Protein family review

The Rab GTPase family

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Summary

The Rab family is part of the Ras superfamily of small GTPases. There are at least 60 Rab genes in the human genome, and a number of Rab GTPases are conserved from yeast to humans. The different Rab GTPases are localized to the cytosolic face of specific intracellular membranes, where they function as regulators of distinct steps in membrane traffic pathways. In the GTP-bound form, the Rab GTPases recruit specific sets of effector proteins onto membranes. Through their effectors, Rab GTPases regulate vesicle formation, actin- and tubulin-dependent vesicle movement, and membrane fusion.

The compartmentalization of eukaryotic cells requires the transport of lipids and proteins between distinct membrane-bounded organelles. This transport is tightly regulated and typically occurs through transport vesicles that bud from a donor compartment and fuse with an acceptor compartment. Rab GTPases ('Ras-related in brain' [1]), which belong to the Ras superfamily of small GTPases, have emerged as central regulators of vesicle budding, motility and fusion. Like other regulatory GTPases, the Rab proteins switch between two distinct conformations, one GTP-bound and the other GDP-bound (see Figure 1). The GTP-bound conformation is generally regarded as 'active' [2], as this is the form that interacts with downstream effector proteins [3].

Gene organization and evolutionary history

A recent analysis of the sequenced human genome and expressed sequence tags indicates that humans have at least 60 different Rab family members (Figure 2) [4]. This must be regarded as a minimum estimate, as a small part of the genome still remains to be sequenced and sequence annotations are still incomplete. Rab genes are widely distributed over the human chromosomes [4]. Rab GTPases have been found in all eukaryotes investigated, including *Saccharomyces cerevisiae* (11 members), *Caenorhabditis elegans* (29 members) and *Drosophila melanogaster* (26

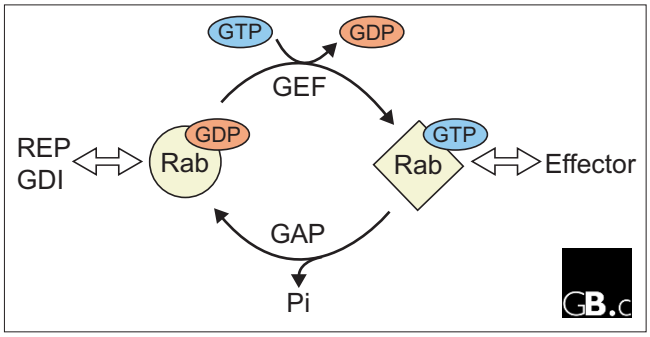

Figure 1
The Rab GTPase cycle. The Rab GTPase switches between GDP- and GTP-bound forms, which have different conformations. Conversion from the GDP- to the GTP-bound form is caused by nucleotide exchange, catalyzed by a GDP/GTP exchange factor (GEF). Conversion from the GTP- to the GDP-bound form occurs by GTP hydrolysis, facilitated by a GTPase-activating protein (GAP). The GTP-bound form interacts with effector molecules, whereas the GDP-bound form interacts with Rab escort protein (REP) and GDP dissociation inhibitor (GDI). Pi, inorganic phosphate.

