Rab GTPases in vesicular transport

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Specificity and directionality are two features shared by the numerous steps of membrane transport that connect cellular organelles. By shuttling between specific membrane compartments and the cytoplasm, small GTPases of the Rab family appear to regulate membrane traffic in a cyclical manner. The restriction of certain Rab proteins to differentiated cell types supports a role for these GTPases in defining the specificity of membrane trafficking.


Introduction

Eukaryotic cells are divided into distinct membrane-bound organelles. While this functional compartmentalization has the advantage of both efficiency and regulation, it also requires controlled interactions between the various organelles. Exchange of constituents between organelles is thought to occur through vesicular or tubular intermediates that selectively deliver proteins and lipids from one compartment to another [1-3]. Specific molecules control this complex trafficking network. Over the past few years, a large number of small GTPases of the Rab family have been described and localized to various intracellular compartments. Through this mechanism they have been proposed to ensure fidelity in the process of docking and/or fusion of vesicles with their correct acceptor compartment [4]. The cycle of GTP binding and hydrolysis would allow multiple rounds of vesicular transport and confer vectoriality. This review focuses on the involvement of Rab proteins in individual trafficking routes between intracellular compartments.

Function of Rab proteins in membrane traffic

The role of Rab GTPases in membrane traffic was first demonstrated by studies on Sec4p and Ypt1p, which function in the exocytic pathway of Saccharomyces cerevisiae [2*]. Rab proteins, the mammalian counterparts of yeast Sec4p and Ypt1p [5], are localized to distinct intracellular compartments (Table 1) and a combination of in vitro and in vivo studies has emphasized their role in the regulation of different exocytic and endocytic transport processes.

In the exocytic pathway, in vitro studies have shown that the Rab1b protein is involved in endoplasmic reticulum (ER) to Golgi transport [6] and work in intact cells has indicated that this transport process requires also Rab1a and Rab2 proteins [7]. The participation of three different Rab proteins in ER-Golgi transport might reflect sequential steps of vesicular transport or, alternatively, the concerted function of several Rab GTPases at the same step. Rab proteins also function in regulated secretion. Synthetic peptides corresponding to the effector region of Rab3a stimulate regulated exocytosis [8-10].

In the endocytic pathway, both Rab4 and Rab5 proteins are associated with early endosomes (Rab5 is also on the plasma membrane) [11,12]. However, they have distinct activities. An increase in the level of Rab5 stimulates endocytosis and expands the size of the early endosomes, whereas expression of a Rab5 GTP-binding defective mutant has the opposite effect [13*]. These morphological alterations are consistent with a role of Rab5 in the fusion between early endosomes in vitro [14]. In contrast, overexpression of Rab4 increases the reflow of endocytic markers to the cell surface and induces the accumulation of endocytic tubules and tubule clusters that could correspond either to structurally altered early endosomes or even to a distinct recycling compartment [15*].

Another example of distinct function of Rab proteins in the same organelle is provided by Rab7 and Rab9. Both proteins have been localized to late endosomes [12,16], but only Rab9 specifically functions in transport from this compartment to the trans-Golgi network (TGN) in vitro [16]. While the role of mammalian Rab7 remains unknown, functional studies on a structural homologue in S. cerevisiae, Ypt7p, suggest that this protein may function at a late step of the endocytic pathway [17*]. Ypt7 null mutants are viable and exhibit normal exocytosis and α-factor internalization, but α-factor degradation is inhibited and the vacuole is fragmented. By analogy

