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Rab GTPases as regulators of transport through endosomes

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Summary. Early endocytic compartments are a highly dynamic, heterogeneous class of prelysosomal organelles that receive internalized proteins from the plasma membrane and sort these to various intracellular destinations. Several monomeric Rab GTPases are associated with the cytoplasmic surface of endosomes and regulate the dynamics of this endomembrane system. We discuss the endosomal Rab proteins and their effector proteins and how they might control vesicular transport through the endocytic pathway.

Keywords: Endocytosis; Rab proteins; Effectors.

Receptor-mediated endocytosis

Eukaryotic cells internalize solutes and macromolecules from their environment through the invagination of the plasma membrane. Various modalities of this vesicular transport mechanism exist and are collectively known as (receptor-mediated) endocytosis (for a review, see Mellman 1996). Extracellular molecules bind to cell-surface receptors and laterally move into plasma membrane specializations called clathrin-coated pits. Alternatively ligand-receptor complexes are triggered to concentrate in clathrin-coated pits. Coated pits bud off the plasma membrane to form clathrin-coated vesicles that rapidly shed their clathrin coat and fuse with early endosomes. Early endosomes are mildly acidic (pH 6.2–6.5) which causes dissociation of pH-sensitive receptor-ligand complexes. Receptors, such as the low-density lipoprotein receptor, are recycled to the cell surface whereas ligands enter the degradative pathway to late endosomes and lysosomes. Thus the low internal pH of early endosomes provides a mechanism to sort ligands from their receptors. Other ligands remain bound to their receptor in early endosomes, e.g., endocytosed lysosomal enzymes bound to the cation-independent mannose 6-phosphate receptor (CI-MPR) are transported from early endosomes to late endosomes. In the lumen of the late endosome (pH 5.0–5.5), lysosomal enzymes dissociate from the CI-MPR. The receptor is recycled back to the trans Golgi network (TGN) and the ligand is delivered to lysosomes (for a review, see Kornfeld and Mellman 1989).

A novel sorting mechanism for plasma membrane proteins destined to be degraded in lysosomes was recently defined by Emr and colleagues in *Saccharomyces cerevisiae*. They observed phosphoinositide-dependent sorting of cargo molecules into internal vesicles of multivesicular bodies (Odorizzi et al. 1998). This process involves the invagination of the limiting membrane of the multivesicular body. Upon fusion of multivesicular bodies with lysosomes, the internal vesicles are thought to be released in the lumen of lysosomes and finally degraded within this organelle. A similar mechanism is likely to occur in multivesicular bodies of mammalian cells (Felder et al. 1990, Fernandez-Borja et al. 1999).

Early endosomes are heterogeneous

Early endosomes are a heterogeneous collection of endocytic organelles. Morphological and kinetic studies of Maxfield and co-workers revealed the existence of multiple prelysosomal organelles with distinct internal pH (Yamashiro et al. 1984). This was initially demonstrated in pulse chase experiments with fluo-

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rescently labeled transferrin that shortly after uptake traverses crescent-shaped organelles in the perinuclear area. In these so-called sorting endosomes, internalized transferrin is sorted away from low-density lipoprotein and transported to recycling endosomes, compact organelles juxtaposed to the nucleus (Mayor et al. 1993). Thus the sorting endosome in which ligand-receptor complexes dissociate is biochemically distinct from the perinuclear recycling endosome. In this organelle intracellular fluorescein isothiocyanate-transferrin accumulates 10–15 min after internalization before it is recycled back to the cell surface. As such, the heterogeneity of the early endosome is analogous to that of the Golgi complex where the different cisternae have distinct biochemical functions (for a review, see Mellman and Simons 1992). The mean intracellular residence time of endocytosed transferrin is less than 15 min in a variety of biochemical assays. As this is appreciably shorter than the time required for transferrin to accumulate in recycling endosomes, this means that there is a short circuit via which internalized transferrin is recycled with faster kinetics than via the recycling compartment to the cell surface. Accordingly, there are at least two pathways for transferrin recycling to the plasma membrane. Recently Moore and co-workers (Teter et al. 1998) demonstrated the existence of several recycling endosomes with distinct acidification and ion transport properties. Apart from a role in directing internalized transferrin receptor molecules back to the cell surface, recent evidence suggests that recycling endosomes are also traversed by endocytosed Shiga toxin B chain (Mallard et al. 1998) and TGN38 (Ghosh et al. 1998) from the cell surface to the TGN, providing a link between the endocytic and biosynthetic pathways.

