

Ypt and Rab GTPases: insight into functions through novel interactions

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Ypt/Rab GTPases are key regulators of vesicular transport in eukaryotic cells. During the past two years, a number of new Ypt/Rab-interacting proteins have been identified and shown to serve as either upstream regulators or downstream effectors. Proteins that interact with these regulators and effectors of Ypt/Rabs have also been identified, and together they provide new insights into Ypt/Rab mechanisms of action. The picture that emerges from these studies suggests that Ypt/Rabs function in multiple and diverse aspects of vesicular transport. In addition, not only are Ypt/Rabs highly conserved, but their functions and interactions are as well. Interestingly, crosstalk among Ypt/Rabs and between Ypt/Rabs and other signaling factors, suggest the possibility of coordination of the individual vesicular transport steps and of the protein transport machinery with other cellular processes.

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Abbreviations

ER	endoplasmic reticulum
GAP	GTPase-activating protein
GEF	guanine nucleotide exchange factor
PI(3)K	phosphatidylinositol 3-kinase
PI(3)P	phosphatidylinositol 3-phosphate
TGN	<i>trans</i> -Golgi network

Introduction

In the exocytic and endocytic pathways of eukaryotic cells, proteins and membranes are transported through a series of compartments. Transport between successive compartments is mediated by vesicles and tubules that bud from a donor compartment and fuse with an acceptor compartment (Figure 1, bottom). In anterograde transport, cargo proteins move from the endoplasmic reticulum (ER), through the Golgi apparatus to the plasma membrane, whereas in retrograde transport, resident proteins and membrane components are retrieved from an acceptor compartment and returned to the appropriate donor compartment. Vesicular transport is regulated on at least three levels. First, in each transport step regulation is required as cargo is selectively sorted at a donor compartment into budding vesicles that are specifically targeted to and fuse with an acceptor compartment. Second, coordination of anterograde and retrograde transport is required to maintain compartment morphology in a dynamic equilibrium. Third, protein transport is most likely to be regulated by other cellular processes as it is critical for the interaction of cells with their environment. In the past decade, GTPases of the Ypt/Rab family have emerged as key regulators of protein transport at all three of these levels.

The Ypt/Rabs constitute the largest group within the Ras GTPase superfamily. A role in the regulation of the exocytic pathway by two members of this family, Sec4 and Ypt1, was first discovered in yeast through both *in vivo* [1,2] and *in vitro* studies [3,4]. The role of Ypt1 was shown to be conserved in mammalian cells [2]. While Sec4 does not have a functional mammalian homologue, its closest homologue, Rab8 [5], plays a role at a similar, more specialized, transport step (Table 1). Members of the Ypt/Rab family are now known to function in both constitutive and regulated exocytosis, as well as in endocytosis and transcytosis. With the completion of sequencing of the yeast and human genomes, we now know the full complement of Ypt and Rab GTPases. There are 11 Ypts (Yeast protein transport), of which nine have a defined role in different steps of the yeast protein transport pathway [6]. Four Ypts, Ypt1, the Ypt31/32 pair and Sec4, function in the exocytic pathway and are essential for cell viability. Of the other five, which are not required for cell viability, four (Ypt51/52/53 and Ypt7) participate in endocytosis, while the specific function of the fifth, Ypt6, is still controversial [7]. Using sequence analysis of the 37 known mammalian Rabs (Ras genes from rat brain), five Rab-family specific motifs were defined. This allowed the identification of additional Rab family members, resulting in a total of 52 mammalian Rabs ([8*]; see also Update). The proposed site of action of the known Ypts and Rabs is summarized in Table 1. The nine yeast Ypts that were shown to have a role in protein transport share 54–71% identity with their closest mammalian Rab homologues. This conservation also extends to their function as well, as Ypts and their Rab homologues act in similar intracellular compartments and, frequently, in similar transport steps. The fact that there are approximately five-fold more Rabs than Ypts can be attributed to the involvement of mammalian Rabs in specialized cell functions and in various stages of development.

Comprehensive reviews of the Ypt/Rab GTPases and their critical role in protein trafficking were recently published elsewhere [7,9,10]. Here, I summarize progress that has been made during the past two years in our understanding of Ypt/Rab function, through their interactions with regulators and effectors. These newly identified interactors reveal that Ypt/Rabs are involved in a greater number of vesicular transport aspects than was, until recently, believed. This new knowledge expands the Ypt/Rab repertoire from vesicle targeting and docking to vesicle formation and membrane remodeling and fusion. To accommodate these multifarious functions, many different Ypt/Rab-interacting proteins have emerged to provide specificity, and neither upstream positive regulators nor downstream effectors of individual Ypts and Rabs share homology. These specific regulators and effectors, however, are conserved from yeast to man.