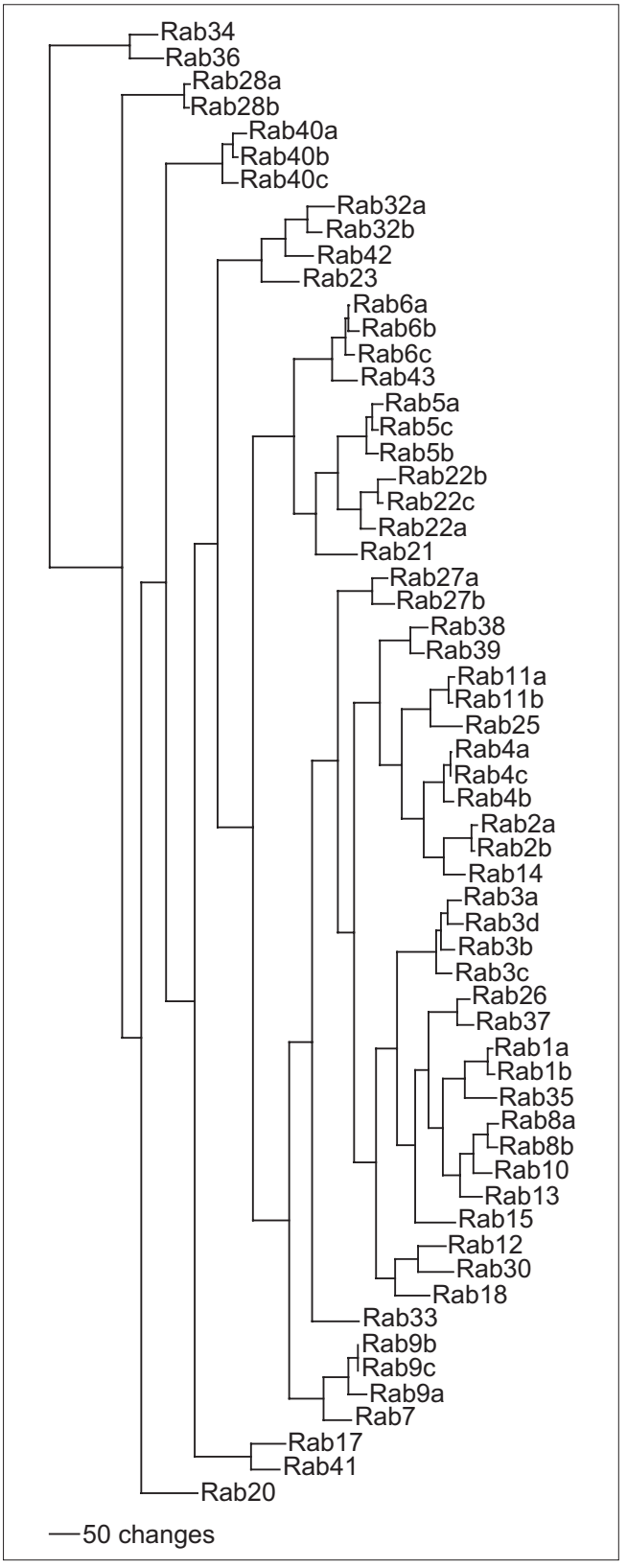

Figure 2
Phylogenetic tree of human Rab GTPases. Adapted with permission from [4].

members) [4]; this large number and wide distribution underlines their importance in eukaryotic cell biology. Most, but not all, of the yeast Rab GTPases (mostly called Ypt proteins) have one or more putative mammalian homologs. In several cases, a mammalian Rab can functionally replace its yeast counterpart, demonstrating conservation of functions of the proteins within the eukaryotes.

Many Rab GTPases seem to be products of gene duplications, given that several subfamilies of closely related Rab GTPases ('isoforms') with 75-95% sequence identity and overlapping functions can be identified (Figure 2) [4]. Ten subfamilies - Rab1, Rab3, Rab4, Rab5, Rab6, Rab8, Rab11, Rab22, Rab27 and Rab40 - have also been defined based on distinct subfamily-specific sequence motifs, but a number of Rab proteins cannot be grouped in these subfamilies [5,6] (see later). In general, Rab GTPases differ most in their carboxyl termini, which have been implicated in subcellular targeting [7], whereas regions involved in guanine-nucleotide binding (see below) are most conserved. Furthermore, mammalian Rab genes generally consist of several exons, and alternative splicing has been reported [8].

Characteristic structural features

High-resolution structural information obtained by X-ray crystallography currently available for four Rab GTPases: mouse Rab3a [9,10], *Plasmodium falciparum* Rab6 [11], and *S. cerevisiae* Ypt51p [12] and Sec4p [13]. The Rabs share a fold that is, in gross terms, common to all small GTPases of the Ras superfamily. The fold consists of a six-stranded β sheet, comprising five parallel strands and one antiparallel one, surrounded by five α helices. In these structures the elements responsible for guanine nucleotide and Mg^{2+} binding, as well as GTP hydrolysis, are located in five loops that connect the α helices and β strands. The amino-acid residues that come together in space to form this active site are closely associated with either the phosphate groups of the bound nucleotide and Mg^{2+} or the guanine base (Figure 3) and are highly conserved within the entire Ras superfamily; they can easily be used to recognize any small GTPase.

