Critical Reviews in Biochemistry and Molecular Biology, 38(2):121–142 (2003)

Regulation of Membrane Transport by rab GTPases

*Magdalena Deneka, Maaike Neeft, and Peter van der Sluijs**

Department of Cell Biology and Institute of Biomembranes, University Medical Center Utrecht, 3584 CX Utrecht, The Netherlands

* Address correspondence to: Peter van der Sluijs, Department of Cell Biology, UMC Utrecht, AZU Room G02.525, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. Phone: +31 30 2507574 Telefax: +31 30 2541797. Email: pvander@knoware.nl

TABLE OF CONTENTS

1040-9238/03/$.50

ABSTRACT: Membrane flow through the cell is a highly dynamic process in which intracellular compartments communicate via tubulo-vesicular structures shuttling cargo molecules to their destinations. Transport carriers are formed at a donor compartment and navigate through the cytoplasm to the target organelle, on which they subsequently dock and fuse. Many of these events are regulated by the cooperative action of monomeric rab GTPases and their effector proteins. Research in recent years resulted in the identification of many rab effectors, providing first glimpses how the GTPase switch of individual rab proteins is utilized in discrete transport steps.

KEY WORDS: membrane traffic, rab proteins, effectors.

I. INTRODUCTION

The complex compartmentalization of eukaryotic cells requires highly specific transport processes between organelles to maintain their identity and architectural integrity. In the endocytic pathway, many receptors with their ligands are internalized from the cell surface and brought to the early endosomal compartment where sorting events take place. Dissociated ligands are destined for degradation in the late endosome/lysosome, while receptors are often recycled back to the plasma membrane for reutilization. In the biosynthetic pathway, nascent proteins are translocated into the endoplasmic reticulum (ER), transported through the Golgi complex and sorted from the trans Golgi network in secretory vesicles that fuse with the plasma membrane. Proteins with an intracellular function are either sorted to late endosomes/lysosomes, retained in the ER, or retrieved to the Golgi complex. The lifetime of transport vesicles is short, in the range of a few seconds. One of the longstanding questions in cell biology is how compartments are generated and how their biochemical identity is maintained in the face of heavy vesicular traffic in forward (antegrade) and reverse (retrograde) transport pathways connecting them. When a transport vesicle approaches its destination, it is thought to be 'caught' by tethering proteins on the target compartment that loosely attach the transport vesicle on the target membrane. This is the first level of specificity to ensure that a vesicle reaches the correct target compartment. The next stages are docking, which secures the vesicle on the target, and finally fusion between the bilayers of the vesicle and target membrane, which is brought about by the pairing of cognate SNAREs (soluble *N*-ethylmaleimide-sensitive factor receptor protein) (see Chen and Scheller, 2001). SNARE proteins are enriched in certain organelles, which minimizes the number of nonspecific fusion events. Because SNAREs are transmembrane proteins, they will be distributed through many of the compartments of the central vacuolar system. The consequence of which is that any given organelle may contain SNAREs that must remain inactive until they reach the site for their specific function. This requires a second level of specificity control to ensure that trans SNARE complexes form at the right time and place. The role of rabGTPases as specificity determinants in membrane tethering and membrane fusion is discussed below.

II. RAB PROTEINS AND THEIR EFFECTORS IN MEMBRANE BIOLOGY

The ras superfamily of small GTPases can be divided in five families ras, rho/rac, rab, arf, and ran (Valencia *et al*., 1991a). The rab family is the largest and presently more than 60 genes encoding rab proteins have been identified in mammals, some of which are alternatively spliced (see Pereira-Leal and Seabra, 2000). Members of this family are found in all eukaryotes, including yeast, plants and mammals, and they regulate membrane transport between intracellular compartments of the vacuolar system (Plate 2*). Interestingly, rab32 was reported recently to associate with mitochondria and to regulate mitochondrial membrane dynamics (Alto *et al*., 2002). Variants of synaptojanin-2 (Nemoto and De Camilli, 1999) and dynamin (Bleazard *et al*., 1999) as well as VAMP-1 (Isenmann *et al*., 1998) have been found also on mitochondria. Since these proteins are members of conserved families that regulate fission and fusion in the vacuolar system, it is likely that there will be more similarities between autonomous organelles as mitochondria and peroxisomes, and the vacuolar system, than was traditionally perceived.

* Plates appear following page 122.

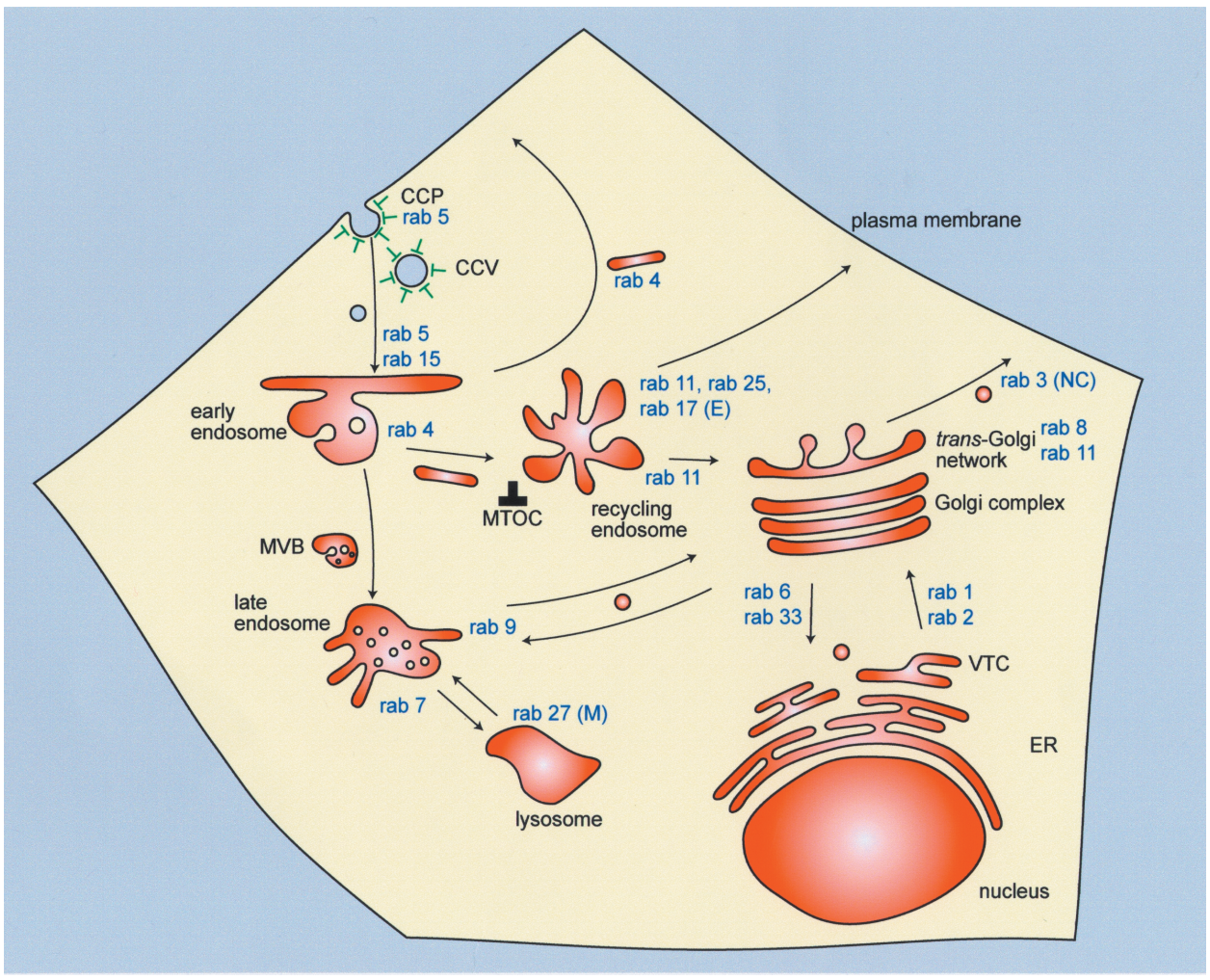

PLATE 1. Intracellular localization of rab proteins. Overview of rab protein localization in mammalian cells. CCP, clathrin coated pit; CCV, clathrin coated vesicle; M, melanosomes; E, epithelial cell type specific expression; NC, neuronal cell specific; VTC, vesiculo-tubular cluster; MVB, multivesicular body; MTOC, microtubule organizing center.

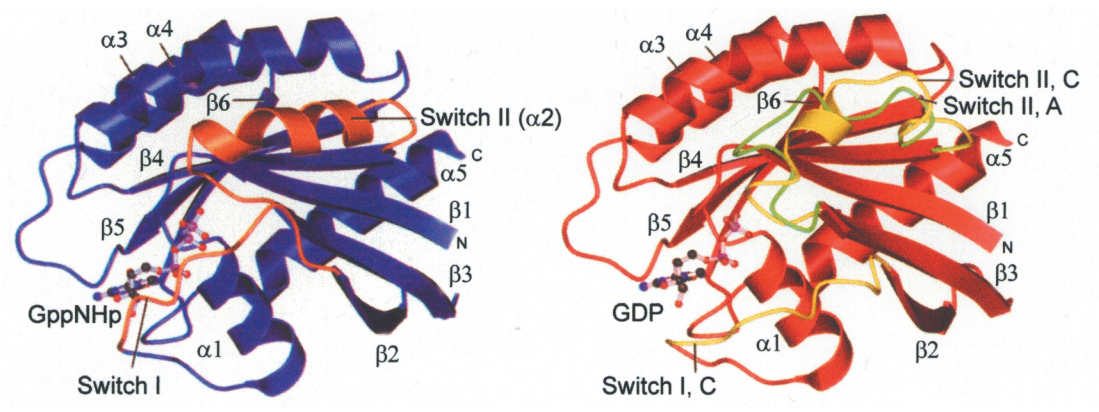

PLATE 2. Crystal structures of Sec4p in the GTP and GDP-bound forms. (left) Ribbon diagram of Sec4p-GppNHp with secondary structure elements labeled. Invariant regions with respect to Sec4p-GDP are shown in blue. Switch I and switch II regions are presented in orange (right). Ribbon diagram of Sec4p-GDP. Regions of structural similarity between 4 molecules (A,B,C,D) in the unit cell and the same with respect to Sec4p-GppNHp are in red. Switch II region of A is in green, while switch I and switch II regions of C are in yellow. (Figure adapted with permission of Elsevier Science from Stroupe and Brunger, 2000.)

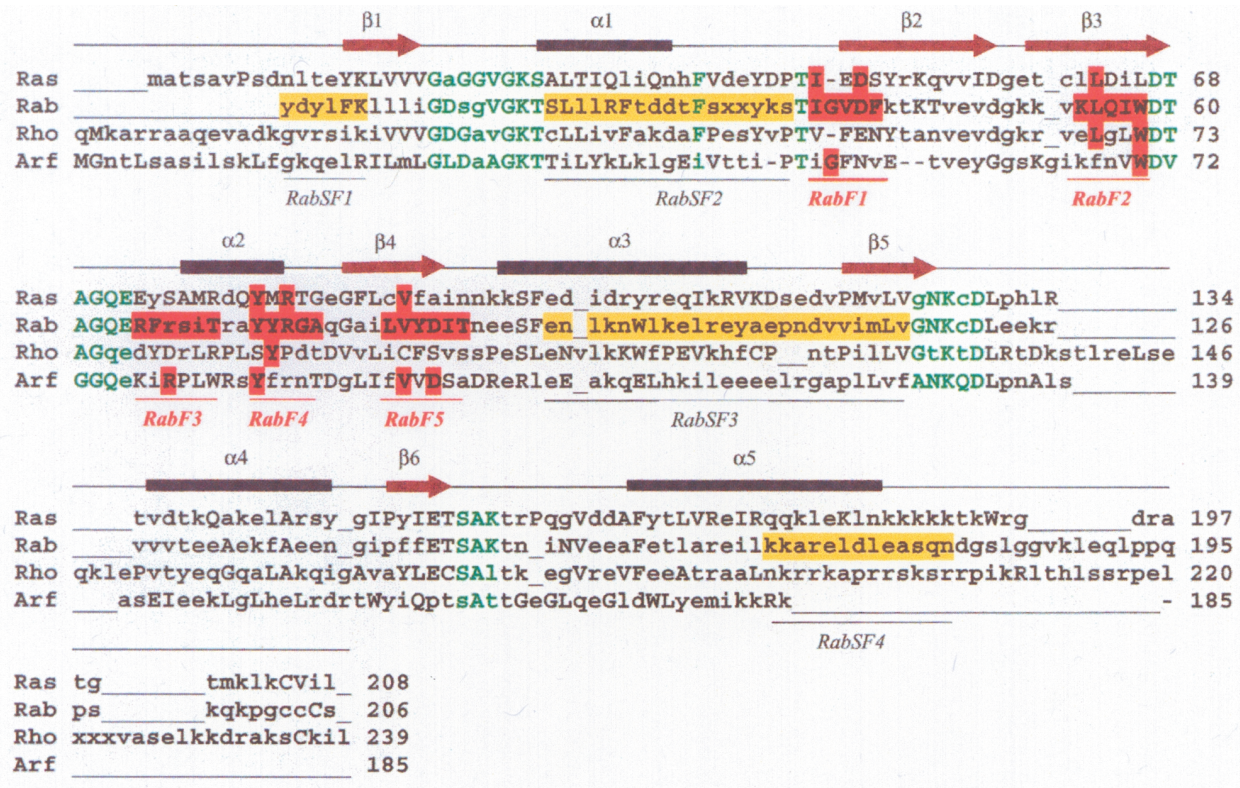

PLATE 3. Alignment of ras family members. Amino acid stretches specific for rab are colored red (RabF1-5). Green characters denote conserved guanine nucleotide binding motifs (PM/G). Yellow regions represent sequences that are highly conserved in rab subfamilies (RabSF1-4). (Figure adapted with permission of Elsevier Science from Pereira-Leal and Seabra, 2000.)