Regulatory proteins in the endocytic pathway

Because endocytic organelles are highly dynamic structures involved in a plethora of vesicle budding and fusion reactions, it is clear that these events have to be tightly regulated to maintain structural and functional integrity. In the past decade the first proteins were identified for membrane transport in the endocytic pathway. The best characterized examples include members of the Rab family of small GTPases that were shown to be critically involved in transport between compartments of the early endocytic pathway (for a review, see Olkkonen and Stenmark 1997). Although much has been learnt about the role of Rab proteins in endocytosis, it is not clear yet how GTP hydrolysis is wired into the mechanism of membrane fusion. Several other proteins including dynamin (Damke et al. 1994, Herskovits et al. 1993), Eps15 (Benmerah et al. 1998, Van Delft et al. 1997), Eps15R (Coda et al. 1998), epsin (H. Chen et al. 1998), amphiphysin (David et al. 1996), and members of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) family were more recently identified on early endocytic compartments (Advani et al. 1998; McMahon et al. 1993; Prekeris et al. 1998; Tang et al. 1998; Wong et al. 1998a, b), a description of their function is however beyond the scope of this review.

The Rab GTPase molecular switch

Today more than 40 mammalian Rab GTPases are known, many of which have been localized to the cytoplasmic surface of intracellular compartments, where they appear to be involved in one or more vesicular transport steps (Olkkonen and Stenmark 1997). After synthesis, Rab proteins associate with the cytosolic Rab escort protein (REP) (Andres et al. 1993). REP-bound Rab is then delivered to geranylgeranyltransferase type II and isoprenylated on both cysteines in the CXC, CC, and CCXY motifs. Geranylgeranyl groups provide the requisite hydrophobicity for reversible membrane association of Rab proteins (Alexandrov et al. 1994, Wilson et al. 1996). Rab proteins with the carboxy-terminal CXC motif are further modified on the last cysteine by carboxymethyl transferase, this modification however is not required for membrane association. The doubly geranylgeranylated Rab protein is thought to remain associated with REP, which delivers the GTPase to a specific organelle or transport vesicle (for a review, see Shen and Seabra 1996). Upon binding to unknown receptors, REP is dissociated and the RabGDP is converted by a guanine nucleotide exchange protein to the GTP-bound state. Membrane-bound RabGTP is stabilized by the recruitment of cytosolic effector proteins that are thought to regulate the activity of a downstream vesicle (v) and target (t) SNARE complex. Possibly, the RabGTP-dependent assembly of docking factors confers vectoriality to vesicular transport between two compartments. At some point, GTP hydrolysis returns the active Rab back to the GDP-bound form. Membrane-bound RabGDP is not capable to interact with effector proteins and Rab-effector complexes are

thought to dissociate. Membrane-bound RabGDP is then retrieved by the GDP dissociation inhibitor (GDI) and translocated to the cytosol as a Rab-GDI complex (Ullrich et al. 1993). GDI now assists in the initiation of a new cycle by targeting RabGDP to a specific organelle (Ayad et al. 1997, Dirac-Svejstrup et al. 1997, Ullrich et al. 1994) or transport vesicle analogous to REP in the delivery of newly synthesized Rab to the organelle.

Multiple Rab proteins are localized to organelles of the early endocytic pathway underscoring the highly dynamic and complex nature of this vesicular route. For instance, Rab4 and Rab5 are associated with early endosomes and differentially regulate membrane transport through this compartment (Chavrier et al. 1990, van der Sluijs et al. 1991). Rab11 is localized to recycling endosomes (Ullrich et al. 1996) and the TGN (Urbe et al. 1993) and regulates recycling of the transferrin receptor to the plasma membrane (Ren et al. 1998, Ullrich et al. 1996).