Crystallographic analysis of $p21^{Ras}$ [14] and also recently of the yeast Rab Sec4p [13] in the GDP- and GTP-bound states shows that the proteins adopt two different conformations, with the major nucleotide-induced differences occurring in regions denoted switch I and switch II [15]. In the amino-acid sequence these switch regions are located in the loop 2 region and the loop 4-α2-loop 5 region, respectively, and in the three-dimensional structure they are found on the surface of the molecule. Numerous mutagenesis studies have shown that the putative switch regions are crucial for the interaction of Rab proteins with regulatory protein partners such as GDP/GTP exchange factors and GTPase-activating proteins (see 'Localization and function'). Furthermore, the

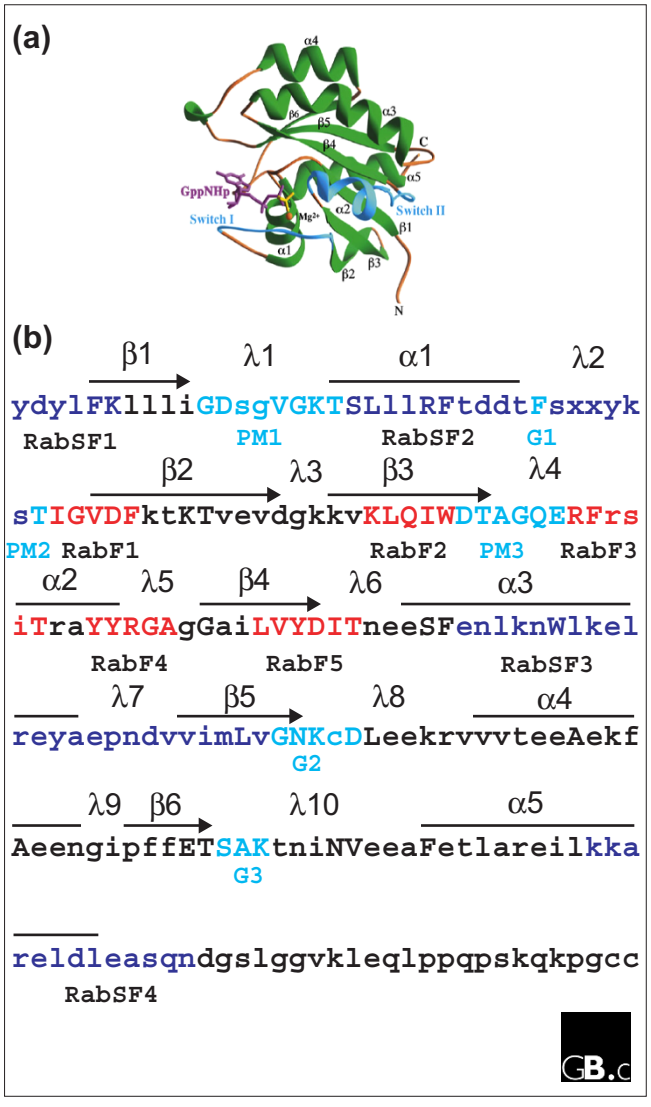

Figure 3
Structural features of Rab GTPases. **(a)** Ribbon drawing of Rab3A complexed with the GTP analog GppNHp (reproduced with permission from [9]). Purple, bound nucleotide; orange sphere, Mg^{2+} ion; blue, switch I and II regions; green, α helices and β sheets; yellow, loops. **(b)** A profile amino-acid sequence of the Rab GTPase subfamily generated using the hidden Markov model (HMM) method (modified from [6]). The uppercase/lowercase coding represents outcome of the profile HMM method. Uppercase characters, residues found in the profile with a probability of $p > 0.5$; red, Rab-specific residues (RabF1-5); dark blue, subfamily-specific motifs (RabSF1-4); cyan, highly conserved nucleotide-binding motifs; G, guanine-base-binding motif; PM, phosphate/magnesium-binding motif. The secondary structure units (α helices, β strands, and loops, λ) are indicated above the sequence.

crystal structure of a complex of Rab3a and its effector molecule rabphilin-3a shows that the switch regions form an important part of the binding interface between the two proteins [10].

