HHS Public Access

Author manuscript

Crit Rev Biochem Mol Biol. Author manuscript; available in PMC 2016 February 23.

Published in final edited form as:

Crit Rev Biochem Mol Biol. 2015 ; 50(3): 203–211. doi:10.3109/10409238.2015.1014023.

Critical Reviews in Biochemistry and Molecular Biology:

Ypt/Rab GTPases: Principles Learned from Yeast

Zhanna Lipatova, **Adelaide U. Hain**, **Volodymyr Y. Nazarko**, and **Nava Segev**[#]

Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago IL

Abstract

Ypt/Rab GTPases are key regulators of all membrane trafficking events in eukaryotic cells. They act as molecular switches that attach to membranes via lipid tails to recruit their multiple downstream effectors, which mediate vesicular transport. Originally discovered in yeast as Ypts, they were later shown to be conserved from yeast to humans, where Rabs are relevant to a wide array of diseases. Major principles learned from our past studies in yeast are currently accepted in the Ypt/Rab field including: i) Ypt/Rabs are not transport-step specific, but are rather compartment specific, ii) stimulation by nucleotide exchangers, GEFs, is critical to their function, whereas GTP hydrolysis plays a role in their cycling between membranes and the cytoplasm for multiple rounds of action, iii) they mediate diverse functions ranging from vesicle formation to vesicle fusion, and iv) they act in GTPase cascades to regulate intracellular trafficking pathways. Our recent studies on Ypt1 and Ypt31/Ypt32 and their modular GEF complex TRAPP raise three exciting novel paradigms for Ypt/Rab function: (a) coordination of vesicular transport substeps, (b) integration of individual transport steps into pathways, and (c) coordination of different transport pathways. In addition to its amenability to genetic analysis, yeast provides a superior model system for future studies on the role of Ypt/Rabs in traffic coordination due to the smaller proteome that results in a simpler traffic grid. We propose that different types of coordination are important also in human cells for fine-tuning of intracellular trafficking, and that coordination defects could result in disease.

Keywords

Ypt; Rab; GTPase; secretion; autophagy

Introduction

Ypt/Rab GTPases together with their upstream regulators and downstream effectors comprise about one percent of the human proteome. They are found in all eukaryotes and regulate all membrane-bound intracellular trafficking pathways (Li and Segev, 2013). The Ypt/Rab GTPases are small “molecular switches” of ~200 amino acids that can be turned “on” and “off” by guanine-nucleotide exchange factor (GEF) activators and GTP hydrolysis activator (GAP) inhibitors, respectively. In addition, Ypt/Rabs cycle between the cytoplasm

[#]Corresponding Author: Nava Segev, Department of Biochemistry and Molecular Genetics, College of Medicine, University of Illinois at Chicago, Molecular Biology Research Building, 900 South Ashland Avenue/ Chicago, Illinois 60607, Phone: 312-355-0142; Fax 312-413-0353; nava@uic.edu.

and membranes, to which they attach through a lipid tail (Segev, 2011b). When associated with membranes in their GTP-bound form, or “on” state, Ypt/Rabs interact with downstream effectors. Each Ypt/Rab can interact with multiple effectors, which in turn mediate the multiple steps of vesicle transport, from vesicle formation at the donor compartment, through their movement and tethering, to their fusion with the acceptor compartment (Segev, 2001a).

Multiple questions regarding the mechanisms by which Ypt/Rabs mediate all these different processes are still unresolved. Several mechanisms were suggested from studies on the mammalian Rabs; e.g., together with their effectors, Rabs are considered membrane domain organizers (Zerial and McBride, 2001), and Rab conversion was suggested to coincide with endosome maturation (Rink et al., 2005). Other open questions include: how are sequential interactions of a single Ypt/Rab with its multiple effectors and sequential functions of multiple Ypt/Rabs orchestrated?

While many questions about Ypt/Rab function are still unresolved, it is clear that they are crucial not only for the cell wellbeing, but also for human health. Rabs are associated with a range of human diseases; from acquired diseases like cancer and neurodegenerative to rare genetic disorders (Mitra et al., 2011). Therefore, the roles of Ypt/Rabs can range from essentiality for viability to fine-tuning of the highly complicated cellular trafficking network. Here, we summarize the major milestones of the Ypt/Rab field since its emergence 25 years ago, with emphasis on contributions of studies in yeast to the establishment of past and present Ypt/Rab paradigms.

I. Past lessons from the yeast Ypts

In the late 1980s, the known GTPases – heterotrimeric G proteins and monomeric Ras oncogenes – played a role in signaling across the plasma membrane (PM) (Barbacid, 1987, Stryer and Bourne, 1986). Moreover, the apparent dissimilarities between the functions of the two yeast Ras proteins (Toda et al., 1986) and the mammalian Ras oncogenes (Barbacid, 1987), raised concerns about the validity of yeast as a model system for studying GTPases. The first two members of the Ypt/Rab family, Ypt1 and Sec4, were identified and characterized in yeast (Goud et al., 1988, Salminen and Novick, 1987, Segev and Botstein, 1987, Segev et al., 1988). Sec4 was shown to be required for secretion in Golgi-to-PM transport (Salminen and Novick, 1987). Ypt1 is the first monomeric GTPase that was shown to function inside the cell on an intracellular organelle, the Golgi apparatus, and to regulate intracellular trafficking and not signaling across the PM. Importantly, in the same paper, we showed that, like the yeast Ypt1, a mammalian homolog also resides on the Golgi, suggesting that the Ypt1 function is conserved (Segev et al., 1988). Later, the functional conservation of Ypt1 and its closest mammalian homolog, Rab1, was established (Haubruck et al., 1989).

The evolutionary conservation between yeast and humans is not limited to sequence similarity and intracellular localization. For example, the functions of the the closest human homologs of Ypt1 and Ypt31/Ypt32, Rab1 and Rab11, respectively, are also conserved (Taussig et al., 2012). So are the functions of the human homologs of Vps21 and Ypt7, Rab5

and Rab7, respectively, in endocytosis (Cantalupo et al., 2001, Schimmoller and Riezman, 1993, Singer-Kruger et al., 1995). In addition, Ypt/Rab regulators and effectors identified in yeast are conserved from yeast to humans (Segev, 2001a). The opposite is understandibly not true, as there are so many more Rabs than Ypts, ~70 versus 11, and accordingly significantly more Rab accessory factors; e.g., the human family of 18 DENN-domain Rab GEFs does not exist in yeast (Marat et al., 2011).

Knowledge from the heavily studied Ras oncogenes helped the emerging Ypt/Rab field move quickly. Because of sequence similarity to the Ras GTP-binding domains, mutations defective in nucleotide binding and GTP hydrolysis could be readily made and their effects on Ypt/Rab function determined. In addition, the concepts from the Ras field that GEFs and GAP stimulate nucleotide exchange and GTP hydrolysis, respectively, served as a road map for studying the newcomer GTPase family. Using these tools and concepts together with genetics in yeast enabled us to establish the following basic principles about Ypt mode of action, which are currently accepted in the Ypt/Rab field.

a) Ypt/Rabs are not transport-step specific, but probably compartment specific

Initially, the field focused on determining the intracellular localization and regulated transport step of each Ypt/Rab. Early studies assigned Ypts and Rabs to the different steps of the exocytic and endocytic pathways. For example, Ypt1, the functional pair of Ypt31/Ypt32 (only one of them is required), and Sec4 were assigned to the different steps of the yeast secretory pathway, endoplasmic reticulum (ER)-to-Golgi transport, exit from the trans Golgi and fusion of trans-Golgi vesicles with the PM, respectively (Goud et al., 1988, Jedd et al., 1997, Segev et al., 1988). Therefore, it was proposed that Ypt/Rabs may provide specificity to transport steps (Pfeffer, 1996).

However, our early studies with Ypts did not fit this mold. Both Ypt1 and Ypt31/32 were shown to mediate more than one transport step. We have since shown that Ypt1 regulates ER-to-Golgi, intra-Golgi and ER-to-autophagosome transport (Jedd et al., 1995, Lipatova et al., 2012, Segev, 1991, Segev and Botstein, 1987, Segev et al., 1988). We have also shown that Ypt31/Ypt32 regulate trans Golgi-to-PM and endosome-to-Golgi transport (Chen et al., 2005, Jedd et al., 1997). Therefore, we proposed that Ypt/Rabs are specific to cellular compartments and not to transport steps. For example, Ypt1 and Ypt31/Ypt32 are specific to the ER-derived membranes and trans Golgi, respectively (Segev, 2001b). Support for this idea came from studies that assigned Vps21 (Ypt51) and Ypt7 to early and late endosomes, respectively, in both endocytosis and autophagy (Chen et al., 2014, Kirisako et al., 1999, Schimmoller and Riezman, 1993, Singer-Kruger et al., 1994). While the idea that Ypt/Rab themselves do not provide transport-step specificity is accepted in the field (Pfeffer, 2013), the compartmental assignment of Ypt1 is still controversial (Sclafani et al., 2010, Segev, 1991) and will be further discussed below.

b) Upstream regulation

The first paradigm for the regulation of Ypt nucleotide cycling was based on analogy to another GTP-binding protein, elongation factor Tu (EfTU), which regulates protein synthesis. This dogma, which made its way to textbooks, stated that Ypt function is

dependent on GTP hydrolysis for energy and irreversibility (Bourne, 1988). Extrapolation of an observation that a *sec4* mutation results in partial defects in GTP hydrolysis and secretion, was taken as support to this notion (Walworth et al., 1992).

