Journal of Cell Science 113, 183-192 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JCS0707

COMMENTARY

Rab GTPases coordinate endocytosis

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Published on WWW 13 January 2000

SUMMARY

Endocytosis is characterized by vesicular transport along numerous pathways. Common steps in each pathway include membrane budding to form vesicles, transport to a particular destination, and ultimately docking and fusion with the target membrane. Specificity of vesicle targeting is rendered in part by associated Rab GTPases. This review summarizes current knowledge about Rab GTPase functions in the endocytic pathways and provides insight into the regulation of Rab GTPase activity and mechanisms of Rab protein function. Functional assays have identified some Rab proteins that operate on individual pathways, but Rab proteins in several pathways remain controversial or have not been identified. Control of Rab GTPase activity is exerted through multiple levels of regulation. Significant new information pertaining to Rab protein function in regulating transport has emerged. Remarkably, Rab5 GTPase links budding, cytoskeletal transport and docking/fusion activities. This paradigm will most likely be generally applicable to other Rab GTPase pathways. Together with the cross-talk between different Rab proteins and their effectors, this may provide an integrated system for the general coordination of endocytic pathways to maintain organelle homeostasis.

Key words: Endocytic traffic, Cytoskeleton and transport, Vesicular transport, Membrane fusion

INTRODUCTION

Rab proteins are small GTPases that regulate vesicular transport in endocytosis and exocytosis, where they have been implicated principally in the control of vesicle docking and fusion (Gonzalez and Scheller, 1999; Mohrmann and van der Sluijs, 1999; Schimmöller et al., 1998). To date, ~40 distinct Rab proteins have been identified, and each is believed to be specifically associated with a particular organelle or pathway. Thus far, the functions of only a fraction of known Rab proteins have been characterized in detail. Efforts to elucidate the molecular basis of Rab protein function have identified diverse proteins, which range from protein kinases to cytoskeletal proteins, associated with individual Rab proteins (Christoforidis et al., 1999b; Echard et al., 1998; Gournier et al., 1998; Nielsen et al., 1999; Ren et al., 1996; Sasaki et al., 1997; Shirataki et al., 1993; Simonsen et al., 1998; Stenmark et al., 1995; Zeng et al., 1999). Consequently, it has been difficult to arrive at a consensus regarding the function of Rab effector proteins in membrane transport.

Emerging data indicate that a single activated Rab protein can selectively bind to a multitude of effector proteins to facilitate discrete steps in membrane transport. Through sequential interactions, it is likely that Rab proteins temporally and spatially coordinate vesicular transport (Christoforidis et al., 1999a,b). Initially Rab proteins are recruited to and activated on the donor membrane, where they are important in vesicle budding (Jedd et al., 1997; Jones et al., 1999; McLauchlan et al., 1998; Nuoffer et al., 1994; Riederer et al., 1994). New data show that Rab proteins subsequently facilitate transport along the cytoskeleton (Echard et al., 1998; Nielsen et al., 1999) and finally participate in docking and fusion (Gonzalez and Scheller, 1999; Mohrmann and van der Sluijs, 1999; Schimmöller et al., 1998). Thus, Rab proteins may be viewed as central regulators of a pathway that integrate events at each step of vesicular transport (Fig. 1). In keeping with this idea, enzymatically active effectors help to regulate protein recruitment and provide motility (Christoforidis et al., 1999b; Echard et al., 1998; Nielsen et al., 1999). Other Rab effector proteins fulfill adhesive or tethering functions, bringing appropriate membranes into close contact (Gournier et al., 1998; Mohrmann and van der Sluijs, 1999; Simonsen et al., 1998; Stenmark et al., 1995; Wurmser et al., 1999). Finally, effector and/or Rab protein interactions with components of the SNARE machinery initiate fusion (Bean et al., 1997; McBride et al., 1999; Peterson et al., 1999; Sapperstein et al., 1996; Sato and Wickner, 1998).

Multiple regulatory circuits control each aspect of membrane transport. The Rab GTPases are themselves tightly regulated by accessory proteins that modulate Rab protein activity by controlling membrane association, nucleotide binding and hydrolysis (Mohrmann and van der Sluijs, 1999). Another level of regulation is exerted by protein kinases that control individual Rab protein activities or downstream

effector functions (Ayad et al., 1997; Bailly et al., 1991; Barbieri et al., 1998; Chiariello et al., 1999; Fitzgerald and Reed, 1999; Numata et al., 1994; Ren et al., 1996; van der Sluijs et al., 1992). Finally, opposing membrane transport pathways governed by distinct Rab proteins must be balanced to ensure organelle homeostasis.

A number of endocytic Rab proteins have been characterized in the past two years. In this review, we consider the regulation and function ascribed to individual Rab proteins in endocytosis. We address mechanistic data supporting Rab proteins as the link between vesicle budding, transport and docking/fusion. Finally, we briefly discuss the coordinate regulation of multiple Rab-controlled endocytic pathways that effects homeostasis of membrane flux.

OVERVIEW OF ENDOCYTIC RAB PROTEINS

Ubiquitous Rab GTPases control specific endocytic pathways

Twelve Rab proteins have been localized to the endocytic pathway of mammalian cells; eight have been functionally characterized, and four are epithelial specific (Table 1). Current understanding of ubiquitous Rab proteins that regulate distinct endocytic pathways is illustrated in Fig. 2.

In the first step of internalization, ligands are sequestered into clathrin-coated pits. Activated Rab5 is important for sequestering ligands into clathrin-coated pits and subsequent fusion of vesicles with early endosomes (also called sorting endosomes; Fig. 2; Christoforidis et al., 1999a; McLauchlan et al., 1998). The presence of Rab5 on early endosomes is also essential for their homotypic fusion in vitro and in vivo (Barbieri et al., 1996; Bucci et al., 1992; Gorvel et al., 1991). The localization of Rab22 and the consequences of its overexpression suggest that Rab22 regulates internalization, but the precise step and mechanisms are unclear (Olkkonen et al., 1993). Recent characterization of Rab15 function in the uptake of transferrin suggests that it inhibits initial internalization events (Zuk and Elferink, 1999). Rab15 inhibitory activity could be attributed to stimulation of a pathway operating in the opposing direction or to direct negative regulation. Since Rab proteins generally are stimulatory, it will be interesting to explore the possibility that a class of inhibitory Rab proteins functions in a similar way to the inhibitory heterotrimeric GTPases. Considering the likely existence of inhibitory Rab GTPases is important for understanding endosome homeostasis.

Molecules exit early endosomes along several different pathways. A direct pathway for recycling receptors to the plasma membrane depends on Rab4 (Daro et al., 1996). Recent findings also implicate Rab4 in recycling via recycling endosomes (see Fig. 2; Mohrmann and van der Sluijs, 1999). Whether Rab4 controls recycling along two pathways or another GTPase is involved remains an open question. In any event, it is likely that Rab5 and Rab4 act together to control influx into and efflux out of early endosomes respectively, since the two proteins exhibit concerted effector binding (Mohrmann and van der Sluijs, 1999) and have opposing effects on an early endosome fusion assay (Chavrier et al., 1997).

A slow recycling route traversed by transferrin receptors and recycling membrane lipids leads from early endosomes through recycling endosomes back to the plasma membrane (Mukherjee et al., 1997). Mounting evidence suggests that recycling endosomes constitute a distinct endocytic compartment characterized by a discrete protein composition and function (Daro et al., 1996; Mukherjee et al., 1997; Sheff et al., 1999). Rab11, concentrated on recycling endosomes, was initially demonstrated to be important for transferrin transport through recycling endosomes in non-polarized cells (Ren et al., 1998; Ullrich et al., 1996). Functional analyses of Rab11 employing the Rab11Q70L mutant that was used in these studies must be evaluated with caution, since this mutant is not GTPase defective as originally presumed (Casanova et al., 1999). In epithelial cells Rab11 is critical for exit from apical recycling endosomes to the plasma membrane (Calhoun et al., 1998; Duman et al., 1999; Fig. 3). On the basis of the cumulative data, we think that Rab11 regulates the return of recycling receptors to the plasma membrane (Figs 2 and 3). Rab11 is also implicated in exocytic membrane transport from the Golgi (Chen et al., 1998; Jedd et al., 1997; Urbé et al., 1993). New data suggest it controls passage from the Golgi through endosomes (W. Chen and A. Wandinger-Ness, unpublished). Thus, Rab11 may be important for controlling the intersection of endocytic and exocytic pathways and for the homeostasis of the recycling endosome. In this view, the observed accumulation of transferrin (Ullrich et al., 1996) and its receptor (Chen et al., 1998) in early endosomes in cells expressing dominant negative Rab11S25N might be an indirect consequence of perturbed recycling that alters endosome structure and function. Such a perturbation could be caused by a block in export from the Golgi to recycling endosomes in the face of continued transport in the opposing direction (Ullrich et al., 1996).

Molecules transported to the trans-Golgi network from endosomes follow at least two different routes (Fig. 2). In one case, defined by internalized Tac-TGN38 and the bacterial toxins, transport occurs from early or recycling endosomes to the trans-Golgi network (Ghosh et al., 1998; Mallard et al., 1998). The Rab GTPase(s) responsible for Golgi transport from early and/or recycling endosomes remain undefined. A second pathway to the Golgi, followed by cation-independent mannose 6-phosphate receptor and furin, occurs via late endosomes and is regulated by Rab9 (Lombardi et al., 1993; Mallet and Maxfield, 1999; Riederer et al., 1994).