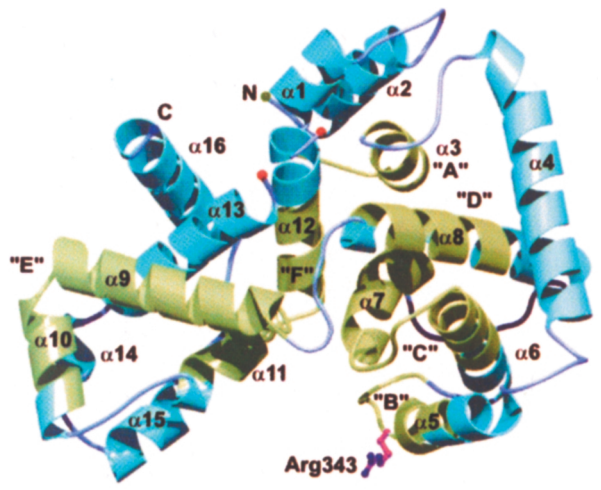

PLATE 4. Three-dimensional structure of the Ypt-GAP domain of Gyp1p. The ribbon diagram displays the secondary structure elements and the active site arginine in ball-and-stick representation. The seven shared sequence motifs are indicated in green. (Figure adapted with permission of EMBO from Rak *et al.*, 2000.)

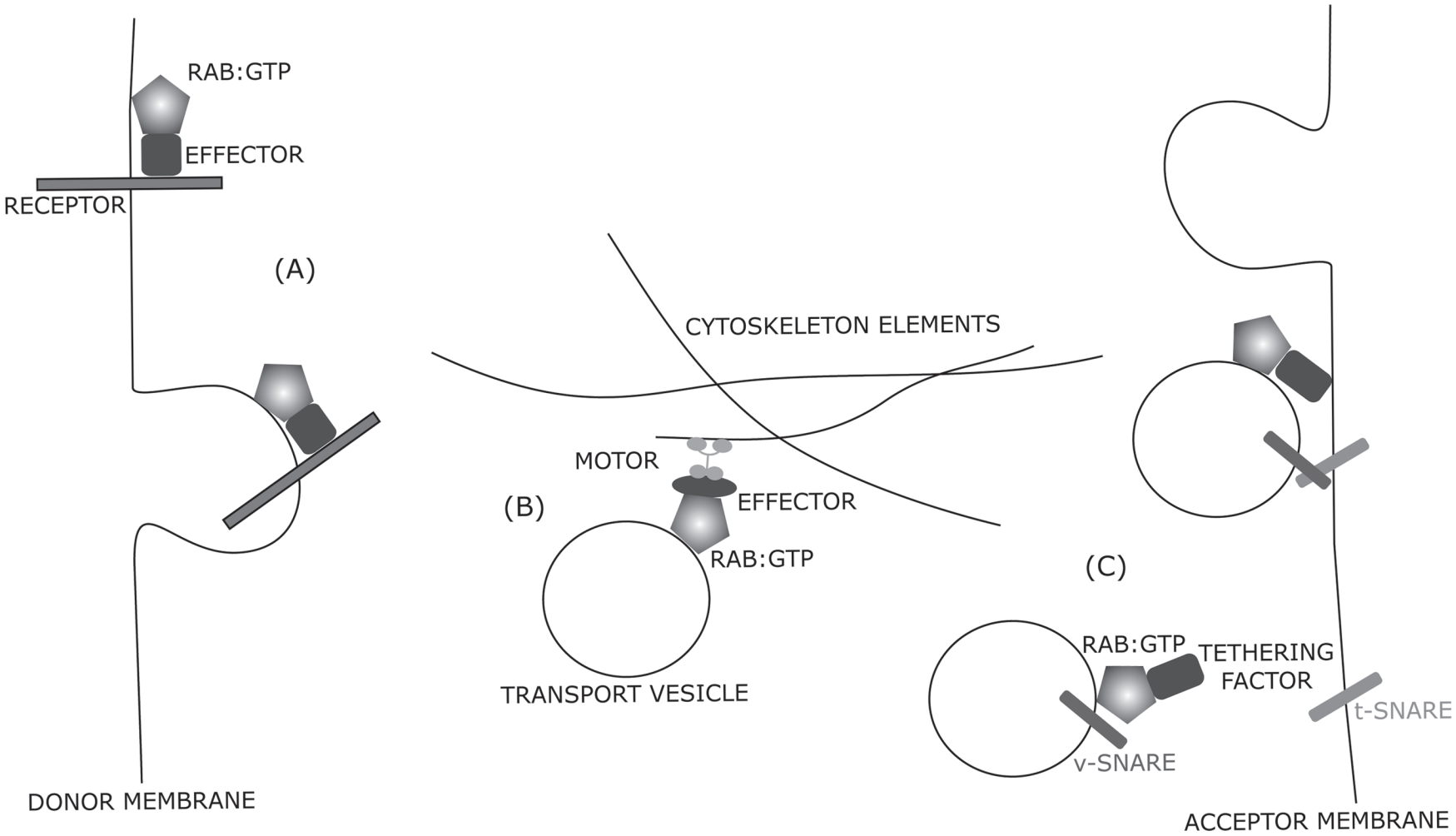

FIGURE 1. Functions of rab GTPases. (a) rab proteins and their effectors are involved in cargo sorting and transport vesicle formation, e.g., rab9 and TIP47. (b) rab proteins in combination with motor proteins play a role in the movement on transport vesicle along the cytoskeleton, e.g., rab27 and Myosin Va. (c) rab proteins associate with tethering factors required for vesicle docking and fusion, e.g., rab1 and p115.

Most rab proteins are ubiquitously expressed, while some have a more restricted tissue distribution. For instance, rab3a is specifically expressed in neuronal tissue (Mollard *et al*., 1990), rab17 in epithelial cells (Zacchi *et al*., 1998), while rab27a occurs predominantly in cells of the hematopoietic lineage and neuroendocrine cells (Hume *et al*., 2001; Stinchcombe *et al*., 2001; Yi *et al*., 2002). Rab proteins function as molecular switches because GTP hydrolysis and guanine nucleotide exchange allows them to continuously cycle between the structurally distinct inactive GDP, and active GTP-bound states (Figure 1). The rab GTPase switch regulates a number of cellular events, including vesicle budding, interactions between motile vesicles and the cytoskeleton, fusion between transport vesicles and target organelles, and organelle architecture (see Plate 3*).

A. Structural Aspects

Rab proteins have a highly conserved domain structure that encompasses six motifs that are involved in guanine nucleotide (designated G1-G3) and phosphate/Mg^{2+} (designated PM1-PM3) binding (Valencia *et al*., 1991b). As these domains occur in all small GTP binding proteins, they are not useful to discriminate between rab GTPases and other members of the ras superfamily. Sequence alignment revealed that five conserved short stretches can be discerned that are diagnostic for the rab family (RabF1-F5). RabF1 (IGVDF) is in the effector domain (loop2-β2) in the switch I region. The so-called switch regions change conformation upon GTP-binding and hydrolysis and therefore are likely to be involved in binding to effectors and regulators of rab proteins. RabF2 (KLQIW) is situated in β3. RabF3 (RFrsiT) is in loop4. RabF4 (YYRGA) localizes to α2-loop5 and RabF5 (LVYDIT) localizes to β4-loop6. The RabF signature sequences, with the exception of RabF1, are grouped between β3 and β4, which also includes the switch II region (see Pereira-Leal and Seabra, 2000).

Phylogenetic analysis of the mammalian rab gene family reveals the presence of subfamilies of related rab proteins. Within these subfamilies, some rab proteins have an unusual high homology and are named isoforms. The amino acid conservation within rab subfamilies (RabSF) is confined to three regions designated RabSF2, RabSF3, and RabSF4 corresponding to α1/loop2, α3/loop7, and α5, respectively. A fourth RabSF (RabSF1) with lower overall identitity can be discerned immediately N-terminal of PM1. Sequence identity within these regions in subfamilies is higher than overall homology, which is particularly clear for RabSF4 that is in the C-terminal hypervariable region. This is the region where rab proteins have the least sequence conservation (14%), while in subfamilies this amounts to 58% (Pereira-Leal and Seabra, 2000). Interestingly, RabSF3 and RabSF4 correspond to complementarity determining regions (CDRs) II and III, respectively. CDRs were identified within the crystal structure of rab3a-GTP in complex with rabphilin-3a (Ostermeier and Brunger, 1999). It is thought that the CDRs contribute to form a pocket for binding to effector proteins. Mapping of the RabSF regions onto the crystal structure of rab3A suggests that RabSF1, RabSF3, and RabSF4 form a surface on one side of the molecule, while RabSF2 is located on the opposite side. The existence of subfamily specific surfaces argues for the case that rab isoforms might interact with the same type of effectors via the subfamily specific regions.

B. Membrane Association

Rab proteins are localized to the cytoplasmic surface of distinct compartments in the cell, although a given rab protein may occur on several locations. For instance, rab5 is associated with the plasma membrane, coated vesicles and early endosomes, in agreement with its function in clathrin coated vesicle formation, heterotypic-coated vesicle-early endosome fusion, and homotypic early endosome fusion. Membrane association of rab proteins requires the post-translational modification at the C-terminus with geranylgeranyl groups that occurs in the cytoplasm (see Casey and Seabra, 1996). Experiments with chimeric rab proteins revealed that the hypervariable region, the two switch regions and α-helix3/loop7 are important for targeting a rab protein to its correct compartment, while switchII and α-helix3/loop7 are also required for membrane association (Brennwald and Novick, 1993; Dunn *et al*., 1992; Stenmark *et al*., 1994). Since the hypervariable region contains rabSF4, it is possible that rab-effector interactions and rab-membrane binding are dependent events.

After GTP hydrolysis, rab is recycled back to its origin by a cytosolic chaperone called rabGDI. rabGDI has a strong preference for the GDP-bound form of a rab protein and has the ability to extract rab-GDP

* Plates appear following page 122.

from the target membrane (Garrett *et al*., 1994). GDI-mediated retrieval is likely to be more critical to heterotypic fusion reactions than for homotypic fusion as in the latter case the GTPase can undergo multiple cycles of GTP hydrolysis and GDP-GTP exchange without the need for membrane-dissociation and GDI-mediated rebinding. In each heterotypic fusion event, where the rab protein is present on a transport vesicle or tubule, it needs to be recycled back to its origin for reuse in a new round of transport. Most likely this occurs via a cytoplasmic rab-rabGDI complex, although recycling via a retrograde vesicular transport pathway theoretically is also possible. The recruitment of a rab protein to a target membrane is also mediated by GDI (see Alory and Balch, 2001). Compartmental specificity of a rab-rabGDI complex with the proper target membrane is entirely contained within sequence determinants of this complex. After binding of the rab-rabGDI complex to the membrane, a GDI displacement factor activity (GDF) (Dirac-Svejstrup *et al*., 1997) catalyzes dissociation of the complex and GDI is relocalized to the cytosol where it can bind another rab molecule. A membrane localized chaperone complex consisting of hsp90, hsp70, and cysteine string protein (CSP) might potentially serve as GDF in membrane association of cytoplasmic rab-rabGDI complex (Sakisaka *et al*., 2002). It has been postulated that the heat shock protein complexes facilitates transfer of the geranylgeranyl groups to the bilayer and stabilizes rabGDI on the membrane. Membrane-bound rabGDP is next converted to the active form by a guanine nucleotide exchange factor (GEF) (Soldati *et al*., 1994; Ullrich *et al*., 1994). In subsequent steps the chaperone complex may coordinate the function of rabGDI with extraction of rab following vesicle fusion.

III. UPSTREAM REGULATORS

A. Guanine Nucleotide Exchange Factors

The ability of rab proteins to cycle between GTP and GDP conformations is essential for their function as regulators of membrane transport. The replacement of GDP for GTP is accelerated by GEFs, which serve in this sense as positive regulators. The very low intrinsic GTPase activity of rab proteins is likely catalyzed by GTPase activating proteins (GAPs) that act as negative regulators of rab protein function. A number of GEFs and GEF activities have been identified that are required for rab protein function. These include rabex-5 (Horiuchi *et al*., 1997), its yeast counterpart Vps9p (Burd *et al*., 1996), Sec2p (Walch-Solimena *et al*., 1997) and the human homolog rabin8 (Hattula *et al*., 2002), the Ric1p-Rigp1p complex (Siniossoglou *et al*., 2000), the HOPS complex (Wurmser *et al*., 2000) and the highly conserved TRAPP complex (Sacher *et al*., 1998; Wang *et al*., 2000), that act on rab5, Ypt51p, Sec4p, rab8, Ypt6p, Ypt7p, and Ypt1p, respectively. The known ypt GEFs do not share obvious sequence similarity, nevertheless a few human homologs have been identified. Rabex-5 is homologous to Vps9p and acts as a GEF for rab5. Rabin8 has GEF activity toward rab8 and shares homology with Sec2p and GRAB, a GEF for rab3 (Luo *et al*., 2001). In contrast, hVam6 which is homologous to Vps39p of the yeast HOP complex, more likely acts as a tethering protein than a GEF for rab7 in lysosome tethering and fusion (Caplan *et al*., 2001). Interestingly, some GEFs are heterodimeric or heterooligomeric complexes in which GEF activity is either contained within a subunit (rabex-5 in the rabaptin5-rabex-5 complex) or within the entire complex as for Ric1p-Rigp1p and TRAPP. Likely the latter possibility reflects the relative instability of the individual components in the absence of a partner subunit. Alternatively, the physical interaction of a GEF and effector protein within a complex may provide an efficient molecular machine to integrate regulation and performance of a rab protein. The use of GEF/effector complexes is not unique for rab proteins. The rac1 and cdc42 effector PAK stimulates the intrinsic GEF activity of PIX and binding of PAK to PIX is essential for the recruitment of the effector to membranes (Manser *et al*., 1998).