Our studies suggested that this dogma does not fit the regulation of Ypt1 nucleotide cycling. Because Ypt1 mediates ER-to-Golgi transport, the then-conventional dogma postulated that GTP hydrolysis would be required for ER-to-Golgi transport and that its GAP would reside on the cis Golgi. However, a *ypt1* mutation that confers a severe GTP-hydrolysis defect, did not seem to affect Ypt1-mediated intracellular trafficking (Richardson et al., 1998). Moreover, a Ypt1 GAP activity was identified not on the Golgi, but on the PM (Jones et al., 1998). In contrast, using *ypt1* mutations, we found that GDP-to-GTP nucleotide exchange is required for Ypt1-mediated intracellular trafficking (Jones et al., 1995) and that the Ypt1-GEF activity was localized to the Golgi (Jones et al., 1998). Based on these findings, we proposed that nucleotide exchange stimulated by GEFs is critical for Ypt/Rab-mediated vesicular transport, whereas GAP-stimulated GTP hydrolysis plays a role in their cycling between membranes and the cytoplasm for multiple rounds of action (Jones et al., 1998). In agreement, whereas Sec2, the GEF for Se4 is required for the function of Sec4 in exocytosis, its GAPs, Msb4 and Msb4, are only required rendering this transport step efficient (Walch-Solimena et al., 1997, Gao et al., 2003).

Currently, this paradigm is accepted in the Ypt/Rab field (Figure 1). In addition to the GEFs and GAPs, two other types of Ypt/Rab accessory factors were identified: GDP-dissociation inhibitor (GDI) and GDI-dissociation factor (GDF). These two factors regulate cycling of the GTPases between membranes and the cytoplasm. The single yeast GDI serves as a “Ypt-specific chaperone” that enables prenylated Ypts to exist in the hydrophilic environment of the cytoplasm, whereas GDF, a more elusive factor, serves as a specificity component to recruit a specific Ypt/Rab to the right membrane (Segev, 2011b).

c) Ypt/Rabs regulate diverse functions ranging from vesicle formation to vesicle fusion

Early studies of Ypt1 and Sec4 showed that they regulate fusion of ER and trans-Golgi vesicles, respectively, with the relevant acceptor compartment (Goud et al., 1988, Segev, 1991, Segev et al., 1988). In addition, the first identified Rab effectors belonged to the “tethers” group. Therefore, in the early stages of the field it was assumed that Ypt/Rabs regulate fusion of transport vesicles with the acceptor compartment (Pfeffer, 1996). However, our studies with the functional pair Ypt31/Ypt32 revealed that it regulates exit of vesicles from the trans Golgi (Jedd et al., 1997). Therefore, we proposed that Ypt/Rabs can regulate diverse steps of vesicular transport, from vesicle formation, like Ypt31/Ypt32 at the trans Golgi (Jedd et al., 1997), to vesicle fusion, like Ypt1 in ER-to-Golgi transport (Segev, 1991).

Diverse Ypt/Rab effectors that mediate vesicle formation, motility, targeting and fusion have since been identified and shown to be effectors not only of different Ypt/Rabs but effectors of individual Ypt/Rabs as well (Segev, 2001a, Stenmark and Olkkonen, 2001). For example, we identified two very different types of Ypt31/Ypt32 effectors. The first is a molecular motor, Myo2, which is required for trans-Golgi vesicle motility towards the PM of the polarized bud (Lipatova et al., 2008). The second is Rcy1, a F-box protein required for

ubiquitination of cargo recycled from endosomes to the Golgi (Chen et al., 2005, Chen et al., 2011). Likewise, multiple effectors were identified for Sec4, the exocyst complex and Sro7 (Grosshans et al., 2006, Guo et al., 1999), and for Vps21, the CORVET complex and Vac1 (Peplowska et al., 2007, Tall et al., 1999).

d) Ypt GTPase cascades

It is reasonable to assume that the multiple steps of the intracellular trafficking pathways are coordinated to ensure uninterrupted transport flow. GTPases are in a perfect position to execute this task. Arf is another GTPase family that regulates intracellular trafficking, primarily vesicle coat formation (Kahn, 2009). Based on genetic interactions between the Golgi Ypts and Arf GEFs, we proposed that a GTPase cascade coordinates the multiple steps of the secretory pathway (Jones et al., 1999). This idea gathered momentum and GTPase cascades were proposed to coordinate transport steps and pathways (Segev, 2011a). For example, three cascades that involve activated Ypt32 were proposed to regulate the exocytic pathawy. The first is an activation cascade in which Ypt32 recruits Sec2, the GEF for Sec4, to secretory vesicles (Ortiz et al., 2002). The other two are inactivation cascades in which Ypt32 recruits the GAPs for Ypt1 and Ypt6 to the Golgi (Suda et al., 2013, Rivera-Molina and Novick, 2009). In the endocytic pathway, Rab5 to Rab7 conversion, which is dependent on the Rab7 GEF Mon1-CCz1 was proposed to regulate endosome maturation in mammalian and *C.elegans* cells (Poteryaev et al., 2010, Rink et al., 2005). The next section elaborates on this topic.

Summary—Currently, principles learned from yeast that are accepted in the Ypt/Rab field include: upstream regulation, diverse downstream functions and cascades.

II. New paradigms emerging from current yeast Ypt studies

Our recent studies of Ypt1, Ypt31/Ypt32 and their accessory factors reveal a number of trafficking junctions coordinated by these proteins.

a) Coordination of vesicular transport substeps

Every step of the cellular trafficking pathways can be divided into multiple vesicular transport substeps: vesicles form at the donor compartment and move to the acceptor compartment where they dock and fuse. These substeps are mediated by specific molecular machinery components: coats, motors, tethers and SNAREs, sequentially. All these types of machinery components were identified as Ypt/Rab effectors, namely, they interact with Ypt/Rabs in their GTP-bound form. Therefore, perhaps Ypt/Rabs coordinate the sequential vesicular transport substeps. Based on our studies of Ypt31/Ypt32 and their effectors we proposed that this GTPase pair coordinates the substeps of trans-Golgi vesicles transport to the PM. First, Ypt31/Ypt32 were shown to play a role in trans-Golgi vesicle formation based on the observation that *ypt31*Δ/*ypt32ts* mutant cells accumulate enlarged Golgi structures at the non-permissive temperature (Jedd et al., 1997).

Based on genetic interactions between Ypt31/Ypt32 and Sec7, an Arf GEF, we suggested that a vesicle biogenesis component is a Ypt31/32 effector (Jones et al., 1999). Recently, the Arf-GEF activity of Sec7 was shown to be dependent on Ypt31/Ypt32 (McDonold and

Fromme, 2014). Second, we identified the myosin V type motor Myo2 as a Ypt31/Ypt32 effector and showed that cells expressing *myo2* mutant proteins, defective specifically in the interaction with Ypt31/Ypt32, accumulate un-polarized vesicles (Lipatova et al., 2008). Thus, we propose that sequential interactions of Ypt31/Ypt32 with their effectors couple vesicle formation and motility (Lipatova et al., 2008). Moreover, effectors of Sec4, the GTPase that mediates trans-Golgi vesicle tethering and fusion, include the tethering complex exocyst and Sro7, which interacts with the PM tSNARE Sec9 (Grosshans et al., 2006). The identification of Sec2, the GEF for Sec4, as a Ypt32 effector (Ortiz et al., 2002) suggests that Ypt31/Ypt32 orchestrate all the Golgi-to-PM vesicle transport substeps (Figure 2).

b) Integration of individual transport steps into pathways

It is tempting to assume that the different steps of a pathway are coordinated to allow unobstructed transport flow. In the endocytic pathway, transition from Rab5- marked early endosomes to Rab7-marked late endosomes was proposed to be coupled by a dual-function molecule that acts as an effector for the first and a GEF for the second (Rink et al., 2005). In the exocytic pathway, where the Golgi serves as the sorting compartment, we proposed a different mechanism for Golgi maturation. We have termed Ypt1 and the functional pair Ypt31/Ypt32 the “Golgi gatekeepers” because they regulate entry into and exit from the Golgi, respectively (Taussig et al., 2012). TRAPP, a conserved protein complex that resides on the Golgi, was characterized in yeast (Sacher et al., 1998). Based on the identification of TRAPP as the GEF for both these GTPases, we proposed that sequential activation of the Golgi gatekeepers by the same basic complex coordinates Golgi entry and exit (Jones et al., 2000). The specific mechanism we envision is conversion of TRAPP I, the Ypt1 GEF, to TRAPP II, the Ypt31/Ypt32 GEF (Morozova et al., 2006).

The TRAPP I complex, which resides on early Golgi, contains five small subunits. TRAPP II, which resides on the late Golgi, contains the TRAPP I subunits plus two larger subunits, Trs120 and Trs130 (Sacher et al., 2008). The idea that TRAPP I and TRAPP II act as GEFs for Ypt1 and Ypt31/Ypt32, respectively, is based on in vitro GEF assays, the effect of TRAPP mutations on the localization of their cognate Ypts, and genetic interactions (Morozova et al., 2006, Taussig et al., 2013, Zou et al., 2012). We propose that conversion of TRAPP I to TRAPP II on the Golgi, perhaps during cisternal maturation, coordinates sequential activation of the Golgi Ypts, thereby integrating the different steps of the exocytic pathway (Figure 3). We identified TRAPP subunits required for conversion of TRAPP I to TRAPP II: Trs20 and one of the two nonessential TRAPP subunits, Trs65 or Trs33 (Liang et al., 2007, Taussig et al., 2013, Tokarev et al., 2009). Dynamic analysis of TRAPP complexes in vivo, using mutations in genes that regulate TRAPP conversion, is needed to test this idea.

c) Coordination of different trafficking pathways

Cells have multiple trafficking pathways including the exocytic (secretory), endocytic, autophagic and recycling of PM proteins (for multiple rounds of action). It is appealing to speculate that these pathways are coordinated, possibly by Ypt/Rabs. We further propose that to coordinate either the merging or branching of two separate pathways, a single Ypt/

Rab, and not multiple, could function at the junction. We delineated two ways by which a single Ypt can coordinate separate pathways: merging pathways through a single GEF-Ypt-effector module, or branching pathways through multiple modules.