Components destined for degradation are delivered from the plasma membrane to early endosomes, where they are segregated into endocytic carrier vesicles for transport first to late endosomes and then to lysosomes (Gruenberg et al., 1989; Gruenberg and Maxfield, 1995). A dominant negative Rab7 mutant strongly inhibited transport from early to late endosomes, which indicates that Rab7 is essential for this pathway (Feng et al., 1995; Mukhopadhyay et al., 1997). Although small GTPases have been implicated in transport from late endosomes to lysosomes and in lysosome-lysosome fusion, the Rab proteins important in the terminal endocytic stages remain undefined (Mullock et al., 1994; Ward et al., 1997). The observation that a GTPase-defective Rab7 mutant exhibits enhanced association with lysosomes led investigators to suspect a role for Rab7 in transport from late endosomes to lysosomes (Méresse et al., 1995). An assay that distinguishes sequential transport steps demonstrated that Rab7 is required

only for transport from early to late endosomes and not for subsequent transport to lysosomes (Y. Feng, B. Press and A. Wandinger-Ness, unpublished). Late endosomes form hybrid organelles with lysosomes (Bright et al., 1997; Mellman, 1996; Mullock et al., 1998). Therefore, in retrospect the apparent localization of the GTPase-deficient form of Rab7 on lysosomes could be the result of the dynamic fusion between late endosomes and lysosomes and a failure of the mutant protein to recycle from lysosomes.

RAB GTPASES IN EPITHELIAL TRANSCYTOSIS

The endocytic and transcytotic pathways of polarized cells share many features in common with pathways of non-polarized cells, but also display some unique specializations and Rab proteins (Table 1; Fig. 3). The apical recycling endosome is a specialized epithelial organelle, similar to recycling endosomes in non-polarized cells, that facilitates polarized recycling and transcytosis (Apodaca et al., 1994; Barroso et al., 1995; Knight et al., 1995; Sheff et al., 1999). Rab11 is present primarily on apical recycling endosomes of epithelial cells (Casanova et al., 1999; Goldenring et al., 1996), where it regulates apical recycling of H^+/K^+ ATPase in gastric parietal cells (Calhoun et al., 1998; Duman et al., 1999), and polymeric IgA receptor in MDCK cells (J. E. Casanova and J. R. Goldenring, unpublished). Basolateral to apical transcytosis and basolateral recycling, possibly from apical recycling endosomes, are also controlled by Rab11 (J. E. Casanova and J. R. Goldenring, unpublished). Functional analyses of Rab5 and Rab11 in polarized (Bucci et al., 1994; Duman et al., 1999) and non-polarized cells (Bucci et al., 1992; Chen et al., 1998; Ullrich et al., 1996) suggest that the ubiquitous endocytic Rab proteins may regulate analogous pathways in both cell types.

In addition to the network of ubiquitous Rab GTPases, a set of epithelia-specific Rab proteins, including Rab17, Rab18, Rab20 and Rab25, facilitates endocytic and transcytotic transport to the apical and basolateral plasma membranes (Table 1; Casanova et al., 1999; Hunziker and Peters, 1998; Lütcke et al., 1994; Zacchi et al., 1998). Little is known about the functions of Rab18 and Rab20 (Lütcke et al., 1993; McMurtrie et al., 1997). Recent functional characterization of Rab17 and Rab25 indicates that they regulate aspects of polarized sorting and receptor mediated transcytosis. Rab17 is important for apical recycling and transcytosis to the apical membrane (Hunziker and Peters, 1998; Zacchi et al., 1998). In one system, overexpression of wild-type Rab17 inhibited basolateral to apical transcytosis (Hunziker and Peters, 1998); yet in another system two mutant forms of Rab17 stimulated this pathway together with the apical recycling pathway (Zacchi et al., 1998). These seemingly disparate data may be reconciled if Rab17 is viewed as an inhibitory rather than a stimulatory GTPase on the apical recycling pathway (Fig. 3) or if Rab17 is specific for the differential trafficking of individual receptors (Hunziker and Peters, 1998; Zacchi et al., 1998). In a similar scenario, overexpression of wild-type Rab25 in MDCK cells decreased apical recycling and basolateral to apical transcytosis of IgA, while the dominant negative Rab25T26N had no effect on either pathway (Casanova et al., 1999). These data suggest a negative

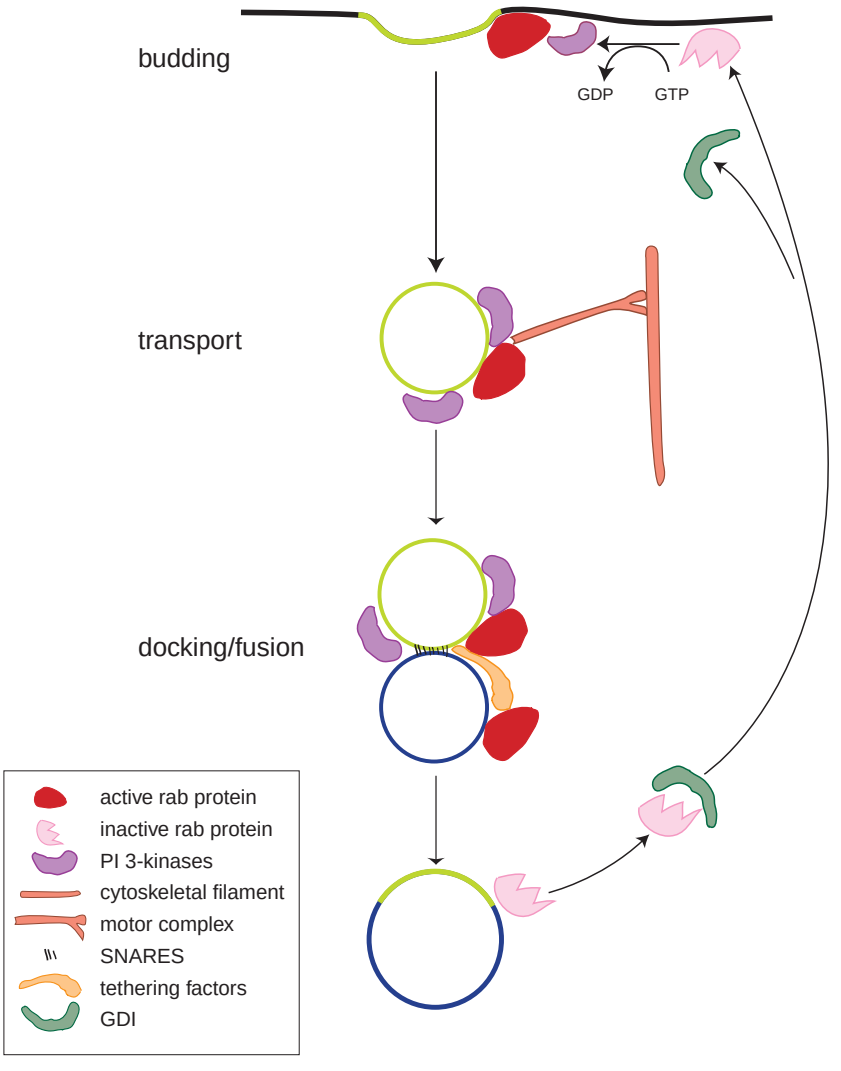

Fig. 1. Coordinate regulation of vesicular transport by Rab proteins. Active, membrane-bound Rab GTPases first participate in budding (possibly contributing to cargo selection, actin cytoskeleton rearrangements or control of coat components), subsequently coordinate cytoskeletal transport through interaction with motor proteins and finally orchestrate docking and fusion with the appropriate target. At the end of the completed transport cycle, GAP-facilitated GTP-hydrolysis inactivates the Rab and promotes its recycling in association with Rab GDI for a new round of transport. To reinitiate recruitment of Rab GTPases to the membrane, the Rab GDI that serves as its cytosolic escort protein is displaced and Rab protein activation through GDP/GTP exchange occurs.

Table 1. Endosomal Rab proteins

Rab	Intracellular Localization	Function	References
Rab4	Early and recycling endosomes	Endocytic recycling to plasma membrane	Daro et al., 1996; Mohrmann and van der Sluijs, 1999
Rab5	Clathrin coated vesicles and early endosomes	Endocytic internalization and early endosome fusion	Bucci et al., 1992; Gorvel et al., 1991
Rab7	Late endosomes	Transport from early to late endosomes	Feng et al., 1995; Gorvel et al., 1991; Mukhopadhyay et al., 1997; Vitelli et al., 1997
Rab9	Late endosomes	Transport from late endsomes to the trans-Golgi	Lombardi et al., 1993; Riederer et al., 1994
Rab11	Golgi and recycling endosomes	Export from the Golgi via endosomes, apical and basolateral endocytic recycling	Chen et al., 1998; Duman et al., 1999; Goldenring et al., 1996; Ren et al., 1998; Ullrich et al., 1996; Urbé et al., 1993
Rab15	Early and recycling endosomes	Inhibitor of endocytic internalization	Zuk and Elferink, 1999
Rab17	Epithelial specific; apical recycling endosome	Transport through apical recycling endosomes (see also text)	Hunziker and Peters, 1998; Zacchi et al., 1998
Rab18	Epithelial specific; kidney dense apical tubules and basolateral domain of intestine	Uncharacterized	Lütcke et al., 1994
Rab20	Epithelial specific; kidney dense apical tubules	Uncharacterized	Lütcke et al., 1994
Rab22	Endosomes and plasma membrane	Uncharacterized	Olkkonen et al., 1993
Rab24	Endoplasmic reticulum, Golgi and late endosomes	Uncharacterized	Olkkonen et al., 1993
Rab25	Epithelial specific; apical recycling endosome	Transport through apical recycling endosomes (see also text)	Casanova et al., 1999

regulatory role for Rab25 in exit from the recycling endosome (Casanova et al., 1999), but could also be explained if Rab25 functions in retrograde trafficking from recycling endosomes to the Golgi (Fig. 3). If so, the closely related Rab11 and Rab25 GTPases could cooperatively regulate the homeostasis of the recycling endosome (Fig. 3). Localization of at least three different Rab proteins (Rab11, Rab17 and Rab25) to apical recycling endosomes is a testament to the complexity of the endocytic recycling system, and further study is essential if we are to decipher the precise inter-relationships between these proteins.