B. GTPase Activating Proteins

In addition to GEFs, a number of rab GAPs has been identified, including Gyp1p, Gyp2p, Gyp3p, Gyp4p, Gyp6p, Gyp7p in yeast (Rak *et al*., 2000), and GAPCenA (Cuif *et al*., 1999), and RN-Tre (Lanzetti *et al*., 2000) in mammals. Rab GAPs contain short fingerprint sequences that are conserved between species and which also occur in GAPs for other GTPase families (Furge *et al*., 1998; Neuwald, 1997). In contrast to GEFs, the rabGAPs appear to have a more broad substrate specificity *in vitro*. For instance, Gyp1p, Gyp6p, and Gyp7p are active on Sec4p, Ypt6p, and Ypt7p. Likewise, mammalian

GAPCenA acts on both rab6 and rab4, and although RN-tre has been reported to be specific for rab5, it will be interesting to asses its GAP activity toward rab22 that also acts in the early endocytic pathway and interacts with the rab5 effector EEA1 (Kauppi *et al*., 2002). The limited substrate specificity of Gyps in yeast is in agreement with the observations that they are not essential for growth. Specificity, however, might be increased by pairing the localization of a GAP and its cognate substrate. For instance, Gyp1p localizes to the Golgi complex, suggesting that its substrates are Golgi-localized GTPases (Du and Novick, 2001). Of the known Gyp1p substrates, Ypt1p is the only one that is predominantly localized to the Golgi complex (Segev *et al*., 1988), and that genetically interacts with Gyp1p (Du and Novick, 2001), arguing for the case that Gyp1p is the principal GAP for Ypt1p *in vivo*.

The catalytic domain of Gyp1p has been crystallized, and its structure reveals the presence of an invariant arginine residue that is essential for the catalytic activity (Albert *et al*., 1999; Rak *et al*., 2000). This arginine is in a comparable position as the arginine finger in rasGAP (Scheffzek *et al*., 1997) and cdc42/rhoGAP (Rittinger *et al*., 1997), which suggests that Gyp1p GAP activity is based on the same mechanistic principle. Sequence alignment of GAP domains of the six Gyps reveals several regions of the α-helical GAP fold that are heterogeneous in sequence and size of the loops. These less-conserved regions therefore may serve as specificity determinants for interactions with other proteins, including substrate GTPases.

IV. DOWNSTREAM EFFECTORS

The active form of rab proteins recruits soluble factors to relay the GTPase signal to downstream effector systems that mediate discrete steps in membrane transport. An increasing number of rab effector proteins have been identified. In contrast to the high degree of sequence conservation in rab proteins, most effector proteins have unrelated amino acid sequences. As originally discovered for ras, it is also clear now that a single rab protein can interact with multiple effectors, which testifies to the notion that a given rab may regulate multiple biochemical reactions at distinct sites in a transport pathway between two organelles as was shown for rab5. The challenge is to identify their components and to understand the molecular signals that regulate the spatiotemporal assembly of rab effector complexes.

A. Vesicle Formation

Several studies in different experimental systems show that rab proteins and effectors are involved in generating transport vesicles. Rab1 regulates COPII-mediated transport between the ER and Golgi complex. Rab1-GTP has been shown to recruit the tethering factor p115 during budding of COPII vesicles, which in turn interacted with a complex consisting of membrin, rbet1, and syntaxin5 (Allan *et al*., 2000). This complex of SNARE proteins, although not directly interacting with rab1, is needed for targeting the COPII vesicles to the Golgi complex and their fusion with the Golgi membrane. Possibly the recruitment of this complex onto the forming vesicle already programs it for the next stage in its itinerary. Ypt1p, the yeast homolog of rab1, is not essential for COPII vesicle formation but is required at the target Golgi membrane (Cao and Barlowe, 2000), suggesting that additional mechanisms are involved in ER to Golgi transport. Rab1-GTP also binds to the Golgi matrix protein GM130. Interestingly, the interaction between rab1 and GM130 does not seem to involve p115 (Moyer *et al*., 2001; Weide *et al*., 2001), the significance of which, however, remains to be further explored with respect to the known functions of p115 and GM130 (Sönnichsen *et al*., 1996).

In vitro studies document a role for rab5 in vesicle budding from the cell surface. A cytosolic rab5-rabGDI complex has been purified as a critical component for sequestering transferrin receptor into clathrin coated pits in permeabilized cells (McLaughlan *et al*., 1998). Although the transferrin receptor is not known to directly interact with rab5, binding of this GTPase to the cytoplasmic tail of other cell surface receptor has been reported. The cytoplasmic tail of the angiotensin II type 1A receptor ($AT_{1A}R$) interacts directly with the GTP-bound form of rab5 *in vivo* and *in vitro* on an endocytic compartment, and positively regulates transport of $AT_{1A}R$. Interestingly, the interaction was enhanced by occupation of the receptor with angiotensin II, suggesting that bound ligand may be instrumental in recruiting a guanine nucleotide exchanger activating rab5 (Seachrist *et al*., 2002). An opposing function of a direct interaction between receptors and rab proteins is suggested by the association of rab3b in its GTP-bound form with the transcytosing polymeric Ig recep-

tor (pIgR) (van IJzendoorn *et al*., 2002). Dissociation of the complex is brought about by rab3b-GTP hydrolysis and is catalyzed by binding of dimeric IgA to the receptor. The expression of a GTP hydrolysis-deficient rab3b mutant inhibits transcytosis of the dIgA-pIgR complex. Collectively, these data suggest that the interaction of rab3b with the cargo molecule pIgR modulates the intracellular itinerary of the receptor by preventing exocytosis, possibly through the recruitment of a GAP.

An insight into how rab proteins may assist in sequestering cargo molecules into budding vesicles was provided by studies on rab9. Rab9 is predominantly localized on late endosomes and like its effector TIP47 is required for transport of mannose 6-phosphate receptors from endosomes to the TGN (Diaz and Pfeffer, 1998; Lombardi *et al*., 1993). TIP47 forms a ternary complex with rab9 and the cytoplasmic tail of the cation-independent mannose 6-phosphate receptor (CI-MPR) on endosomes and serves as a linker between rab9 and CI-MPR (Carroll *et al*., 2001). *In vivo* experiments showed that the cooperative action of the three components forming the complex is required for transport from endosomes to the TGN. Interestingly, rab9 enhanced binding of TIP47 to CI-MPR, while, vice versa, CI-MPR increased the affinity of rab9 to TIP47, documenting that rab9 likely enhanced TIP47 recruitment to endosomes and cargo capture.

B. Vesicle Motility Along Actin Cytoskeleton

The actin and microtubule cytoskeleton are essential determinants of organelle architecture and serve as tracks along which vesicular transport occurs through the cytoplasm. Long distance transport of transport vesicles along microtubules is powered by motor proteins such as cytoplasmic dynein (minus end directed) and members of the kinesin family (plus end directed). The first indication that rab proteins are involved in vesicle motility came from Novick's lab, who found that MYO-V interacted genetically with SEC4 (Govindan *et al*., 1995) and with a subset of late acting sec genes that also genetically interacts with SEC4. Others subsequently reported that the product Myo2p colocalized with Sec4p (Schott *et al*., 1999) and that the two proteins form a complex (Wagner *et al*., 2002). As the actin cytoskeleton provides the major track for vesicular transport in yeast, these observations might explain why secretory vesicles in yeast are transported along actin cables to the growing bud. Similarly, rab3d in the exocrine pancreas might regulate the transfer of zymogen granules across the actin terminal web to their docking site on the apical cell surface (Valentijn *et al*., 2000). Rab8 has also been implicated in actin-dependent motility via its effector FIP-2 (Hattula and Peränen, 2000). Rab8 regulates biosynthetic transport from the TGN to the plasma membrane in polarized epithelial cells and to the dendrites in neurons (Huber *et al*., 1993a; Huber *et al*., 1993b) and has the ability to reorganize the actin cytoskeleton (Peranen *et al*., 1996). FIP-2 interacts with the Huntington protein (Faber *et al*., 1998), which via Huntington interacting protein HIP-1 may link membrane vesicles to the actin cytoskeleton (Wanker *et al*., 1997). In addition, rab8 has also been implicated to control transferrin recycling in conjunction with Myo-Vc (Rodriguez and Cheney, 2002). Another nonconventional myosin, Myo-Vb, directly interacts with active rab11 that regulates membrane recycling from early endosomes (Lapierre *et al*., 2001). The expression experiments with a dominant negative Myo-Vb tail mutant inhibited transferrin recycling, suggesting a role for rab11 in the regulation of the actin-dependent recycling from early endosomes (Lapierre *et al*., 2001). A family of rab11 interacting proteins (FIPs) has been identified (Hales *et al*., 2001; Prekeris *et al*., 2001), and several of its members colocalize with Myo-Vb, while FIP2 has been shown to directly interact with Myo-Vb (Hales *et al*., 2002). Possibly the FIPs act as adaptors that contribute to the anchoring of rab11a containing vesicles with the actin cytoskeleton. None of the above-mentioned studies so far addressed the significance of those interactions in organelle motility assays.

The best evidence for a role of rab proteins in actin-based motility comes from studies in *dilute* and *ashen* mice. These mouse pigmentation mutants lack functional Myo-Va (Mercer *et al*., 1991) and rab27a (Wilson *et al*., 2000), respectively, and may serve as models for Griscelli syndrome a rare inherited human disorder featuring pigmentary dilution of the skin and hair. Most of the Griscelli patients have a mutation in rab27a and not in Myo-Va (Menasche *et al*., 2000). Both mouse strains have reduced hair coloration caused by clustering of melanosomes in the perinuclear region. This location does not allow transfer of melanin pigment from melanocytes to adjacent

keratinocytes, because melanosomes either cannot be unidirectionally transported along the actin cytoskeleton into the dendritic spines (Wu *et al*., 1998), or cannot be tethered to the actin cytoskeleton in the cell periphery. The phenotype of both mutants is suppressed by the semidominant suppressor *dsu*, and mice homozygous for both mutations have the same reduced coat color as the individual mutants (Moore *et al*., 1988). These genetic observations suggest that the dil and ash gene products act in the same or overlapping pathways. In normal melanocytes rab27a and Myo-Va colocalize on melanosomes (Bahadoran *et al*., 2001; Hume *et al*., 2001; Wu *et al*., 2001) and can be co-immunoprecipitated in the same complex (Hume *et al*., 2001) but do not directly interact with each other. In *ashen* melanocytes Myo-Va does not localize on melanosomes, suggesting that rab27a serves to anchor the actin motor on melanocytes (Bahadoran *et al*., 2001; Hume *et al*., 2001; Wu *et al*., 2001). The indentification of the missing link was facilitated by the cloning of melanophilin, the gene that is defect in the *leaden* mouse (Matesic *et al*., 2001). The *leaden* mouse has a coat color defect that is indistinguishable from ashen and dilute, and the phenotype is also suppressed by *dsu* (Moore *et al*., 1988). Melanosomes containing rab27a accumulate in the perinuclear region in *leaden* melanocytes, while Myo-Va is not targeted to melanosomes (Wu *et al*., 2002), a phenotype that can be rescued by transfection with melanophilin. Together with biochemical binding assays that revealed simultaneous binding of rab27a to the N-terminus, and Myo-Va to the C-terminal half of melanophilin (Fukuda *et al*., 2002), these data convincingly show that melanophilin serves to link Myo-Va via rab27 a to melanosomes. Melanophilin was independently cloned as slac2-a (Fukuda *et al*., 2002) and is a member of the synaptotagmin-like protein (Slp) family. The Slp family contains two conserved Slp homology domains SHD1 and SHD2 in their N termini and often C-type tandem phospholipid binding C2 domains. SHD1 and SHD2 may either be linked together, or be separated by a sequence containing two zinc finger motifs. SHD2 includes a motif that is similar to the rab3 binding domain in rabphilin-3a (Kuroda *et al*., 2002). Other members of the Slp family include granuphilin and the rab3 effectors rabphilin-3A, Noc2, and RIM. Granuphilin also interacts with rab27 and regulates exocytosis of dense core granules in pancreatic β cells that secrete insulin (Wang *et al*., 1999; Yi *et al*., 2002), suggesting that the ununderstood process of pigment transfer from melanocytes to keratinocytes is mechanistically related to the regulated secretory pathway. Finally, MyRIP, a novel protein, was identified in retinal pigment epithelium, which links Myo-VIIa to rab27 and enables Myo-VIIa to be recruited to retinal melanosomes (Amraoui *et al*., 2002). MyRIP is not a member of the Slp family, and the region that binds Myo-VIIa is not homologous to the binding site of Myo-Va on melanophilin. MyRIP, however, does contain the rab binding domain. Possibly the loss of a functional Myo-VIIa, MyRIP, rab27a complex may account for the perinuclear accumulation of retinal melanosomes in Myo-VIIa-defective *shaker-1* mice (Liu *et al*., 1998).