The regulation of autophagy by Vps21 (Ypt51), a known endocytic Ypt, is an example of the first way. Autophagy is a recycling pathway in which cellular components are delivered via the double-membrane autophagosome to the lysosome (vacuole in yeast) for degradation and reuse of their building blocks, especially under stress. In addition to the autophagy-specific proteins, Atgs, autophagy also requires the membrane trafficking machinery. A role for the endocytic pathway in autophagosome formation has been previously proposed, however, the mechanism underlying this role was not clear (Tooze et al., 2014). We have recently shown that in addition to its role in endocytosis, Vps21 also regulates autophagy. Importantly, Vps21 regulates autophagy in the context of the same module that mediates endocytosis, using the same GEF, Vps9, and the same effectors (Chen et al., 2014). Therefore, we proposed that the two pathways that lead to the vacuole, endocytosis and autophagy, merge in a Vps21-dependent manner.

We have elucidated two examples for the second way: Ypt1 and Ypt31/Ypt32 coordinate the exocytic pathway with two different types of recycling processes, autophagy and PM recycling, respectively, each in the context of different modules (Lipatova and Segev, 2014). We proposed that Ypt1 coordinates early steps of the exocytic and the autophagic pathways, ER-to- Golgi and pre-autophagosomal structure (PAS) formation, respectively. One of the first characterized phenotypes of the *ypt1-1* mutation was a defect in cell viability under nitrogen starvation (Segev and Botstein, 1987), a hallmark of an autophagy defect. Recently, we identified Atg11, a PAS organizer, as an autophagy-specific Ypt1 effector (Lipatova et al., 2012, Lipatova and Segev, 2012). We have also shown that the two functions of Ypt1, in ER-to-Golgi transport and autophagy, can be separated by mutations and are stimulated by two different GEFs, TRAPP I and the Trs85-containing TRAPP III (Lipatova et al., 2013, Taussig et al., 2014). Thus, we proposed that a single Ypt/Rab can coordinate different trafficking steps emanating from the same compartment. In this case, depending on the cargo, Ypt1 regulates transport from ER-derived membranes either to the exocytic or to the autophagic pathway in the context of two different “modules” that contain different GEFs and effectors (Figure 4).

Ypt31/Ypt32 also coordinate two trafficking pathways using separate modules. In this case, Ypt31/Ypt32 regulate a late step of the secretory pathway, exit from the Golgi towards the PM, by recruiting effectors like Sec7, Myo2 and Sec2. In addition, Ypt31/Ypt32 regulate a step of recycling of PM proteins, endosome-to-Golgi, using Rcy1 as an effector. The only GEF currently known for Ypt31/Ypt32 is TRAPP II, which resides in the trans Golgi. It is possible that the same GEF stimulates Ypt31/Ypt32 in these two transport steps, which merge at the trans Golgi. However, it is possbile that there is a still an uncharacterized GEF for Ypt31/Ypt32 (Lipatova and Segev, 2014).

Summary—Based on our studies, we have suggested a number of models for Ypt/Rab-mediated coordination of intracellular trafficking at different levels. All the players and the

functions are conserved from yeast to humans (Bennett and Scheller, 1993). The challenge now is to elucidate their importance and mechanisms in both systems.

III. Future challenges

a) Lingering unresolved questions from the past

In a field that rushes into dogmas, sometimes prematurely, even basic accepted assumptions are controversial or unsupported; e.g., intracellular localization, compartmental specificity and assignment of full set of GEF/effectors/GAP to specific Ypt/Rabs.

1) Ypt/Rab localization and compartment specificity—The localization of Ypt/Rabs to certain cellular compartments was visualized mainly using fluorescently tagged Ypt/Rabs and compartmental markers. Unfortunately, most of these studies, even in yeast, used over-expressed tagged proteins, sometimes non-endogenous and frequently did not establish functionality. Some discrepancies about Ypt/Rab localization need to be re-visited using more rigorous standards; for example, in yeast the localization of Ypt1 to the late Golgi (Sclafani et al., 2010), as well as localization of mammalian Rabs using live-cell microscopy.

2) Assigning roles for Ypt/Rabs—The assignment of Ypt/Rab function to specific compartments cannot be inferred based solely on their localization. Roles are usually established using mutations or manipulating protein expression. However, it is not always obvious that observed phenotypes are direct, especially if the protein functions in a pathway. This led to controversies and in many cases Ypt/Rab roles must be reconsidered. For example, two early studies assigned roles for Ypt1 in microtubule organization and intracellular calcium regulation. These studies used expression shutdown and dominant-interfering mutations leading to indirect effects (Schmitt et al., 1988, Schmitt et al., 1986).

In yeast, gene deletions or mutations have been used to determine function. For example, to establish the roles of Ypt1 and Ypt31/Ypt32 in the exocytic pathway, we used rapidly inactivating conditional recessive mutations (Jedd et al., 1997, Jedd et al., 1995). To define multiple roles of the same Ypt in different processes, we used conditions or mutations that affected specifically one role and not the other. For example, the role of the Ypt31/Ypt32 interaction with the Myo2 motor was shown using a *myo2* mutation that specifically affects its interaction with Ypt31/Ypt32. Moreover, we established that the phenotype of this mutation is suppressed by fusing the mutant protein to Ypt31 (Lipatova et al., 2008). In order to establish a role for Ypt1 in autophagy, we used mutations that do not affect its function in the exocytic pathway. Here too, fusing the autophagy-specific effector, Atg11, to the Ypt1-mutant protein, bypassed the autophagy phenotypes of this mutation (Lipatova et al., 2013).

Still, there is an apparent discrepancy in the literature regarding Ypt1 function. Using *ypt1* mutations not defective in ER-to-Golgi transport, Sclafani et al., reported a role for Ypt1 at the late Golgi, specifically in the endosome-to-Golgi transport step (Sclafani et al., 2010). The problem is that intracellular accumulation of the marker used for this study, GFP-tagged Snc1, can be caused by a defect either in endosome-to-Golgi transport or in autophagy.

Using the best-characterized mutation from the Sclafani et al. study and four rigorous assays, we recently established that this mutation confers an ER-autophagy and not a endosome-to-Golgi transport defect (Lipatova and Segev, 2014, Lipatova et al., 2013). Therefore, we consider Ypt1 as a regulator specific to dispatching cargo from the ER to the exocytic or autophagic pathways, and Ypt31/Ypt32 as trans-Golgi specific regulators that control transport to this compartment from endosomes, and from this compartment to the PM.

In mammalian cells, dominant mutations and gene knockdown are often used to determine Rab function. Each approach has its limitations. The problem with dominant Ypt/Rab mutations is that mechanisms by which they block function are not always specific (Feig, 1999). Because knockdown experiments require days to silence a gene, the observed phenotypes are not necessarily direct. Because a single Ypt/Rab can regulate multiple pathways, the question whether they are specific for compartments is still open. To resolve this issue, improved methods and more rigorous criteria should be used for intracellular localization and for role assignment of Ypt/Rabs.

3) GEF-Ypt/Rab-effector modules—Ypt/Rab GEFs have been tough to identify, because, with the exception of the 18 human DENN-domain GEFs (Marat et al., 2011), GEFs for different Ypt/Rabs do not typically share sequence similarity. Therefore, Ypt/Rab GEFs are identified by their activity. Even for the longtime studied Ypt1 and Ypt31/Ypt32, there is still a controversy regarding the identity of their GEFs at the Golgi. While our published data provide biochemical, genetic and cell biological evidence that TRAPP I and TRAPP II act as GEFs for Ypt1 and Ypt31/Ypt32, respectively (see above, Section IIb), another group claims that all TRAPP complexes act as GEFs for Ypt1 and not for Ypt31/Ypt32 (Barrowman et al., 2010). This idea is based on two types of evidence. First, negative biochemical evidence for lack of GEF activity and co-precipitation of Ypt31/Ypt32 with TRAPP II (Cai et al., 2008, Yip et al., 2010). However, both these studies used GST-tagged Ypt31/Ypt32, whereas all our studies that show activity and co-precipitation used untagged Ypts (Jones et al., 2000, Morozova et al., 2006). The second type of evidence relies on the suggestion that Ypt1 acts at the late Golgi where TRAPP II resides (Sclafani et al., 2010). However, we recently established that Ypt1 does not function in the late Golgi (Lipatova and Segev, 2014, Lipatova et al., 2013). Therefore, the major reason for assigning TRAPP II to Ypt1 as a GEF is disputed.

As for Ypt/Rab effectors, while the field as a whole identified multiple and diverse Ypt/Rab effectors, the search is still on for assigning effectors for specific Ypt/Rabs, including Ypt1 and Ypt31/Ypt32.

Because we identified two separate functions for Ypt1, in ER-to-Golgi transport and in autophagy, and each function involves a different GEF and effector, we termed them “GEF-Ypt/Rab-effector modules” (Lipatova and Segev, 2012, Lipatova et al., 2013). In remains to be seen whether more such modules will surface.