Although many Rab-regulated pathways in endocytosis have been identified, information about specific Rab proteins associated with almost half of the known pathways is lacking (Figs 2 and 3).

REGULATION OF ENDOCYTOSIS BY RAB PROTEINS

Rab protein activity is affected by multiple factors and through multiple effectors, influences endocytic pathways at the level of budding, cytoskeletal transport and fusion. The combination of specific Rab regulators and specific effectors provides a platform for coordinate regulation of endocytic pathways.

Control of active, GTP-bound and membrane-bound Rab protein

Rab activity is partly controlled through membrane association and the status of bound nucleotides. Specific membrane recruitment is dependent on the hypervariable, isoprenylated C-terminus (Alexandrov et al., 1994; Stenmark et al., 1994). Rab proteins cycle between the membrane and the cytosol depending on the activity of Rab GTP-dissociation inhibitor (GDI; Pfeffer et al., 1995; Wu et al., 1996). Rab protein, complexed to GDI, is presented to the membrane where dissociation of GDI may be facilitated by a displacement factor (Dirac-Svejstrup et al., 1997). Nucleotide exchange occurs upon Rab protein recruitment to the membrane and is regulated by specific guanine nucleotide exchange factors (GEFs; Horiuchi et al., 1997). Following membrane fusion, GTPase activating factors (GAPs) interact with Rab proteins to stimulate GTP hydrolysis and Rab protein inactivation. Tuberin is a GAP that specifically inactivates Rab5 and thereby inhibits fluid-phase endocytosis (Xiao et al., 1997). Interestingly, two proteins that regulate Rab5 nucleotide binding and hydrolysis (Rabex5 and tuberin) also interact with a Rab5 effector, Rabaptin5α (Horiuchi et al., 1997; Xiao et al., 1997), which suggests coordinate control of Rab activation and effector binding. Nucleotide hydrolysis inactivates the Rab protein and allows recognition by GDI for release from membranes and recycling. Since each Rab GTPase is controlled by discrete regulators of nucleotide exchange and hydrolysis, differences in nucleotide-bound status are likely and may contribute to the differential recycling of individual Rab proteins by GDI (Chen et al., 1998; Luan et al., 1999).

In some cases Rab protein synthesis and activation may be regulated by activated signal transduction cascades (Alvarez-Dominguez and Stahl, 1998; Barbieri et al., 1998; Liu and Li, 1998; Xu et al., 1996). Protein kinase B/Akt, coupled to Ras signal transduction pathway, regulates Rab5 in macrophages (Barbieri et al., 1998) and the Ras GAP, p120, interacts with Rab5 to stimulate GTPase activity (Liu and Li, 1998). In B cells, receptor signaling triggers membrane recruitment of several small GTPases to compartments involved in antigen presentation (Xu et al., 1996). Thus, multiple inter-related regulatory cascades influence the levels of active, membrane-bound Rab protein.

Regulation of membrane budding

Membrane budding to form vesicles requires complex interactions among multiple proteins, including the ARF GTPases, coatomer subunits, adaptins and clathrin (Marsh and McMahon, 1999). Accumulating evidence shows that active Rab proteins are also required for vesicle budding, although their precise roles remain to be determined (Schimmöller et al., 1998). Initial evidence for Rab protein involvement in budding was suggested by the absence of accumulated vesicles when

membrane transport was inhibited by expression of dominant negative Rab proteins (Nuoffer et al., 1994; Riederer et al., 1994). Subsequently, active Rab5 was shown to be required for ligand sequestration into clathrin coated pits (McLauchlan et al., 1998). Given that Rab5 is not involved in coated pit formation per se, these data hint that Rab proteins facilitate cargo selection. A novel cargo selection protein called TIP47 is recruited to membranes in a GTP-dependent manner, where it binds to the cation-independent mannose-6-phosphate receptor and facilitates transport of the receptor from endosomes to the trans-Golgi network (Diaz and Pfeffer, 1998). Given that Rab9 is important for transport of the mannose-6-phosphate receptor along this pathway, there may be a link between TIP47, Rab9 and cargo recruitment. One last piece of evidence for involvement of Rab proteins in vesicle budding stems from the demonstrated genetic interaction between the ARF GEFs, participants in coat protein recruitment, and yeast Rab proteins (Jones et al., 1999). These data, together with the fact that Rab proteins are recruited to and activated on the donor membrane prior to vesicle budding (Soldati et al., 1994), make a compelling case for Rab protein involvement in transport beginning at the vesicle budding stage.

Regulation of cytoskeletal transport

Membrane vesicles are dynamically transported within the cell to allow delivery and recycling of proteins and lipids. The importance of the cytoskeleton in organellar transport and morphology was recognized decades ago (Sheetz, 1999). More recently, endocytic transport along the actin cytoskeleton and microtubules has been examined in depth. This has led to the identification of novel motor proteins and the recognition that Rab proteins are intimately associated with cytoskeletal proteins for the regulation of transport along microtubules.

In the early stages of endocytosis, the actin cytoskeleton plays a prominent role. This was elegantly demonstrated by genetic studies in yeast, where endocytosis mutants exhibited deficiencies in actin and myosin (Riezman et al., 1996). Disruption of the actin cytoskeleton in mammalian cells with reagents that sequester actin monomers inhibits transferrin clustering into clathrin-coated pits and subsequent endocytosis (Lamaze et al., 1997). Several regulators of endocytosis and recycling (dynamin I, clathrin and a member of the NSF/sec18 family) associate with profilins, which denotes an interaction between endocytic compartments and the actin cytoskeleton (Witke et al., 1998).

In addition to the established functions of the Rho GTPases in actin remodeling and endocytic transport (Murphy et al., 1996), it is necessary to consider the less well-characterized functional links between the Rab GTPases and the actin cytoskeleton. Rab5 and its effectors bind to actin (Kato et al., 1996; Kurzchalia et al., 1992; Ohya et al., 1998) and, under some circumstances, Rab5 is actively required for the reorganization of actin stress fibers (Imamura et al., 1998). Analyses of green fluorescent protein (GFP)-Rab11-labeled endosomes reveal an intimate interaction between Rab11 and the actin cytoskeleton that is critical for transferrin recycling (Sönnichsen and Zerial, 1998). The reorganization of actin into comet-like tails is important for micropinocytosis (Merrifield, 1999) and remodeling of membrane-associated actin on endosomes might be generally important for membrane budding (Harder et al., 1997; Lamaze et al., 1997).

In spite of these tantalizing links, the function of interactions between Rab proteins and the actin cytoskeleton remains unclear. Could the function of Rab proteins in cargo selection and vesicle budding be linked to their associations with actin for transport? Could microfilaments interact with myosin based motors present on endosomes and lysosomes and serve as tracks for directed vesicle transport (Raposo et al., 1999; Simon and Pon, 1996)? Could Rab proteins bind to myosin motors to mediate transport on the actin cytoskeleton much as Rab-associated kinesins mediate transport along microtubules (Echard et al., 1998; Nielsen et al., 1999)? Finally, studies on Rab8 and its yeast orthologue Sec4p indicate that interactions between Rab proteins and actin may also be important for vesicle docking and fusion (Finger et al., 1998; Guo et al., 1999; Peränen et al., 1996). Thus, investigation of the links between Rab proteins and the actin cytoskeleton should help us better understand the molecular and functional basis for this interaction.

Microtubules and associated dynein and kinesin motor proteins have been implicated in multiple endocytic transport steps (Aniento et al., 1993; Apodaca et al., 1994; D'Arrigo et al., 1997; Gruenberg et al., 1989; Itin et al., 1999; Santama et al., 1998). Although the CLIP family of proteins control membrane association of motor proteins (Pierre et al., 1992), it remains unclear how the activities of motor proteins are coordinated to bring about membrane transport. The recent recognition that active Rab proteins can bind novel kinesin motor proteins (Echard et al., 1998; Nielsen et al., 1999) and regulate motor protein activity (Nielsen et al., 1999) defines a new paradigm for the coordinate control of membrane transport. Movement of GFP-Rab5 labeled early endosomes on microtubules depends on active Rab5, the hVPS34 lipid kinase and a minus-end directed kinesin (Nielsen et al., 1999). Specificity was ascertained by the inability of Rab7 or another Rab5 associated lipid kinase (p85α/p110β) to stimulate early endosome movement. Furthermore, Rab5-stimulated microtubule motility is separable from Rab5-dependent endosome docking and fusion. These data lend strong support to a model in which activated Rab proteins serve as a scaffold for multiple effectors. Through the temporal control of effector protein binding, individual Rab GTPases may control multiple events necessary for the transport of cargo between compartments.