Rab5

Three Rab5 isoforms have been identified (Bucci et al. 1995) and named Rab5a, Rab5b, and Rab5c, of which Rab5a is best characterized. Most likely the three isoforms have the same intracellular localization although this issue has not been addressed so far. Although initially identified on early endosomes (Chavrier et al. 1990), Rab5a later was also found on the plasma membrane and clathrin-coated vesicles (Bucci et al. 1992). In *S. cerevisiae*, the Rab5 homologs Ypt51p, Ypt52p, and Ypt53p are associated with early endosomes and required for transport to this organelle (Singer-Krüger et al. 1994) and protein sorting to the vacuole (Horazdovski et al. 1994). The *ypt51*, *ypt52*, and *ypt53* genes are not essential. While disruption of *ypt52* and/or *ypt53* has little effects on intracellular transport, the disruption of *ypt51* leads to defects in endocytosis and growth at high temperature. These defects are enhanced by the triple knockout (Singer-Krüger et al. 1994). In contrast to *S. cerevisiae*, only a single nonessential *ypt5* gene product is present in fission yeast with similar functions as canine Rab5a (Armstrong et al. 1993).

Rab5a and the Rab5aQ79L GTP hydrolysis-deficient mutant stimulate homotypic early-endosome fusion in vitro (Stenmark et al. 1994). In contrast, Rab5aN133I, with a low affinity for guanine nucleotides, and the Rab5aS34N GDP mutant inhibit early endosome fusion (Gorvel et al. 1991, Stenmark et al. 1994). Since the XTP-binding mutant Rab5aD136N stimulates early-endosome fusion when bound to XTPγS, homotypic early-endosome fusion does not appear to require XTP or GTP hydrolysis (Barbieri et al. 1994, Rybin et al. 1996). It has therefore been proposed that Rab5 may act as a timer in controlling homotypic early-endosome fusion (Rybin et al. 1996). In contrast to other Rab proteins, Rab5 exhibits fast intrinsic GTP hydrolysis. It remains to be seen therefore whether the timer model also applies in vivo and for other Rab proteins regulating homotypic and/or heterotypic membrane fusion. On a different level, this model of Rab5 function implicitly assumes that the entire pool of GTP-hydrolyzing Rab5 molecules participate in regulating a membrane fusion event. This prerequisite however has not been experimentally addressed.

Overexpression of Rab5a and Rab5aQ79L in mammalian cells results in the expansion of early endocytic organelles, whereas expression of Rab5aS34N and Rab5aN133I causes fragmentation of early endosomes. Wild-type Rab5a and Rab5aQ79L selectively increase the rate of fluid-phase endocytosis and transferrin uptake, while Rab5aS34N and Rab5aN133I inhibit transferrin endocytosis (Bucci et al. 1992, Stenmark et al. 1994) suggesting that Rab5 is involved in the fusion of coated vesicles with early endosomes. In polarized Mardin-Darby canine kidney cells transfected with Rab5, the small GTPase localized to apical and basolateral endosomes and enhanced fluid-phase endocytosis from both plasma membrane domains (Bucci et al. 1994). This indicates that Rab5 is involved in apical as well as basolateral endocytosis. Consistent with parallels in membrane transport between polarized MDCK cells and cultured hippocampal neurons (Dotti and Simons 1990), Rab5a colocalizes with synaptophysin-labeled structures and somatodendritic and axonal early endosomes (de Hoop et al. 1994). Apart from a role in coated-vesicle fusion, Rab5 also appears to regulate the formation of transport vesicles. In search for novel cytosolic proteins required for coated-pit invagination at the plasma membrane, McLaughlan et al. (1998) purified a plasma membrane budding-stimulating activity that turned out be the Rab5-GDI complex. Possibly, coordination of the activity of Rab proteins at both vesicle budding and fusion sites provides a control mechanism to ensure that the amount of membrane that is consumed through fusion of transport vesicles with a

target organelle is balanced by the amount of membrane that is formed from the donor organelle.