4) Ypt/Rab GAP specificity—Ypt/Rab GAPs were relatively easy to identify because they share a domain termed GYP in yeast and TBC in mammlian cells (Barr and Lambright,

2010). However, the apparent substrate promiscuity in vitro of Ypt GAPs and the fact that they are not essential stalled progress in understanding their importance (Taussig et al., 2012). Insight about their function came from work on mammalian Rab GAPs, which established biochemical Rab specificity (Haas et al., 2007). In addition to establishing Ypt-GAP relationships, other important questions include whether Ypt/Rab GAPs can also serve as effectors, and whether they are important components of Ypt/Rab modules.

b) Open Questions about Ypt/Rabs and traffic coordination

Ypt/Rabs regulate different trafficking pathways as well as multiple steps within the same pathway. It has therefore been suggested that Ypt/Rabs coordinate progression of transport steps within a pathway and between separate pathways. However, confirmation and mechanisms for such coordination are still missing.

1) Coordination proofs—Current evidence tells us that Ypt/Rabs and their GEFs or GAPs could coordinate transport steps and pathways because individual Ypt/Rabs regulate multiple steps and pathways. Several cascades have been suggested in which the effector of one Ypt/Rab acts as a GEF or GAP for the downstream or upstream Ypt/Rab, respectively (see section I,d). However, this propsal is based on observing Ypt/Rab conversion microscopically and showing that the middle factor can act as an effector to one and a regulator for the other (Nottingham and Pfeffer, 2009) (Figure 5). While suggestive, observing a cascade of events microscopically, demonstrating an interaction, and showing an effect when omitting the middle factor, is not enough. To establish a Ypt/Rab-mediated coordination, there is a need to separate the other roles of the Ypt/Rab or the middle factor from the coordination. Alternatively, the choice of effectors might be determined not by the Ypt/Rab, but by the effector being at the right place at the right time.

2) Mechanisms of coordination—Three possible Ypt/Rab coordination paradigms are described above (Section II). The first paradigm envisions coordination of sequential substeps within a single transport step. For example, Ypt31/Ypt32 use multiple effectors (Sec7, Myo2, and Sec2) to mediate multiple substeps of Golgi-to-PM transport. The question is, how are sequential interactions of a single Ypt/Rab with its multiple effectors orchestrated?

The second paradigm is coordination of multiple transport steps of a single pathway. For sequential function of multiple Ypts in the same pathway there are currently two models. The first suggests that an effector of the first Ypt/Rab acts as a GEF for the next Ypt/Rab: for example, Rab5-to-Rab7 conversion on endosomes (Rink et al., 2005) (Figure 5). However, the question is how sequential interactions of Rab5 are orchestrated so that Rab5 can execute all its functions in early endosomes before recruiting the Rab7 GEF, which would result in its own exclusion and conversion of the early endosome to late endosome. We proposed a second model for sequential function of multiple Ypt/Rabs: GEF conversion, TRAPP I-to-TRAPP II, to sequentially activate Ypt1 and Ypt31/Ypt32 at the Golgi (Morozova et al., 2006) (Figure 3). However, proof that such coordination occurs and has a role is still missing.

The third paradigm (Section IIc) envisions coordination of two different pathways by a single Ypt/Rab. We have shown that a single Ypt/Rab can function in the context of two separate modules. However, the question is if the two functions are coordinated. This question is related to a more basic question about monomeric GTPase function in general: can an activated GTPase "diffuse" in membranes to interact with their effectors in a random manner? This would be very inefficient (Kahn, 2014). If not, what is the mechanism for ensuring that a Ypt/Rab GTPase binds a specific effector in a specific location at a specific time? One possibility is that different GEFs control not only the Ypt/Rab activation, but also their attachment to a specific compartment and the specific effectors with which it interacts; namely, assembly of Ypt/Rab "modules" is not random (Figure 6).

3) Ypt/Rab coordination conservation—Complete loss of function of the exocytic Ypts, Ypt1 and Ypt31/Ypt32, results in cell lethality and the same is true for their GEF complex TRAPP. In humans, for the ubiquitous Rabs, it is expected that a complete loss of function would result in early development lethality. The disease connection of the closest homologues, Rab1, Rab11, and mTRAPP, was established with subtle mutations or differences in expression (Mitra et al., 2011). We propose that Ypt/Rab-dependent coordination plays a role in fine-tuning of the intracellular trafficking network. Therefore, its disruption is expected to result in human disease, especially in systems that depend on efficient and timely trafficking, like neurons.

IV. Why yeast is a good model system to address future questions?

While the Ypt/Rabs were first discovered in yeast and principles of upstream regulation and downstream function were first established in this model organism, many discoveries about their function emerged from studies in mammalian cells. These include the Rab domains, Rab conversion (Rink et al., 2005, Sonnichsen et al., 2000) and GAP specificity (Haas et al., 2007). Most importantly, work from mammalian cells pointed to their relevance to human health and disease (Mitra et al., 2011). So, why continue using yeast to study Ypt/Rabs?

Whereas mammalian cells have been more tractable than yeast in microscopy and biochemistry studies due to their bigger size and easier protein isolation, yeast still provide a superior system for discovering and establishing new paradigms of Ypt/Rab function for two reasons: amenability to genetic analysis and a smaller proteome.

Genetic analysis is crucial for establishing the significance of suggested mechanisms. For example, two interesting findings shown a decade ago with mammalian Rabs are still at the level of phenomena: Rab domains and Rab cascades (Rink et al., 2005, Sonnichsen et al., 2000). To date, their role and importance are not clear because of lack of mutants that disrupt these phenomena specifically without affecting other functions of the Rab GTPases or their regulators. While construction of mutations in endogenous mammalian genes has become easier in the last decade, determining direct phenotypes, as opposed to indirect phenotypes, still lags behind yeast, as does analysis of multiple mutations.

Moreover, the smaller proteome of yeast is of a huge advantage when principles of intracellular traffic coordination are being studied. Whereas the human proteome contains

~70 Rabs, the yeast proteome has only 11 Ypts. The whole secretory pathway requires three Ypts and the endocytic pathway needs two Ypts. Thus, yeast has a major advantage for inquiring how the different steps of these pathways are coordinated by Ypts. The same is true for inquiring about coordination of the secretory pathway with other cellular pathways, like autophagy and PM recycling (Lipatova and Segev, 2014).

The question is whether what we find in yeast about coordination of trafficking networks by Ypts would pertain to Rabs in human cells. In retrospect, the early finding that Rab1 can functionally replace Ypt1 (Haubruck et al., 1989) is striking considering what we know today about Ypt/Rabs and intracellular trafficking. Ypt/Rabs do not function solo. Instead, they function as parts of welloiled molecular machines. Functional conservation of a Ypt/Rab implies that other components of these machines are conserved as well. Thus, we propose that the basic principles discovered in yeast about coordination of intracellular traffic by Ypts will pertain to the more complicated Rab circuits.

Acknowledgments

We thank J. Kim and U. Majumdar for text editing. This research was supported by grant GM-45444 from NIH to N. Segev.

References

Barbacid M. ras genes. Annu Rev Biochem. 1987; 56:779–827. [PubMed: 3304147]

Barr F, Lambright DG. Rab GEFs and GAPs. Curr Opin Cell Biol. 2010; 22:461–470. [PubMed: 20466531]

Barrowman J, Bhandari D, Reinisch K, Ferro-Novick S. TRAPP complexes in membrane traffic: convergence through a common Rab. Nat Rev Mol Cell Biol. 2010; 11:759–763. [PubMed: 20966969]

Bennett MK, Scheller RH. The molecular machinery for secretion is conserved from yeast to neurons. Proc Natl Acad Sci U S A. 1993; 90:2559–2563. [PubMed: 8096639]

Bourne HR. Do GTPases direct membrane traffic in secretion? Cell. 1988; 53:669–671. [PubMed: 2836065]

Cai Y, Chin HF, Lazarova D, Menon S, Fu C, Cai H, Sclafani A, Rodgers DW, De la cruz EM, Ferro-Novick S, Reinisch KM. The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. Cell. 2008; 133:1202–1213. [PubMed: 18585354]

Cantalupo G, Alifano P, Roberti V, Bruni CB, Bucci C. Rab-interacting lysosomal protein (RILP): the Rab7 effector required for transport to lysosomes. Embo J. 2001; 20:683–693. [PubMed: 11179213]

Chen SH, Chen S, Tokarev AA, Liu F, Jedd G, Segev N. Ypt31/32 GTPases and their novel F-box effector protein Rcy1 regulate protein recycling. Mol Biol Cell. 2005; 16:178–192. [PubMed: 15537705]

Chen SH, Shah AH, Segev N. Ypt31/32 GTPases and their F-Box effector Rcy1 regulate ubiquitination of recycling proteins. Cell Logist. 2011; 1:21–31. [PubMed: 21686101]

Chen Y, Zhou F, Zou S, Yu S, Li S, Li D, Song J, Li H, He Z, Hu B, Bjorn LO, Lipatova Z, Liang Y, Xie Z, Segev N. A Vps21 endocytic module regulates autophagy. Mol Biol Cell. 2014; 25:3166–3177. [PubMed: 25143401]

Feig LA. Tools of the trade: use of dominant-inhibitory mutants of Ras-family GTPases. Nat Cell Biol. 1999; 1:E25–E27. [PubMed: 10559887]

Gao XD, Albert S, Tcheperegine SE, Burd CG, Gallwitz D, Bi E. The GAP activity of Msb3p and Msb4p for the Rab GTPase Sec4p is required for efficient exocytosis and actin organization. J Cell Biol. 2003; 162:635–646. [PubMed: 12913108]

Goud B, Salminen A, Walworth NC, Novick PJ. A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. Cell. 1988; 53:753–768. [PubMed: 3131018]

Grosshans BL, Andreeva A, Gangar A, Niessen S, Yates JR 3rd, Brennwald P, Novick P. The yeast lgl family member Sro7p is an effector of the secretory Rab GTPase Sec4p. J Cell Biol. 2006; 172:55–66. [PubMed: 16390997]