Rab proteins present on other pathways probably also coordinate microtubule-based motility. In vivo, GFP-Rab7 labeled late endosomes move bidirectionally along microtubules (Y. Feng and A. Wandinger-Ness, unpublished). Given that Rab7-controlled endocytic vesicle transport to late endosomes depends on dynein (Aniento et al., 1993), it will be of interest to determine the possible connection between these two factors. Rab9-dependent transport from late endosomes to the trans-Golgi depends on microtubules and dynein, which suggests that Rab9 coordinates microtubule-dependent motility as well. The exquisite sensitivity of Rab11 and Rab25 localization to agents that alter microtubule stability suggests that the connections between Rab proteins and the cytoskeleton also extend to epithelial cells (Casanova et al., 1999).

Regulation of docking and fusion

It has been appreciated for several years that Rab proteins provide specificity for membrane docking and fusion

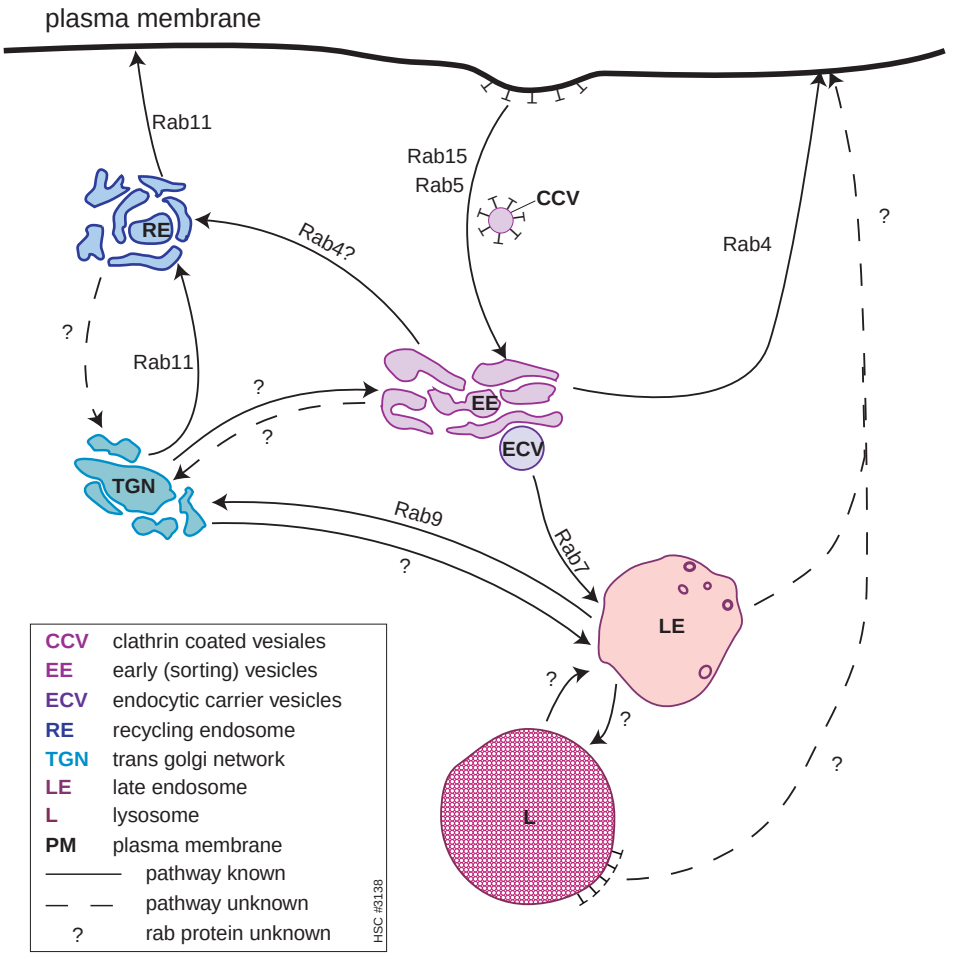

Fig. 2. Rab-regulated endocytic pathways in non-polarized cells. Molecules internalized via clathrin coated pits are first delivered to early endosomes. Recycling receptors are returned to the plasma membrane along two different routes, one passing through recycling endosomes. Molecules can also be targeted to lysosomes for degradation or be sorted at various points for transport to the Golgi. Functionally characterized Rab proteins known to regulate transit from one organelle to the next are indicated. Although multiple isoforms of several Rab proteins have been identified, functional distinctions remain elusive (Mohrmann and van der Sluijs, 1999). Therefore, we have not designated specific isoforms. Pathways that are not defined are shown as dashed lines and those with unidentified Rab proteins are labeled with question marks. Late endosome-lysosome hybrids formed from the dynamic fusion of late endosome and lysosomes are not shown.

(Mohrmann and van der Sluijs, 1999; Schimmöller et al., 1998; Ungermann et al., 1998). Recently, several groups have shown that EEA1 and SNAREs suffice for homotypic early endosome docking and fusion (Christoforidis et al., 1999a; McBride et al., 1999; Simonsen et al., 1998). Active Rab5 functions upstream, transiently binding the hVPS34 lipid kinase and generating a localized microdomain that recruits EEA1 (Christoforidis et al., 1999a; Patki et al., 1997). Following membrane recruitment, EEA1 becomes part of a high molecular mass oligomer that includes Rabaptin, Rabex5 and NEM-sensitive factor (NSF) (McBride et al., 1999). EEA1 both tethers the incoming vesicle and mediates the transient incorporation of a t-SNARE essential for endosome fusion into the oligomeric complex (McBride et al., 1999). Thus, through the assembly of this oligomeric complex, active Rab5 and its effectors can coordinate the docking of an incoming vesicle and NSF/SNARE-mediated fusion (McBride et al., 1999). A large macromolecular complex is also assembled in a temporally and spatially controlled manner to promote homotypic vacuole fusion in yeast (Mayer and Wickner, 1997; Sato and Wickner, 1998; Ungermann et al., 1998; Xu et al., 1998).

The requirement for phosphoinositide (PI) 3-kinases in other endocytic transport steps has been tested using inhibitors such as wortmannin. With the exception of transport from late endosomes to the trans-Golgi (Nakajima and Pfeffer, 1997), PI 3-kinases are required for most endocytic pathways examined (Brown et al., 1995; Davidson, 1995; Mallet and Maxfield, 1999; Reaves et al., 1996; Shpetner et al., 1996). Distinct PI 3-kinases are important for different endocytic events. Rab5 binds to two different PI 3-kinases, hVPS34/p150 and p85α/p110β (Christoforidis et al., 1999b). Since the p85α/p110β complex (in contrast to hVPS34/p150) affects neither microtubule motility nor early endosome fusion events, it is speculated to be important for budding (Christoforidis et al., 1999b; Nielsen et al., 1999). Although distinct anti-PI 3-kinase IgGs microinjected into cells can differentially affect endocytic pathways (Siddhanta et al., 1998), our view is that PI 3-kinases are not pathway specific but rather specify the recruitment of different categories of effectors. We base this view on indications that hVPS34 functions in multiple steps, e.g. promoting transport of vacuolar proteins from the yeast Golgi to endosomes (Peterson et al., 1999) and in Rab7 regulated transport to late endosomes (Y. Feng and A. Wandinger-Ness, unpublished). The specificity of effector recruitment in response to the hVPS34 kinase most likely lies in the combined recognition of the active Rab protein and specific membrane lipids, as is the case for EEA1 (Stenmark and Aasland, 1999). EEA1 is a member of a family of proteins that have the FYVE finger signature motif specifying lipid binding (Burd and Emr, 1998; Kutateladze et al., 1999). Therefore, localization and characterization of the known FYVE domain proteins should allow for identification of those that function on the endocytic pathway. The class-I p85α/p110β kinase that also associates with active Rab5 has a different substrate specificity and preferentially produces bis- and tris-phosphorylated inositol lipids. This challenges us to discover downstream effectors of these Rab/lipid kinase complexes and to determine how they differ from those of the Rab/hVPS34 kinase complex.