C. Vesicle Motility Along Microtubule Cytoskeleton

Rab proteins have as well been implicated in the regulation of long-range microtubule-based organelle motility, as initially found through the identification of rabkinesin6 as an effector of rab6a (Echard *et al*., 1998). Rab6a controls retrograde transport from early endosomes via the Golgi complex (Mallard *et al*., 2002) to the ER (White *et al*., 1999) and is important for membrane dynamics of the Golgi complex (Martinez *et al*., 1997; Martinez *et al*., 1994). Rabkinesin6 is a kinesin-like protein that directly interacts with rab6a, binds microtubules, and has microtubule-activated ATPase activity. It colocalizes with rab6a in the Golgi complex in interphase cells. The overexpression of a GFP-tagged wild-type protein causes a dosage-dependent dispersal of the Golgi complex throughout the cytoplasm. Although these features suggest that rabkinesin6 might act as a plus-end directed motor, this notion will need to be confirmed in motility assays. Rabkinesin6 does not bind to the splice variant rab6a′, in which a TV motif close to the switch II region is altered to AA (Echard *et al*., 2000). Structural considerations show that this region is important for interactions of rab proteins with regulators and effectors, which may account for the loss of binding of rabkinesin6 to rab6a′. Equally interesting, however, is the suggestion that a putative phosphorylation motif is abolished by the substitution. The expression level of human rabkinesin6, which is 86% identical to mouse mouse rabkinesin6, is subject to regulation during the cell cycle, and increases to maximal levels in mitosis (Fontijn *et al*., 2001; Hill *et*

al., 2000). During prophase rabkinesin6 is in the nucleus and does not colocalize with rab6 on Golgi fragments. Antibody injection or overexpression of rabkinesin6 interfered with cell division, suggesting that rabkinesin6 has an important function during cytokinesis. As rabkinesin6 has 50% homology with the mitotic kinesin MKLP-1 (Nislow *et al*., 1992), further research will be needed to understand rabkinesin6 function in membrane transport. The role of rab6 in a COP-I independent Golgi-ER retrograde transport pathway (Girod *et al*., 1999; White *et al*., 1999) has become more clear through the identification of the Bicaudal-D proteins BICD1 and BICD2 as effectors of rab6 (Matanis *et al*., 2002; Short *et al*., 2002). BICDs localize in a rab6 dependent manner via their C-terminus to the Golgi complex, TGN and rab6-containing vesicles. Because the N-terminus of BICD binds to the dynein-dynactin complex, BICD serves as a linker between the retrograde microtubule motor and rab6 on the Golgi. The overexpression of a C-terminal BICD fragment inhibits association of full length BICD to membranes and enhanced the velocity of minus end-directed motility of rab6-labeled vesicles along microtubules. In addition, the duration of minus end-directed motility and pausing between plus-end and minus-end motility was decreased. Possibly, expression of this trunction construct uncouples the interaction of rab6 vesicles and cytoplasmic dynein.

A second example of rab proteins regulating microtubule-dependent organelle motility is provided by rab7. Rab7 is localized to late endocytic compartments, including lysosomes (Bucci *et al*., 2000; Chavrier *et al*., 1990). Initially, rab7 was thought to control transport between early endosomes and late endosomes (Feng *et al*., 1995; Press *et al*., 1998). A reevaluation of its function, however, established that rab7 more likely regulates fusion of late endocytic structures/lysosomes (Bucci *et al*., 1994; Lebrand *et al*., 2002). The overexpression of rab7 inhibits motility of CD63-labeled late endocytic structures, causing their accumulation in the perinuclear region, possibly due to enhanced minus end directed motility. Two labs independently identified the effector protein RILP that controls lysosomal transport by enhancing recruitment of functional dynein-dynactin complex detected (Cantalupo *et al*., 2001; Jordens *et al*., 2001). RILP is a novel protein with limited similarity to CLIP-170, an intermediate filament associated protein. CLIP-170 links endocytic vesicles to microtubules, and to $p150^{Glued}$, which is part of the dynactin complex that is important for the binding of motor protein complexes to organelles. It is not clear how the rab7-RILP interaction talks to the dynein-dynactin complex since RILP does not appear to bind to the complex. Possibly, rab7-RILP may modify the lipid distribution or composition on the cytoplasmic surface of late endocytic organelles, which could provide a scaffold for protein recruiting the dynein-dynactin complex.

Rab4 and rab5 have been implicated in microtubule-dependent motility of early endocytic structures. Rab5 stimulates minus end directed motility along microtubules and enhances binding of early endosomes to microtubules through unknown motor and linker proteins (Nielsen *et al*., 1999). The active form of rab4, on the other hand, binds to the cytoplasmic dynein light chain and partially colocalizes with it (Bielli *et al*., 2001). A functional evaluation of this interaction has not been reported yet.

D. Vesicle Tethering

The initial contact between a transport vesicle, and the target compartment it fuses with is called tethering. Most likely the contact between a vesicle and a target needs to be established over a considerable distance. In a simple model for which there is not too much experimental evidence, one could envisage that a protein recognition complex associated with the target compartment continuously surveys the surrounding space in the cytoplasm for transport vesicles containing cargo to be delivered (see Pfeffer, 1999; Pfeffer, 2001; Whyte and Munro, 2002). After recognition, the vesicle is trapped at the end of other end of the complex bringing it in the close proximity of the membrane. Although SNARE proteins are the mediators of membrane fusion, experimental evidence suggests that tethering is not accomplished by trans SNARE complexes on the vesicle and target membrane. For instance, SNARE proteins do not appear to be concentrated in the areas of docking and fusion (Garcia *et al*., 1995), secondly treatment of cells with neurotoxins prevents SNARE complex formation, but does not block vesicle docking (Broadie *et al*., 1995), and, finally, the size of a SNARE complex is simply too small to bridge the distance with a transport vesicle (Sutton *et al*., 1998). An increasing number of proteins has been identified that seems to be involved in vesicle tethering and although the actual mechanisms

of tethering are not clear, some tethering factors bind to rab proteins, which suggests that the rabGTPase switch may be an important regulators of vesicle tethering (see Pfeffer, 2001; Whyte and Munro, 2002).

Two general classess of proteins have been suggested to serve as tethering factors, a group consisting of extended coiled-coil proteins and a class of oligomeric complexes. The known examples of the first group act in in the Golgi complex and the endocytic pathway. Typical examples include Uso1p (Seog *et al*., 1994) and its mammalian homolog p115 (Nelson *et al*., 1998; Sapperstein *et al*., 1995) that are required for transport through the Golgi complex. p115 is anchored via GM130 and GRASP65 to the Golgi membrane (Barr *et al*., 1997), and it binds via its other end to giantin on a COPI vesicle (Sönnichsen *et al*., 1998). Although intuitively attractive, the simultaneous interaction of GM130 and giantin to p115 has been called into question by others who found a common binding site for the two proteins on p115 (Linstedt *et al*., 2000). Both p115 and GM130 bind directly to rab1 (cf. above). Rab1 also binds to golgin-84, a coiled coil membrane protein of the cis-Golgi network (CGN) that has an essential function in Golgi ribbon formation (Diao *et al*., 2003). As golgin-84 does not interact with the Golgi matrix proteins GM130 and p115, it is likely that its interaction of rab1 serves a different purpose than the interaction of rab1 with the matrix proteins. Possibly, golgin-84 might have a tethering function in the incorporation of VTCs in the cis Golgi, promoting fusion of rab1 containing membranes with the CGN. As such this transport step might be parallel to the one regulated by the matrix proteins and rab1, or at a different location. Finally, rab2 binds to the matrix protein GRASP55 (Short *et al*., 2001) and rab33 to GM130 (Valsdottir *et al*., 2001). The increasing number of tethering complexes in the Golgi likely reflects the many transport pathways that either bring or retrieve membrane to this compartment. EEA1 and the rabaptins are also long coiled coil proteins, these may serve as tethering proteins in the endocytic pathway and interact with rab5 and rab22 (Kauppi *et al*., 2002; Simonsen *et al*., 1998), and rab5 and rab4, respectively (Nagelkerken *et al*., 2000; Vitale *et al*., 1998).

Oligomeric tethering complexes can be divided into two subgroups (see Whyte and Munro, 2002). One in which one or more of the subunits have a common domain in the N-terminus, while the second group does not have this feature. Members of the first group are named quatrefoil complexes because they appear to consist of multiples of four subunits. The best understood quatrefoil tethering complex is the exocyst, originally identified as a sec4 effector complex in yeast (TerBush *et al*., 1996; TerBush and Novick, 1995) and later as sec6/8 complex in the mammalian system (Grindstaff *et al*., 1998; Kee *et al*., 1997). The complex consists of eight subunits (sec5, sec6, sec8, sec10, sec15, exo70, and exo84) and is localized to the plasma membrane in a sec3-dependent manner at sites where polarized secretion occurs (Finger *et al*., 1998). Sec10 and sec15 bind to each other and can be coimmunoprecipitated with sec4, suggesting that a subcomplex localizes on a Golgi vesicle that associates with sec3 on the cell surface (Guo *et al*., 1999). The other components are then thought to be recruited from the cytosol to establish a tether between the secretory vesicle and the plasma membrane.

A second example of a tetrafoil complex is the COG (conserved oligomeric Golgi) complex (Ungar *et al*., 2002) previously known as the sec34/35 complex (van Rheenen *et al*., 1999). Like the exocyst, the COG complex also consists of eight subunits (COG1-8) (Ungar *et al*., 2002; Whyte and Munro, 2001). A mammalian version of the COG complex was originally purified as the GTC complex, which is identical to the ldlC complex (Chatterton *et al*., 1999). Sec34 (Cog3) and sec35 (Cog2p) were the first characterized subunits of the complex and are essential proteins in the early secretory pathway in yeast. The COG complex is likely to be involved in retrograde transport in the Golgi complex and recycling from endosomes to the Golgi complex given the proteins that it interacts with and results from functional assays. The COG complex interacts directly with Ypt1p, the intra Golgi t-SNARE sed5, and v-SNAREs Gos1p, Ykt6p, and Sec22p (Suvorova *et al*., 2002). The COG complex also associates with the COPI coat via γ-COP, but not with COPII. A deletion mutant of Cog1p is defective in N- and O-linked Golgi glycosylation, while the Cog1p mutant at the permissive temperature has no effect in ER to Golgi transport. It has been proposed that the COG complex may be evolutionary and functionally related to the exocyst (Whyte and Munro, 2001). Indeed, both complexes consist of eight subunits with similar molecular weight, they share many biochemical and biophysical properties, and the N-termini of a number of subunits of the complexes share a common domain of predicted

coiled-coils or amphipatic helix. Nevertheless, their structures are dissimilar by deep freeze rotary shadowing EM, which neccesitates further work to define the relationships between the two complexes (Ungar *et al*., 2002).

The third known quatrefoil complex is the VFT (Vps Fifty Three) complex that has been identified in yeast (Conibear and Stevens, 2000). In contrast to the exocyst and COG complexes, VFT consists of four subunits Vps51, 52, 53, 54. It localizes to the TGN and is required for retrograde transport from endosomes to the TGN. The VFT complex binds directly to the GTP-bound form of Ypt6p on Golgi membranes and the SNARE Tlg1p on endosome-derived membranes (Siniossoglou and Pelham, 2001). It is likely that Ypt6p is important for the specific localization of the VFT complex on the Golgi. Interestingly, Vps52p has significant homology to the N-terminus of Sec3p (Whyte and Munro, 2001), the protein that provides the spatial landmark for docking the exocyst on the plasma membrane. Possibly, Vps52p may play a similar role for the VFT complex as Sec3p does for the exocyst.

The TRAPP complex and HOPS (or class C Vps) complex are two examples of nonquatrefoil tethering complexes. The TRAPP complex was identied as a large protein complex consisting of seven subunits that bound to Bet3p (Sacher *et al*., 1998). Bet3p is part of the complex and the other subunits are Trs85, Trs31, Trs33, Trs23, Trs20, and Bet5. Recently, it became clear that there is a second TRAPP complex containing three additional subunits, Trs120, Trs 130, and Trs65 (Sacher *et al*., 2001). The TRAPP complexes localize to cis Golgi membranes and the cytoplasm (Barrowman *et al*., 2000). The Golgi localization likely occcurs via Bet3p, because this subunit stably associates with an unknown receptor on the Golgi complex, even under conditions in which forward transport from the ER to the Golgi complex is inhibited. TRAPP I is required for ER to Golgi transport, and the complex binds to COP II vesicles generated from purified components in the absence of cytosol and Golgi membranes. In the absence of Bet3p COP II vesicles do not tether on the Golgi, showing that TRAPP I is required and sufficient to serve as a docking site for COP II vesicles. Uso1p, a typical extended coiled-coil tethering protein, is also required for binding of COP II vesicles to the Golgi complex *in vitro* (Cao *et al*., 1998). It is not clear why the cell would use two modes of tethering in the same pathway, but possibly the TRAPP complex and Uso1p cooperate. TRAPP I and TRAPP II act as guanine nucleotide exchange factors for the small GTPase Ypt1p. Recently, TRAPP complexes were also reported to serve as exchangers for the late Golgi proteins Ypt31 and Ypt32 (Jones *et al*., 2000), although this would be inconsistent with the localization of these complexes to the cis Golgi and a function in anterograde transport in the early biosynthetic pathway (Jedd *et al*., 1997).

The HOPS (Class C Vps) complex is involved in homotypic and heterotypic fusion events of the vacuole, and transport from the Golgi and endosomes to the vacuole (Price *et al*., 2000a; Price *et al*., 2000b; Sato *et al*., 2000). Class C vacuole proteins sorting (vps) mutants lack normal vacuoles. The core of the HOPS complex consists of four subunits Vps11p, Vps16p, Vps18p, Vps33p, while on the vacuole the complex recruits the additional subunits Vps39p (Vam6) and Vps41p (Vam2p) (see Wickner, 2002). Vps18p also interacts with Vac1p, a class D Vps mutant, which explains why mutations in Vps18 produce pleiotropic phenotypes that go beyond the accumulation of multivesicular bodies and show that the HOPS complex acts at multiple stages of the vacuolar transport pathway (Peterson and Emr, 2001). Although the precise protein-protein interactions between the subunits are not worked out, it is thought that Vps11p, Vps16p, and Vps 18p form a subcomplex, in which Vps16p recruits Vps33p to the vacuole (Rieder and Emr, 1997). Following Vps33p recruitment, the HOPS complex binds via Vps33p to the unpaired t-SNARE Vam3p, an association that is stabilized by Vps18p (Price *et al*., 2000a; Sato *et al*., 2000). Possibly, the HOPS complex may function in maintaining Vam3p in an inactive state by preventing formation of nonproductive cis SNARE complexes on the vacuole. This protective function of the HOPS complex is based on the structural considerations derived from the nsec-1/syntaxin-1A interaction (Yang *et al*., 2000). Since Vps39p is a guanine nucleotide exchanger for Ypt7p (Wurmser *et al*., 2000), it may activate Ypt7p on a transport vesicle (or other vacuole as in homotypic vacuole fusion) that might allow the complex to act as a tether (Price *et al*., 2000a; Price *et al*., 2000b), release Vamp3p from the HOPS complex, permitting it to assemble with VAM7p into a trans SNARE complex (Sato *et al*., 1998).