Abbreviations

CHM—choroideremia; DSS4—dominant suppressor of Sec4; ER—endoplasmic reticulum; GAP—GTPase activating protein; GDI—GDP dissociation inhibitor; Mss4—mammalian suppressor of Sec4; NSF—N-ethylmaleimide-sensitive fusion protein; SNAP—soluble NSF attachment protein; SNARE—SNAP receptor; TGN—trans-Golgi network.
Table 1. Summary of the localization, functional properties and interacting components of Ypt1, Sec4 and Rab proteins in yeast and mammalian cells.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Localization</th>
<th>Function</th>
<th>Interacting components</th>
</tr>
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<tbody>
<tr>
<td>Ypt1 (yeast)</td>
<td>ER–Golgi</td>
<td>Fusion of post-ER vesicles with plasma membrane</td>
<td>DSS4*, MSS4*</td>
</tr>
<tr>
<td>Sec4 (yeast)</td>
<td>Secretory vesicles–plasma membrane</td>
<td>Fusion of post-Golgi vesicles with plasma membrane</td>
<td>Rabphilin-3A</td>
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<tr>
<td>Rab1a</td>
<td>ER–Golgi</td>
<td>ER–Golgi transport in yeast and mammalian cells</td>
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<tr>
<td>Rab1b</td>
<td>ER–Golgi</td>
<td>ER–cis-Golgi and cis–medial</td>
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<tr>
<td>Rab2</td>
<td>ER–Golgi intermediate compartment</td>
<td>ER–Golgi transport</td>
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</tr>
<tr>
<td>Rab3a</td>
<td>Synaptic vesicles</td>
<td>Regulated exocytosis in pancreatic acinar cells, adrenal chromaffin cells and mast cells</td>
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<tr>
<td>Rab4a</td>
<td>Early endosomes</td>
<td>Early endosomes–plasma membrane recycling pathway</td>
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<td>Rab5a</td>
<td>Plasma membrane, clathrin-coated vesicles, early endosomes</td>
<td>Plasma membrane–early endosome transport and homotypic fusion between early endosomes</td>
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<tr>
<td>Rab6</td>
<td>Middle Golgi–TGN</td>
<td>Not determined</td>
<td></td>
</tr>
<tr>
<td>Ypt7 (yeast)</td>
<td>Not determined</td>
<td>Transport in endocytic pathway</td>
<td></td>
</tr>
<tr>
<td>Rab7</td>
<td>Late endosomes</td>
<td>Not determined</td>
<td></td>
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<tr>
<td>Rab8</td>
<td>Post-Golgi basolateral secretory vesicles</td>
<td>Golgi–plasma membrane transport</td>
<td></td>
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<tr>
<td>Rab9</td>
<td>Late endosomes, TGN</td>
<td>Transport from late endosomes to TGN</td>
<td></td>
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<tr>
<td>Rab17</td>
<td>Apical dense tubules, basolateral plasma membrane</td>
<td>Not determined</td>
<td></td>
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</table>

*Regulatory factors active on different Rab proteins. Rab GDI is not listed among the interacting components, as it interacts with most Rab proteins. See text for references.

With mammalian endocytic pathway organization and localization of Rab7 to late endosomes, Ypt7p is thought to control transport between early and late endosomal compartments in yeast [17*]. This interpretation is consistent with the existence of kinetically and biochemically distinct endocytic organelles in yeast [18]. Certainly, these observations suggest that the yeast endocytic machinery shares features with the mammalian endocytic apparatus.

A vesicle transport process can be typically divided into several steps: budding, uncoating, docking and fusion [1–3*]. While Rab proteins are implicated in the regulation of distinct membrane traffic events, the exact step controlled by each protein is not clear. Both Sec4p and Rab5 appear to control vesicle docking/fusion. However, while yeast Ypt1p is thought to function in the same step [2*], its mammalian-related protein, Rab1b, is thought to be essential for vesicle formation [6]. The interpretation of in vivo studies is complicated by the fact that transport between compartments may occur in both directions, forwards and backwards, and affecting one direction is likely to influence the other. Overexpression of Rab5 stimulates endocytosis but also accelerates recycling from the early endosomes to the plasma membrane [13*]. Likewise, inhibition of retrograde transport could, in principle, indirectly affect anterograde movement between the ER and the Golgi, and vice versa. Clearly, these studies have shown that changes in the balance between vesicles coming from and returning to a compartment have profound effects on organelle organization. Similar effects have also been observed with other components of the trafficking machinery. For example, the shibire mutation in a dynamin-related protein of Drosophila melanogaster is thought to block coated vesicle budding from the plasma membrane [19–21]. This causes an increase in its surface area, an accumulation of elongated coated pits and a decrease in the number of endocytic tubular elements [19]. Thus, similar to the overexpression of Rab4 and Rab5, the mutation induces drastic morphological changes. Altogether, these biochemical and morphological studies only represent a first step towards a more detailed dissection of Rab protein function.
Complexity of the Rab protein family