Table 1**Identified Ypts and Rabs and their proposed site of action.**

Transport step*	Ypts†	Rabs‡
Exocytosis		
ER-to-Golgi	Ypt1	Rab1; Rab1b; Rab2
Golgi-to-ER		Rab6; Rab6b
Intra-Golgi	Ypt1; Ypt6(?)	Rab1; Rab6; Rab6b
TGN-to-PM	Ypt31; Ypt32; Sec4	Rab11a,b
Golgi associated (unknown step)		Rab10; Rab12; Rab30; Rab33b
Endocytosis		
PM-to-EE		Rab5a,b,c
EE-to-PM recycling		Rab4; Rab15; Rab18
EE-to-LE	Ypt51/Vps21; Ypt52; Ypt53	Rab7
TGN-to-LE	Ypt51/Vps21, Ypt52, Ypt53	
LE-to-vacuole/lysosome	Ypt7	Rab7
LE-to-TGN	Ypt6	Rab9; Rab11a,b
Endosome associated (unknown step)		Rab22a,b; Rab25
Specialized roles		
Regulated secretion		
Synaptic vesicle fusion with PM		Rab3a,b,c,d
Secretory granules exocytosis		Rab8b
Secretory granules associated		Rab26; Rab37
Polarized secretion		
TGN-to-basolateral membrane		Rab8
TGN-to-apical PM (EC)		Rab21
Tight junction (EC)		Rab13
Transcytosis (EC)		Rab17
Phagosome associated		Rab14
Melanosome transport		Rab27a,b
Unknown roles	Ypt10, Ypt11	Rab4b; Rab19; Rab20; Rab24; Rab28; Rab7L1/Rab29; Rab30; Rab31; Rab32; Rab33a; Rab/Rab34; Rab35; Rab36; Rab38; Rab39; CAB09136/Rab40a; AAA17031/Rab40b

*For details regarding proposed site of action, see [7,9,10]. †Ypts, as identified in YPD™ [5]. ‡Rabs, as identified in LocusLink [90]; except for: Rab15, Rab34, and Rab40a and b, which are identified in

GenBank [91]. TGN, *trans*-Golgi network; PM, plasma membrane; EE, early/sorting endosome; LE, late/recycling endosome; EC, epithelial cells; (?) controversial.

Upstream regulation of Ypt/Rabs

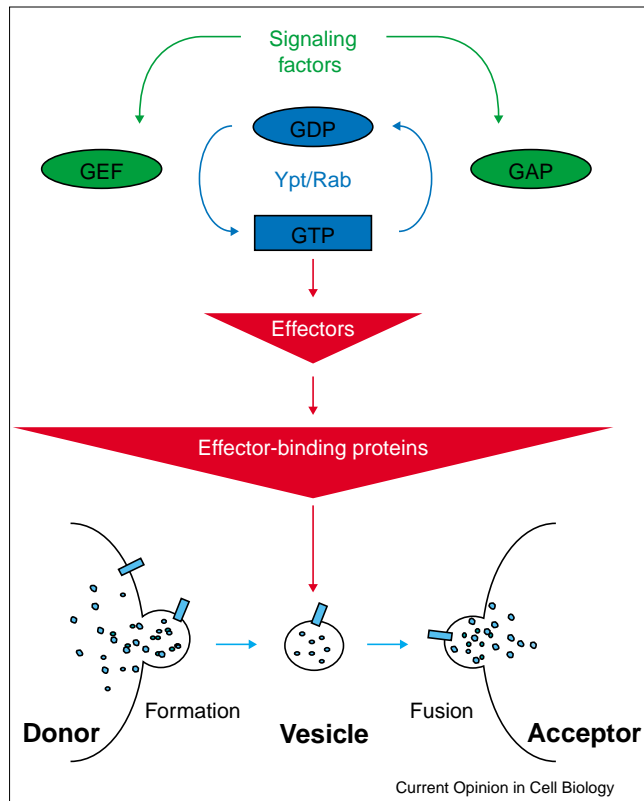
GTPases cycle between GDP- and GTP-bound forms, with the latter being considered to be the active or 'on' form of these molecular switches. Crystal structures of three GTP-bound Ypt/Rabs, Rab3A, Ypt51, and Sec4, as well as one of a GDP-bound Sec4, have been determined [11–13]. These studies reveal that the overall structure of Ypt/Rabs is similar to that of Ras, as are the observed conformational changes between the GDP-bound and the GTP-bound forms. Switching of all GTPases from the GDP-bound to the GTP-bound form is achieved by an endogenous nucleotide exchange activity, stimulated by GEFs. Cycling back to the GDP-bound form is accomplished by endogenous GTP hydrolysis, stimulated by GAPs (Figure 1). Two other factors, GDI and GDF, regulate the cycling of Ypt/Rabs between membranes and also affect Ypt/Rab nucleotide cycling (Figures 2 and 3). GDI, a GDP-dissociation inhibitor, extracts GDP-bound Ypt/Rabs from membranes to recycle them to their donor compartments. GDF, a GDI-displacement factor, recruits the Ypt/Rabs to specific donor membranes. Although GDIs

are well characterized [14], much less is known about GDFs. One candidate for such a factor has been identified recently: Rab5ip is an endosomal membrane protein that binds the GDP-bound form of Rab5 [15]. In this review, I focus on the recently characterized GEFs and GAPs for Ypt/Rabs, and the new insights that they provide into Ypt/Rab function.