The human homolog of Vps39 (hVps39) has been cloned and contains a clathrin homology (CLH) do-

main that is not present in Vps39. The CLH domain has been suggested to act as a protein interaction domain involved in homo- and heterooligomerization. Indeed hVps39 is present as a homooligomer, which likely is of functional significance since homo-oligomerization is essential for its localization to late endosomes/lysosomes. The CLH domain has also been identified in Vps11, Vps18, and Vps41 subunits of the HOPS complex. It is not known whether hVps39 can act as a GEF for rab7. The overexpression of hVps39 induces massive clustering and fusion of late endosomes/lysosomes, which is reminiscent of phenotypes seen after the overexpression of a GTP hydrolysis-deficient rab7 mutant. Consistent with this, phenotypes seen after overexpression of both hVps39 and rab7 are not additive, while overexpressed hVps39 bypasses the fragmented lysosome phenotype caused by the overexpression of dominant negative rab7, suggesting that it may act downstream of rab7. hVps39, however, does not directly interact with rab7 nor with RILP and human hVps41. Possibly, hVps39 and hVps41 may enter the HOPS complex only after its association with the lysosomal membrane, as has been observed for the vacuole in yeast.

Although it is not known how tethering of two membranes may promote their fusion, it could be speculated that the act of bringing them in each others vicinity enhances the likelihood of SNARE pairing. Alternatively, or simultaneously, oligomeric tethering complexes may remove inhibitory proteins from t-SNAREs, allowing them to engage in productive pairing in trans SNARE complexes. As with the functions of rab effector proteins, it is clear that there is no common mechanism for rab-dependent membrane tethering. For some membrane fusion events, rab proteins appear to act upstream of the tethering molecules or complexes as in case of the long coiled-coil tethers or the exocyst. At other locations the oligomeric tethering complexes contain a guanine nucleotide exchange factor that acts upstream of the rab protein as in the HOPS and TRAPP complexes.

E. Domain Formation

Rab proteins also appear to contribute to the formation of subdomains in organelle membranes. Heterogeneity in biological membranes often arises from interactions between proteins and protein-lipid interactions, as originally found in model membranes *in vitro*, but for which there is now also firm evidence *in vivo*. Our understanding of the function of rab proteins in subdomain formation and maintenance derives almost entirely from studies on rab5 and its effectors by Zerial's laboratory. Through the engagement of rab5 with the rabaptin5–rabex-5 complex, it is activated to the GTP-bound form on early endosomes. The precise mechanism how the rabaptin5–rabex-5 GEF complex is recruited to early endosomes is not entirely clear since rabex-5 can bind independently of rab5 to early endosomes, and rabaptin5 only interacts with rab5 in its GTP-bound form (Lippe *et al*., 2001). Nevertheless, the presence of rabaptin 5 within the complex is required for significant GEF activity toward rab5 (Lippe *et al*., 2001). Active GTP-bound rab5 can then recruit the wortmannin-sensitive PI3-kinase, hVPS34-p150 to early endosomes, allowing for the localized generation and deposition of its product PI(3)P (Christoforidis *et al*., 1999) in endosomal membranes. Although the interaction with the regulatory subunit p150 is direct, it remains to be established whether rab5 is required and sufficient to target hVPS34-p150 to early endosomes. Since PI(3)K p110β/p85α, a type 1 PI(3) kinase that phosphorylates PI(4)P and PI(4,5)P_2 is also a rab5 effector, which is enriched in clathrin-coated vesicles and not present on early endosomes (Christoforidis *et al*., 1999), it is likely that in addition to rab5 other proteins are required to target hVPS34-p150 to early endosomes.

PI(3)P produced by hVPS34-p150 may locally recruit PX domain containing proteins, or in combination with rab5 the FYVE finger proteins EEA1 and rabenosyn-5 (Nielsen *et al*., 2000). EEA1 binds directly to the t-SNAREs syntaxin6 and syntaxin13 and serves as a tethering factor in heterotypic fusion between incoming clathrin-coated vesicles and early endosomes (Rubino *et al*., 2000) and in homotypic early endosome fusion (Simonsen *et al*., 1998). Rabenosyn-5 is complexed to the nsec-1 homolog hVps45 and has a more complex function since it also regulates membrane recycling as well as transport of newly synthesized lysosomal enzymes (de Renzis *et al*., 2002; Nielsen *et al*., 2000). After rab5 is activated, it thus appears to recruit a number of effector proteins in a carefully orchestrated and interdependent fashion to early endosomes. Some of these occur together in a dynamic heterooligomeric complex that drives early endosome fusion (McBride *et al*., 1999). Other rab5-regulated events like minus end-directed endosome movement along microtubules also depend

on PI(3)P (Nielsen *et al*., 1999), suggesting that linker or motor proteins might be recruited in a PI(3)P-dependent manner. Although this has not been demonstrated yet for PI(3)P, PI(4,5)P2 was recently shown to form a docking site for Unc104. This PH domain containing kinesin was sufficient to move synthetic vesicles containing PI(4,5)P2 along microtubules *in vitro* (Klopfenstein *et al*., 2002). Thus, rab5 functions as a platform to which many key proteins are recruited and that may serve as a subdomain organizer of early endosomes. Live cell imaging experiments using GFP variants of rab5, rab4, and rab11 are in agreement with the notion that rab proteins may define endosomal subdomains (Sönnichsen *et al*., 2000). Although the three rab proteins colocalize to a significant extent in the endosomal system, they do not mix in the plane of the membrane, but occupy distinct areas that are differentially affected by drugs like BFA, nocodazole, and wortmannin. Rab5 and rab4 occur mostly in early endosomes, while rab4 and rab11 domains are found in recycling endosomes. The domains defined by rab5, rab4, rab11 are in dynamic equilibrium as endocytosed transferrin is passing sequentially through rab5, rab4, and rab11 domains (Sönnichsen *et al*., 2000). The communication between the rab5 and rab4 domains is likely organized through bivalent effector proteins such as the rabaptins and rabenosyn-5 that can bind simultaneously to rab5 and rab4 (de Renzis *et al*., 2002). The rab4 and rab11 domains might be connected through proteins like RCP that bind to rab4 and rab11, although rab11 binding occurs independent of its guanine nucleotide status (Lindsay *et al*., 2002).

RIN1 is a recently identified GEF for rab5. It contains the Vps9p catalytic domain of rabex-5, which is claimed to be present in a wide variety of multidomain proteins (Tall *et al*., 2001). RIN1 exchange activity is enhanced through signaling via the epidermal growth factor receptor/ras pathway, and enhances receptor-mediated endocytosis of epidermal growth factor. RIN1 does not appear to be involved in endocytosis of transferrin receptor. An important question is whether RIN1 selectively acts as a GEF for cell surface-localized rab5 molecules or whether it activates rab5 on early endosomes like the rabaptin5–rabex-5 complex, or both. It will also be interesting to know which proteins are associated with activated rab5 on the cell surface, and if they form subdomains as well.

V. INTEGRATION AND CROSSTALK OF SMALL GTPASE PATHWAYS

After the initial discovery of rab proteins, research has been focussed on their functional characterization with respect to the regulation of a particular transport step. This also provided convenient tools for others to elucidate transport pathways of certain ligands, especially in mammalian systems. The advent of new technologies in the early 1990s, like the two hybrid system and protein mass spectrometry greatly facilitated progress in the field and permitted the identification of many novel proteins interacting with, or required for, rab protein function. This set the stage for issues concerning the coordination of the activities of individual rab proteins acting on the same compartment, and how rab proteins talk to other signaling pathways. Rab5 activity in the endocytic pathways is at least regulated at two levels by receptor tyrosine kinase signaling. Epidermal growth factor receptor interacts via eps8 to RN-tre, a rab5-GAP (Lanzetti *et al*., 2000). RN-tre activity is downregulated after receptor occupation, causing an increase in the level of rab5GTP (Lanzetti *et al*., 2000; Matoskova *et al*., 1996; Scita *et al*., 1999). The overexpression of RN-tre inhibits constitutive endocytosis of transferrin receptor endocytosis, but not the regulated internalization of epidermal growth factor receptor. In eps 8 –/– cells, overexpressed RN-tre still inhibited transferrin endocytosis, while uptake of epidermal growth factor receptor was not affected. These results clearly link epidermal growth factor signaling to ligand-dependent, but not constitutive endocytosis. Epidermal growth factor signaling also activates ras via the Grb2–Sos pathway, while active ras is long known to increase fluid phase endocytosis (Bar-Sagi and Feramisco, 1986). Possibly this effect is due to ras effector RIN1, which is an exchange factor for rab5 and that is activated by ras signaling (Tall *et al*., 2001). RIN1 enhances endosome fusion *in vitro* and internalization of epidermal growth factor and horseradish peroxidase *in vivo*. Whether RIN1 provides a negative feedback to ras, or stimulates signaling from endosomes is an issue that remains to be demonstrated.

Another exciting example of crosstalk between GTPase systems and rab proteins concerns the exocyst sec4 effector complex. Different labs reported that active ral interacts with the sec5 subunit (Brymora *et al*., 2001; Moskalenko *et al*., 2002; Sugihara *et al*., 2002). Ral signaling function has been implicated before in membrane transport since ral regulates

epidermal growth factor endocytosis via eps15 and the EH domain protein POB1 (Nakashima *et al*., 1999; Yamaguchi *et al*., 1997). In recent studies, the overexpression of constitutively active ral randomized polarized delivery of proteins to the basolateral surface in MDCK cells, while apical targeting remained unaffected (Moskalenko *et al*., 2002). Knockdown experiments with siRNAs showed that ral is essential for exocyst assembly in MDCK cells (Moskalenko *et al*., 2002). Others found that a dominant negative ral mutant reduced the ready releasable pool of synaptic vesicles in PC12 cells, suggesting a role for ral in the exocyst-dependent tethering, or maturation of synaptic vesicles (Polzin *et al*., 2002). The exocyst is also regulated by rho GTPases. Rho1p in the GTP-bound form binds directly to N-terminus of sec3p in yeast (Guo *et al*., 2001). Sec3p is required for its localization to the cell surface and spatial positioning of the secretion apparatus to the bud in yeast. Sec3p truncation mutants lacking the rho1p binding site are mislocalized in the cytoplasm. Another yeast rho protein, rho3p, binds directly to the exocyst subunit exo70p (Adamo *et al*., 1999; Robinson *et al*., 1999). A rho3p mutant that fails to bind exo70p exhibits constitutive exocytic defects, while secretory vesicles accumulate in the bud at 25°C, suggesting an effect at the docking stage and not in actin-dependent secretory transport that is also regulated by rho3p.

Cross-talk between between two rab proteins has been described in the secretory pathway in yeast. Ypt31p/ypt32p (83% identical) are nonessential rab proteins implicated in intra-Golgi transport and in budding secretory vesicles from the TGN. As such they act upstream of sec4p, which is required for polarized transport of exocytic vesicles from the Golgi to the bud. Novick et al. identified *YPT31*/*YPT32* in a search for high copy suppressors of *sec2-78*, a point mutant of the exchange factor sec2p that fails to be localized properly to membranes. In binding studies ypt32p was found to interact directly with sec2p and that ypt32p and sec4p bind independently to different regions on sec2p. The overexpression of ypt32p partially restored the polarized localization of sec4 and sec2-78p, implying that ypt32p might serve as a membrane attachment site for sec2p. These observations suggest that the activities of ypt32p and sec4p may be linked in a regulatory cascade via sec2p. When sec2p is recruited to secretory vesicles by ypt32p it may in turn activate its own rab protein sec4p and recruit it to secretory vesicles, regulating the next stage of transport to the bud.

VI. CONCLUSIONS AND PERSPECTIVES

A role for rab proteins in membrane transport has been firmly established in a large number of studies, and it is clear that rabs are engaged in multiple aspects of membrane biology, including regulation of vesicle formation, motility along cytosketal systems, and membrane fusion. A single rab protein generally has more than one effector that directly interacts with it, and that in turn may bind to a variety of other proteins. Although some rab effector proteins are related to each other, many of them have no sequence homology, which may contribute to the wide array of biological events that is controlled by the effector network of a given rab protein. Progress to the next level of understanding requires the elucidation of the signaling pathways that activate rabs. Other important questions include how rab proteins talk to other small GTPases regulating membrane transport such as ARF and rho proteins and how this relates to cytoskeleton dynamics. Several genetic (Stearns *et al*., 1990; Walch-Solimena and Novick, 1999; Zhang *et al*., 2002) and biochemical studies (Eitzen *et al*., 2001; Imamura *et al*., 1998; Müller *et al*., 2001) indicate that such links exist; however, the mechanistic principles remain to be worked out. Genomic and proteomic experiments have already provided some clues and will be of value in the future to chart the protein networks that rab proteins participate in (Gavin, 2002; Seeley *et al*., 2002; Uetz *et al*., 2000). It will also be important to determine how rab proteins contribute to the features of an organelle they localize to, and how this affects the biophysical properties of organelle membranes. Some progress in this area has been made for rab5-defined microdomains in the endosomal system, but more research is clearly needed to establish whether related principles also apply to rabs that are located elsewhere in the cell. Ultimately, this reductionistic approach will lead to successful reconstitution of the particular events that are regulated by rab proteins on a given organelle and an understanding how they collectively determine membrane dynamics of that organelle in the cell.

ACKNOWLEDGMENTS

We thank Rene Scriwanek and Marc van Peski for artwork. This work was supported by the ALW branch of the Netherlands Foundation for Research.

REFERENCES

Adamo, J.E., Rossi, G., and Brennwald, P. (1999) The rho GTPase rho3 has a direct role in exocytosis that is distinct from its role in actin polarity. *Mol. Biol. Cell*, **10**, 4121–4133.