Guo W, Roth D, Walch-solimena C, Novick P. The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. Embo J. 1999; 18:1071–1080. [PubMed: 10022848]

Haas AK, Yoshimura S, Stephens DJ, Preisinger C, Fuchs E, Barr FA. Analysis of GTPase-activating proteins: Rab1 and Rab43 are key Rabs required to maintain a functional Golgi complex in human cells. J Cell Sci. 2007; 120:2997–3010. [PubMed: 17684057]

Haubruck H, Prange R, Vorgias C, Gallwitz D. The ras-related mouse ypt1 protein can functionally replace the YPT1 gene product in yeast. Embo J. 1989; 8:1427–1432. [PubMed: 2670553]

Jedd G, Mulholland J, Segev N. Two new Ypt GTPases are required for exit from the yeast trans-Golgi compartment. J Cell Biol. 1997; 137:563–580. [PubMed: 9151665]

Jedd G, Richardson C, Litt R, Segev N. The Ypt1 GTPase is essential for the first two steps of the yeast secretory pathway. J Cell Biol. 1995; 131:583–590. [PubMed: 7593181]

Jones S, Jedd G, Kahn RA, Franzusoff A, Bartolini F, Segev N. Genetic interactions in yeast between Ypt GTPases and Arf guanine nucleotide exchangers. Genetics. 1999; 152:1543–1556. [PubMed: 10430582]

Jones S, Litt RJ, Richardson CJ, Segev N. Requirement of nucleotide exchange factor for Ypt1 GTPase mediated protein transport. J Cell Biol. 1995; 130:1051–1061. [PubMed: 7657691]

Jones S, Newman C, Liu F, Segev N. The TRAPP complex is a nucleotide exchanger for Ypt1 and Ypt31/32. Mol Biol Cell. 2000; 11:4403–4411. [PubMed: 11102533]

Jones S, Richardson CJ, Litt RJ, Segev N. Identification of regulators for Ypt1 GTPase nucleotide cycling. Mol Biol Cell. 1998; 9:2819–2837. [PubMed: 9763446]

Kahn R. Is the model of signal amplification by GPCRs/GEFs activating multiple GTPases relevant to a broad spectrum of heterotrimeric and RAS superfamily GTPases? Cellular Logistics. 2014; 4

Kahn RA. Toward a model for Arf GTPases as regulators of traffic at the Golgi. FEBS Lett. 2009; 583:3872–3879. [PubMed: 19879269]

Kirisako T, Baba M, Ishihara N, Miyazawa K, Ohsumi M, Yoshimori T, Noda T, Ohsumi Y. Formation process of autophagosome is traced with Apg8/Aut7p in yeast. J Cell Biol. 1999; 147:435–446. [PubMed: 10525546]

Li, G.; Segev, N. Ypt/Rab GTPases and Intracellular Membrane Trafficking: An Overview. In: Li, G.; Segev, N., editors. Rab GTpases and membrane trafficking. Bentham Science; 2013.

Liang Y, Morozova N, Tokarev AA, Mulholland JW, Segev N. The role of Trs65 in the Ypt/Rab guanine nucleotide exchange factor function of the TRAPP II complex. Mol Biol Cell. 2007; 18:2533–2541. [PubMed: 17475775]

Lipatova Z, Belogortseva N, Zhang XQ, Kim J, Taussig D, Segev N. Regulation of selective autophagy onset by a Ypt/Rab GTPase module. Proc Natl Acad Sci U S A. 2012; 109:6981–6986. [PubMed: 22509044]

Lipatova Z, Segev N. A Ypt/Rab GTPase module makes a PAS. Autophagy. 2012; 8:1271–1272. [PubMed: 22669536]

Lipatova Z, Segev N. Ypt/Rab GTPases regulate 2 intersections of the secretory and endosomal/lysosomal pathways. Cellular Logistics. 2014; 4

Lipatova Z, Shah AH, Kim JJ, Mulholland JW, Segev N. Regulation of ER-phagy by a Ypt/Rab GTPase module. Mol Biol Cell. 2013; 24:3133–3144. [PubMed: 23924895]

Lipatova Z, Tokarev AA, Jin Y, Mulholland J, Weisman LS, Segev N. Direct interaction between a myosin V motor and the Rab GTPases Ypt31/32 is required for polarized secretion. Mol Biol Cell. 2008; 19:4177–4187. [PubMed: 18653471]

Marat AL, Dokainish H, Mcpherson PS. DENN domain proteins: regulators of Rab GTPases. J Biol Chem. 2011; 286:13791–13800. [PubMed: 21330364]

Mcdonold CM, Fromme JC. Four GTPases Differentially Regulate the Sec7 Arf-GEF to Direct Traffic at the trans-Golgi Network. Dev Cell. 2014; 30:759–767. [PubMed: 25220393]

Mitra S, Cheng KW, Mills GB. Rab GTPases implicated in inherited and acquired disorders. Semin Cell Dev Biol. 2011; 22:57–68. [PubMed: 21147240]

Morozova N, Liang Y, Tokarev AA, Chen SH, Cox R, Andrejic J, Lipatova Z, Sciorra VA, Emr SD, Segev N. TRAPPII subunits are required for the specificity switch of a Ypt-Rab GEF. Nat Cell Biol. 2006; 8:1263–1269. [PubMed: 17041589]

Nottingham RM, Pfeffer SR. Defining the boundaries: Rab GEFs and GAPs. Proc Natl Acad Sci U S A. 2009; 106:14185–14186. [PubMed: 19706500]

Ortiz D, Medkova M, Walch-solimena C, Novick P. Ypt32 recruits the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles; evidence for a Rab cascade in yeast. J Cell Biol. 2002; 157:1005–1015. [PubMed: 12045183]

Peplowska K, Markgraf DF, Ostrowicz CW, Bange G, Ungermann C. The CORVET tethering complex interacts with the yeast Rab5 homolog Vps21 and is involved in endo-lysosomal biogenesis. Dev Cell. 2007; 12:739–750. [PubMed: 17488625]

Pfeffer SR. Transport vesicle docking: SNAREs and associates. Annu Rev Cell Dev Biol. 1996; 12:441–461. [PubMed: 8970734]

Pfeffer SR. Rab GTPase regulation of membrane identity. Curr Opin Cell Biol. 2013; 25:414–419. [PubMed: 23639309]

Poteryaev D, Datta S, Ackema K, Zerial M, Spang A. Identification of the switch in early-to-late endosome transition. Cell. 2010; 141:497–508. [PubMed: 20434987]

Richardson CJ, Jones S, Litt RJ, Segev N. GTP hydrolysis is not important for Ypt1 GTPase function in vesicular transport. Mol Cell Biol. 1998; 18:827–838. [PubMed: 9447979]

Rink J, Ghigo E, Kalaidzidis Y, Zerial M. Rab conversion as a mechanism of progression from early to late endosomes. Cell. 2005; 122:735–749. [PubMed: 16143105]

Rivera-molina FE, Novick PJ. A Rab GAP cascade defines the boundary between two Rab GTPases on the secretory pathway. Proc Natl Acad Sci U S A. 2009; 106:14408–14413. [PubMed: 19666511]

Sacher M, Jiang Y, Barrowman J, Scarpa A, Burston J, Zhang L, Schieltz D, Yates JR 3rd, Abeliovich H, Ferro-Novick S. TRAPP, a highly conserved novel complex on the cis-Golgi that mediates vesicle docking and fusion. Embo J. 1998; 17:2494–2503. [PubMed: 9564032]

Sacher M, Kim YG, Lavie A, Oh BH, Segev N. The TRAPP complex: insights into its architecture and function. Traffic. 2008; 9:2032–2042. [PubMed: 18801063]

Salminen A, Novick PJ. A ras-like protein is required for a post-Golgi event in yeast secretion. Cell. 1987; 49:527–538. [PubMed: 3552249]

Schimmoller F, Riezman H. Involvement of Ypt7p, a small GTPase, in traffic from late endosome to the vacuole in yeast. J Cell Sci. 1993; 106(Pt 3):823–830. [PubMed: 8308065]

Schmitt HD, Puzicha M, Gallwitz D. Study of a temperature-sensitive mutant of the ras-related YPT1 gene product in yeast suggests a role in the regulation of intracellular calcium. Cell. 1988; 53:635–647. [PubMed: 3286011]

Schmitt HD, Wagner P, Pfaff E, Gallwitz D. The ras-related YPT1 gene product in yeast: a GTP-binding protein that might be involved in microtubule organization. Cell. 1986; 47:401–412. [PubMed: 3094963]

Sclafani A, Chen S, Rivera-molina F, Reinisch K, Novick P, Ferro-Novick S. Establishing a role for the GTPase Ypt1p at the late Golgi. Traffic. 2010; 11:520–532. [PubMed: 20059749]

Segev N. Mediation of the attachment or fusion step in vesicular transport by the GTP-binding Ypt1 protein. Science. 1991; 252:1553–1556. [PubMed: 1904626]

Segev N. Ypt and Rab GTPases: insight into functions through novel interactions. Curr Opin Cell Biol. 2001a; 13:500–511. [PubMed: 11454458]

Segev N. Ypt/rab gtpases: regulators of protein trafficking. Sci STKE. 2001b; 2001:re11. [PubMed: 11579231]

Segev N. Coordination of intracellular transport steps by GTPases. Semin Cell Dev Biol. 2011a; 22:33–38. [PubMed: 21130177]

Segev N. GTPases in intracellular trafficking: an overview. Semin Cell Dev Biol. 2011b; 22:1–2. [PubMed: 21145981]

Segev N, Botstein D. The ras-like yeast YPT1 gene is itself essential for growth, sporulation, and starvation response. Mol Cell Biol. 1987; 7:2367–2377. [PubMed: 3302675]

Segev N, Mulholland J, Botstein D. The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. Cell. 1988; 52:915–924. [PubMed: 3127057]

Singer-Kruger B, Stenmark H, Dusterhoft A, Philippsen P, Yoo JS, Gallwitz D, Zerial M. Role of three rab5-like GTPases, Ypt51p, Ypt52p, and Ypt53p, in the endocytic and vacuolar protein sorting pathways of yeast. J Cell Biol. 1994; 125:283–298. [PubMed: 8163546]

Singer-kruger B, Stenmark H, Zerial M. Yeast Ypt51p and mammalian Rab5: counterparts with similar function in the early endocytic pathway. J Cell Sci. 1995; 108(Pt 11):3509–3521. [PubMed: 8586662]

Sonnichsen B, De renzis S, Nielsen E, Rietdorf J, Zerial M. Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of Rab4, Rab5, and Rab11. J Cell Biol. 2000; 149:901–914. [PubMed: 10811830]

Stenmark H, Olkkonen VM. The Rab GTPase family. Genome Biol. 2001; 2 REVIEWS3007.