Rab4

Two isoforms of Rab4 have been cloned from mammalian cells and named Rab4a and Rab4b. Interestingly, homologs with a similar function as Rab4, have not been identified so far in both fission yeast and budding yeast. Using free-flow electrophoresis and immunofluorescence microscopy, we showed that Rab4a is localized to early endosomes (Bottger et al. 1996, van der Sluijs et al. 1991). Overexpression of wild-type Rab4 reduces the intracellular accumulation of the fluid-phase tracer horse radish peroxidase, and transferrin receptors are redistributed from endosomes to the plasma membrane (van der Sluijs et al. 1992b). Furthermore the discharge of iron from transferrin in early endosomes is inhibited, suggesting that internalized transferrin is mistargeted to a population of nonacidic vesicles and tubules. Rab4 thus appears to control the function or formation of endosomes involved in recycling of internalized transferrin back to the plasma membrane. The fact that Rab4N121I inhibits receptor-mediated antigen processing when transfected in the mouse A20 B cell line (Lazzarino et al. 1998) is consistent with this notion. Transfection of the same Rab4 mutant in 3T3-L1 adipocytes reduced the recruitment of the insulin-dependent glucose transporter GLUT4 from an intracellular storage compartment to the plasma membrane (Vollenweider et al. 1997), and microinjection of a peptide corresponding to the hypervariable region of Rab4 inhibits insulin action on glucose transport in rat adipocytes (Shibata et al. 1996).

In pulse chase experiments using fluorescein isothiocyanate-labeled transferrin, we showed at early time points that the fluorescent label first appeared in Rab4-containing endocytic organelles before it was transported to a tubulovesicular organelle in the area of the microtubule-organizing center (Daro et al. 1996). We found this compartment to be enriched in the v-SNARE cellubrevin, which is involved in regulating transferrin recycling (Galli et al. 1994). Thus Rab4-containing endosomes in the transferrin-recycling route are biochemically distinct from recycling endosomes (Daro et al. 1996).

As Rab4 is relatively depleted from recycling endosomes it is difficult to assess whether it controls transport from sorting endosomes to recycling endosomes or direct recycling from sorting endosomes back to the plasma membrane. Several lines of evidence however suggest that the indirect recycling pathway from early endosomes to recycling endosomes is regulated by Rab4. First, in vivo, Rab4Q67L, which is thought to be stabilized in its association with the target organelle, is associated with a perinuclear compartment (our unpubl. results). Second, cotransfection of Rab4 and its effector rabaptin4 causes the expansion of a perinuclear compartment that is enriched in cellubrevin, and contains the majority of internalized Cy3-labeled transferrin at steady state. These are hallmarks of recycling endosomes and suggest that Rab4 acts in the indirect recycling pathway (our unpubl. results). Finally, Rab4 is not found on the plasma membrane by immunogold electronmicroscopy on frozen sections.

Although it is thought that isoforms of ubiquitously expressed Rab proteins have similar functions, it is unknown whether these isoforms have the same or distinct spatiotemporal expression patterns in tissues of multicellular organisms. For instance, Rab4 appears to be upregulated during development in the rat pancreas (Valentijn et al. 1997). Presently it is not clear whether one of the Rab4 isoforms, or both, is subject to developmental regulation and whether regulation occurs at the transcriptional or at the protein level. Rab4a and Rab4b differ in at least one important aspect. The hypervariable region of Rab4a but not of Rab4b contains a consensus sequence for phosphorylation by $p34^{cdc2}$ kinase. Indeed Rab4a is reversibly phosphorylated on Ser196 during mitosis by $p34^{cdc2}$ kinase (van der Sluijs et al. 1992a). Phosphorylation occurs on cytoplasmic and membrane-bound Rab4a. Interestingly, phosphorylated Rab4 is complexed with the peptidyl prolyl isomerase Pin1 in the cytoplasm during mitosis (our unpubl. results), possibly preventing Rab4 to participate in endosomal functions.

Membrane transport is coordinately inhibited in mammalian cells during mitosis, and several intracellular compartments, including the nuclear envelope, the endoplasmic reticulum, and the Golgi apparatus, are disassembled or fragmented at this stage of the cell cycle (Birky 1983, Warren 1993). Fragmentation of organelles during mitosis might ensure their equal partitioning between mother and daughter cells and is thought to occur by inhibition of vesicle fusion in the face of ongoing transport vesicle budding (Lowe et al. 1998, Warren 1993, Warren and Wickner 1996). For

the Golgi complex, these fragments are tethered to kinetochore microtubules, increasing the partitioning-fidelity between the mother and the daughter cell (Shima et al. 1998). In telophase, homotypic vesicle fusion is reactivated prior to vesicle budding, causing the reassembly of the fragmented organelles. The molecular mechanisms responsible for the inhibition of vesicular transport during cell division are incompletely understood. It is clear however that protein modification by mitotic kinases is a key event in this process. Any one of the proteins required for or involved in the vesicle transport machinery might be regulated through phosphorylation by mitotic kinases such as $p34^{cdc2}$ kinase or NIMA kinase. Indeed, in addition to Rab4, epsin and Eps15, two proteins involved in clathrin-mediated endocytosis, are phosphorylated by $p34^{cdc2}$ kinase and do not associate with their interphase partner alpha-adaptin (H. Chen et al. 1999). From these examples the concept emerges that several regulatory proteins in endocytosis are targeted for phosphorylation during mitosis, and that not a single molecule or mechanism is responsible for the inhibition of endocytosis in dividing cells.