Albert, S., Will, E., and Gallwitz, D. (1999) Identification of the catalytic domains and their functionally critical arginine residues of two yeast GTPase activating proteins specific for ypt/rab transport GTPases. *EMBO J.*, **18**, 5216–5225.

Allan, B.B., Moyer, B.D., and Balch, W.E. (2000) Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. *Science*, **289**, 444–448.

Alory, C. and Balch, W.E. (2001) Organization of the Rab-GDI/CHM superfamily: the functional basis for choroideremia disease. *Traffic*, **2**, 532–543.

Alto, N.M., Soderling, J., and Scott, J.D. (2002) Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics. *J. Cell Biol.*, **158**, 659–668.

Amraoui, A.E., Schonn, J.S., Andermann, P.K., Blanchard, S., Desnos, C., Henry, J.P., Wulfrum, U., Darchen, F., and Petit, C. (2002) MyRIP, a novel rab effector, enables myosin VIIa recruitment to retinal melanosomes. *EMBO Rep.*, **3**, 463–470.

Bahadoran, E.K., Aberdam, E., Mantoux, F., Busca, R., Bille, K., Yalman, N., de Saint-Basili, G., Casaroli-Marano, R., Ortonne, J.P., and Ballotti, R. (2001) Rab27a: a key to melanosome transport in human melanocytes. *J. Cell Biol.*, **102**, 843–850.

Bar-Sagi, D. and Feramisco, J.R. (1986) Induction of membrane ruffling and fluid-phase endocytosis in quiescent fibroblasts by ras proteins. *Science*, **233**, 1061–1068.

Barr, F.A., Puype, M., VandeKerckhove, J., and Warren, G. (1997) GRASP65, a protein involved in the stacking of Golgi cisternae. *Cell*, **91**, 253–262.

Barrowman, J., Sacher, M., and Ferro-Novick, S. (2000) TRAPP stably associates with the Golgi and is required for vesicle docking. *EMBO J.*, **19**, 862–869.

Bielli, A., Thörnqvist, P.O., Hendrick, A.G., Finn, R., Fitzgerald, K., and McCaffrey, M.W. (2001) The small GTPase rab4a interacts with the central region of cytoplasmic dynein light intermediate chain. *Biochem. Biophys. Res. Comm.*, **281**, 1141–1153.

Bleazard, W., McCaffery, J., King, E., Bale, S., Mozdy, A., Tieu, Q., Nunnari, J., and Shaw, J. (1999) The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast. *Nat. Cell. Biol.*, **1**, 298–304.

Brennwald, P. and Novick, P. (1993) Interaction of three domains that distinguishing the Ras related GTP binding proteins Ypt1 from SEC4. *Nature*, **362**, 557–560.

Broadie, K., Prokop, A., Bellen, H.J., O'Kane, C.J., Schulze, K.L. and Sweeney, S.T. (1995) Syntaxin and synaptobrevin function downstream of vesicle docking in *Drosophila*. *Neuron*, **15**, 663–673.

Brymora, A., Valova, V.A., Larsen, M.R., Roufogalis, B.D., and Robinson, P.J. (2001) The brain exocyst complex interacts with RalA in a GTP-dependent manner: identification of a novel mammalian sec3 gene and a second sec15 gene. *J. Biol. Chem.*, **276**, 29792–29797.

Bucci, C., Thomsen, P., Nicoziani, P., McCarthy, J., and van Deurs, B. (2000) Rab7: a key to lysosome biogenesis. *Mol. Biol. Cell*, **11**, 467–480.

Bucci, C., Wandinger-Ness, A., Lütcke, A., Chiarello, 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. Natl. Acad. Sci. USA*, **91**, 5061–5065.

Burd, C.G., Mustol, P.A., Schu, P.V., and Emr, S.D. (1996) A yeast protein related to a mammalian ras-binding protein, vps9p, is required for localization of vacuolar proteins. *Mol. Cell Biol.*, **16**, 2369–2377.

Cantalupo, G., Alifano, P., Roberti, V., Bruni, C.B., and Bucci, C. (2001) Rab-interacting lysosomal protein (RILP): the rab7 effector required for transport to lysosomes. *EMBO J.*, **20**, 683–693.

Cao, X., Ballew, N., and Barlowe, C. (1998) Initial docking of ER-derived vesicles requires Uso 1p and Ypt1 but is independent of SNARE proteins. *EMBO J.*, **17**, 2156–2165.

Cao, X. and Barlowe, C. (2000) Asymmetric requirements for a rabGTPase and SNARE proteins in fusion of COPII vesicles with acceptor membranes. *J. Cell Biol.*, **149**, 55–65.

Caplan, S., Hartnell, L.M., Aguilar, R.C., Naslavsky, N., and Bonifacino, J.S. (2001) Human Vam6p promotes lysosome clustering and fusion in vivo. *J. Cell Biol.*, **154**, 109–122.

Carroll, K.S., Hanna, J., Simon, I., Krise, J., Barbero, P., and Pfeffer, S.R. (2001) Role of rab9 GTPase in facilitating receptor recruitment by TIP47. *Science*, **292**, 1373–1376.

Casey, P.J. and Seabra, M.C. (1996) Protein prenyltransferases. *J. Biol. Chem.*, **271**, 5289–5292.

Chatterton, J.E., Hirsch, D., Schwartz, J.J., Bickel, P.E., Rosenberg, R.D., Lodish, H.F., and Krieger, M. (1999) Expression cloning of LDLB, a gene essential for normal Golgi function and assembly

of the ldlC complex. *Proc. Natl. Acad. Sci. USA*, **96**, 915–920.

Chavrier, P., Parton, R.G., Hauri, H.P., Simons, K., and Zerial, M. (1990) Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. *Cell*, **62**, 317–329.

Chen, Y.A. and Scheller, R.H. (2001) SNARE-mediated membrane fusion. *Nature Rev. Mol. Cell Biol.*, **2**, 98–106.

Christoforidis, S., Miaczynska, M., Ashman, K., Wilm, M., Zhao, L., Yip, S.C., Waterfield, M.D., Backer, J.M., and Zerial, M. (1999) Phosphatidylinositol-3–OH kinases are rab5 effectors. *Nature Cell Biol.*, **1**, 249–252.

Conibear, E. and Stevens, T.H. (2000) Vps52p, vps53p, and vps54p form a novel multisubunit complex required for protein sorting at the yeast late Golgi. *Mol. Biol. Cell*, **11**, 305–323.

Cuif, M.H., Possmayer, F., Zander, H., Bordes, N., Jollivet, F., Courteille, A.C., Lerosey, I.J., Langsley, G., Bornens, M., and Goud, B. (1999) Characterization of GAPC en A, a GTPase activating protein for rab6, part of which associates with the centrosome. *EMBO J.*, **18**, 1772–1782.

de Renzis, S., Sönnichsen, B., and Zerial, M. (2002) Divalent rab effectors regulate the sub-compartmental organization and sorting function of early endosomes. *Nature Cell Biol.*, **4**, 124–133.

Diao, A., Rahman, D., Pappin, D.J.C., Lucocq, J., and Lowe, M. (2003) The coiled-coil membrane protein Golgin-84 is a novel rab effector required for Golgi ribbon formation. *J. Cell Biol.*, **160**, 201–212.

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.

Du, J.L. and Novick, P.J. (2001) Yeast rab GTPase-activating protein Gyp1p localizes to the Golgi apparatus and is a negative regulator of Ypt1p. *Mol. Biol. Cell*, **12**, 1215–1226.

Dunn, B., Stearns, T., and Botstein, D. (1992) Specificity domains distinguishing the ras-related GTPases YPT1 and SEC4. *Nature*, **362**, 560–563.

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.

Echard, A., Opdam, F.J.M., de Leeuw, H.J.P.C., Jollivet, F., Savelkoul, P., Hendriks, W., Voorberg, J., Goud, B., and Fransen, J.A.M. (2000) Alternative splicing of the human rab6A gene generates two close but functionally different isoforms. *Mol. Biol. Cell*, **11**, 3819–3832.

Eitzen, G., Thorngren, N., and Wickner, W. (2001) Rho1p and cdc42p act after Ypt7p to regulate vacuole docking. *EMBO J.*, **20**, 5650–5656.

Faber, P.W., Barnes, G.T., Srinidhi, J., Chen, J., Gusella, J.F., and MacDonald, M.F. (1998) Huntington interacts with a family of WW domain proteins. *Hum. Mol. Genet.*, **7**, 1463–1474.

Feng, Y., Press, B., and Wandinger Ness, A. (1995) Rab7: 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.

Fontijn, R.D., Goud, B., Echard, A., Jollivet, F., van Marle, J., Pannekoek, H., and Horrevoets, A.J.G. (2001) The human kinesin-like protein RB6K is under tight cell cycle control and is essential for cytokinesis. *Mol. Cell Biol.*, **21**, 2944–2955.

Fukuda, M., Kuroda, T.S., and Mikoshiba, K. (2002) Slac2–a/Melanophilin, the missing link between rab27 and Myosin Va: implications of a tripartite protein complex for melanosome transport. *J. Biol. Chem.*, **277**, 12432–12436.

Furge, K.A., Wong, K., Armstrong, J., Balasubramanian, M., and Albright, C.F. (1998) Byr4 and cdc16 form a two component GTPase activating protein for the Spg1 GPTase that controls septation in fission yeast. *Curr. Biol.*, **8**, 947–954.

Garcia, E.P., McPherson, P., Chilcote, T.J., Takei, K., and De Camilli, P. (1995) Rbsec1A and B colocalize with syntaxin 1 and SNAP25 throughout the axon, but are not in a stable complex with syntaxin. *J. Cell Biol.*, **129**, 105–120.

Garrett, M.D., Zahner, J.E., Cheney, C.M., and Novick, P.J. (1994) GDI1 encodes a GDP dissociation inhibitor that plays an essential role in the yeast secretory pathway. *EMBO J.*, **13**, 1718–1728.

Gavin, A.C. (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature*, **415**, 141–147.

Girod, A., Storrie, B., Simpson, J.C., Johannes, L., Goud, B., Roberts, L.M., Lord, J., Nilsson, T., and Pepperkok, R. (1999) Evidence for a COP-I-independent transport route from the Golgi complex to the endoplasmic reticulum. *Nature Cell Biol.*, **1**, 423–430.

Govindan, B., Bowser, R., and Novick, P. (1995) The role of Myo 2, a yeast class V myosin, in vesicular transport. *J. Cell Biol.*, **128**, 1055–1068.

Grindstaff, K.K., Yeaman, C., Anandasabapathy, N., Hsu, S.C., Rodriguez-Boulan, E., Scheller, R.H., and Nelson, W.J. (1998) Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell*, **93**, 731–740.

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**, 1071–1081.

Guo, W., Tamanoi, F., and Novick, P. (2001) Spatial regulation of the exocyst complex by RhoI GTPase. *Nature Cell Biol.*, **3**, 353–360.

Hales, C.M., Griner, R., Hobdy-Henderson, C., Dorn, M.C., Hardy, D., Kumar, R., Navarre, J., Chan, E.K.L., Lapierre, L.A., and Goldenring, J.R. (2001) Identification and characterization of a family of rab11–interacting proteins. *J. Biol. Chem.*, **276**, 39067–39075.

Hales, C.M., Vaerman, J.P., and Goldenring, J.R. (2002) Rab11 family interacting protein 2 associates with myosin Vb and regulates plasma membrane recycling. *J. Biol. Chem.*, **277**, 50415–50421.

Hattula, K., Furuhjem, J., Arffman, A., and Peränen, J. (2002) A rab8–specific GDP/GTP exchange factor is involved in actin remodeling and polarized membrane transport. *Mol. Biol. Cell*, **13**, 3268–3280.

Hattula, K. and Peränen, J. (2000) FIP-2, a coiled coil protein, links Huntington to rab8 and modulates cellular morphogenesis. *Curr. Biol.*, **10**, 1603–1606.

Hill, E., Clarke, M., and Barr, F. (2000) The rab6–binding kinesin, rab6–KIFL, is required for cytokinesis. *EMBO J.*, **119**, 5711–5719.

Horiuchi, H., Lippe, R., McBride, H.M., Rubino, M., Woodman, P., Stenmark, H., Rybin, V., Wilm, M., Ashman, K., Mann, M., and Zerial, M. (1997) A novel rab5 GDP/GTP exchange factor complexed to rabaptin-5 links nucleotide exchange to effector recruitment and function. *Cell*, **90**, 1149–1159.

Huber, L.A., deHoop, M.J., Dupree, P., Zerial, M., Simons, K., and Dotti, C. (1993a) Protein transport to the dendritic plasma membrane of cultured neurons is regulated by rab8p. *J. Cell Biol.*, **123**, 47–56.

Huber, L.A., Pimplikar, S., Parton, R.G., Virta, H., Zerial, M., and Simons, K. (1993b) Rab8, a small GTPase involved in vesicular traffic between the TGN and the basolateral plasma membrane. *J. Cell Biol.*, **123**, 35–46.

Hume, A.N., Collinson, L.M., Rapak, A., Gomes, A.Q., Hopkins, C.R. and Seabra, M.C. (2001) Rab27a regulates the peripheral distribution of melanosomes in melanocytes. *J. Cell Biol*, **152**, 795–808.

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.

Isenmann, S., Khew-Goodall, Y., Gamble, J., Vadas, M., and Wattenberg, B.W. (1998) A splice-isoform of vesicle-associated protein-1 (VAMP-1) contains a mitochondrial targeting sequence. *Mol. Biol. Cell*, **9**, 1649–1660.

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., Newman, C., Liu, F., and Segev, N. (2000) The TRAPP complex is a nucleotide exchanger for ypt1 and ypt31/32. *Mol. Biol. Cell*, **11**, 4403–4411.

Jordens, I., Fernandez-Borja, M., Marsman, M., Dusseljee, S., Janssen, L., Calafat, J., Janssen, H., Wubbolts, R., and Neefjes, J. (2001) The rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors. *Curr. Biol.*, **11**, 1680–1685.