Novel Ypt/Rab GEFs

Two years ago, Sec2, which acts as a GEF for Sec4, was the only known Ypt GEF [16]. Since then, four other GEFs for Ypts have been identified and now we have a full complement of GEFs assigned to all nine Ypts (Table 2). The TRAPP complex was identified as a GEF for both Ypt1 [17*,18**] and the Ypt31/32 pair [18**]. Vps9 acts as a GEF for Ypt51 (and most likely for its functional homologues Ypt52/53) [19], the Ric1/Rgp1 complex is a GEF for Ypt6 [20], and Vps39, a component of the HOPS complex, acts as a GEF for Ypt7 [21**]. Only two mammalian GEFs are known to date, one for Rab5 and one for Rab3 (Table 2). The Ypt GEFs do not share sequence homology, but they

Figure 1

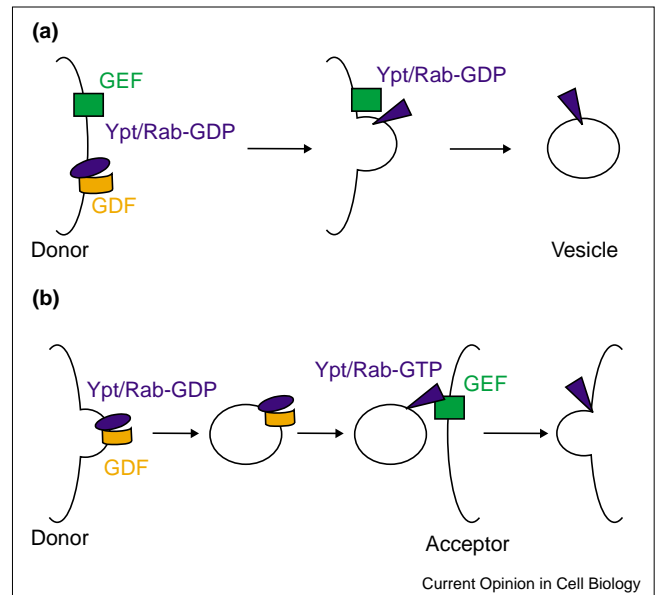


The Ypt/Rab GTPase signaling pathway. Ypt/Rabs cycle between a GDP-bound and a GTP-bound form. This cycling is controlled by positive and negative regulators, GEF and GAP, respectively, that are probably themselves regulated by upstream signaling factors. The GTP-bound form of the Ypt/Rab signals through multiple effectors to downstream effector-binding proteins that mediate the various steps of vesicle transport.

each have mammalian homologues; for example, Vps9 is homologous to Rabex-5, the GEF for the Ypt51 homologue, Rab5. Therefore, the search for Rab GEFs can rely, at least in part, on homology with the Ypt-GEFs.

The newly identified GEFs provide significant insight into the role of these positive regulators of Ypt/Rabs. First, GEF function is required for Ypt/Rab activity in vesicular transport. This requirement was suggested originally using *YPT1* mutations that specifically inhibit Ypt1-GEF activity [22]. This finding has now been confirmed by the demonstration that the GEF for Ypt1, TRAPP, is required for ER-to-Golgi transport [23], a step mediated by Ypt1. A requirement for GEFs in other Ypt-mediated protein transport steps was also shown for both Sec2 [24] and HOPS [25••], GEFs for Sec4 and Ypt7, respectively. Second, in all cases that have been examined, Ypt/Rab GEFs are part of large protein complexes. The significance of this finding is not clear, but it is possible that these multi-subunit complexes have other functions in addition to their roles as Ypt/Rab GEFs. This idea is supported by two major findings. Two Ypt/Rab-GEF complexes were

Figure 2



Regulation of Ypt/Rab nucleotide exchange. Nucleotide exchange, stimulated by GEF, occurs at the Ypt/Rab site of action. (a) A model for Ypt/Rab nucleotide exchange during vesicle formation: Ypt/Rab, in its GDP-bound form, is recruited to an appropriate donor compartment via a receptor, GDF. A GEF, which also resides on this compartment, stimulates the conversion of the Ypt/Rab to its GTP-bound form. Ypt/Rab-GTP acts to induce vesicle budding at this site. (b) A model for nucleotide exchange during vesicle targeting/fusion: Ypt/Rab, in its GDP-bound form, is recruited to the appropriate donor compartment via a receptor, GDF. GEF, which resides on the acceptor compartment, stimulates nucleotide exchange by the Ypt/Rab only when the vesicle reaches the relevant acceptor compartment. The Ypt/Rab then acts to induce vesicle tethering or fusion. For example, in Ypt1-mediated ER-vesicle targeting, the Ypt1-GEF localizes to the acceptor membrane, the *cis*-Golgi [17,18]. The role of the GDF receptor in these cases is to ensure non-reversible recruitment of the Ypt/Rab to the membrane from the cytosolic Ypt/Rab-GDI pool.

shown to also act as effectors for their cognate Ypt/Rabs (i.e. HOPS for Ypt7 [25••], and Rabaptin-5/Rabex-5 for Rab5 [26]). In addition, AEX-3, the *Caenorhabditis elegans* homologue of the GEF for Rab3A, was shown to have second function that is Rab3-independent [27•]. It remains to be determined whether this theme is more general. Third, experiments that show genetic interactions and co-precipitation have demonstrated that the same basic TRAPP complex can act as a GEF for both Ypt1 and Ypt31/32 [18••]. On the basis of size and detergent sensitivity, differences between a Ypt1-GEF activity that does not act on Ypt31/32, and TRAPP that does, it is suggested that TRAPP is a modular complex that functions at different transport steps in the Golgi. It is proposed that at specific steps particular TRAPP subunits associate and allow TRAPP to act as a GEF for one or other of the two distinct Ypts [18••,28]. Although other Ypt/Rab GEF complexes are considered specific for their Ypt/Rab substrates, this assumption should be revisited with the idea of modular GEF complexes in mind. In the context of coordination