A recent extensive sequence-analysis study shows the presence of five distinct amino-acid stretches that are characteristic of the Rab GTPases (Figure 3b) [6]. These so-called RabF regions (shown in red in Figure 3b) cluster in and around switch regions I and II and are suggested to provide a means of unequivocally identifying Rab proteins. In addition, four regions (RabSF regions, shown in dark blue in Figure 3b) have been identified that can be used to define the ten subfamilies of Rab GTPases mentioned above [6,7]. The RabSF regions are on two different surfaces of the GTPases; they probably allow specific binding of downstream effector molecules, which must recognize a specific Rab or Rab subfamily in addition to detecting the nucleotide-binding state. In support of this, the crystallographic study by Ostermeier and Brunger [10] showed that the well-characterized effector of Rab3a, rabphilin-3A, occupies two major binding interfaces on the surface of the GTPase. These regions of Rab3a have been named Rab complementarity-determining regions, and they involve both the switch regions and Rab superfamily-specific motifs [6]. A model has therefore been suggested in which effectors and regulators bind both to the RabF motifs in the switch I and II regions, to discriminate between active and inactive conformations, and to RabSF regions for specificity.

Localization and function

Localization and regulation

Some Rabs are expressed ubiquitously in human tissues, whereas others are tissue-specific (Table 1). Within cells, they are localized to the cytosolic face of distinct intracellular membranes (see Figure 4 and Table 1). Their reversible membrane localization depends on the post-translational modification of a cysteine motif at the very carboxyl terminus (CXXX, CC, CXC, CCXX or CCXXX where X is any amino acid), with one or two highly hydrophobic geranylgeranyl groups [16]. This post-translational modification requires the initial recognition of the newly synthesized Rab protein by a Rab escort protein (REP), which presents the Rab protein to the geranylgeranyl transferase. REP then functions as a chaperone that keeps the hydrophobic, geranylgeranylated Rab soluble and delivers it to the appropriate membrane [17]. The specific targeting of Rab GTPases is thought to rely on membrane receptors that recognize the complex between REP and specific Rabs [18], but so far no such receptors have been identified at the molecular level.

The REP-associated Rab GTPases are thought to be in the GDP-bound form, whereas membrane delivery is accompanied by the exchange of GDP with GTP, catalyzed by a GDP/GTP exhange factor (GEF), and the release of REP [17]. Upon GTP hydrolysis, which is catalyzed by a GTPase-activating protein (GAP), the Rab GTPase may be released from the membrane. This is mediated by Rab GDP-dissociation inhibitor (GDI), which is capable of retrieving the geranylgeranylated, GDP-bound Rab from intracellular membranes