Kauppi, M., Simonsen, A., Bremnes, B., Vieira, A., Callaghan, J., Stenmark, H., and Olkkonen, V.M. (2002) The small GTPase rab22 interacts with EEA1 and controls endosomal trafficking. *J. Cell Sci.*, **115**, 899–911.

Kee, Y., Yoo, J.S., Hazuka, C., Peterson, K.E., Hsu, S.C., and Scheller, R.H. (1997) Subunit structure of the mammalian exocyst complex. *Proc. Natl. Acad. Sci. USA*, **94**, 14438–14443.

Klopfenstein, D., Tomishige, M., Stuurman, N., and Vale, R.D. (2002) Role of phosphatidylinositol(4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor. *Cell*, **109**, 347–358.

Kuroda, T.S., Fukuda, M., Ariga, H., and Mikoshiba, K. (2002) The Slp homology domain of synaptotagmin-like proteins1–4 and slac2 functions as a novel rab27a binding domain. *J. Biol. Chem.*, **277**, 9212–9218.

Lanzetti, L., Rybin, V., Malabarba, M.G., Christoforidis, S., Scita, G., Zerial, M., and Di Fiore, P.P. (2000) The Eps8 protein coordinates EGF receptor signaling through rac and trafficking through rab5. *Nature*, **408**, 374–377.

Lapierre, L.A., Kumar, R., Hales, C.M., Navarre, J., Bhartur, S.G., Burnette, J.O., Provance Jr, D.W., Mercer, J.A., Bähler, M., and Goldenring, J.R. (2001) Myosin Vb is associated with plasma membrane recycling systems. *Mol. Biol. Cell*, **12**, 1843–1857.

Lebrand, C., Corti, M., Goodson, H., Cosson, P., Cavalli, V., Mayran, N., Faure, J., and Gruenberg, J. (2002)

Late endosome motility depends on lipids via the small GTPase rab7. *EMBO J.*, **21**, 1289–1300.

Lindsay, A.J., Hendrick, A.G., Cantalupo, G., Matuglia, F.S., Goud, B., Bucci, C., and McCaffrey, M.W. (2002) Rab coupling protein (RCP), a novel rab4 and rab11 effector protein. *J. Biol. Chem.*, **277**, 12190–12199.

Linstedt, A.D., Jesch, S.A., Mehta, A., Lee, T., Mata, R.G., Nelson, D.S., and Sztul, E. (2000) Binding relationships of membrane tethering components: the giantin N-terminus and the GM130 N-terminus compete for binding to the p115 C-terminus. *J. Biol. Chem.*, **275**, 10196–10201.

Lippe, R., Miaczynska, M., Rybin, V., Runge, A., and Zerial, M. (2001) Functional synergy between rab5 effector rabaptin-5 and exchange factor rabex-5 when physically associated in a complex. *Mol. Biol. Cell*, **12**, 2219–2228.

Liu, X.L., Ondek, B., and Williams, D.S. (1998) Mutant myosin VII causes defective melanosome distribution in the retinal pigment epithelium of shaker-1 mice. *Nature Genet.*, **19**, 117–118.

Lombardi, D., Soldati, T., Riederer, M., Zerial, M., and Pfeffer, S. (1993) Rab 9 functions in transport between late endosomes and the trans Golgi network. *EMBO J.*, **12**, 677–682.

Luo, H.R., Saiardi, A., Nagata, E., Ye, K., Yu, H., Jung, T.S., Luo, X., Jain, S., Sawa, A., and Snyder, S.H. (2001) GRAB: A physiologic guanine nucleotide exchange factor for rab3A, which interacts with inositol hexakisphosphate kinase. *Neuron*, **31**, 439–451.

Mallard, F., Tang, B.L., Galli, T., Tenza, D., A., S.-P., Yue, X., Antony, C., Hong, W., Goud, B., and Johannes, L. (2002) Early/recycling endosomes-to-TGN transport involves two SNARE complexes and a rab6 isoform. *J. Cell Biol.*, **156**, 653–664.

Manser, E., Loo, T.H., Koh, C.G., Zhao, Z.S., Chen, X.Q., Tan, L., Tan, I., Leung, T., and Lim, L. (1998) PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. *Mol. Cell*, **1**, 183–192.

Martinez, O., Anthony, C., Arnaudet, G.P., Berger, E.G., Salamero, J., and Goud, B. (1997) GTP bound forms of rab6 induce the redistribution of Golgi proteins to the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA*, **94**, 1828–1833.

Martinez, O., Schmidt, A., Salamero, J., Hoflack, B., Roa, M., and Goud, B. (1994) The small GTP binding protein rab6 functions in intra Golgi transport. *J. Cell Biol.*, **127**, 1575–1588.

Matanis, T., Akhamanova, A., Wulf, P., Del Nery, E., Weide, T., Stepanova, T., Galjart, N., Grosveld, F., Goud, B., de Zeeuw, C.I., Barnekow, A., and Hoogenraad, C.C. (2002) Bicaudal-D regulates COPI-independent Golgi-ER transport by recruiting the dynein-dynactin motor complex. *Nature Cell Biol.*, **4**, 986–992.

Matesic, L.E., Yip, R., Reuss, A.E., Swing, D.A., O'Sullivan, T.N., Fletcher, C.F., Copeland, N.G., and Jenkins, N.A. (2001) Mutations in Mlph, encoding a member of the rab effector family, cause the melanosome transport defects observed in leaden mice. *Proc. Natl. Acad. Sci. USA*, **98**, 10238–10243.

Matoskova, B., Wong, W.T., Nomura, N., Robbins, K.C., and di Fiore, P. (1996) RN-tre specifically binds to the SH3 domain of eps8 with high affinity and confers growth advantage to NIH3T3 upon carboxyterminal truncation. *Oncogene*, **12**, 2679–2688.

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.

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

Menasche, H., Pastural, E., Feldmann, J., Certain, S., Ersoy, F., Dupuis, S., Wulffraat, N., Bianchi, D., Fischer, A., Le Deist, F., and de Saint Basile, G. (2000) Mutations in rab27a cause Griscelli's syndrome associated with haemophagocytic syndrome. *Nature Genet.*, **25**, 173–176.

Mercer, J.A., Seperack, P.K., Strobel, M.C., Copeland, N.G., and Jenkins, N.A. (1991) Novel myosin heavy chain encoded by murine dilute coat color locus. *Nature*, **349**, 709–713.

Mollard, G.F., Mignery, G.A., Baumert, M., Perin, M.S., Hanson, T.J., Burger, P.M., Jahn, R., and Südhof, T.C. (1990) rab3 is a small GTP binding protein exclusively localized to synaptic vesicles. *Proc. Natl. Acad. Sci. USA*, **87**, 1988–1992.

Moore, K.J., Swing, D.A., Rinchik, E.M., Mucenski, M.L., Buchberg, A., Copeland, N.G., and Jenkins, N.A. (1988) The murine dilute suppressor gene dsu suppresses the coat color phenotype of three pigment mutations that alter melanocyte morphology. *Genetics*, **119**, 933–941.

Moskalenko, S., Henry, D.O., Rosse, C., Mirey, G., Camonis, J.H., and White, M.A. (2002) The exocyst is a ral effector complex. *Nature Cell Biol.*, **4**, 66–72.

Moyer, B.D., Allan, B.B., and Balch, W.E. (2001) Rab1 interaction with GM130 effector complex regulates COPII vesicle cis-Golgi tethering. *Traffic*, **2**, 268–276.

Müller, O., Johnson, D.I., and Mayer, A. (2001) Cdc42p functions at the docking stage of yeast acuole membrane fusion. *EMBO J.*, **20**, 5647–5665.

Nagelkerken, B., van Anken, E., van Raak, M., Gerez, L., Mohrmann, K., van Uden, N., Holthuizen, J., Pelkmans, L., and van der Sluijs, P. (2000) Rabaptin4, a novel effector of rab4a, is recruited to perinuclear recycling vesicles. *Biochem. J.*, **346**, 593–601.

Nakashima, S., Morinaka, K., Koyama, S., Ikeda, M., Kishida, M., Okawa, K., Iwamatsu, A., Kishida, S., and Kikuchi, A. (1999) Small G protein ral and its downstream molecules regulate endocytosis of EGF and insulin receptors. *EMBO J.*, **18**, 3629–3642.

Nelson, D.S., Alvarez, C., Gao, Y., Mata, R.G., Fialkowski, E., and Sztul, E. (1998) The membrane transport factor TAP/p115 cycles between the Golgi and earlier secretory compartments and contains distinct domains required for its localization and function. *J. Cell Biol.*, **143**, 319–331.

Nemoto, Y. and De Camilli, P. (1999) Recruitment of an alternatively spliced form of synaptojanin 2 to mitochondria by the interaction with the PDZ domain of a mitochondrial outer membrane protein. *EMBO J.*, **18**, 2991–3006.

Neuwald, A.F. (1997) A shared domain between a spindle assembly checkpoint protein and Ypt/rab-specific GTPase activators. *Trends Biochem. Sci.*, **22**, 243–244.

Nielsen, E., Christoforidis, S., Utenwiler-Joseph, S., Miaczynska, M., Dewitte, F., Wilm, M., Hoflack, B., and Zerial, M. (2000) Rabenosyn-5, a novel rab5 effector, is complexed with hVPS45 and recruited to endosomes through a FYVE finger domain. *J. Cell Biol.*, **151**, 601–612.

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

Nislow, C., Lombillo, V., Kuriyama, R., and McIntosh, J.R. (1992) A plus end directed motor enzyme that moves antiparallel microtubules *in vitro* localizes to the interzone of mitotic spindles. *Nature*, **359**, 543–547.

Ostermeier, C. and Brunger, A.T. (1999) Structural basis of rab effector specificity: crystal structure of the small G protein rab3A complexed with the effector domain of rabphilin-3A. *Cell*, **96**, 363–374.

Peranen, 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**, 153–168.

Pereira-Leal, J.B. and Seabra, M.C. (2000) The mammalian rab family of small GTPases: definition of family and subfamily sequence motifs suggests a mechanism for fundamental specificity in the ras superfamily. *J. Mol. Biol.*, **301**, 1077–1087.

Peterson, M.R. and Emr, S.D. (2001) The class C vps complex functions at multiple stages of the vacuolar transport pathway. *Traffic*, **2**, 476–486.

Pfeffer, S.R. (1999) Transport vesicle targeting: tethers before SNAREs. *Nature Cell Biol.*, **1**, E17–E22.

Pfeffer, S.R. (2001) Constructing a Golgi complex. *J. Cell Biol.*, **155**, 873–875.

Polzin, A., Shipitsin, M., Goi, T., Feig, L.A., and Turner, T.J. (2002) Ral-GTPase influences the regulation of the readily releasable pool of synaptic vesicles. *Mol. Cell Biol.*, **22**, 1714–1722.

Prekeris, R., Davies, J.M., and Scheller, R.H. (2001) Identification of a novel rab11/25 binding domain present in eferin and rip proteins. *J. Biol. Chem.*, **276**, 38966–38970.

Press, B., Feng, Y., Hoflack, B., and Wandinger-Ness, A. (1998) Mutant rab7 causes the accumulation of cathepsin D and cation independent mannose 6–phosphate receptor in an early endocytic compartment. *J. Cell Biol.*, **140**, 1075–1089.

Price, A., Seals, D., Wickner, W., and Ungermann, C. (2000a) The docking stage of yeast vacuole fusion requires the transfer of proteins from a cis-SNARE complex to a rab/ypt protein. *J. Cell Biol.*, **148**, 1231–1238.

Price, A., Wickner, W., and Ungermann, C. (2000b) Vacuole protein sorting (Vps) proteins needed for transport vesicle budding from the Golgi are also required for the docking step of homotypic vacuole fusion. *J. Cell Biol.*, **148**, 1223–1230.

Rak, A., Fedrov, A., Alexandrov, K., Albert, S., Goody, R.S., and Gallwitz, D. (2000) Crystal structure of the GAP domain of Gyp1p: first insights into the interaction with Ypt/rab proteins. *EMBO J.*, **19**, 5105–5113.

Rieder, S.E. and Emr, S.D. (1997) A novel RING finger protein complex essential for a late step in protein transport to the yeast vacuole. *Mol. Biol. Cell*, **8**, 2307–2327.

Rittinger, K., Walker, P.A., Eccleston, J.F., Nurmahomed, K., Owen, D., Laue, E., Gamblin, S.J., and Smerdon, S.J. (1997) Crystal structure of a small G protein in complex with the GTPase-activating proein rhoGAP. *Nature*, **388**, 693–697.

Robinson, N.G., Guo, L., Imai, J., Toh-e, A., Matsui, Y., and Tamanoi, F. (1999) Rho3 of *Saccharomyces cerevisiae*, which regulates the actin cytoskeleton and exocytosis, is a GTPase which interacts with Myo2 and Exo70. *Mol. Cell Biol.*, **19**, 3580–3587.

Rodriguez, O.C. and Cheney, R.E. (2002) Human myosin-Vc is a novel class myosin expressed in epithelial cells. *J. Cell Sci.*, **115**, 991–1004.

Rubino, M., Miaczynska, M., Lippe, R., and Zerial, M. (2000) Selective membrane recruitment of EEA1 suggests a role in directional transport of clathrin coated vesicles to early endosomes. *J. Biol. Chem.*, **275**, 3745–3748.

Sacher, M., Barrowman, J., Wang, W., Horecka, J., Zhang, Y., Pypaert, M., and Ferro-Novick, S. (2001) TRAPP is implicated in the specificity of tethering in ER to Golgi transport. *Mol. Cell*, **7**, 433–442.

Sacher, M., Jiang, Y., Barrowman, J., Scarpa, A., Burston, J., Zhang, L., Schieltz, D., Yates, J.R., Abeliovich, H., and Ferro-Novick, S.F. (1998) TRAPP, a highly conserved novel complex on the cis-Golgi that mediates vesicle docking and fusion. *EMBO J.*, **17**, 2494–2504.