The large number of Rab GTPases identified in mammalian cells (30 different members) [22-24] led to the hypothesis that each step of vesicular traffic is regulated by at least one Rab protein [4]. Nevertheless, the implications of this complexity are not clear. First, the picture is complicated by the existence of subgroups of Rab proteins sharing high sequence identity (e.g. Rab1a, Rab1b, Rab3a, Rab3b, Rab3c, Rab3d). The high sequence conservation suggests that proteins belonging to one subgroup may have a similar function, as supported by the finding that both Rab1a and Rab1b regulate ER–Golgi transport [7]. However, this simple interpretation is countered by the fact that members of a subgroup may have different biochemical properties: both the exocytic Rab1a and the endocytic Rab4a proteins contain a phosphorylation site for p34cdc2 kinase [25,26*] whereas Rab1b and Rab4b do not [27]. Anti-Rab5a specific antibodies block early endosome fusion in vitro, despite the presence of the Rab5b and Rab5c proteins [28]. Different members of the Rab3 subgroup carry out distinct functions (see below). Altogether, these differences question the idea that Rab isoforms are functionally redundant. Instead, the diversity might be responsible for fine tuning intracellular transport by the coordinated activity of several Rab isoforms.

Second, recent morphological data indicate that several Rab proteins localize to the same organelle. Early endosomes provide a striking example: they contain at least three additional Rab proteins besides the Rab4 and Rab5 subgroups (VM Olkkonen, P Dupree, I Killisch, A Lutcke, M Zerial, et al. and A Lütcke, A Valencia, VM Olkkonen, G Griffiths, RG Parton, et al., unpublished data). What are the roles played by these different Rab proteins? One possibility is that they function in either of the transport routes that meet in this compartment: transport from and to the cell surface, and from the TGN to late endosomes [28]. Alternatively, several distinct Rab proteins might be required in a given transport process. These proteins might carry out either the same function (e.g. vesicle docking) or different functions (e.g. budding, uncoating, docking or fusion).

Cell type specific Rab proteins

The notion that Rab proteins control distinct intracellular transport processes suggests that cell type specific steps in membrane traffic also require specific Rab proteins. Several Rab proteins that are cell type or tissue-specific have been identified. For instance, neurons, exocrine and endocrine cells, which display regulated secretion, specifically express the Rab3a protein [29-31]. The expression of cell type specific Rab proteins is regulated during differentiation. Expression of Rab3d increases during differentiation of 3T3-L1 cells into adipocytes. This protein might control the insulin-dependent exocytosis of vesicles containing glucose transporter in these specialized cells [32]. Recent data indicate that epithelial cells, which have distinct apical and basolateral transport pathways, and transcytotic routes (reviewed in [33,34]), also express specific Rab proteins once they become polarized. In the developing kidney, Rab17 is absent from the mesenchymal precursors but is induced upon their differentiation into epithelial cells. In the adult organ, the protein is associated with the basolateral plasma membrane and with apical tubules [35*]. Both expression and localization suggest that Rab17 may function in transcytosis, an epithelial cell specific transport process [33].