Table 2**Recently identified Ypt/Rab regulators.**

Regulator	GTPase substrate*	Regulator comments	References
Yeast GEFs			
TRAPP	Ypt1	~800 kDa 10-subunit complex	[17*,18**]
TRAPP	Ypt31/32	As above	[18**]
Vps9	Ypt51		[19]
Ric1/Rgp1	Ypt6	Heterodimer	[20]
Vps39	Ypt7	HOPS complex	[21**]
Mammalian GEFs			
Rab3A-GEF	Rab3	~295 kDa	[92]
Rabex-5	Rab5	Complexed with Rabaptin	[26]
Yeast GAPs			
Gyp2	Ypt6, Sec4	Identified as Cdc42-interacting (Mdr1)	[31]
Gyp3	Sec4, Ypt6,51,31,32,1	As above (Msb3)	[31]
Gyp4	Sec4, Ypt6,7	As above (Msb4 [38])	[32]
Gyp7	Ypt7,31,32,1		[33]
Mammalian GAPs			
GAPCenA	Rab6,4,2	Stimulates microtubule nucleation	[34]

*GAP substrate specificity listed in order of preference.

between Ypt/Rabs, the finding that Ypt1 and Ypt31/32 share a GEF is discussed further below.

Novel Ypt/Rab GAPs

Two years ago only two Ypt GAPs were known, Gyp6 [29] and Gyp1 [30]. On the basis of sequence similarity with these two Gypts, four other Gypts have been identified: Gyp2,3,4,7 ([31,32,33]; Table 2). The yeast Gypts share sequence similarity with a novel mammalian Rab GAP, GAPCenA [34*]. Therefore, unlike GEFs, all the identified yeast Ypt GAPs share sequence similarity, and are conserved. However, one mammalian Rab GAP, tuberlin, a GAP for Rab5, does not share sequence similarity with any of the currently identified Gypts or GAPCenA [35]. In addition, a GAP that acts preferentially on Ypt1 and Ypt31/32 has not yet been identified, even though a Ypt1-GAP activity has been described [28]. Together, these findings raise the possibility that more Ypt/Rab GAPs may exist.

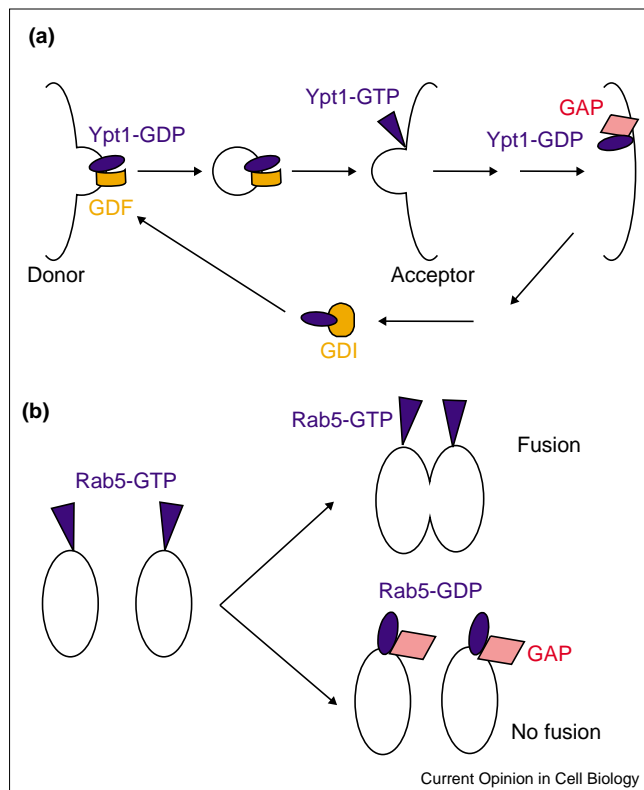
New insights into the role and mechanism of action of the Ypt/Rab GAPs are provided by studies of these recently identified proteins. First, the crystal structure of the GAP domain of Gyp1 has been solved [36]. The active site of Gyp1 exhibits structural similarities to that of the GAPs for Ras and Cdc42, including an arginine finger motif, suggesting a common activation mechanism by GAPs for these three GTPase families [36]. Second, although the newly identified GAPs show some substrate preference,

they do not exhibit absolute substrate specificity *in vitro*, suggesting that they might also be promiscuous *in vivo*. Third, deletion of the *GYP* genes, individually or in combination, does not affect yeast cell viability [30,37,38], suggesting that the function of these proteins is non-essential for Ypt-mediated protein transport. Fourth, some GAPs might have functions in addition to stimulation of GTP hydrolysis by Ypt/Rabs. For example, three of the Gypts were originally found to interact with Cdc42, a Rho-family GTPase involved in actin polarization in yeast [38]. The significance of this finding is not clear, but might point to a connection between the Ypt Gypts and polarized transport. Interestingly, the Rab GAP, GAPCenA, associates with the centrosome, possibly implying a role in directional transport [34*]. Questions regarding the function, intracellular localization, and specificity of Ypt/Rab GAPs are still open.