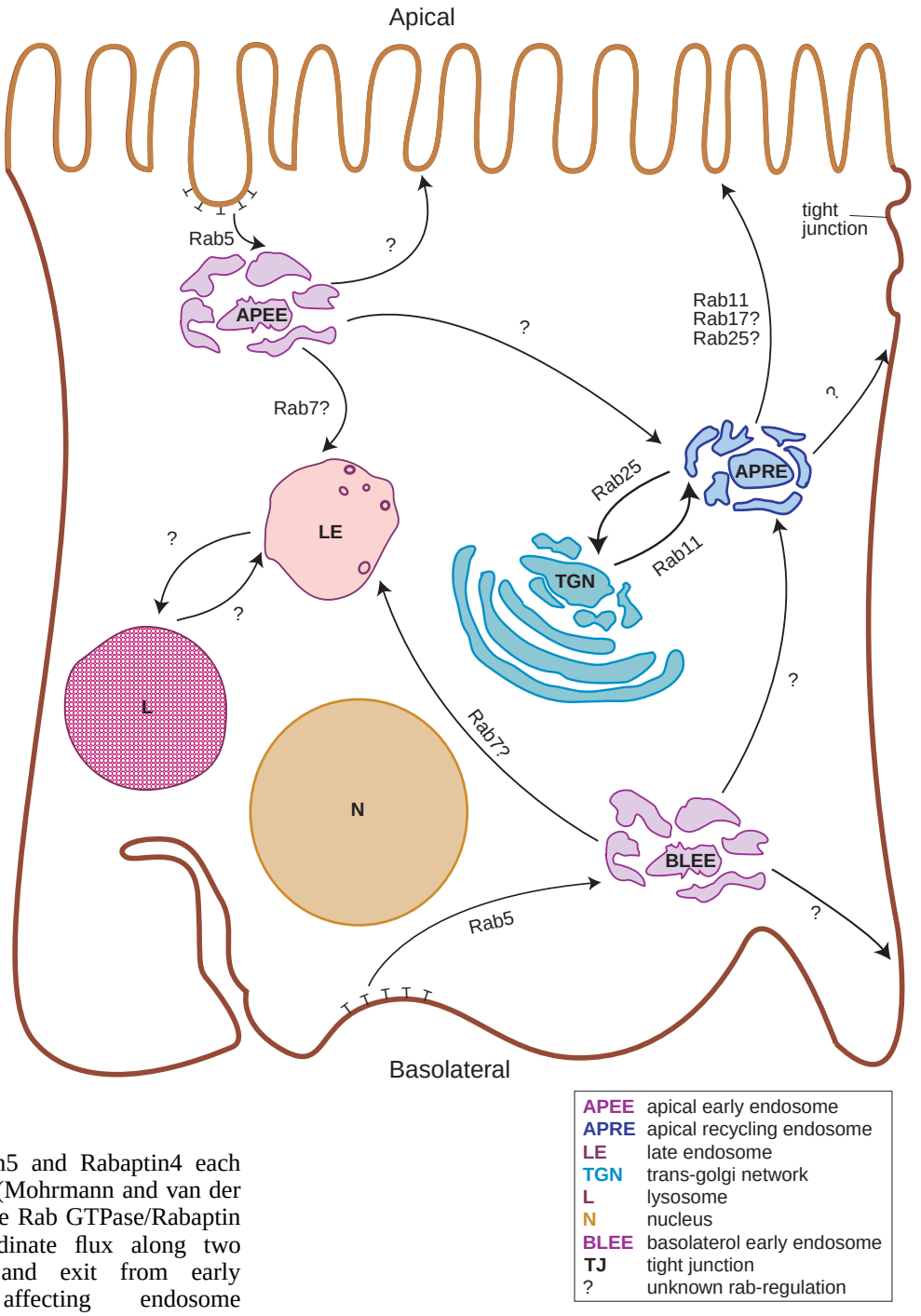

Fig. 3. Rab-regulated endocytosis and transcytosis in polarized cells. In polarized cells molecules may be internalized from the apical or basolateral plasma membrane domains into distinct early endosome populations. In some cases molecules in apical or basolateral early endosomes are transported to the apical recycling endosome where they are sorted for recycling or transcytosis to the opposite plasma membrane domain. Ubiquitous and unique Rab proteins involved in endocytosis and transcytosis are labeled on the pathways. Question marks denote ubiquitous Rab proteins not yet functionally tested in polarized cells or uncertainty regarding the site of Rab function.

Coordinate control of endocytosis orchestrated by Rab proteins

Endocytic vesicles transit diverse pathways to deliver and retrieve cargo from organelles. When transport is inhibited, dramatic structural alterations occur within minutes (Coimbra et al., 1983; Knapp and Swanson, 1990; Parton et al., 1992). Maintaining homeostasis in the face of continuous membrane transport requires precise coordination of transport pathways so that delivery to an organelle equals exit from it. Cross-talk between Rab proteins operative on distinct pathways may facilitate coordinate control of endocytosis. For example, Rabaptin5 and Rabaptin4 each associate with both Rab5 and Rab4 (Mohrmann and van der Sluijs, 1999; Vitale et al., 1998). The Rab GTPase/Rabaptin pair may thus differentially coordinate flux along two endocytic pathways (transit to and exit from early endosomes), simultaneously affecting endosome homeostasis. There are several interfaces between the biosynthetic and endocytic pathways that are important for balance between new synthesis and molecular recycling. Currently, only Rab9 is known to function on these circuits, but undoubtedly there are others. Interconnections between the endo- and exocytic pathways may even be controlled by the same GTPases that are important for endocytic recycling (Rab11, Rab4 and ARF6), as evidenced by function on both pathways (Chen et al., 1998) or presence on regulated secretory vesicles and endosomes (Millar et al., 1999; Ohnishi et al., 1999; Urbé et al., 1993). Signal transduction cascades affecting the Rab GTPases may additionally serve to coordinate control of multiple pathways and impact homeostasis (Barbieri et al., 1998; Fitzgerald and Reed, 1999). Therefore, delineating the localization, regulation, effectors and functions of endocytic Rab GTPases is necessary not only for understanding how Rab proteins function, but also for elucidating the mechanisms underlying endosome homeostasis and coordinate control of membrane transport.

CONCLUSION

Rab proteins are important intermediaries that are regulated by

GTP binding and hydrolysis, by effector protein interactions and by signal transduction pathways. Rab proteins in turn coordinate vesicular transport from the initial stages of budding from one membrane, to transport along the cytoskeleton and, finally, to docking/fusion with an acceptor membrane. Rab proteins specifically regulate each of these processes by serving as a scaffold for binding unique effectors to conduct each function. Therefore Rab proteins can be considered master-regulators of transport.

A.W.-N. gratefully acknowledges support from an NSF CAREER grant (MCB-9507206, MCB-9996127), an award from NIDDK (R01-DK50141), start-up funds from a HHMI Research Resources for Medical Schools Award (76296-550501) and a NATO travel award (CRG 940772). The authors thank Drs Carol Murphy and James Goldenring for helpful discussions. We are grateful to Mark Goodman for the preparation of illustrations and to Debra Matchers for general assistance in document preparation.

REFERENCES

Alexandrov, K., Horiuchi, H., Steele-Mortimer, O., Seabra, M. C. and Zerial, M. (1994). Rab escort protein-1 is a multifunctional protein that accompanies newly prenylated rab proteins to their target membranes. *EMBO J.* **13**, 5262-5273.

Alvarez-Dominguez, C. and Stahl, P. D. (1998). Interferon-gamma selectively induces Rab5a synthesis and processing in mononuclear cells. *J. Biol. Chem.* **273**, 33901-33904.

Aniento, F., Emans, N., Griffiths, G. and Gruenberg, J. (1993). Cytoplasmic dynein-dependent vesicular transport from early to late endosomes [published erratum appears in *J. Cell Biol.* (1994) **124**, 397]. *J. Cell Biol.* **123**, 1373-1387.

Apodaca, G., Katz, L. A. and Mostov, K. E. (1994). Receptor-mediated transcytosis of IgA in MDCK cells is via apical recycling endosomes. *J. Cell Biol.* **125**, 67-86.

Ayad, N., Hull, M. and Mellman, I. (1997). Mitotic phosphorylation of rab4 prevents binding to a specific receptor on endosome membranes. *EMBO J.* **16**, 4497-4507.

Bailly, E., McCaffrey, M., Touchot, N., Zahraoui, A., Goud, B. and Bornens, M. (1991). Phosphorylation of two small GTP-binding proteins of the Rab family by p34cdc2. *Nature* **350**, 715-718.

Barbieri, M. A., Roberts, R. L., Mukhopadhyay, A. and Stahl, P. D. (1996). Rab5 regulates the dynamics of early endosome fusion. *Biocell* **20**, 331-338.

Barbieri, M. A., Kohn, A. D., Roth, R. A. and Stahl, P. D. (1998). Protein kinase B/akt and Rab5 mediate Ras activation of endocytosis. *J. Biol. Chem.* **273**, 19367-19370.

Barroso, M., Nelson, D. S. and Sztul, E. (1995). Transcytosis-associated protein (TAP)/p115 is a general fusion factor required for binding of vesicles to acceptor membranes. *Proc. Nat. Acad. Sci. USA* **92**, 527-531.

Bean, A. J., Seifert, R., Chen, Y. A., Sacks, R. and Scheller, R. H. (1997). Hrs-2 is an ATPase implicated in calcium-regulated secretion. *Nature* **385**, 826-829.

Bright, N. A., Reaves, B. J., Mullock, B. M. and Luzio, J. P. (1997). Dense core lysosomes can fuse with late endosomes and are re-formed from the resultant hybrid organelles. *J. Cell Sci.* **110**, 2027-2040.

Brown, W. J., DeWald, D. B., Emr, S. D., Plutner, H. and Balch, W. E. (1995). Role for phosphatidylinositol 3-kinase in the sorting and transport of newly synthesized lysosomal enzymes in mammalian cells. *J. Cell Biol.* **130**, 781-796.

Bucci, C., Parton, R. G., Mather, I. H., Stunnenberg, H., Simons, K., Hoflack, B. and Zerial, M. (1992). The small GTPase Rab5 functions as a regulatory factor in the early endocytic pathway. *Cell* **70**, 715-728.