Sakisaka, T., Meerlo, T., Matteson, J., Plutner, H., and Balch, W.E. (2002) Rab-alphaGDI activity is regulated by a hsp90 chaperone complex. *EMBO J.*, **21**, 6125–6135.

Sapperstein, S., Walter, D.M., Grosvenor, A.R., Heuser, J.E., and Waters, M.G. (1995) p115 is a general vesicular transport factor related to the yeast endoplasmic reticulum to Golgi transport factor uso1p. *Proc. Natl. Acad. Sci. USA*, **92**, 522–526.

Sato, T.K., Darsow, T., and Emr, S.D. (1998) Vam7p, a SNAP-25 like molecule, and Vam3p, a syntaxin homolog, function together in yeast vacuolar protein trafficking. *Mol. Cell Biol.*, **18**, 5308–5319.

Sato, T.K., Rehling, P., Peterson, M.R., and Emr, S.D. (2000) Class Vps protein complex regulates vacuolar SNARE pairing and is required for vesicle docking/fusion. *Mol. Cell*, **6**, 661–671.

Scheffzek, K., Ahmadian, M.R., KAbsch, W., Wiesmüller, L., Lautwein, A., Schmitz, F., and Wittinghofer, A. (1997) The Ras-rasGAP complex: basis for GTPase activation and its loss in oncogenic ras mutants. *Science*, **277**, 333–338.

Schott, D., Ho, J., Pruyne, D., and Bretscher, A. (1999) The COOH terminal domain of Myo2p, a yeast myosin V has a direct role in secretory vesicle targeting. *J. Cell Biol.*, **147**, 791–807.

Scita, G., Nordstrom, J., Carbone, R., Tenca, P., Giardina, G., Gutkind, S., Bjarnegard, M., Betsholtz, C., and di Fiore, P. (1999) Eps8 and E3B1 transduce signals from ras to rac. *Nature*, **401**, 290–293.

Seachrist, J.L., Laporte, S.A., Dale, L.B., Babwah, A.V., Caron, M.G., Anborgh, P.H., and Ferguson, S.S. (2002) Rab5 association with the angiotensin II type 1A receptor promotes Rab5 GTP binding and vesicular fusion. *J. Biol. Chem.*, **277**, 679–685.

Seeley, E.S., Kato, M., Margolis, N., Wickner, W., and Eitzen, G. (2002) Genomic analysis of homotypic vacuole fusion. *Mol. Biol. Cell*, **13**, 782–794.

Segev, N., Mullholland, J., and Botstein, D. (1988) The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell*, **52**, 915–924.

Seog, D.H., Kito, M., Igarashi, K., and Yamasaki, M. (1994) Molecular characterization of the USO1 gene product which is essential for vesicular transport in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Comm.*, **200**, 647–653.

Short, B., Preisinger, C., Körner, R., Kopajtich, R., Byron, O. and Barr, F.A. (2001) A GRASP55–rab2 effector complex linking Golgi structure to membrane traffic. *J. Cell Biol.*, **155**, 877–883.

Short, B., Preisinger, C., Schaletzky, J., Kopajtich, R., and Barr, F.A. (2002) The rab6 GTPase regulates recruitment of the dynactin complex to Golgi membranes. *Curr. Biol.*, **12**, 1792–1795.

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.

Siniossoglou, S. and Pelham, H.R.B. (2001) An effector of Ypt6p binds the SNARE Tlg1p and mediates selective fusion of vesicles with late Golgi membranes. *EMBO J.*, **20**, 5991–5998.

Siniossoglou, S., S.Y.P., C., and Pelham, H.R.B. (2000) Ric1p and Rgp1p form a complex that catalyzes nucleotide exchange on Ypt6p. *EMBO J.*, **19**, 4885–4894.

Soldati, T., Shapiro, A.D., Svejstrup, A.B.D., and Pfeffer, S. (1994) Membrane targeting of the small GTPase rab9 is accompanied by nucleotide exchange. *Nature*, **369**, 76–78.

Sönnichsen, B., De Renzis, S., Nielsen, E., Rietdorf, J., and Zerial, M. (2000) Distinct membrane domains on endosomes in the recycling pathway visualized by multicolor imaging of rab4, rab5, and rab11. *J. Cell Biol.*, **149**, 901–913.

Sönnichsen, B., Lowe, M., Levine, T., Jämsä, E., Dirac-Svejstrup, B., and Warren, G. (1998) A role for giantin in docking COPI vesicles to Golgi membranes. *J. Cell Biol.*, **140**, 1013–1021.

Sönnichsen, B., Watson, R., Clausen, H., Misteli, T., and Warren, G. (1996) Sorting of COP-I coated vesicles under interphase and mitotic conditions. *J. Cell Biol.*, **134**, 1411–1426.

Stearns, T., Willingham, M.C., Botstein, D., and Kahn, R.A. (1990) ADP-ribosylation factor is functionally and physicaly associated with the Golgi complex. *Proc. Natl. Acad. Sci. USA*, **87**, 1238–1242.

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.

Stinchcombe, J.C., Barral, D.C., Mules, E.H., Booth, S., Hume, A.N., Machesky, L.M., Seabra, M.C., and Griffiths, G.M. (2001) Rab27a is required for regulated secretion in cytotoxic T lymphocytes. *J. Cell Biol.*, **152**, 825–834.

Stroupe, C. and Brunger, A. (2000) Crystal structures of a rab protein in its inactive and active conformations. *J. Mol. Biol.*, **304**, 585–598.

Sugihara, K., Asano, S., Tanaka, K., Iwamatsu, A., Okawa, K., and Ohta, Y. (2002) The exocyst complex binds the small GTPase ralA to mediate filopodia formation. *Nature Cell Biol.*, **4**, 73–78.

Sutton, R.B., Fasshauer, D., Jahn, R., and Brunger, A.T. (1998) Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. *Nature*, **395**, 347–353.

Suvorova, E.S., Duden, R., and Lupashin, V.V. (2002) The Sec34p/Sec35p complex, a Ypt1p effector required for retrograde intra-Golgi trafficking, interacts with Golgi SNAREs and COPI vesicle coat proteins. *J. Cell Biol.*, **157**, 631–643.

Tall, G.G., Barbieri, M.A., Stahl, P.D., and Horazdovsky, B.F. (2001) Ras-activated endocytosis is mediated by the rab5 guanine nucleotide exchange activity of RIN1. *Dev. Cell*, **1**, 73–82.

TerBush, D.R., Maurice, T., Roth, D., and Novick, P. (1996) The exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO J.*, **15**, 6483–6494.

TerBush, D.R. and Novick, P. (1995) Sec6, sec8, and sec15 are components of a multisubunit complex which localizes to small bud tips in *Saccharomyces cerevisiae*. *J. Cell Biol.*, **130**, 299–312.

Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S., and Rothberg, J.M. (2000) A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. *Nature*, **403**, 623–627.

Ullrich, O., Horiuchi, H., Bucci, C., and Zerial, M. (1994) Membrane association of rab5 mediated by GDP-dissociation inhibitor and accompanied by GDP/GTP exchange. *Nature*, **368**, 157–160.

Ungar, D., Oka, T., Brittle, E.E., Vasile, E., Lupashin, V.I., Chatterton, J.E., Heuser, J.E., Krieger, M., and Waters, M.G. (2002) Characterization of a mammalian Golgi localized protein complex, COG, that is required for normal Golgi morphology and function. *J. Cell Biol.*, **157**, 405–415.

Valencia, A., Chardin, P., Wittinghofer, A., and Sander, C. (1991a) The ras protein family: evolutionary tree and role of conserved amino acids. *Biochemistry*, **30**, 4637–4648.

Valencia, A., Kleldgaard, M., Pai, E.F., and Sander, C. (1991b) GTPase domains of ras p21 oncogene protein and elongation factor Tu: analysis of three-dimensional structures, sequence families, and functional sites. *Proc.Natl. Acad. Sci. USA*, **88**, 5443–5447.

Valentijn, J.A., Valentijn, K., Pastore, L., and Jamieson, J.D. (2000) Actin coating of secretory granules during regulated exocytosis correlates with the release of rab3D. *Proc. Natl. Acad. Sci. USA*, **97**, 1091–1095.

Valsdottir, R., Hashimoto, H., Ashman, K., Koda, T., Storrie, B., and Nilsson, T. (2001) Identification of rabaptin-5, rabex-5, and GM130 as putative effectors of rab33b, a regulator of retrograde traffic between the Golgi apparatus and ER. *FEBS Lett.*, **508**, 201–209.

van IJzendoorn, S.C.D., Tuvim, M.J., Weimbs, T., Dickey, B.F., and Mostov, K.E. (2002) Direct interaction between rab3b and the polymeric IgG receptor controls ligand-stimulated transcytosis in epithelial cells. *Dev. Cell*, **2**, 219–228.

van Rheenen, S.M., X., C., Sapperstein, S.K., Chiang, E.C., Lupashin, V.V., Barlowe, C., and Waters, M.G. (1999) Sec34p, a protein required for vesicle tethering to the yeast Golgi apparatus, is in a complex with Sec35p. *J. Cell Biol.*, **147**, 729–742.

Vitale, G., Rybin, V., Christoforidis, S., Thornqvist, P.O., 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.

Wagner, W., Bielli, P., Wacha, S., and Ragnini-Wilson, A. (2002) Mlc1p promotes septum closure during cytokinesis via the IQ motifs of the vesicle motor Myo2p. *EMBO J.*, **21**, 6397–6408.

Walch-Solimena, C., Collins, R.N., and Novick, P.J. (1997) Sec2p mediates nucleotide exchange on sec4p and is involved in polarized delivery of post Golgi vesicles. *J. Cell Biol.*, **137**, 1495–1509.

Walch-Solimena, C. and Novick, P. (1999) The yeast phosphatidylinositol-4–OH kinase Pik1 regulates secretion at the Golgi. *Nature Cell Biol.*, **1**, 523–525.

Wang, J., Takeuchi, T., Yokata, H., and Izumi, T. (1999) Novel rabphilin-3–like protein associates with insulin-containing granules in pancreatic beta cells. *J. Biol. Chem.*, **274**, 28542–28548.

Wang, W., Sacher, M., and Ferro-Novick, S. (2000) TRAPP stimulates guanine nucleotide exchange on Ypt1p. *J. Cell Biol.*, **151**, 289–296.

Wanker, E.E., Rovira, C., Scherzinger, E., Hasenbank, R., Walter, S., Tait, D., Colicelli, J., and Lehrach, H. (1997) HIP-I: a huntington interacting protein isolated by the yeast two hybrid system. *Hum. Mol. Genet.*, **6**, 487–495.

Weide, T., Bayer, M., Köster, M., Siebrasse, J.P., Peters, R., and Barnekow, A. (2001) The Golgi matrix protein GM130: a specific interacting partner of the small GTPase rab1b. *EMBO rep.*, **2**, 336–341.

White, J., Johannes, L., Mallard, F., Girod, A., Grill, S., Reinsch, S., Keller, P., Tzschaschel, B., Echard, A., Goud, B., and Stelzer, E.H.K. (1999) Rab6 coordinates a novel Golgi to ER retrograde transport pathway in live cells. *J. Cell Biol.*, **147**, 743–759.

Whyte, J.R.C. and Munro, S. (2001) The Sec34/Sec35 Golgi transport complex is related to the exocyst, defining a family of complexes involved in multiple steps of membrane traffic. *Dev. Cell*, **1**, 527–537.

Whyte, J.R.C. and Munro, S. (2002) Vesicle tethering complexes in membrane transport. *J. Cell Sci.*, **115**, 2627–2637.

Wickner, W. (2002) Yeast vacuoles and membrane fusion. *EMBO J.*, **21**, 1241–1247.

Wilson, S.M., Yip, R., Swing, D.A., O'Sullivan, N., Zhang, Y., Novak, E.K., Swank, R.T., Russell, L.B., Copeland, N.G., and Jenkins, N.A. (2000) A mutation in rab27a causes the vesicle transport defects observed in ashen mice. *Proc. Natl. Acad. Sci. USA*, **97**, 7933–7938.

Wu, X., Bowers, B., Rao, K., Wei, Q., and Hammer III, J.A. (1998) Visualization of melanosome dynamics within wild-type and dilute melanocytes suggests a paradigm for myosin V function in vivo. *J. Cell Biol.*, **143**, 1899–1918.

Wu, X., Rao, K., Bowers, M.B., Copeland, N.G., Jenkins, N.A., and Hammer III, J.A. (2001) Rab27a enables myosin Va-dependent melanosome capture by recruiting the myosin to the organelle. *J. Cell Sci.*, **114**, 1091–1100.

Wu, X.S., Rao, K., Zhang, H., Sellers, J.R., Matesic, L.E., Copeland, N.G., Jenkins, N.A., and Hammer III, J.A. (2002) Identification of an organelle receptor for myosin Va. *Nature Cell Biol.*, **4**, 1–8.

Wurmser, A.E., Sato, T.K., and Emr, S.D. (2000) New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE dependent docking and fusion. *J. Cell Biol.*, **151**, 551–562.

Yamaguchi, A., Urano, T., Goi, T., and Feig, L. (1997) An eps homology (EH) domain protein that binds to the Ral-GTPase target, RalBP1. *J. Biol. Chem.*, **272**, 31230–31234.

Yang, B., Steegmaier, M., Gonzalez Jr., L.C., and Scheller, R.H. (2000) nsec1 binds a closed conformation of syntaxin1A. *J. Cell Biol.*, **148**, 247–252.

Yi, Z., Yokota, H., Torii, S., Aoki, T., Hosaka, M., Zhao, S., Takata, K., Takeuchi, T., and Izumi, T. (2002) The rab27a/granuphilin complex regulates the exocytosis of insulin-containing dense-core vesicles. *Mol. Cell Biol.*, **22**, 1858–1867.

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

Zhang, C.J., Bowzard, J.B., Greene, M., Anido, A., Sterans, K., and Kahn, R.A. (2002) Genetic interactions kink ARF1, YPT31/YPT32 and TRS130. *Yeast*, **19**, 1075–1108.