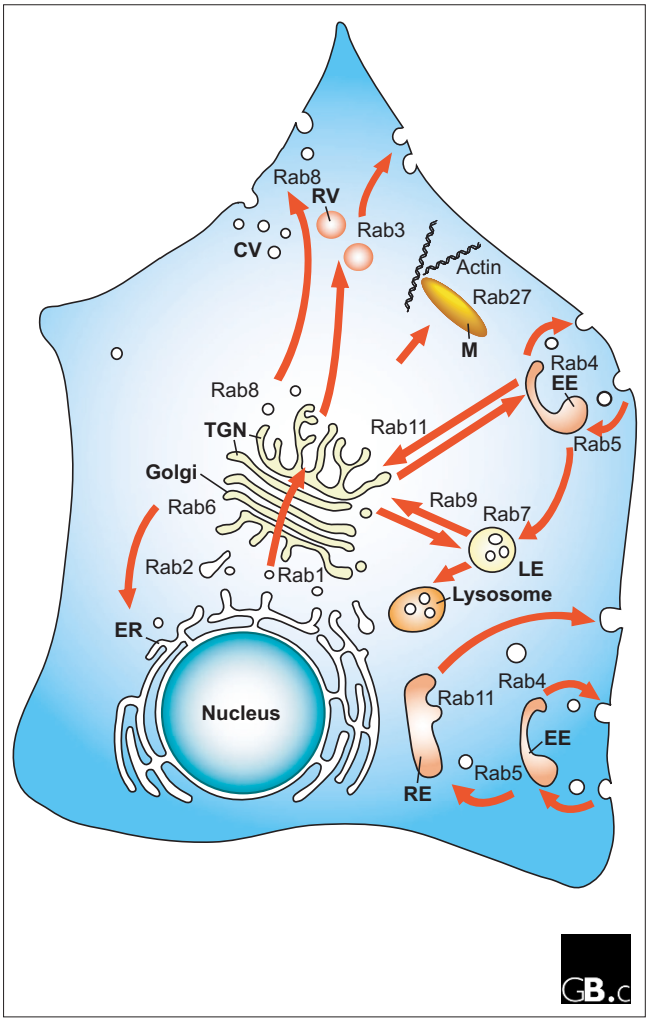

Figure 4
Intracellular vesicle transport pathways and localization of selected Rabs. The biosynthetic pathway transports proteins from the endoplasmic reticulum (ER) through the Golgi complex to the cell surface. In the *trans*-Golgi network (TGN), molecules can enter either constitutive secretory vesicles (CV) or regulated secretory granules/vesicles (RV). In specialized cells melanosomes (M) are a lysosome-related compartment that moves within the cell in an actin- and myosin-dependent manner, generating pigmentation. Material internalized from outside the cell reaches the early endosomes (EE) first and can be recycled back to the surface, either directly or via a perinuclear recycling endosome (RE) compartment, or transported to late endosomes (LE) and lysosomes. The biosynthetic and endocytic circuits (arrows) exchange material at the level of the Golgi apparatus and the endosomal elements. The localization of selected mammalian Rab proteins in the membrane compartments participating in these transport processes is indicated.

[19]. GDI has structural similarity to REP [20] and, like REP, GDI can present geranylgeranylated, GDP-bound Rab proteins to specific membranes [21]. GDI, which is more abundant than REP, thus serves as a recycling factor that allows several rounds of membrane association and retrieval of the Rab GTPases.

Function

A wealth of genetic and biochemical studies indicate that Rab GTPases function as regulators of specific intracellular traffic pathways (for a recent review, see [3]). The key to their function is the recruitment of effector molecules that bind exclusively to their GTP-bound form. Rab effectors are a very heterogeneous group of proteins: some are coiled-coil proteins involved in membrane tethering or docking, while others are enzymes or cytoskeleton-associated proteins. Two-hybrid screening for protein interactions and affinity chromatography have revealed that the endosomal GTPase Rab5a has several different effectors, and this is probably true for other Rabs as well [22-24]. This means that a Rab GTPase may be capable of regulating several molecular events at a restricted membrane location. For example, although initial studies showed that Rab5a regulates endocytic vesicle tethering and fusion, more recent evidence suggests that it also controls vesicle formation at the plasma membrane and microtubule-dependent motility of endocytic structures [25-27]. Even though effectors for many Rab GTPases have been identified, the identification and functional characterization of Rab effectors is still in an early phase. The introduction of an efficient affinity-chromatography protocol promises to speed up the identification of new effectors [24].

Important mutants

Gene knock-out studies in yeast have shown that some Rab GTPases are essential, whereas others are dispensable [28]. The only mammalian Rab knockout so far, that of the neuronally expressed Rab3a, resulted only in minor phenotypic changes in mice [29]. Several genetic diseases have been found to involve Rab GTPases or their interacting proteins, however [30,31].

Griscelli syndrome is an autosomal recessive disorder that causes partial albinism. There are two variants of this disease, one that is associated primarily with immunological defects and one associated with neurological dysfunctions. The syndrome with immunological defects is caused by missense mutations in the gene encoding Rab27a [32]. This GTPase regulates the movement of melanosomes to the cell periphery of melanocytes, and it also regulates the secretion of lytic granules in cytotoxic T lymphocytes [33,34]. The lack of Rab27a thus causes pigment anomalies and dysfunctional T lymphocytes, in agreement with the defects observed in the patients. The Griscelli syndrome with neurological symptoms is caused by mutations in the gene encoding the motor protein myosin Va [35], a putative Rab27a effector that drives the peripheral distribution of melanosomes along actin filaments [33]. As myosin Va does not participate in the exocytosis of lytic granules, the inactivation of this protein does not lead to immunological symptoms.