Rab11

In addition to Rab5 and Rab4, another Rab protein is associated with early endocytic organelles. Rab11 is localized to the TGN and recycling endosomes (Ullrich et al. 1996). Rab11S25N inhibits transport of internalized transferrin from the recycling endosome to the plasma membrane and causes fragmentation of this compartment into the cytoplasm. Transfection of Rab11Q70L leads to compaction of the recycling endosome, but also inhibits recycling of transferrin (Ullrich et al. 1996). Through the use of a low-temperature block to inhibit transport between sorting endosomes and the recycling endosome, Ren et al. (1998) recently showed that Rab11S25N inhibits transferrin recycling, whereas Rab11Q70L and wild-type Rab11 do not show this inhibitory effect. This suggests that activation of Rab11 by GTP is required for the exit of the transferrin receptor, regardless whether this occurs towards the recycling endosome, from the recycling endosome to the TGN, or directly from the early sorting endosomes to the plasma membrane. In polarized MDCK cells, Rab11 is associated with the apical endosomal system involved in membrane recycling. Rab25 is an epithelium-specific Rab protein with 68% identity to the ubiquitously expressed Rab11. In Rab25 MDCK transfectants, Rab25 colocalizes to a large extent with Rab11 in subapical vesicles (Casanova et al. 1999). However, similar to Rab4 and Rab5 that are both associated with early endosomes, Rab11 and Rab25 do not have identical distributions. Although the function of Rab11 has not been defined in MDCK cells, Rab25 appears to regulate transcytosis and/or apical membrane recycling in this polarized epithelial cell line (Casanova et al. 1999). In addition to the endosomal system, Rab11 also localizes to the Golgi complex and the TGN. Recent studies of the laboratory of Wandinger-Ness clearly documented that Rab11 is required for transport of vesicular stomatitis virus G glycoprotein between the TGN and the plasma membrane. Conversely overexpression of the Rab11S25N mutant did not effect delivery of influenza virus hemagglutinin from the TGN to the cell surface (W. Chen et al. 1998). Collectively these data suggest that Rab11 is an important regulatory molecule for a subset of transport vesicles that deliver proteins from the TGN to the basolateral domain equivalent of polarized epithelial cells in fibroblasts (Yoshimori et al. 1996).

In conclusion, it is not entirely clear yet which effects of transfected Rab11 on membrane transport are primary and secondary. Since Rab11 is involved in biosynthetic transport of membrane proteins from the TGN to cell surface, a role of this protein in transferrin recycling might however be expected. Nevertheless the picture emerges that at least two Rab proteins, Rab4 and Rab11, are required for recycling of transferrin from sorting endosomes to the plasma membrane.

Rab17

Rab17 is localized to the basolateral plasma membrane of kidney proximal tubule epithelial cells and the expression of Rab17 is induced during cell polarization in the developing kidney (Lütcke et al. 1993). Recently it was shown that Rab17 colocalized with internalized transferrin in recycling endosomes of BHK-21 cells and with apical recycling endosomes in the Eph4 mammary epithelial cell line (Zacchi et al. 1998). Basolateral to apical transport of the transferrin receptor and of a chimeric Fc receptor was increased in cells transfected with Rab17N131I, or Rab17Q77L, suggesting a role for Rab17 in re-

gulating traffic through the apical recycling endosome (Zacchi et al. 1998). Although the same group also found Rab17 on the basolateral plasma membrane (Lütcke et al. 1993), its function at this location is not established and awaits further studies. Hunziker and Peters (1998) transfected Rab17 in the MDCK epithelial cell line and showed an inhibition of basolateral to apical transcytosis of the polymeric immunoglobulin A receptor. The results of these two studies however cannot be directly compared because different Rab17 constructs were expressed in these cell lines, and since the Eph cell line only recently has been explored for membrane transport studies and is not well characterized in terms of its intracellular transport routes.