Rab8, a ubiquitously expressed protein sharing high sequence homology with Sec4 [27], functions in traffic from the TGN to the basolateral surface in polarized MDCK cells and to the somatodendritic plasma membrane in hippocampal neurons [36,37]. These two domains are thought to correspond to the plasma membrane of non-polarized cells [38]. This suggests that Rab8 does not function in an epithelial cell specific pathway. In contrast, another as yet unidentified GTP-binding protein is found on immuno-isolated apical exocytic vesicles and is implicated in apical exocytosis, the epithelial cell specific transport step [36]. Collectively, these data suggest that specialized Rab proteins are part of the machinery regulating cell type specific transport processes. The localization and function of the Rab proteins must depend on other cell type specific regulatory components, for example, factors that regulate their GTP–GDP cycle.

Regulatory factors for Rab proteins

The cycle of GTP binding and hydrolysis of Rab proteins has been postulated to ensure directionality in the vesicle docking/fusion process [4]. According to a common view [4,39], a specific Rab protein is recruited on the donor membrane or on nascent transport vesicles in its GTP-bound form. Hydrolysis of GTP by the Rab protein is thought to trigger fusion of the vesicle with the acceptor membrane [4,40*]. Subsequently, the GDP bound Rab protein is released into the cytosol and returned to the donor membrane. There, a GDP–GTP exchange protein catalyzes GDP release and GTP binding and allows the cycle to continue.

Proteins that promote GDP–GTP exchange by members of the Rab protein family have been characterized in yeast and mammalian cells [41,42,43*]. Do these factors regulate the activity of specific Rab proteins? In vitro, yeast Dss4 stimulates GDP dissociation of both Sec4p and Ypt1p [42*]. Mammalian suppressor of Sec4, Mss4 protein, that acts on Sec4p, also stimulates GDP release from Ypt1p and mammalian Rab3a [43*]. Thus, exchange proteins appear to be active on multiple Rab proteins. If Rab proteins associate with their respective compartments in the GTP-bound form [39], then exchange factors might function in association with organelle-specific components. In contrast, GTPase-activating proteins (GAPs) appear to be more selective as the recently identified GAP
of Ypt6p (Gyp6) does not act efficiently on other Ypt proteins [44*].

Association of Rab proteins with membranes requires geranylgeranyl groups on the carboxyl-terminal cysteine motif [45,46]. This process is reversed by a cytosolic factor, Rab GDI, originally purified as a GDP dissociation inhibitor for Rab3a [47]. Rab GDI is able to recognize several geranylgeranylated Rab proteins and extract them from membranes in vitro [48-52]. These combined properties suggest that Rab GDI could function to remove Rab proteins from membranes after GTP hydrolysis and to return them to the donor membrane. As predicted from such a function, cytosolic Rab proteins are found in complex with Rab GDI [50-52] and a small but significant fraction of Rab GDI is associated with various organelles [50]. Thus, Rab GDI appears to be an essential component that ensures the cyclical and vectorial function of Rab proteins.

Is the function of GDI regulated? Analysis of the quartet mutation in D. melanogaster suggests that it might be. Mutations in the quartet locus are associated with pleiotropic developmental defects in the larvae and distinguished by the presence of a basic shift in the isoelectric point of three abundant proteins, one of which has been identified as a Drosophila homologue of Rab GDI [53]. This suggests that Drosophila Rab GDI is post translationally modified or demodified by the wild-type quartet gene product. Although there is no evidence that quartet encodes a kinase, it is interesting to note that the cytosolic form of mammalian GDI seems to be highly phosphorylated (J Gruenberg, personal communication). Phosphorylation or other post-translational modifications of Rab GDI might be necessary to inhibit re-association of Rab proteins with the target membrane and direct them to the donor compartment.

Other components may also be involved in the Rab protein cycle. Geranylgeranylation of Rab proteins is catalyzed by geranylgeranyl transferase II, an enzyme that, in contrast to other known isoprenyl transferases [54], requires sequences amino-terminal to the cysteine motif [55-57]. This peculiarity may be due to a third enzyme subunit, component A [54]. Perhaps component A recognizes Rab proteins and presents them to component B, the catalytic part of the transferase.