Ypt/Rab nucleotide cycling regulation

Current models depict the stimulation of nucleotide exchange by GEF as occurring at a donor compartment, while stimulation of GTP hydrolysis by GAP is thought to take place at the acceptor membrane. This latter event has been proposed to be important for membrane fusion or for its timing [39,40]. However, accumulating evidence suggests an alternative model. First, the identified GEF for Ypt1, TRAPP, localizes to the Golgi, which serves as the acceptor compartment in Ypt1-mediated ER-vesicle

Figure 3



Regulation of Ypt/Rab GTP hydrolysis. GTP hydrolysis, stimulated by GAP, might regulate Ypt/Rab function differently in heterotypic and homotypic membrane fusion: (a) GTP hydrolysis promotes Ypt/Rab cycling between membranes in heterotypic membrane fusion. For example, while GTP hydrolysis is not important for Ypt1-mediated ER-to-Golgi transport [37], it might play a role in GDI-mediated Ypt1 recycling to the donor membrane, allowing for multiple rounds of transport [28]. In this recycling, GDI extracts Ypt/Rab-GDP from membranes and presents it to GDF, a specific membrane receptor. (b) In homotypic fusion GTP hydrolysis turns off Ypt/Rab function. For example, GTP hydrolysis is important for inhibiting continuous Rab5-mediated homotypic endosome fusion [42].

targeting [17•,18••,28,41]. This finding suggests that Ypt/Rabs are activated by their GEFs at the site of function, probably to ensure Ypt/Rab activity at the right place and time (Figure 2). According to this hypothesis, the site of GEF action in Ypt/Rab-mediated vesicle formation is at the donor compartment (Figure 2a), whereas in vesicle targeting it acts at the acceptor compartment (Figure 2b). Second, although GTP hydrolysis stimulated by GAP is important for turning off Rab5 function in homotypic endosomal fusion [42]; Figure 3b), it seems to be dispensable for Ypt1 function in heterotypic fusion in ER-to-Golgi transport [37]. It has been suggested that the function of GTP-hydrolysis and GAP in Ypt/Rab-mediated heterotypic fusion might be to promote GDI-dependent recycling of Ypt/Rabs to donor membranes ([28]; Figure 3a). Recycling of Ypt/Rabs, which allows their participation in multiple rounds of transport, can also be achieved by GDI-independent processes (e.g. retrograde transport). The fact that the identified Ypt GAPs are dispensable for both protein

transport and cell viability supports this hypothesis. However, these ideas have yet to be tested directly.

Ypt/Rab functions and downstream effectors

GTPases transmit signals to downstream effectors in a GTP-dependent manner, activating and/or recruiting effectors to sites of action (Figure 1). Studies of Ypt/Rab function, together with analysis of Ypt/Rab downstream effectors and their effector-binding proteins, suggest that diverse mechanisms exist for Ypt/Rab action.

Ypt/Rabs function in multiple transport steps and manifold aspects of vesicular trafficking

Accumulating evidence suggests that individual Ypt/Rabs play a role in multiple protein transport steps in both yeast and mammalian cells. In yeast, Ypt1 is required for two successive steps: ER to *cis*-Golgi and *cis*-to-medial Golgi [43]. More recently, Ypt51 was also shown to function in two steps: early endosome to late endosome and *trans*-Golgi network to late endosome [44]. In both of these cases, there is one compartment that is common to the two transport steps, the *cis*-Golgi for Ypt1, and the late endosome for Ypt51. In mammalian cells, Rab1, Rab5 and Rab11 have all been implicated in more than a single transport step (see [7]). It has been proposed that Ypt/Rabs regulate transport to and from a particular compartment, rather than being specific for an individual transport step [43].

Ypt/Rabs were originally believed to act after vesicle formation, in the subsequent targeting and docking of these vesicles to their acceptor membranes [1,45]. However, it has become evident that these GTPases play a role in multiple and various aspects of vesicular transport at individual traffic steps, as enumerated below (Figure 4). First, involvement of Ypt/Rabs in the formation of vesicles was suggested both by *in vivo* studies in yeast and by *in vitro* studies in mammalian cells. *In vivo*, the Ypt31/32 functional pair was shown to be required for exit from the *trans*-Golgi [46], whereas *in vitro*, Rab5 was shown to be required for sequestration of receptor-bound ligands into clathrin-coated vesicles (CCVs) [47••]. Second, a role for Ypt/Rabs in vesicle motility has been suggested by the finding that Rab5 stimulates both endosome association with and movement along microtubules [48•]. Third, through the interaction of Rab5 with PI(3) kinase (PI(3)K), which generates PI(3)P, a role for Ypt/Rabs in membrane remodeling has been proposed. This Rab-mediated membrane remodeling is achieved by the increase of the local concentration of PI(3)P [49••]. Fourth, the traditional role of Ypt/Rabs in vesicle docking (see above) is currently suggested to involve 'tethering' of adjacent membranes prior to their fusion [50]. Fifth, more recent evidence is now pointing to a direct role for Ypt/Rabs in the event of membrane fusion itself, via regulation of SNARE complex formation (see below).

Can one Ypt/Rab function in all the various aspects of vesicle trafficking? Rab5 has been implicated in all of the

Table 3**Recently described Ypt/Rab functions and downstream interactions.**

GTPase	Effector	Effector-binding protein	Comments	References
Vesicle formation				
Rab9	TIP47	MPRs	MPRs: cargo receptors	[60**]
Rab11	Rabphilin-11	Sec13	Sec13: a COPII component	[61*]
Rab5			Cargo sequestration into CCVs	[47]
Rab1	p115		Program budding vesicles for targeting	[63**]
Vesicle motility				
Rab5	PI(3)K		Both: required for EE motility	[48*]
Rab6	Rabkinesin-6		A kinesin-like microtubule motor	[65]
Rab6	GAPCenA	γ -tubulin	GAPCenA: microtubule nucleation	[34]
Membrane remodeling				
Rab5	PI(3)K		PI(3)K generates PI(3)P	[49**]
Vesicle docking				
Rab1	p115	Giantin, GM130	p115 giantin, GM130: tethering factors	[63**,67,68]
Rab5	EEA1		FYVE domain, binds PI(3)P	[51*,52,53]
Ypt51	Vac1		FYVE domain, binds PI(3)P	[69*]
Sec4	Exocyst	Sec3	Sec3: spacial landmark	[71**]
Ypt7	HOPS complex		HOPS: required for vacuole docking	[25**]
Membrane fusion				
Rab5	EEA1	Syntaxin-6	Syntaxin-6: t-SNARE	[55]
Rab5	EEA1	Syntaxin-13	Syntaxin-13: t-SNARE	[56]
Ypt51	Vac1	Vps45	Vps45: Sec1-like	[58*,69*]
Rab5	Rabenosyn	hVps45	hVps45: Sec1-like	[54]
Ypt7	HOPS complex	Vps33	Vps33: Sec1-like	[25**]