Rab7 and Rab9

Late endosomes are a crossroads for transport of CI-MPR to the TGN and of lysosomal enzymes and material to be degraded to the lysosomes (Kornfeld and Mellman 1989). Not surprisingly, several Rab proteins, including Rab7 (Chavrier et al. 1990) and Rab9 (Lombardi et al. 1993), but not Rab4 and Rab5 (Bottger et al. 1996), have been localized to this compartment. In cells transfected with the Rab7N125I and Rab7S22N, internalized vesicular stomatitis virus G protein becomes trapped in early endosomes (Feng et al. 1995). These mutants also partially inhibit the cleavage of paramyxovirus SV5 hemagglutinin neuraminidase and the degradation of low-density protein and cause the accumulation of cathepsin D and CI-MPR in an early endocytic compartment (Press et al. 1998). Thus mammalian Rab7 is involved in transport from early to late endosomes. In contrast Ypt7p, the *S. cerevisiae* homolog of Rab7, regulates transport from late endosomes to the vacuole (Wichmann et al. 1992), suggesting that transport pathways to late endocytic compartments are not completely conserved between mammals and yeasts. Alternatively, as Rab7 and Ypt7p are not identical, differences in the amino acid composition might confer distinct properties to the two proteins. Rab9 is involved in transport from late endosomes to the TGN. In vitro transport of CI-MPR from late endosomes to the TGN is enhanced by addition of recombinant Rab9 and selectively inhibited by antibodies against Rab9 (Lombardi et al. 1993) or its effector protein p40 (Diaz et al. 1997). In cells transfected with Rab9S21N, recycling of the CI-MPR from late endosomes to the TGN as well as delivery of newly synthesized lysosomal enzymes to late endosomes are inhibited (Riederer et al. 1994).

Effector proteins of early-endosome-associated Rab GTPases

Understanding the mechanism how monomeric RabGTP regulate transport through early endocytic organelles requires the identification of accessory proteins regulating their activity and the identification of their downstream target effector molecules. Several Rab effector proteins were recently identified, including rabphilin3A (Shirataki et al. 1993), rabin3 (Brondyk et al. 1995), rab8ip (Ren et al. 1996), Rim (Wang et al. 1997), p40 (Diaz et al. 1997), rabaptin5 (Stenmark et al. 1995), rabaptin5β (Gournier et al. 1998), and early-endosome-associated autoantigen EEA1 (Simonsen et al. 1998). For the sake of brevity, only the effector proteins of early-endosome-associated Rab proteins will be discussed here.

The groups of Zerial and Stenmark recently identified three effector proteins of Rab5 (Gournier et al. 1998, Simonsen et al. 1998, Stenmark et al. 1995). The first, rabaptin5, is a cytosolic 100 kDa protein with several putative coiled-coil domains and interacts with Rab5GTP. The extensive α-helical coils cause homodimerization of rabaptin5. A fraction of rabaptin5 colocalizes with Rab5 on peripheral early endosomes, and transfection of Rab5Q79L causes recruitment of rabaptin5 to swollen early endosomes. The Rab5-binding site is located in the carboxy-terminal 73 amino acids of rabaptin5 and is sufficient for Rab5-dependent recruitment to early endosomes. Rabaptin5 is required for early-endosome fusion, since cytosol depleted of rabaptin5 inhibits endosome fusion (Stenmark et al. 1995). Alternatively and equally possible, immunodepletion of cytosol with rabaptin5 antibody may also remove factors bound to rabaptin5 and involved in homotypic early-endosome fusion. Rabaptin5 contains a distinct binding domain for Rab4 in the amino terminus (Vitale et al. 1998). This suggests that rabaptin5 may link the activities of Rab4 and Rab5, the two GTPases associated with early endosomes. Rabaptin5 also binds to the *TSC2* gene product, tuberin, which enhances GTP hydrolysis on Rab5 and inhibits endocytosis (Xiao et al. 1997). Mutations in the *TSC2* and *TSC1* genes are associated with the disease tuberosclerosis. Tuberin is present in a high-molecular-weight complex with hamartin (van Slegtenhorst et al. 1998), the product of *TSC1*,

the second gene associated with the disease (van Slegtenhorst et al. 1997). Presently the putative role of the hamartin-tuberin protein complex in the regulation of the endocytic pathway is investigated by several groups. Rabaptin5 also binds to rabphilin3A (Ohya et al. 1998) and GAP-43 (Neve et al. 1998), a precise role of rabaptin5 in synaptic-vesicle recycling however remains to be established.