Stryer L, Bourne HR. G proteins: a family of signal transducers. Annu Rev Cell Biol. 1986; 2:391–419. [PubMed: 3103658]

Suda Y, Kurokawa K, Hirata R, Nakano A. Rab GAP cascade regulates dynamics of Ypt6 in the Golgi traffic. Proc Natl Acad Sci U S A. 2013; 110:18976–18981. [PubMed: 24194547]

Tall GG, Hama H, Dewald DB, Horazdovsky BF. The phosphatidylinositol 3-phosphate binding protein Vac1p interacts with a Rab GTPase and a Sec1p homologue to facilitate vesicle-mediated vacuolar protein sorting. Mol Biol Cell. 1999; 10:1873–1889. [PubMed: 10359603]

Taussig, D.; Chen, S.; Segev, N. The Golgi Gatekeepers: Ypt1-Rab1 and Ypt31/32-Rab11. In: Li, G.; Segev, N., editors. Rab GTPases and Membrane Trafficking. Bentham Science; 2012.

Taussig D, Lipatova Z, Kim JJ, Zhang X, Segev N. Trs20 is required for TRAPP II assembly. Traffic. 2013; 14:678–690. [PubMed: 23465091]

Taussig D, Lipatova Z, Segev N. Trs20 is required for TRAPP III complex assembly at the PAS and its function in autophagy. Traffic. 2014; 15:327–337. [PubMed: 24329977]

Toda T, Broek D, Field J, Michaeli T, Cameron S, Nikawa J, Sass P, Birchmeier C, Powers S, Wigler M. Exploring the function of RAS oncogenes by studying the yeast Saccharomyces cerevisiae. Princess Takamatsu Symp. 1986; 17:253–260. [PubMed: 3332013]

Tokarev AA, Taussig D, Sundaram G, Lipatova Z, Liang Y, Mulholland JW, Segev N. TRAPP II complex assembly requires Trs33 or Trs65. Traffic. 2009; 10:1831–1844. [PubMed: 19843283]

Tooze SA, Abada A, Elazar Z. Endocytosis and autophagy: exploitation or cooperation? Cold Spring Harb Perspect Biol. 2014; 6:a018358. [PubMed: 24789822]

Walch-solimena C, Collins RN, Novick PJ. Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles. J Cell Biol. 1997; 137:1495–1509. [PubMed: 9199166]

Walworth NC, Brennwald P, Kabcenell AK, Garrett M, Novick P. Hydrolysis of GTP by Sec4 protein plays an important role in vesicular transport and is stimulated by a GTPase-activating protein in Saccharomyces cerevisiae. Mol Cell Biol. 1992; 12:2017–2028. [PubMed: 1569938]

Yip CK, Berscheminski J, Walz T. Molecular architecture of the TRAPPII complex and implications for vesicle tethering. Nat Struct Mol Biol. 2010; 17:1298–1304. [PubMed: 20972447]

Zerial M, Mcbride H. Rab proteins as membrane organizers. Nat Rev Mol Cell Biol. 2001; 2:107–117. [PubMed: 11252952]

Zou S, Liu Y, Zhang XQ, Chen Y, Ye M, Zhu X, Yang S, Lipatova Z, Liang Y, Segev N. Modular TRAPP complexes regulate intracellular protein trafficking through multiple Ypt/Rab GTPases in Saccharomyces cerevisiae. Genetics. 2012; 191:451–460. [PubMed: 22426882]

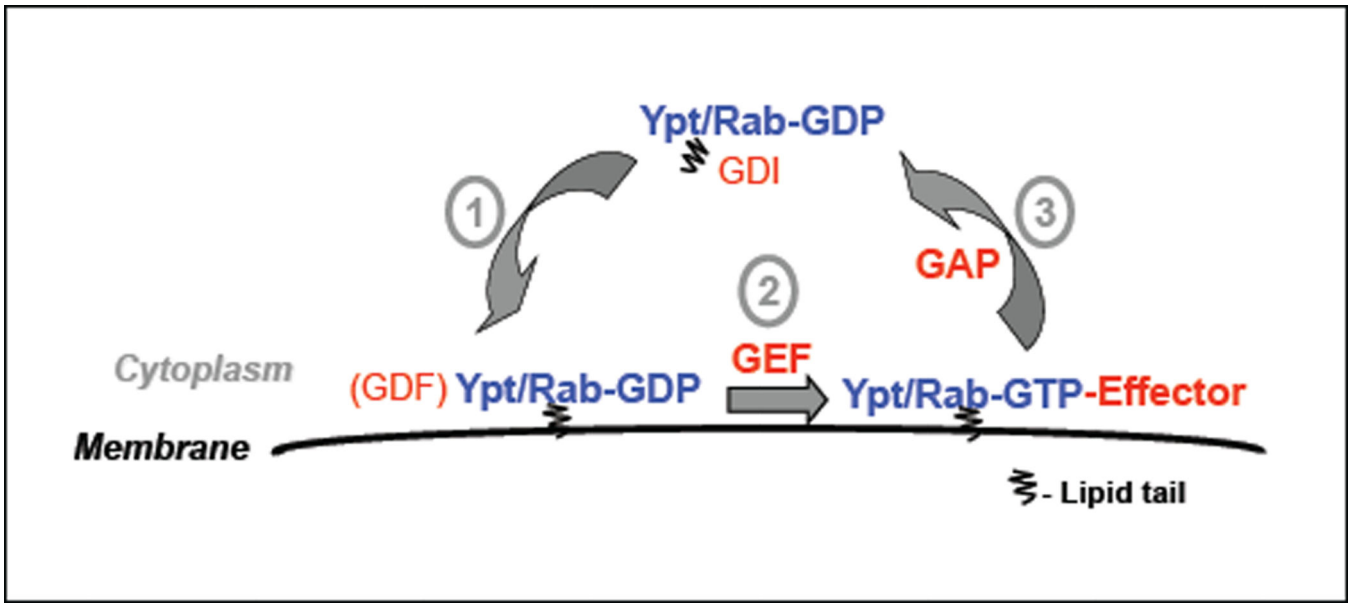

Figure 1. Regulation of Ypt/Rabs nucleotide switching and membrane cycling by their accessory factors

Two inputs contribute to the activation of Ypt/Rabs: (1) attachment to membranes mediated by GDI and possibly a GDF, and (2) nucleotide exchange stimulated by GEFs. (3) GTP-hydrolysis, stimulated by GAPs, allows the recycling of Ypt/Rabs to the cytoplasm by GDI. Ypt/Rab, blue; accessory factors, red. Note: a color version of this figure is available online.