Intriguingly, the gene products of human and mouse choroideremia (CIIM), a disease associated with degeneration of the retina and the underlying vasculature, the choroid, share sequence homology with rat component A [58,59*]. Two additional findings support the possibility that CIIM could be a member of a family of A components. First, in cytosol of lymphoblasts from CIIM patients, geranylgeranylation activity is reduced and isoprenylation of Rab3a is more affected than that of Rab1a [60]. Second, another CIIM-like gene, CHM, has been identified [61]. Interestingly, CHM shares sequence similarity with Rab GDI [62]. This sequence conservation might reflect the ability of both GDI and component A to interact with geranylgeranylated Rab proteins. However, unlike Rab GDI, component A can also recognize the non-prenylated form of Rab proteins, and it interacts with both the GTP- and the GDP-bound forms [59*]. Therefore, it is thought that component A would be more likely to act as a chaperone for Rab proteins during the initial process of membrane association, than to remove Rab proteins from membranes. This function might be necessary to prevent interactions of the hydrophobic geranylgeranyl moiety with non-target membranes.

Rab proteins and the vesicle transport machinery

If Rab proteins are implicated in the regulation of vesicular transport, the real challenge now is to understand how they function. For this task it is important to study the relationship between Rab proteins and other elements of the transport machinery. Different vesicle fusion processes in mammalian cells and in yeast require N-ethylmaleimide-sensitive fusion protein (NSF) and the soluble NSF attachment proteins (SNAPs) [1*]. More recently, a search for SNAP receptors (SNAREs) has yielded four different factors from bovine brain, all associated with the synapse [63**,64*]. VAMP/synaptobrevin-2 is found on transport vesicles (v-SNARE), while syntaxin A and B are on target membranes (t-SNAREs). It is not clear where the fourth protein, SNAP-25 (synaptosome-associated protein of 25 kDa) is localized. These findings have led to the hypothesis that, in general, specificity in the process of vesicle fusion with its target compartment might be mediated by the interaction of a specific v-SNARE with its complementary t-SNARE through the NSF-SNAPs complex (Fig. 1).

Where do Rab proteins fit into this scheme? The answer is not there yet. However, recent progress suggests possible connections between Rab proteins and the vesicle fusion apparatus. Rabphilin-3A, a protein that interacts with Rab3a, has recently been purified and characterized [65,66]. Structural analysis of the deduced amino acid sequence from the corresponding cDNA revealed high homology with synaptotagmin, a synaptic vesicle integral membrane protein [67]. However, unlike synaptotagmin, rabphilin-3A contains neither a signal peptide nor a putative transmembrane domain. The precise function of synaptotagmin is unknown, although it has been postulated to be a regulatory component of calcium-dependent exocytosis in the presynaptic terminal [67,68]. The identification of rabphilin-3A provides a potential link between Rab proteins and t-SNAREs. In fact, syntaxin (a t-SNARE) can be co-immunoprecipitated with synaptotagmin suggesting an association of the two proteins [67], and rabphilin-3a is a synaptotagmin-like molecule. In addition, the SLY2/SEC22 and SLY12/BET1 genes, which are multicopy suppressors of functional loss of YPT1 in S. cerevisiae, encode two members of the synaptobrevin (v-SNARE) family [69-71]. Therefore, it is possible to envisage interactions between a rabphilin and a t-SNARE and between a Rab protein and a v-SNARE (Fig. 1). The specificity of interactions between v-SNAREs and t-SNAREs in a complex with NSF and SNAPs might thus require further regulation by a Rab protein. This regula-
Fig. 1. Model for possible interactions between Rab proteins and known components of the membrane traffic machinery. Proper docking and fusion of a vesicle with its correct target membrane depends on interactions between specific components present on both compartments. (a) NSF and SNAPS interact with v-SNARE- and t-SNARE-type molecules [67]. (b) Interaction between the vesicle-specific v-SNARE and its target-specific t-SNARE is still hypothetical. (c) The vesicle-specific, GTP-bound Rab protein interacts with its corresponding rabphilin, and (d) possibly, to a v-SNARE. (e) Rabphilin might interact with a t-SNARE as suggested by the interaction of syntaxin with synaptotagmin [67]. In this chain of connections, the GTPase activity of Rab proteins could be utilized to proof-read the interaction between the correct pair of SNAREs.

