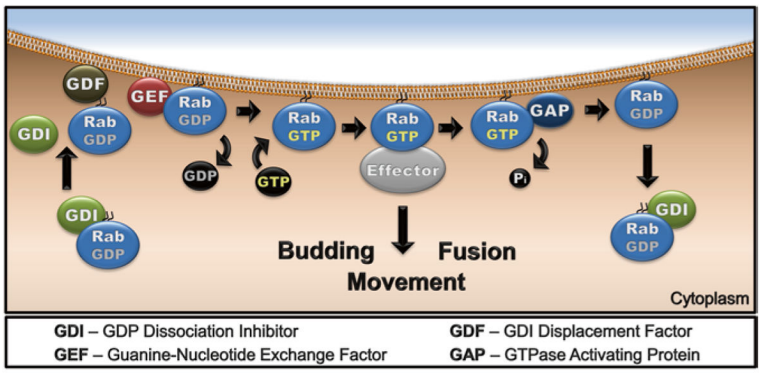

Fig. 3.

The Rab GTPase cycle coupled with membrane targeting. Inactive GDP-bound Rabs are found in the cytosol bound to GDI. Upon approaching the target membrane, GDF may interact with the Rab to facilitate GDI dissociation and Rab insertion into the membrane. On the membrane, GEF catalyzes GDP dissociation, allowing for GTP binding and subsequent activation of the Rab, which in turn interacts with multiple effectors to promote vesicle budding, movement, and fusion. Then GTP hydrolysis by the Rab, accelerated by a cognate GAP, converts it to inactive GDP-bound state. The inactive Rab can be removed from the membrane by GDI and recycled back to the donor compartment

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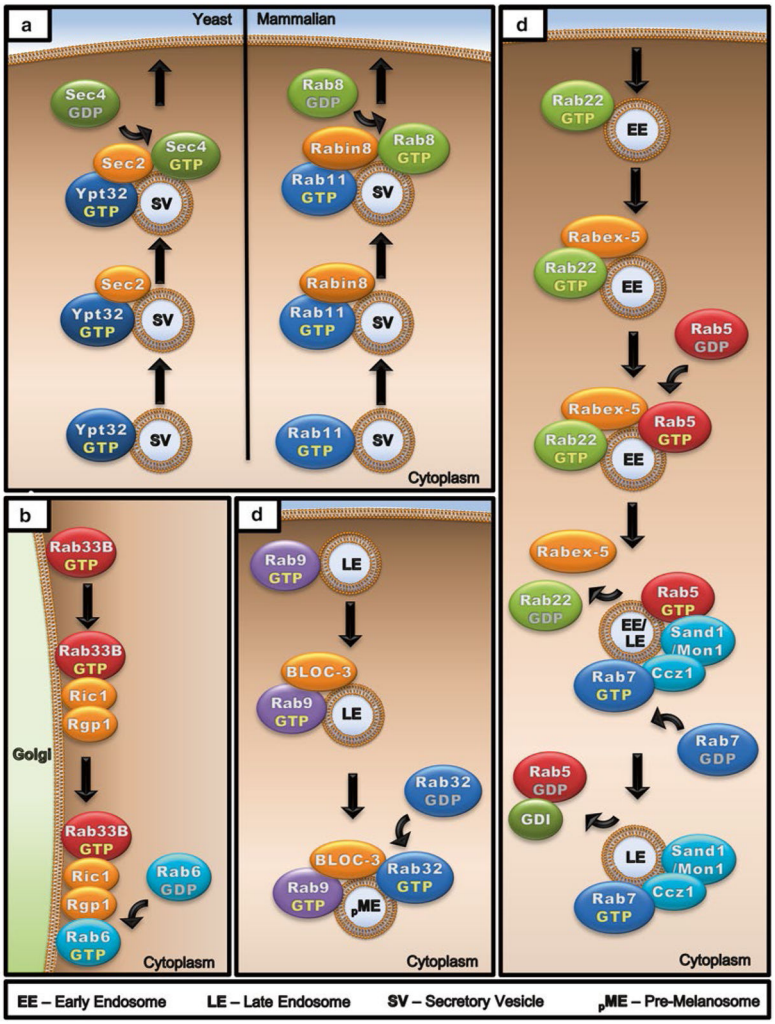

Fig. 4.

Rab activation cascades. The GTP loading and activation of a Rab can be regulated as part of an activation cascade from an upstream Rab. (**a**) Rab activation cascades are evolutionarily conserved from yeast to mammals. During polarized exocytosis in *S. cerevisiae*, activated Ypt32 recruits Sec2, a Sec4 GEF, to the membrane of secretory vesicles destined for exocytosis. Sec2 in turn leads to the recruitment and activation of Sec4. The same cascade is seen in mammalian cells with Rab11, Rabin8 (Rab8 GEF), and Rab8. (**b**) On the Golgi membrane, active Rab33B recruits the Ric1–Rgp1 complex (Rab6 GEF), which activates Rab6. (**c**) On late endosomes, Rab9 recruits Bloc-3 (Rab32/33 GEF) to the membrane and activates Rab32 as they move toward lysosomes or melanosomes. (**d**) Active Rab22 binds and recruits Rabex-5 (Rab5 GEF) and activates Rab5 on early endosomes. Active Rab5 in turn binds Sand1 (Mon1 in Yeast) –Ccz1 complex (Rab7 GEF), which recruits and activates Rab7 to facilitate transition to late endosomes. Upon dissociation of Rab22 and Rabex-5, Rab5 is inactivated by GTP hydrolysis and converted to GDP bound state, which is then removed from the membrane by GDI