CCV, clathrin-coated vesicles; COPII, coat protein complex II; EE, early endosome; MPRs, mannose-6-phosphate receptors.

aspects mentioned above. The data come from either studying Rab5 itself, as was done for its function in vesicle formation and motility [47**,48*], or studying Rab5 effectors that are known to function in membrane remodeling, and vesicle docking and fusion ([48*,49**,51*,52–56]; Table 3). It is now clear that Ypt/Rabs are able to function in multiple aspects of vesicular trafficking as well as in multiple transport steps through their interactions with their many and varied effectors.

Novel Ypt/Rab effectors and effector-binding proteins

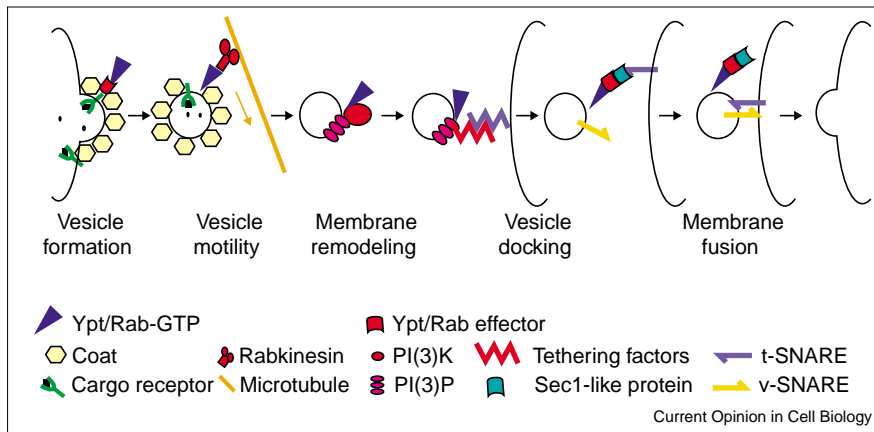
Analysis of recently identified Ypt/Rab effectors (Table 3) leads to two major conclusions. First, in contrast to the high degree of conservation within the Ypt/Rab GTPase family, Ypt/Rab effector sequences are unrelated. This finding is in agreement with the hypothesis that diverse mechanisms of action exist for the Ypt/Rab GTPases. Second, a single Rab GTPase can have more than one effector. For example, 22 proteins were shown to bind specifically to Rab5 in its GTP-bound form [51*]. Thus far, four of these factors (Rabaptin5, EEA1, Rabenosyn and

PI(3)K) have been identified as specific effectors of Rab5 [54,56,57,58*,59]. Taken together, these two observations support the hypothesis that a single Ypt/Rab GTPase can mediate more than one transport step, and that these GTPases are involved in a number of aspects of vesicular transport, over and above their accepted role in vesicle targeting. The newly identified Ypt/Rab effectors and effector-binding proteins provide insight into the mechanisms by which Ypt/Rabs regulate all the various aspects of protein transport mentioned above (Table 3 and Figure 4).

Vesicle formation

Two recent findings suggest a model in which Ypt/Rab effectors play a role in cargo sequestration during vesicle budding. In this model a Ypt/Rab effector interacts with a ligand-bound receptor and/or a coat component, both of which are required for cargo selection and vesicle formation. First, a Rab9 effector, TIP47, binds the cytoplasmic tail of the mannose 6-phosphate cargo receptors [60**]. Second, Rabphilin-11, a Rab11 effector, was shown to bind Sec13, a component of the COPII coat complex [61*];

Figure 4



Models for the mechanisms by which Ypt/Rab GTPases regulate the various steps of vesicle transport: Ypt/Rabs are implicated in the following five different vesicle transport steps: vesicle formation, vesicle motility, membrane remodeling, vesicle docking and vesicle fusion (see examples in Table 3 and text). Individual Ypt/Rabs might function in more than one vesicle transport step by sequential interaction of a Ypt/Rab with its various effectors, mediating the diverse steps. Alternatively, individual Ypt/Rabs might concentrate within the membrane to form microdomains, where multiple molecules of a specific Ypt/Rab could interact with its various effectors.

COPII plays a role in cargo sequestration into sites of vesicle formation on the ER [62]. An alternative model was proposed recently for Rab1 and its effector p115. In this model the role of the Ypt/Rabs in vesicle formation is to program budding vesicles by incorporating proteins necessary for their subsequent docking and fusion [63*].

Vesicle motility

Microtubules play a role in protein transport by serving as rails and using kinesin- and dynein-based motors for the movement of vesicles and organelles [64]. A model for the role of Ypt/Rabs in vesicle movement along microtubules can be inferred from two findings. First, a kinesin-like protein, Rabkinesin-6, was found to be an effector for Rab6 [65]. Second, Rab5 was shown to regulate the movement of early endosomes on microtubules [48*]. In this model, Ypt/Rab-GTP recruits a kinesin-like protein, which serves to link membranous vesicles to microtubules, and to promote their movement along these microtubules (see also Update).