Conclusions

A comprehensive analysis of the molecules implicated in membrane trafficking is beginning to establish which components are common to many transport processes, and which are specific. The data obtained so far suggest that, whereas NSF and SNAPs belong to the first category, Rab proteins belong to the latter one. Although a number of studies have demonstrated the role of these GTPases as regulators of membrane traffic, their precise function remains unknown. Proteins that regulate the membrane association and nucleotide state of Rab proteins have now been identified, and further mechanistic studies will undoubtedly focus on three key questions. First, what brings about the organelle-specific localization of Rab proteins? Second, which are their target molecules? Third, how does the nucleotide state (regulated by GAPs and exchange proteins) control the interactions between Rab proteins and their target molecules? The synaptotagmin-like protein rabphilin-3A is certainly an interesting candidate for a Rab target [66*]. Complicated molecular machineries are often regulated by a number of different GTPases, and Rab proteins are not the only GTPases implicated in intracellular transport. Compelling evidence indicates that members of other subfamilies of GTPases function in regulating different aspects of membrane traffic, such as vesicle budding and vesicle coat assembly and disassembly [28,39]. Future studies will reveal whether there are links between Rab proteins and these other GTPases.

The identification of several tissue- and cell type specific Rab proteins suggests that the complexity of this family of GTPases could still increase, when one considers the high heterogeneity of cell types from different organs. Learning more about the distribution of Rab proteins in various cell types will contribute to understanding the relationship between structural features of organelles and their specialized functions, and between sub-compartments and transport routes.

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References and recommended reading

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

- of special interest
- of outstanding interest


Ypt7 null mutants were characterized by a highly fragmented vacuole. A S. cerevisiae gene encoding Ypt7, a protein with seven GTPase domains, led to an increased rate of endocytosis and to the formation of unusually large early endosomes. By contrast, cells expressing a null allele of YPT7 showed no apparent defects in endocytosis, despite the fact that its degradation was severely inhibited in the null mutants. 

Overexpression of YPT7 also had no effect on the growth properties of yeast cells at various temperatures. However, the overexpression of YPT7 led to increased rates of endocytosis and to the formation of unusually large early endosomes. By contrast, cells expressing a null allele of YPT7 showed no apparent defects in endocytosis, despite the fact that its degradation was severely inhibited in the null mutants.


Transgenic overexpression of wild-type Rab5 in rat hepatoma cells resulted in a decreased rate of endocytosis and to the formation of unusually large early endosomes. By contrast, cells expressing a nucleotide binding-deficient Rab5 mutant displayed a reduced rate of endocytosis and contained numerous small vesicles and tubules close to the plasma membrane. The results indicate that Rab5 is rate-limiting and regulates the membrane traffic between the plasma membrane and the early endosome.


Whereas normal Chinese hamster ovary cells contained only 20% of their transferrin receptors on their surface, this number was raised to 80% in stable transfectants overexpressing Rab4. Overexpression also prevented delivery of internalized transferrin to acidic endosomes, and a large fraction of internalized transferrin was associated with small, tubular clusters incapable of efficient acidification. Overexpression of Rab4 did not affect initial rates of transferrin internalization, but the rate of transferrin recycling and a fluid phase marker to the plasma membrane were increased. The results suggest that Rab5 controls the rate of recycling, possibly by controlling the rate of transport between the early endosome and a putative recycling compartment.