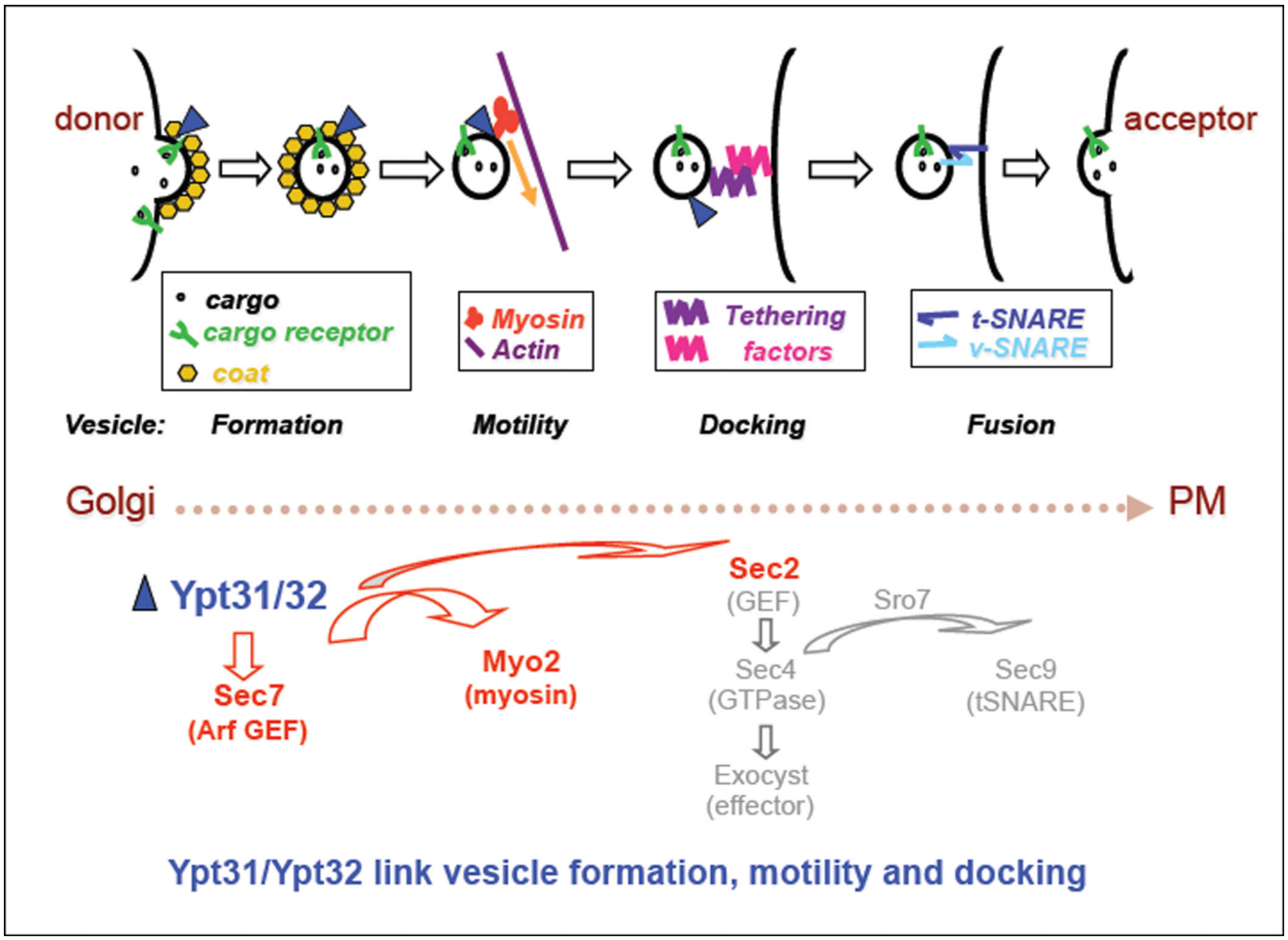

Figure 2. A model for coordination of vesicle transport substeps by Ypt31/Ypt32

Ypt31/Ypt32 (triangle) recruit three different effectors, Sec7, Myo2 and Sec2, which mediate trans-Golgi vesicle formation, motility and docking to the PM, respectively. It is not clear how the sequence of these interactions is regulated (see text for details). Note: a color version of this figure is available online.

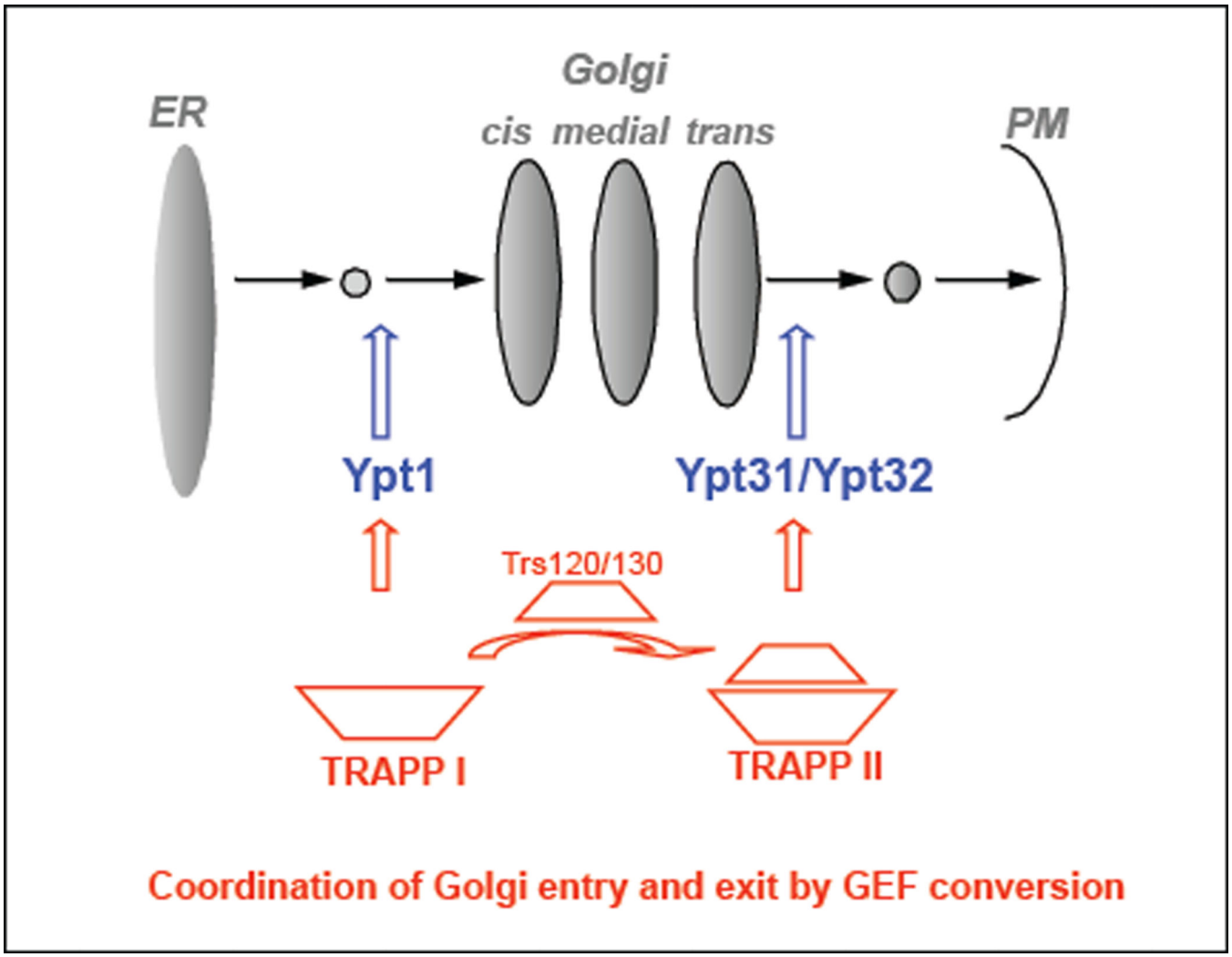

Figure 3. A model for coordination of Golgi entry and exit by the TRAPP GEF complex

TRAPP I, a Ypt1 GEF, converts to TRAPP II, a Ypt31/Ypt32 GEF, by the addition of the two TRAPP II-specific subunits, Trs120 and Trs130. We have proposed that such conversion could couple Ypt1-mediated Golgi entry with Ypt31/Ypt32-mediated Golgi exit (see text for details). Note: a color version of this figure is available online.

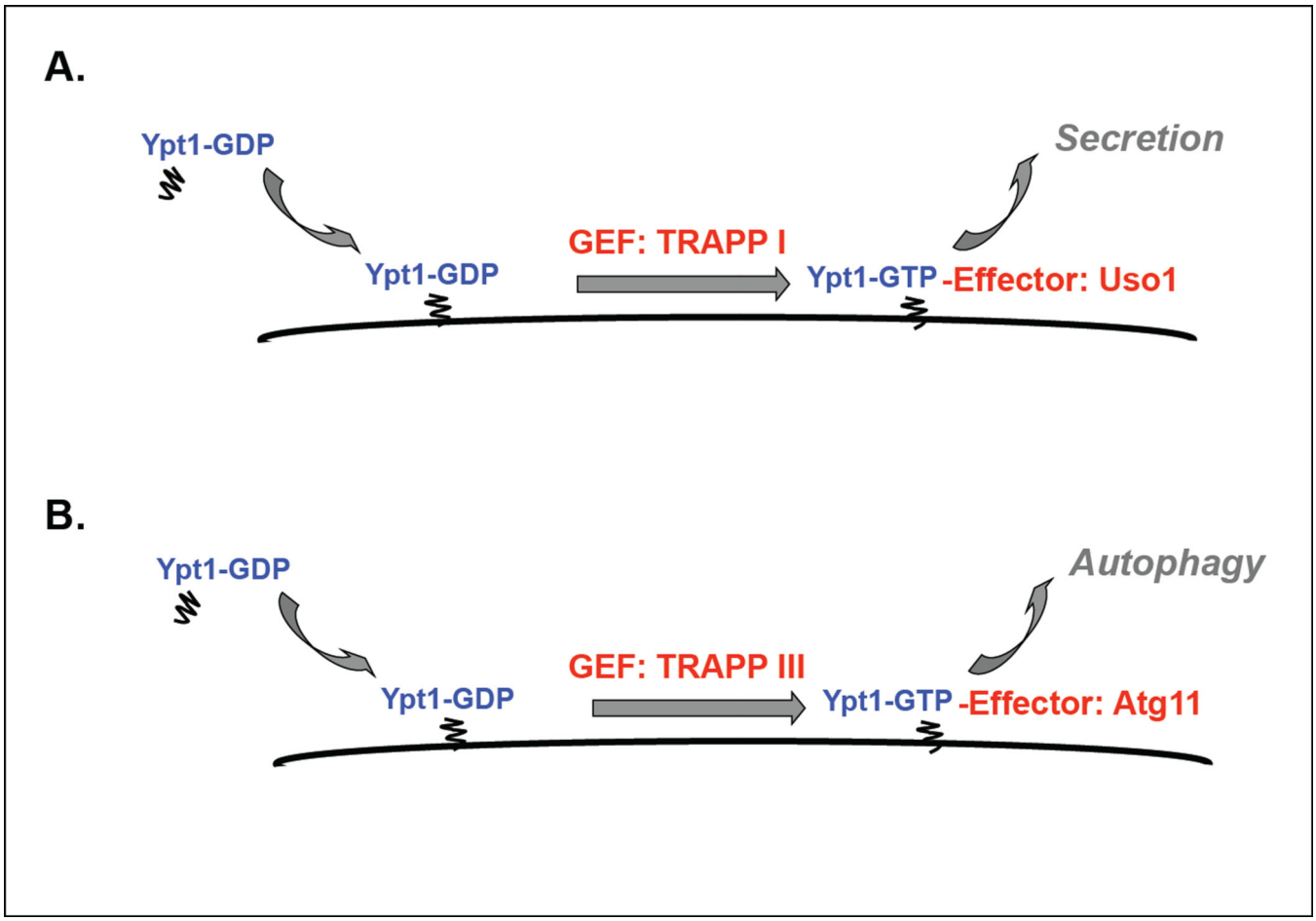

Figure 4. A model for coordination of ER-to-Golgi and ER-phagy by two Ypt1 modules

We have proposed that depending on the cargo, Ypt1 is recruited to the ER-originating membranes by different GEFs, where it recruits different effectors: **A)** with TRAPP I as a GEF, Ypt1 recruit effectors like Uso1 to send the cargo for secretion through the Golgi; **B)** with TRAPP III as a GEF, Ypt1 recruit their autophagy-specific effector Atg11 to deliver the cargo for degradation via the autophagy pathway. Note: a color version of this figure is available online.