Bucci, C., Wandinger-Ness, A., Lutcke, A., Chiariello, M., Bruni, C. B. and Zerial, M. (1994). Rab5a is a common component of the apical and basolateral endocytic machinery in polarized epithelial cells. *Proc. Nat. Acad. Sci. USA* **91**, 5061-5065.

Burd, C. G. and Emr, S. D. (1998). Phosphatidylinositol(3)-phosphate signaling mediated by specific binding to RING FYVE domains. *Mol. Cell.* **2**, 157-162.

Calhoun, B. C., Lapierre, L. A., Chew, C. S. and Goldenring, J. R. (1998). Rab11a redistributes to apical secretory canaliculus during stimulation of gastric parietal cells. *Am. J. Physiol.* **275**, C163-170.

Casanova, J. E., Wang, X., Kumar, R., Bhartur, S. G., Navarre, J., Woodrum, J. E., Altschuler, Y., Ray, G. S. and Goldenring, J. R. (1999). Association of Rab25 and Rab11a with the apical recycling system of polarized Madin-Darby canine kidney cells. *Mol. Biol. Cell.* **10**, 47-61.

Chavrier, P., van der Sluijs, P., Mishal, Z., Nagelkerken, B. and Gorvel, J. P. (1997). Early endosome membrane dynamics characterized by flow cytometry. *Cytometry* **29**, 41-49.

Chen, W., Feng, Y., Chen, D. and Wandinger-Ness, A. (1998). Rab11 is required for trans-Golgi network-to-plasma membrane transport and a preferential target for GDP dissociation inhibitor. *Mol. Biol. Cell* **9**, 3241-3257.

Chiariello, M., Bruni, C. B. and Bucci, C. (1999). The small GTPases Rab5a, Rab5b and Rab5c are differentially phosphorylated in vitro. *FEBS Lett.* **453**, 20-24.

Christoforidis, S., McBride, H. M., Burgoyne, R. D. and Zerial, M. (1999a). The Rab5 effector EEA1 is a core component of endosome docking. *Nature* **397**, 621-625.

Christoforidis, S., Miaczynska, M., Ashma, K., Wilm, M., Zhao, L., Yip, S.-C., Waterfield, M. D., Backer, J. M. and Zerial, M. (1999b). Phosphatidylinositol-3-OH kinases are Rab5 effectors. *Nature Cell Biol.* **1**, 249-252.

Coimbra, T. M., Furtado, M. R., Lachat, J. J. and de Carvalho, I. F. (1983). Effects of administration of cationic and native homologous albumin on the kidney. *Nephron* **33**, 208-215.

D'Arrigo, A., Bucci, C., Toh, B. H. and Stenmark, H. (1997). Microtubules are involved in bafilomycin A1-induced tubulation and Rab5-dependent vacuolation of early endosomes. *Eur. J. Cell Biol.* **72**, 95-103.

Daro, E., van der Sluijs, P., Galli, T. and Mellman, I. (1996). Rab4 and cellubrevin define different early endosome populations on the pathway of transferrin receptor recycling. *Proc. Nat. Acad. Sci. USA* **93**, 9559-9564.

Davidson, H. W. (1995). Wortmannin causes mistargeting of procathepsin D. evidence for the involvement of a phosphatidylinositol 3-kinase in vesicular transport to lysosomes. *J. Cell Biol.* **130**, 797-805.

Diaz, E. and Pfeffer, S. R. (1998). TIP47: a cargo selection device for mannose 6-phosphate receptor trafficking. *Cell* **93**, 433-443.

Dirac-Svejstrup, A. B., Sumizawa, T. and Pfeffer, S. R. (1997). Identification of a GDI displacement factor that releases endosomal Rab GTPases from Rab-GDI. *EMBO J.* **16**, 465-472.

Duman, J. G., Tyagarajan, K., Kolsi, M. S., Moore, H. P. and Forte, J. G. (1999). Expression of rab11a N124I in gastric parietal cells inhibits stimulatory recruitment of the H^+-K^+-ATPase. *Am. J. Physiol.* **277**, C361-C372.

Echard, A., Jollivet, F., Martinez, O., Lacapere, J. J., Rousselet, A., Janoueix-Lerosey, I. and Goud, B. (1998). Interaction of a Golgi-associated kinesin-like protein with Rab6. *Science* **279**, 580-585.

Feng, Y., Press, B. and Wandinger-Ness, A. (1995). Rab 7: An important regulator of late endocytic membrane traffic. *J. Cell Biol.* **131**, 1435-1452.

Finger, F. P., Hughes, T. E. and Novick, P. (1998). Sec3p is a spatial landmark for polarized secretion in budding yeast. *Cell* **92**, 559-571.

Fitzgerald, M. L. and Reed, G. L. (1999). Rab6 is phosphorylated in thrombin-activated platelets by a protein kinase C-dependent mechanism: effects on GTP/GDP binding and cellular distribution. *Biochem. J.* **342**, 353-360.

Ghosh, R. N., Mallet, W. G., Soe, T. T., McGraw, T. E. and Maxfield, F. R. (1998). An endocytosed TGN38 chimeric protein is delivered to the TGN after trafficking through the endocytic recycling compartment in CHO cells. *J. Cell Biol.* **142**, 923-936.

Goldenring, J. R., Smith, J., Vaughan, H. D., Cameron, P., Hawkins, W. and Navarre, J. (1996). Rab11 is an apically located small GTP-binding protein in epithelial tissues. *Am. J. Physiol.* **270**, G515-525.

Gonzalez, L., Jr and Scheller, R. H. (1999). Regulation of membrane trafficking: Structural insights from a Rab/effector complex. *Cell* **96**, 755-758.

Gorvel, J. P., Chavrier, P., Zerial, M. and Gruenberg, J. (1991). RAB5 controls early endosome fusion in vitro. *Cell* **64**, 915-925.

Gournier, H., Stenmark, H., Rybin, V., Lippe, R. and Zerial, M. (1998). Two distinct effectors of the small GTPase Rab5 cooperate in endocytic membrane fusion. *EMBO J.* **17**, 1930-1940.

Gruenberg, J., Griffiths, G. and Howell, K. E. (1989). Characterization of the early endosome and putative endocytic carrier vesicles in vivo and with an assay of vesicle fusion in vitro. *J. Cell Biol.* **108**, 1301-1316.

Gruenberg, J. and Maxfield, F. R. (1995). Membrane transport in the endocytic pathway. *Curr. Opin. Cell Biol.* **7**, 552-563.

Guo, W., Roth, D., Walch-Solimena, C. and Novick, P. (1999). The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. *EMBO J.* **18**, 1071-1080.

Harder, T., Kellner, R., Parton, R. G. and Gruenberg, J. (1997). Specific release of membrane-bound annexin II and cortical cytoskeletal elements by sequestration of membrane cholesterol. *Mol. Biol. Cell* **8**, 533-545.

Horiuchi, H., Lippe, R., McBride, H. M., Rubino, M., Woodman, P., Stenmark, H., Rybin, V., Wilm, M., Ashman, K., Mann, M. et al. (1997). A novel Rab5 GDP/GTP exchange factor complexed to Rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell* **90**, 1149-1159.

Hunziker, W. and Peters, P. J. (1998). Rab17 localizes to recycling endosomes and regulates receptor-mediated transcytosis in epithelial cells. *J. Biol. Chem.* **273**, 15734-15741.

Imamura, H., Takaishi, K., Nakano, K., Kodama, A., Oishi, H., Shiozaki, H., Monden, M., Sasaki, T. and Takai, Y. (1998). Rho and Rab small G-proteins coordinately reorganize stress fibers and focal adhesions in MDCK cells. *Mol. Biol. Cell* **9**, 2561-2575.

Itin, C., Ulitzur, N., Muhlbauer, B. and Pfeffer, S. R. (1999). Mapmodulin, cytoplasmic dynein, and microtubules enhance the transport of mannose 6-phosphate receptors from endosomes to the trans-golgi network. *Mol. Biol. Cell* **10**, 2191-2197.

Jedd, G., Mulholland, J. and Segev, N. (1997). Two new Ypt GTPases are required for exit from the yeast trans-Golgi compartment. *J. Cell Biol.* **137**, 563-580.

Jones, S., Jedd, G., Kahn, R. A., Franzusoff, A., Bartolini, F. and Segev, N. (1999). Genetic interactions in yeast between Ypt GTPases and ARF guanine nucleotide exchangers. *Genetics* **152**, 1543-1556.

Kato, M., Sasaki, T., Ohya, T., Nakanishi, H., Nishioka, H., Imamura, M. and Takai, Y. (1996). Physical and functional interaction of rabphilin-3A with alpha-actinin. *J. Biol. Chem.* **271**, 31775-31778.

Knapp, P. E. and Swanson, J. A. (1990). Plasticity of the tubular lysosomal compartment in macrophages. *J. Cell Sci.* **95**, 433-439.

Knight, A., Hughson, E., Hopkins, C. R. and Cutler, D. F. (1995). Membrane protein trafficking through the common apical endosome compartment of polarized Caco-2 cells. *Mol. Biol. Cell* **6**, 597-610.