Table 1

Localization and function of selected Rab GTPases

Name	Yeast homolog	Localization	Expression	Function
Rab1a	Ypt1p	ER/*cis*-Golgi	U	ER-Golgi transport
Rab2a		ER/*cis*-Golgi	U	Golgi-ER retrograde transport
Rab3a		SV	Neurons	Regulation of neurotransmitter release
Rab4a		EE	U	Endocytic recycling
Rab5a	Ypt51p	EE, CCV, PM	U	Budding, motility and fusion in endocytosis
Rab6a	Ypt6p	Golgi	U	Retrograde Golgi traffic
Rab7	Ypt7p	LE	U	Late endocytic traffic
Rab8a	Sec4p	TGN, PM	U	TGN-PM traffic
Rab9a		LE	U	LE-TGN traffic
Rab11a	Ypt31p	RE, TGN	U	Endocytic recycling via RE and TGN
Rab27a		Melanosomes Granules	Melanocytes Platelets Lymphocytes	Movement of lytic granules and melanosomes towards PM

See [3,4,39] for references and further details. Compartment abbreviations: CCV, clathrin-coated vesicles; EE, early endosomes; ER, endoplasmic reticulum; LE, late endosome; PM, plasma membrane; RE, recycling endosome; SV, synaptic vesicle; TGN, *trans*-Golgi network; U, ubiquitous.

Choroideremia is an X-linked disease that involves the degeneration of the retinal pigment epithelium and the adjacent choroid and retinal photoreceptor cell layers, leading to blindness. The gene mutated in choroideremia is one of the two REP isoforms, REP-1 [36]. Although the other isoform, REP-2, seems to be sufficient for the geranylgeranylation of all Rab GTPases in all tissues except for the retinal pigment epithelium, REP-1 is essential for the efficient geranylgeranylation of Rab27a in this tissue. Thus, a lack of REP-1 leads to a lack of functional Rab27a specifically in the retinal pigment epithelium [37]. The degeneration of this epithelium and its adjacent layers may be due to deficient melanosome transport and consequently a lack of protection against harmful light exposure.

A subgroup of patients with X-linked nonspecific mental retardation have mutations in the gene for one of the GDI isoforms, GDI-α [38]. This isoform is particularly abundant in the brain, and dysfunctional membrane recycling of one or more Rab GTPases in brain synapses, leading to aberrant neurotransmission, is likely to underly the symptoms in this disease.

Frontiers

The Rab GTPases are a large family of proteins with a variety of regulatory functions in membrane traffic. The central role of these proteins has become clear during the past decade, as part of the progress in understanding in detail the mechanistic principles of transport vesicle formation, movement, and fusion. Sequencing of the human genome has allowed us to realize the diversity of the Rab gene family, though the functions of a majority of the gene products are unknown. The availability of complete genomic sequences, as well as important advances in molecular and cell biological methods, promise to bring a significant progress in our understanding of Rab function in the near future.

At the molecular level, the identification of novel GAPs, GEFs and effectors will yield information about the regulation of Rab GTPases and the molecular complexes they control. Crosstalk with regulatory mechanisms involving other members of the Ras GTPase superfamily is already becoming apparent. A key question concerns the targeting of the Rab GTPases. Which 'receptor' molecules determine their specific intracellular distributions? A combination of biochemical and genetic approaches will hopefully illuminate this issue.

At the level of the membrane, several aspects of Rab GTPase function remain to be clarified. Are Rab GTPases confined to restricted membrane domains [3] and, if so, how is this determined? Furthermore, how do Rab GTPases and their effectors regulate membrane budding, motility and fusion? With respect to membrane fusion, the role of Rab effectors as membrane tethers is already being revealed, and it seems realistic to expect that Rab-dependent membrane fusion may be reconstituted *in vitro* from purified components in the near future.

Finally, comprehending the ways in which the regulatory actions of Rabs intertwine with cell-signaling cascades and developmental processes is an enormous task for cell biologists. Here, the natural mutant models provided by human

genetic diseases that have defects in Rabs or their auxiliary proteins, as well as the novel genome-wide approaches for gene expression analysis, will be instrumental.

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