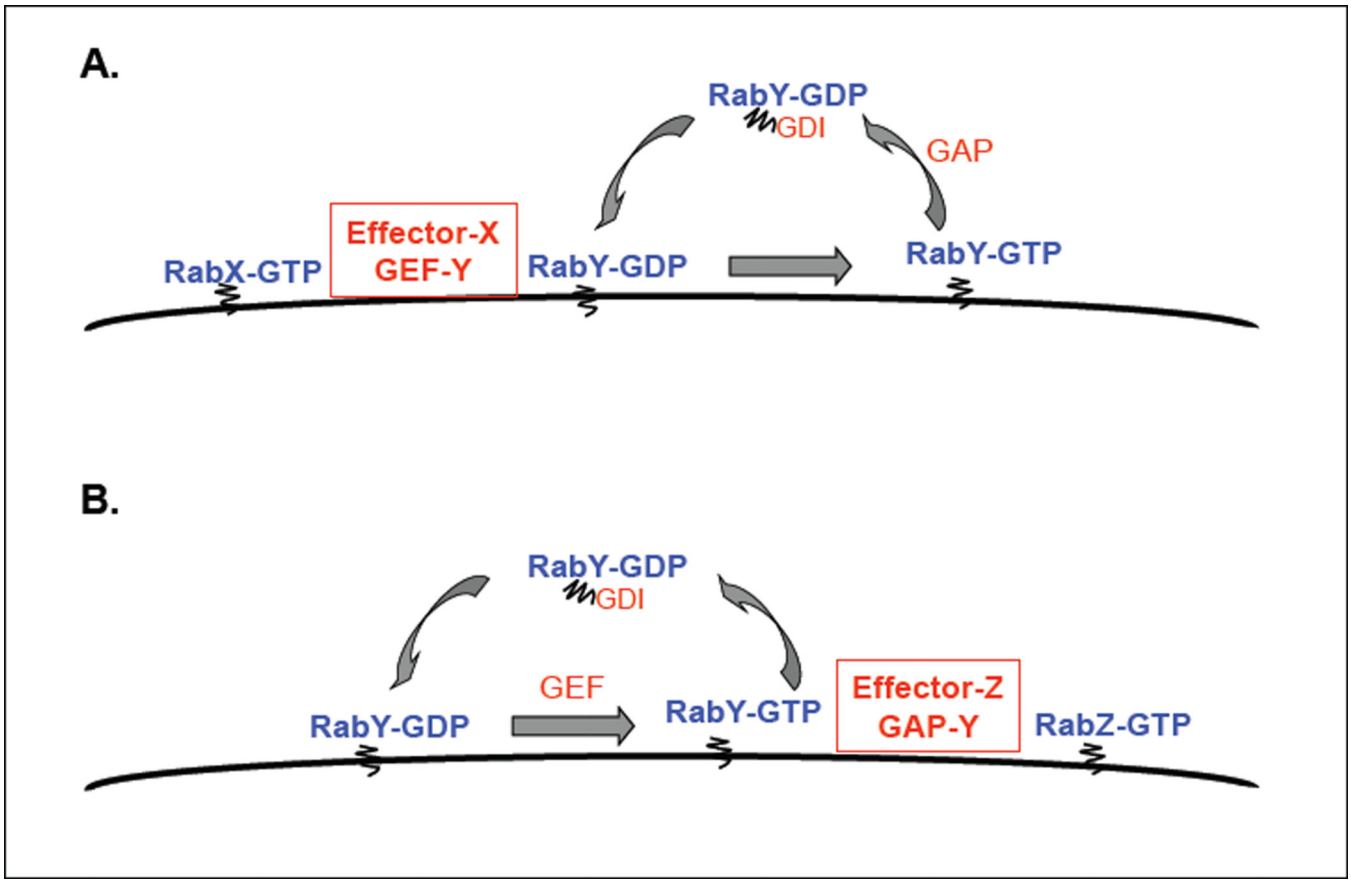

Figure 5. A model for Ypt/Rab cascades

Rab cascades were suggested to function in endosomes and the Golgi either using GEFs or GAPs. **A)** In the GEFcascade model, the effector of RabX acts as a GEF for RabY, which functions in the next step, ensuring forward progression in the cascade. **B)** In the GAP-cascade model, the effector of RabZ acts as a GAP for RabY, which functions in the previous step, ensuring that only one Rab is present on a compartment at any time. Note: a color version of this figure is available online.

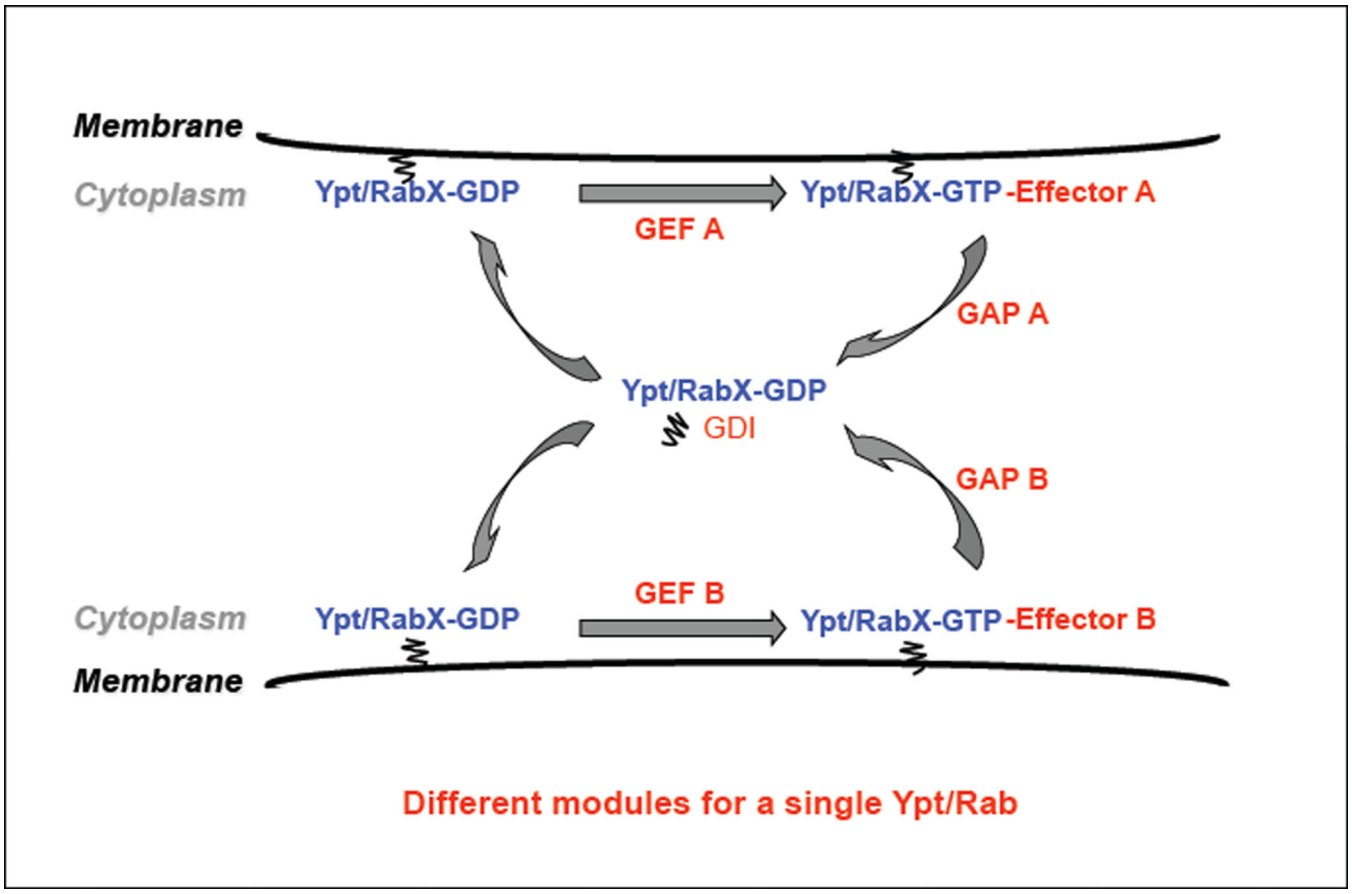

Figure 6. A model for the function of a single Ypt/Rab in the context of different non-random modules

Depending on the cargo, a Ypt/RabX is activated on different membranes by different GEFs: A or B. Depending on the GEF, Ypt/RabX interacts with the cognate effector: A or B. It is possible that GAPs, A and B, are also part of such modules. Note: a color version of this figure is available online.