Membrane remodeling

A role for Ypt/Rabs in membrane remodeling is suggested by two Ypt/Rab effectors. First, PI(3)K, which generates PI(3)P, was identified as a Rab5 effector, and it is thought that Rab5 might recruit PI(3)K to sites of Rab5 action [49**]. Second, EEA1 and Vac1, effectors of Rab5 and Ypt51, respectively, bind directly to PI(3)P through their FYVE domain (a conserved protein domain that binds specifically to PI(3)P) [53,66]. These findings suggest a model in which the recruitment of PI(3)K by Rab5 results in an increase in the local concentration of PI(3)P. This membrane remodeling induces the recruitment of EEA1 to PI(3)P-enriched sites on membranes, and then in turn EEA1 functions in docking of vesicles to target membranes.

Vesicle docking

Docking of vesicles to the acceptor compartment is an established function of Ypt/Rabs (see above). Current models postulate that Ypt/Rabs recruit proteins that form extended structures to bridge adjacent membranes. A number of

Ypt/Rab effectors are implicated in such a function. A coiled coil protein p115, which can form extended rod structures, was shown to be an effector for Rab1 [63**]. p115 may tether vesicles to the Golgi via its interactions with Golgi proteins ([67,68]; see also Update). EEA1 and Vac1, effectors for the homologues Rab5 and Ypt51, respectively, are implicated in vesicle docking [51*,69*]. As EEA1 has two Rab5-binding domains, one on either end [70], it might function by tethering two endosomes through Rab5 molecules. In addition, two Ypt/Rab effector complexes are thought to have a role in vesicle docking. First, the Exocyst complex, an effector for the Ypt Sec4, is thought to tether vesicles to sites of vesicle fusion by its interaction with Sec3, which serves as a spatial landmark for polarized secretion in yeast [71**]. Second, Ypt7 and its effector, the HOPS complex, are thought to function in the docking stage of yeast vacuole fusion, although the specific mechanism of docking is not yet clear [25**,72].

Membrane fusion

Current models suggest that Ypt/Rabs function in vesicle tethering, a step that precedes SNARE-dependent vesicle fusion, but do not act directly in fusion itself [50,73]. However, recent data from studies of the interaction of the various Ypt/Rab effectors with other proteins give new clues regarding their mode of interaction, and suggest that Ypt/Rabs do have a direct role in SNARE complex formation. First, EEA1, a Rab5 effector, was shown to interact with two distinct t-SNAREs, Syntaxin 6 and Syntaxin 13 [55,56]. As the interaction of Ypt/Rabs with SNAREs appears to be promiscuous [74], the significance of these interactions is still controversial. Second, Vac1 and Rabenosyn, effectors for Ypt51 and Rab5, respectively, interact with the Sec1-like proteins Vps45 and h-Vps45 [54,58*,69*]. Similarly, Vps33, a subunit of the HOPS complex, an effector of Ypt7, is also a Sec1-like protein [25**]. Sec1-like proteins are known to be regulators of SNARE complex formation [75]. A model for Ypt/Rab function in vesicle fusion suggests that Ypt/Rabs recruit an effector that in turn, interacts with a Sec1-like protein. This interaction is important for the formation of the SNARE complex, an event that results in membrane fusion.

For at least one Ypt/Rab, Rab5, effectors have been identified that can act at four of the defined aspects of vesicular transport. It is tempting to propose that effectors of individual Ypt/Rabs act cooperatively. Coordination of the various functions of the individual Ypt/Rabs, acting through their multiple effectors, is envisioned by the following models that can exist in different combinations. First, individual Ypt/Rab molecules might interact sequentially with their various effectors. Second, effector complexes with multiple roles might exist. Third, Ypt/Rab clusters may form micro-domains on membranes, interacting with their multiple effectors to create a Ypt/Rab-transport machine. The challenge in the near future will be to sort out which of the multiple interactions between the Ypt/Rabs and their effectors and effector-binding proteins are physiologically relevant. And further to determine the mechanisms by which Ypt/Rabs interact with their multiple effectors. The three-dimensional structure of Rab3A, complexed with one of its effectors, Rabphilin-3A, suggests a basis for effector-binding specificity of the diverse Ypt/Rabs [76•]. Solving the structure of additional Ypt/Rabs complexed with their regulators and effectors will no doubt shed light on the fine details of their many mechanisms of action.

Coordination among transport steps and between the transport machinery and other cellular processes

Coordination of entry into and exit from individual compartments is predicted to be important for maintenance of the morphology of intracellular compartments while allowing bidirectional transport. GTPases are good candidates for playing a key role in such a coordination, as they are known to fulfill an analogous role in other cellular processes, that is, cell morphogenesis and movement [77]. There is now data to support the idea that GTPases couple discrete protein transport steps through their interactions with other GTPases. The examples that currently exist for GTPase cross-talk in protein transport are diverse, and include two cases of coordinated GTPase activation, and two cases of coordinated GTPase function. Coordinated GTPase activation by GEFs is supported by two findings. First, the existence of a common GEF, TRAPP, for the Ypts that regulate entry into and exit from the yeast Golgi (Ypt1 and Ypt31/32, respectively), suggests coordinated activation of the Ypts to ensure Golgi homeostasis [18••]. Secondly, genetic interactions between the exocytic Ypts and the known yeast Arf GEFs suggest the existence of an Ypt–Arf GTPase cascade in the protein transport pathway. Such a cascade might coordinate vesicle coat assembly, which is dependent on Arf activation, with Ypt-mediated vesicle transport [78••]. Coordinated function by effectors is suggested by two findings. Rabaptin-5, an effector of Rab5, was shown to bind Rab4 [79] and, therefore, Rabaptin-5 might couple Rab5-mediated entry into early endosomes, with Rab4-mediated exit. Furthermore, Rabaptin-5 was also shown to interact with Rabphilin-3, a Rab3 effector. As Rab5 and Rab3A function in the endocytic and the exocytic pathways, respectively, interaction between their two effectors might serve to coordinate these pathways [80].