Kurzchalia, T. V., Gorvel, J. P., Dupree, P., Parton, R., Kellner, R., Houthaeve, T., Gruenberg, J. and Simons, K. (1992). Interactions of Rab5 with cytosolic proteins. *J. Biol. Chem.* **267**, 18419-18423.

Kutateladze, T. G., Ogburn, K. D., Watson, W. T., de Beer, T., Emr, S. D., Burd, C. G. and Overduin, M. (1999). Phosphatidylinositol 3-phosphate recognition by the FYVE domain. *Mol. Cell* **3**, 805-811.

Lamaze, C., Fujimoto, L. M., Yin, H. L. and Schmid, S. L. (1997). The actin cytoskeleton is required for receptor-mediated endocytosis in mammalian cells. *J. Biol. Chem.* **272**, 20332-20335.

Liu, K. and Li, G. (1998). Catalytic domain of the p120 Ras GAP binds to Rab5 and stimulates its GTPase activity. *J. Biol. Chem.* **273**, 10087-10090.

Lombardi, D., Soldati, T., Riederer, M. A., Goda, Y., Zerial, M. and Pfeffer, S. R. (1993). Rab9 functions in transport between late endosomes and the trans-Golgi network. *EMBO J.* **12**, 677-682.

Luan, P., Balch, W. E., Emr, S. D. and Burd, C. G. (1999). Molecular dissection of guanine nucleotide dissociation inhibitor function in vivo. Rab-independent binding to membranes and role of Rab recycling factors. *J. Biol. Chem.* **274**, 14806-14817.

Lütcke, A., Jansson, S., Parton, R. G., Chavrier, P., Valencia, A., Huber, L. A., Lehtonen, E. and Zerial, M. (1993). Rab17, a novel small GTPase, is specific for epithelial cells and is induced during cell polarization. *J. Cell Biol.* **121**, 553-564.

Lütcke, A., Parton, R. G., Murphy, C., Olkkonen, V. M., Dupree, P., Valencia, A., Simons, K. and Zerial, M. (1994). Cloning and subcellular localization of novel rab proteins reveals polarized and cell type-specific expression. *J. Cell Sci.* **107**, 3437-3448.

Mallard, F., Antony, C., Tenza, D., Salamero, J., Goud, B. and Johannes, L. (1998). Direct pathway from early/recycling endosomes to the Golgi apparatus revealed through the study of Shiga toxin B-fragment transport. *J. Cell Biol.* **143**, 973-990.

Mallet, W. G. and Maxfield, F. R. (1999). Chimeric forms of furin and TGN38 are transported with the plasma membrane in the trans-Golgi network via distinct endosomal pathways. *J. Cell Biol.* **146**, 345-359.

Marsh, M. and McMahon, H. T. (1999). The structural era of endocytosis. *Science* **285**, 215-220.

Mayer, A. and Wickner, W. (1997). Docking of yeast vacuoles is catalyzed by the Ras-like GTPase Ypt7p after symmetric priming by Sec18p (NSF). *J. Cell Biol.* **136**, 307-317.

McBride, H. M., Rybin, V., Murphy, C., Giner, A., Teasdale, R. and Zerial, M. (1999). Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. *Cell* **98**, 377-386.

McLauchlan, H., Newell, J., Morrice, N., Osborne, A., West, M. and Smythe, E. (1998). A novel role for Rab5-GDI in ligand sequestration into clathrin-coated pits. *Curr. Biol.* **8**, 34-45.

McMurtrie, E. B., Barbosa, M. D., Zerial, M. and Kingsmore, S. F. (1997). Rab17 and rab18, small GTPases with specificity for polarized epithelial cells: genetic mapping in the mouse. *Genomics* **45**, 623-625.

Mellman, I. (1996). Endocytosis and molecular sorting. *Annu. Rev. Cell. Dev. Biol.* **12**, 575-625.

Méresse, S., Gorvel, J. P. and Chavrier, P. (1995). The Rab7 GTPase resides on a vesicular compartment connected to lysosomes. *J. Cell Sci.* **108**, 3349-3358.

Merrifield, C. J., Moss, S. E., Ballestrem, C., Imhof, B. A., Giese, G., Wunderlich, I., Almers, W. (1999). Endocytic vesicles move at the tips of actin tails in cultured mast cells. *Nature Cell Biol.* **1**, 72-74.

Millar, C. A., Powell, K. A., Hickson, G. R., Bader, M. F. and Gould, G. W. (1999). Evidence for a role for ADP-ribosylation factor 6 in insulin-stimulated glucose transporter-4 (GLUT4) trafficking in 3T3-L1 adipocytes [In Process Citation]. *J. Biol. Chem.* **274**, 17619-17625.

Mohrmann, K. and van der Sluijs, P. (1999). Regulation of membrane transport through the endocytic pathway by rabGTPases. *Mol. Membr. Biol.* **16**, 81-87.

Mukherjee, S., Ghosh, R. N. and Maxfield, F. R. (1997). Endocytosis. *Physiol. Rev.* **77**, 759-803.

Mukhopadhyay, A., Funato, K. and Stahl, P. D. (1997). Rab7 regulates transport from early to late endocytic compartments in Xenopus oocytes. *J. Biol. Chem.* **272**, 13055-13059.

Mullock, B. M., Perez, J. H., Kuwana, T., Gray, S. R. and Luzio, J. P. (1994). Lysosomes can fuse with a late endosomal compartment in a cell-free system from rat liver. *J. Cell Biol.* **126**, 1173-1182.

Mullock, B. M., Bright, N. A., Fearon, C. W., Gray, S. R. and Luzio, J. P. (1998). Fusion of lysosomes with late endosomes produces a hybrid organelle of intermediate density and is NSF dependent. *J. Cell Biol.* **140**, 591-601.

Murphy, C., Saffrich, R., Grummt, M., Gournier, H., Rybin, V., Rubino, M., Auvinen, P., Lütcke, A., Parton, R. G. and Zerial, M. (1996). Endosome dynamics regulated by a Rho protein. *Nature* **384**, 427-432.

Nakajima, Y. and Pfeffer, S. R. (1997). Phosphatidylinositol 3-kinase is not required for recycling of mannose 6-phosphate receptors from late endosomes to the trans-Golgi network. *Mol. Biol. Cell* **8**, 577-582.

Nielsen, E., Severin, F., Backer, J. M., Hyman, A. A. and Zerial, M. (1999). Rab5 regulates motility of early endosomes on microtubules. *Nature Cell Biol.* **1**, 376-382.

Numata, S., Shirataki, H., Hagi, S., Yamamoto, T. and Takai, Y. (1994). Phosphorylation of Rabphilin-3A, a putative target protein for Rab3A, by cyclic AMP-dependent protein kinase. *Biochem. Biophys. Res. Commun.* **203**, 1927-1934.

Nuoffer, C., Davidson, H. W., Matteson, J., Meinkoth, J. and Balch, W. E. (1994). A GDP-bound of Rab1 inhibits protein export from the endoplasmic reticulum and transport between Golgi compartments. *J. Cell Biol.* **125**, 225-237.

Ohnishi, H., Mine, T., Shibata, H., Ueda, N., Tsuchida, T. and Fujita, T. (1999). Involvement of Rab4 in regulated exocytosis of rat pancreatic acini. *Gastroenterology* **116**, 943-952.

Ohya, T., Sasaki, T., Kato, M. and Takai, Y. (1998). Involvement of Rabphilin3 in endocytosis through interaction with Rabaptin5. *J. Biol. Chem.* **273**, 613-617.

Olkkonen, V. M., Dupree, P., Killisch, I., Lütcke, A., Zerial, M. and Simons, K. (1993). Molecular cloning and subcellular localization of three GTP-binding proteins of the Rab subfamily. *J. Cell Sci.* **106**, 1249-1261.

Parton, R. G., Schrotz, P., Bucci, C. and Gruenberg, J. (1992). Plasticity of early endosomes. *J. Cell Sci.* **103**, 335-348.

Patki, V., Virbasius, J., Lane, W. S., Toh, B. H., Shpetner, H. S. and Corvera, S. (1997). Identification of an early endosomal protein regulated by phosphatidylinositol 3-kinase. *Proc. Nat. Acad. Sci. USA* **94**, 7326-7330.

Peränen, J., Auvinen, P., Virta, H., Wepf, R. and Simons, K. (1996). Rab8 promotes polarized membrane transport through reorganization of actin and microtubules in fibroblasts. *J. Cell Biol.* **135**, 153-167.

Peterson, M. R., Burd, C. G. and Emr, S. D. (1999). Vac1p coordinates Rab

and phosphatidylinositol 3-kinase signaling in Vps45p-dependent vesicle docking/fusion at the endosome. *Curr. Biol.* **9**, 159-162.

Pfeffer, S. R., Dirac-Svejstrup, A. B. and Soldati, T. (1995). Rab GDP dissociation inhibitor: putting rab GTPases in the right place. *J. Biol. Chem.* **270**, 17057-17059.

Pierre, P., Scheel, J., Rickard, J. E. and Kreis, T. E. (1992). CLIP-170 links endocytic vesicles to microtubules. *Cell* **70**, 887-900.

Raposo, G., Cordonnier, M. N., Tenza, D., Menichi, B., Durrbach, A., Louvard, D. and Coudrier, E. (1999). Association of myosin I alpha with endosomes and lysosomes in mammalian cells. *Mol. Biol. Cell***. 10**, 1477-1494.