A S. cerevisiae gene encoding Ypt7p, a protein with 63% sequence identity to mammalian Rab7, was cloned. Ypt7p and Rab7 have identical effector domains. Gene disruption of Ypt7p had essentially no effect on the growth properties of yeast cells at various temperatures. However, the Ypt7p null mutants were characterized by a highly fragmented vacuole. While uptake of the phenome alpha factor occurred at a normal rate, its degradation was severely inhibited in the null mutants.

Cloning of a mouse cDNA for a novel Rab protein, Rab17, is reported. Northern blot analysis revealed that Rab17 is expressed exclusively in polarized epithelial cells and is thus the first example of an epithelial cell specific Rab protein. In situ hybridization on murine developing kidneys showed that Rab17 expression was turned on concomitantly with the onset of epithelial differentiation. In the adult kidney, Rab17 was found to be associated with the basolateral plasma membrane and with apical tubules. Localization data suggest that Rab17 might be involved in regulating transcytosis.


A mutation (Gln79→Leu) in the Sec4 protein strongly inhibited its intrinsic GTPase activity. A partially purified GAP activity stimulated the hydrolysis rate of the mutant to about 30% of the GAP-stimulated wild-type Sec4. The sec4 Δlev79 allele can function as the only copy of sec4 in yeast cells, but the cells are cold-sensitive, exhibit slowed invaginate secretion and accumulate secretary vesicles. It thus appears that the mutation in Sec4 causes a partial loss of function. This is the best evidence so far that GTP hydrolysis by a member of the Sec4/ypf/Rab family is necessary for vesicle fusion, as has been suggested earlier [41].


DSS4-I was isolated as a dominant suppressor of the temperature-sensitive sec4-8 mutation. The gene product, Dss4-lp, is a protein of 14kDa and has sequence homology with Dss4-lp [41]. Ms2p stimulates GDP release from Sec4p, and to a lesser extent from Rab3a and Ypt1, but was inactive on Ras2. The ms2p gene is expressed in a variety of tissues, suggesting that its natural target is one or more widely expressed Sec4-like proteins.


Yeast cells were transformed with a yeast genomic library on a multicycopy plasmid, and extracts from the individual transformants were assayed for their ability to stimulate the GTPase activity of Ypt6. A single, positive colony was isolated, and from this the GTPase activity of Ypt6 gene was cloned. The gene product, Gyp6, has no significant homology to other known proteins, including other GTPase-activating proteins. Purified Gyp6 strongly stimulated the GTPase activity of Ypt6, and had a weak activity on Ypt7. It did not stimulate the GTPase activity of Ypt1, Ypt4, Ypt3A, H-ras or Rab2. Whereas ypt6 deletion mutants are temperature-sensitive, disruption of gyp6 resulted in no phenotypic alterations.


SNARES ensure specific interactions between vesicles and their target membranes. Of these, VAMP/synaptobrevin 2, is a synaptic vesicle protein, whereas B-SNAP is brain-specific. B-SNAP competes for the same binding site on SNAP receptors as α-SNAP, but unlike α-SNAP it cannot restore cell-free Golgi transport in cytosol from sec17 mutant yeast. It is most abundant in areas of high neuron density and may be involved in neurosecretion.

This paper reports the cloning and sequencing of cDNAs encoding α-, β- and γ-SNAPs. α- and β-SNAPs were found to be 85 % identical to each other, whereas γ-SNAP showed about 25 % identity to the other SNAPs. α- and γ-SNAPs are ubiquitously expressed and act synergistically, whereas β-SNAP is brain-specific. β-SNAP competes for the same binding site on SNAP receptors as α-SNAP, but unlike α-SNAP it cannot restore cell-free Golgi transport in cytosol from sec17 mutant yeast. It is most abundant in areas of high neuron density and may be involved in neurosecretion.