We identified a novel rabaptin in a two-hybrid screen using Rab4Q67L as a bait (our unpubl. results). Rabaptin4 is highly homologous to rabaptin5 and interacts with Rab4GTP. Rabaptin4 contains a Rab4-binding domain in the amino terminus and Rab5-binding sites in the amino terminus as well as the carboxy terminus. Rabaptin4 and rabaptin5 heterodimerize (our unpubl. results) suggesting that rabaptins may provide a scaffold to coordinate the activity of Rab proteins in the early endocytic pathway. Rabaptin4 contains an identical 33-amino-acid carboxy-terminal in-frame deletion as rabaptin5β. This deletion is immediately adjacent to the Rab5-binding site, but does not appear to have a major impact on Rab5 binding. Recently the group of Zerial identified rabaptin5β. This 62 kDa cytosolic protein shares 42% sequence identity with rabaptin5 (Gournier et al. 1998). Rabaptin5β is also recruited to endosomes in a GTP-dependent manner. The relative expression levels of rabaptin5 and rabaptin5β vary among different cell types. Rabaptin5β does not bind to rabaptin5 and forms a distinct complex with rabex5, a Rab5 guanine nucleotide exchange factor (Horiuchi et al. 1997). Cytosol immunodepleted of rabaptin5β only partially inhibits early-endosome fusion in vitro. Stronger inhibition is seen when both rabaptin5 and rabaptin5β are depleted, suggesting that maximal fusion efficiency at least requires both proteins. These results indicate that both effectors cooperate in the regulation of homotypic early-endosome fusion (Gournier et al. 1998). During apoptosis rabaptin5 is proteolytically cleaved by members of the family of apoptotic caspases. The cleavage of rabaptin5 is responsible for the loss of early-endosome fusion activity (Cosulich et al. 1997) in vitro which might relate the mechanism causing membrane fragmentation in apoptotic cells. It is not known whether rabaptin4 and rabaptin5β are subject to caspase-mediated cleavage during apoptosis.

Early-endosome-associated antigen EEA1 (Stenmark et al. 1996) was recently shown to be a third Rab5 effector and to be required for homotypic early-endosome fusion (Mills et al. 1998, Simonsen et al. 1998). In addition to an amino-terminal Rab5-binding domain, EEA1 contains a carboxy-terminal RING-FYVE finger that is required for early-endosome association as well as phosphatidylinositol 3-phosphate binding (Gaullier et al. 1998, Simonsen et al. 1998). The presence of two separate binding domains for Rab5 and phosphatidylinositol 3-phosphate might promote specific recruitment of EEA1 to early endosomes and confer vectoriality to transport through the endocytic pathway. Since Rab5 is localized to the plasma membrane, coated vesicles, and early endosomes, it will become necessary to establish where the distinct Rab5 effectors act in vivo and how their activities are coordinated.

The cysteine- and histidine-rich RING-FYVE domain is present in several other proteins known to regulate vesicular transport, including Vac1p (Weisman and Wickner 1992), Vps27p (Piper et al. 1995), and Fab1p (Odorizzi et al. 1998) in *S. cerevisiae*. Consistent with a role of RING-FYVE domains in binding phosphatidylinositol 3-phosphate and recruiting proteins to specific intracellular locations (Burd and Emr 1998), SARA, a FYVE-domain-containing protein, was recently shown to recruit the signaling mediator Smad2 to TGFβ receptor by mediating the specific subcellular localization of Smad2 (Tsukazaki et al. 1998).

Conclusion

For many years our understanding of the mechanisms regulating transport through the endocytic pathway has been lagging behind that of the biosynthetic route. The identification and functional characterization of multiple Rab proteins and their effectors on endocytic compartments during the last decade already generated, and will continue to provide, important insights in the machinery controlling endocytosis and membrane dynamics of the endocytic system.

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