Reaves, B. J., Bright, N. A., Mullock, B. M. and Luzio, J. P. (1996). The effect of wortmannin on the localisation of lysosomal type I integral membrane glycoproteins suggests a role for phosphoinositide 3-kinase activity in regulating membrane traffic late in the endocytic pathway. *J. Cell Sci.* **109**, 749-762.

Ren, M., Zeng, J., De Lemos-Chiarandini, C., Rosenfeld, M., Adesnik, M. and Sabatini, D. D. (1996). In its active form, the GTP-binding protein Rab8 interacts with a stress-activated protein kinase. *Proc. Nat. Acad. Sci. USA* **93**, 5151-5155.

Ren, M., Xu, G., Zeng, J., De Lemos-Chiarandini, C., Adesnik, M. and Sabatini, D. D. (1998). Hydrolysis of GTP on Rab11 is required for the direct delivery of transferrin from the pericentriolar recycling compartment to the cell surface but not from sorting endosomes. *Proc. Nat. Acad. Sci. USA* **95**, 6187-6192.

Riederer, M. A., Soldati, T., Shapiro, A. D., Lin, J. and Pfeffer, S. R. (1994). Lysosome biogenesis requires Rab9 function and receptor recycling from endosomes to the trans-Golgi network. *J. Cell Biol.* **125**, 573-582.

Riezman, H., Munn, A., Geli, M. I. and Hicke, L. (1996). Actin-, myosin- and ubiquitin-dependent endocytosis. *Experientia* **52**, 1033-1041.

Santama, N., Krijnse-Locker, J., Griffiths, G., Noda, Y., Hirokawa, N. and Dotti, C. G. (1998). KIF2beta, a new kinesin superfamily protein in non-neuronal cells, is associated with lysosomes and may be implicated in their centrifugal translocation. *EMBO J.* **17**, 5855-5867.

Sapperstein, S. K., Lupashin, V. V., Schmitt, H. D. and Waters, M. G. (1996). Assembly of the ER to Golgi SNARE complex requires Uso1p. *J. Cell Biol.* **132**, 755-767.

Sasaki, T., Shirataki, H., Nakanishi, H. and Takai, Y. (1997). Rab3A-rabphilin-3A system in neurotransmitter release. *Advan. Second Messenger Phosphoprotein Res.* **31**, 279-294.

Sato, K. and Wickner, W. (1998). Functional reconstitution of Ypt7p GTPase and a purified vacuole SNARE complex. *Science* **281**, 700-702.

Schimmöller, F., Simon, I. and Pfeffer, S. R. (1998). Rab GTPases, directors of vesicle docking. *J. Biol. Chem.* **273**, 22161-22164.

Sheetz, M. P. (1999). Motor and cargo interactions. *Eur. J. Biochem***. 262**, 19-25.

Sheff, D. R., Daro, E. A., Hull, M. and Mellman, I. (1999). The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions. *J. Cell Biol.* **145**, 123-139.

Shirataki, H., Kaibuchi, K., Sakoda, T., Kishida, S., Yamaguchi, T., Wada, K., Miyazaki, M. and Takai, Y. (1993). Rabphilin-3A, a putative target protein for smg p25A/rab3A p25 small GTP-binding protein related to synaptotagmin. *Mol. Cell Biol.* **13**, 2061-2068.

Shpetner, H., Joly, M., Hartley, D. and Corvera, S. (1996). Potential sites of PI-3 kinase function in the endocytic pathway revealed by the PI-3 kinase inhibitor, wortmannin. *J. Cell Biol.* **132**, 595-605.

Siddhanta, U., McIlroy, J., Shah, A., Zhang, Y. and Backer, J. M. (1998). Distinct roles for the p110alpha and hVPS34 phosphatidylinositol 3′-kinases in vesicular trafficking, regulation of the actin cytoskeleton, and mitogenesis. *J. Cell Biol.* **143**, 1647-1659.

Simon, V. R. and Pon, L. A. (1996). Actin-based organelle movement. *Experientia* **52**, 1117-1122.

Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J. M., Brech, A., Callaghan, J., Toh, B. H., Murphy, C., Zerial, M. and Stenmark, H. (1998). EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. *Nature* **394**, 494-498.

Soldati, T., Shapiro, A. D., Svejstrup, A. B. and Pfeffer, S. R. (1994). Membrane targeting of the small GTPase Rab9 is accompanied by nucleotide exchange. *Nature* **369**, 76-78.

Sönnichsen, B. and Zerial, M. (1998). Membrane dynamics of the perinuclear recycling endosome monitored with Rab11-GFP. *Mol. Biol. Cell* **9**, 464a.

Stenmark, H., Valencia, A., Martinez, O., Ullrich, O., Goud, B. and Zerial, M. (1994). Distinct structural elements of Rab5 define its functional specificity. *EMBO J.* **13**, 575-583.

Stenmark, H., Vitale, G., Ullrich, O. and Zerial, M. (1995). Rabaptin-5 is a direct effector of the small GTPase Rab5 in endocytic membrane fusion. *Cell***. 83**, 423-432.

Stenmark, H. and Aasland, R. (1999). FYVE-finger proteins: effectors of an inositol lipid. *J. Cell Sci.*

Ullrich, O., Reinsch, S., Urbe, S., Zerial, M. and Parton, R. G. (1996). Rab11 regulates recycling through the pericentriolar recycling endosome. *J. Cell Biol.* **135**, 913-924.

Ungermann, C., Sato, K. and Wickner, W. (1998). Defining the functions of trans-SNARE pairs. *Nature* **396**, 543-548.

Urbé, S., Huber, L. A., Zerial, M., Tooze, S. A. and Parton, R. G. (1993). Rab11, a small GTPase associated with both constitutive and regulated secretory pathways in PC12 cells. *FEBS Lett.* **334**, 175-182.

van der Sluijs, P., Hull, M., Huber, L. A., Male, P., Goud, B. and Mellman, I. (1992). Reversible phosphorylation–dephosphorylation determines the localization of rab4 during the cell cycle. *EMBO J.* **11**, 4379-4389.

Vitale, G., Rybin, V., Christoforidis, S., Thornqvist, P., McCaffrey, M., Stenmark, H. and Zerial, M. (1998). Distinct Rab-binding domains mediate the interaction of Rabaptin-5 with GTP-bound Rab4 and Rab5. *EMBO J.* **17**, 1941-1951.

Vitelli, R., Santillo, M., Lattero, D., Chiariello, M., Bifulco, M., Bruni, C. B. and Bucci, C. (1997). Role of the small GTPase Rab7 in the late endocytic pathway. *J. Biol. Chem.* **272**, 4391-4397.

Ward, D. M., Leslie, J. D. and Kaplan, J. (1997). Homotypic lysosome fusion in macrophages: analysis using an in vitro assay. *J. Cell Biol.* **139**, 665-673.

Witke, W., Podtelejnikov, A. V., Di Nardo, A., Sutherland, J. D., Gurniak, C. B., Dotti, C. and Mann, M. (1998). In mouse brain profilin I and profilin II associate with regulators of the endocytic pathway and actin assembly. *EMBO J.* **17**, 967-976.

Wu, S. K., Zeng, K., Wilson, I. A. and Balch, W. E. (1996). Structural insights into the function of the Rab GDI superfamily. *Trends Biochem. Sci***. 21**, 472-476.

Wurmser, A. E., Gary, J. D. and Emr, S. D. (1999). Phosphoinositide 3-kinases and their FYVE domain-containing effectors as regulators of vacuolar/lysosomal membrane trafficking pathways. *J. Biol. Chem.* **274**, 9129-9132.

Xiao, G. H., Shøarinejad, F., Jin, F., Golemis, E. A. and Yeung, R. S. (1997). The tuberous sclerosis 2 gene product, tuberin, functions as a Rab5 GTPase activating protein (GAP) in modulating endocytosis. *J. Biol. Chem.* **272**, 6097-6100.

Xu, X., Press, B., Wagle, N. M., Cho, H., Wandinger-Ness, A. and Pierce, S. K. (1996). B cell antigen receptor signaling links biochemical changes in the class II peptide-loading compartment to enhanced processing. *Int. Immunol.* **8**, 1867-1876.

Xu, Z., Sato, K. and Wickner, W. (1998). LMA1 binds to vacuoles at Sec18p (NSF), transfers upon ATP hydrolysis to a t-SNARE (Vam3p) complex, and is released during fusion. *Cell* **93**, 1125-1134.

Zacchi, P., Stenmark, H., Parton, R. G., Orioli, D., Lim, F., Giner, A., Mellman, I., Zerial, M. and Murphy, C. (1998). Rab17 regulates membrane trafficking through apical recycling endosomes in polarized epithelial cells. *J. Cell Biol.* **140**, 1039-1053.

Zeng, J., Ren, M., Gravotta, D., De Lemos-Chiarandini, C., Lui, M., Erdjument-Bromage, H., Tempst, P., Xu, G., Shen, T. H., Morimoto, T., Adesnik, M. and Sabatini, D. D. (1999). Identification of a putative effector protein for Rab11 that participates in transferrin recycling. *Proc. Nat. Acad. Sci. USA* **96**, 2840-2845.

Zuk, P. A. and Elferink, L. A. (1999). Rab15 mediates an early endocytic event in Chinese hamster ovary cells. *J. Biol. Chem.* **274**, 22303-22312.