Protein trafficking is crucial for all cellular processes, and therefore, it is expected to be coordinated with them. Ypt/Rab GTPases are good candidates for playing a role in such a coordination (Figure 1, top), and there is new evidence to support their involvement. First, stress response and endocytosis are connected through the stress-induced p38 MAP kinase regulation of Rab5 localization, which affects endocytosis [81•]. Second, activated cell surface receptors generate signals that activate the endocytic pathway, resulting in their internalization. Two pieces of data suggest that this activation might occur through Rab GTPases: EGF-receptor stimulation of endocytosis requires the activation of Rab5a [82], and Eps8, a protein that is regulated by the EGF receptor, might coordinate Rab and Rho GTPases function, regulating EGF-receptor signaling [83•]. Third, a role for the endocytic Rabs, Rab5 and Rab7 has recently been suggested in *Drosophila* development through the regulation of a TGF- β homologue [84••].

Conclusions

A picture, summarized in this review, of Ypt/Rab mechanisms of action, is beginning to emerge from the multitudinous details of Ypt/Rab interactions. It is now clear that individual Ypt/Rabs function in multiple transport steps, and regulate the various aspects of vesicular trafficking: vesicle formation, targeting and docking, and membrane remodeling and fusion. Therefore, it is not surprising that Ypt/Rabs can interact with multiple effectors, and that these effectors themselves are diverse in both their structure and function. In addition, GEFs, which seem to activate Ypt/Rabs at the site of action, are also divergent. Despite the variability between effectors and GEFs for individual Ypt/Rabs, these interactors are conserved from yeast to man. On the other hand, most of the known negative regulators of Ypt/Rabs, share sequence similarity. However, they are promiscuous in their substrate interactions, and do not seem to be essential for Ypt/Rab function. Together, these findings have contributed to the development of new models for the regulation of Ypt/Rab nucleotide cycling (Figures 2 and 3), and for the manifold mechanisms by which Ypt/Rabs function through their several effectors (Figure 4). Finally, Ypt/Rabs are emerging also as coordinators of protein transport steps, as well as coordinators between the protein transport pathway and other cellular processes.

A number of key questions are still unanswered regarding Ypt/Rab function in protein trafficking. First, do Ypt/Rabs determine the specificity of intracellular compartments? Second, how is the function of the Ypt/Rabs regulated by their activator complexes, and what is the role of their negative regulators? Third, what are the specific mechanisms by which Ypt/Rab effector complexes function in so many aspects of vesicular transport, and how are these functions coordinated? Fourth, are individual complexes that play dual roles, as activators and effectors of Ypt/Rabs, a common theme? Fifth, do Ypt/Rabs coordinate the individual protein transport steps, as well as the protein transport

pathway with other cellular processes? If this coordination is confirmed, how is it achieved? Finally, what is the molecular basis for Ypt/Rab mechanism of action?

These questions can now be addressed in the context of accumulating knowledge regarding the mechanisms that underlie protein transport in general. To generate a coherent picture of how transport machines are triggered by Ypt/Rab molecular switches, biochemists will continue to explore how the individual parts interact with each other, and try to reconstitute their function *in vitro*. Structural biologists are just beginning to explore the fine details of the Ypt/Rab switches and their interactions with other molecules. Cell biologists and geneticists need to determine which Ypt/Rab interactions are important for each Ypt/Rab function in the context of cells, at three levels: individual transport steps, coupling of these steps, and the coordination of protein transport with other cellular processes.

Update

In a recent analysis of the human genome, a total of 60 human Rabs were identified [85]. In addition, two recently described Rab interactors broaden the spectrum of Ypt/Rabs associations. First, a recent flurry of papers suggests a role for Rab27a in melanosome transport, a process that is dependent on the actin-motor myosin V (summarized in [86]). Hume *et al.* [87] have recently shown a physical association between Rab27a and myosin Va. This Rab-actin motor interaction is in addition to the previously demonstrated Rab-microtubule motor interaction. Second, a novel Rab1 effector complex, which contains the tethering factor GM130, was identified. This complex is known to reside on the *cis*-Golgi. The interaction of GM130 with Rab1 is independent of another identified Rab1 effector, the tethering factor p115, that is important for vesicle formation at the ER. Moyer *et al.* [88*] suggest that Rab1 interacts with these two different tethering effectors on the two separate compartments, to coordinate formation of vesicles at the ER with their subsequent docking at the *cis*-Golgi. A possible mechanism for such a coordination is suggested by the observation that the two Rab1 effectors, p115 and GM130, interact directly [89]. Rab1 could coordinate these two events by creating specific domains on the docking vesicle and on the acceptor membrane that contain these complementary tethering factors primed for interaction.

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