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Control of Vesicular Trafficking by Rho GTPases

Review

Marc Symons[1,2,3] and Nicole Rusk[1]

Although vesicular trafficking is essential for a large variety of cellular processes, the regulation of vesicular trafficking is still poorly understood. Members of the Rho family of small GTPases have recently emerged as important control elements of many stages of vesicular trafficking, providing new insight into the regulation of these events. We will discuss the diverse roles played by Rho proteins in membrane trafficking and focus on the biological implications of these functions.

Introduction

Membrane trafficking plays an important role in a wide range of processes, such as pattern formation, establishment of cell polarity and uptake of nutrients and particles. Over the past few years, evidence has accumulated indicating that Rho GTPases are critical regulators of many stages of vesicular trafficking. In this review, we will discuss some of the salient findings that support the diverse roles played by Rho proteins in membrane trafficking. We also will cover recent progress in our understanding of the Rho-controlled signaling mechanisms that regulate vesicular trafficking.

Like other GTPases, members of the Rho family cycle between a GDP-bound (inactive) and a GTP-bound (active) state. In the GTP-bound state, the GTPase relays signals that are initiated by growth factors, cytokines and adhesion molecules to a large number of downstream effectors. Rho proteins are key regulators of actin dynamics. Notably, individual Rho family members have been shown to control distinct types of actin-based structures. Thus, Rac stimulates the formation of veil-like protrusions of the plasma membrane, called lamellipodia, while Cdc42 induces finger-like extensions, called filipodia. RhoA on the other hand regulates actomyosin-based contractility and stimulates the formation of actin bundles, called stress fibers. In addition, Rho proteins mediate many other functions, including lipid metabolism, transcription and cell proliferation [1]. The GTPase cycle is controlled by three types of regulatory proteins: Guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs) [2]. GEFs catalyze the exchange of GDP for GTP by transiently stabilizing the nucleotide-free protein. GAPs stimulate the intrinsic GTP hydrolysis activity of Rho proteins, rapidly converting them to the 'inactive' GDP-bound form. RhoGDIs preferentially bind to the GDP-bound form of GTPases and prevent spontaneous and GEF-catalyzed release of nucleotide, thereby maintaining the GTPases in the inactive state. Rho proteins are localized to membranous compartments by post-translational modification with a prenyl group that serves as a lipid anchor. The prenylated form of the GTPases is preferentially bound by RhoGDIs, which masks this prenyl group and thereby restricts the GTPases to the cytosol.

The regulation of nucleotide exchange is thought to be the rate-limiting factor for GTPase activation [2]. Recent evidence obtained in a number of different systems has indicated that these regulators are highly localized within the cell and that such spatial control is likely to be relevant for directed vesicle transport events [2,3].

Endocytic Trafficking

Endocytosis, the internalization of plasma membrane as well as extracellular particles and solutes, can occur via several distinct routes and mechanisms. Clathrin-mediated endocytosis involves the concentration of plasma membrane receptors and their ligands in specialized domains of the plasma membrane, termed clathrin-coated pits [4]. Pit formation is mediated in part via direct interaction of the cytoplasmic tails of receptors with a constituent of the adaptor protein (AP) complex. Subsequently, the coated pits invaginate and bud off to form coated vesicles. In addition to clathrin-mediated endocytosis, a number of clathrin-independent endocytic pathways have been described, including macropinocytosis, caveolae-dependent endocytosis and phagocytosis [5]. Macropinocytosis contributes to bulk fluid-phase uptake via the formation of membrane protrusions that trap extracellular fluid and subsequently fuse back with the plasma membrane. Yet another pathway relies on caveoli, plasma membrane invaginations that are enriched in cholesterol and glycosphingolipids. Both clathrin- and caveolae-dependent endocytosis involve dynamin, a GTPase that functions in vesicle fission from the plasma membrane [6]. Phagocytosis refers to the engulfment of large particles (>0.5 μm) and involves extensive reorganization of the actin cytoskeleton.

Macropinocytosis

In many cell types, macropinocytosis is a transient process that is stimulated by growth factors. Pioneering work by Ridley and Hall had implicated Rac in the stimulation of pinocytosis [7]. More recent studies have provided evidence for an important role for the Rac/Cdc42 effector p21-activated kinase (Pak) in the regulation of this process [8]. Rac and Pak co-localize to membrane ruffles and pinocytic vesicles. A

[1]Center for Oncology and Cell Biology, North Shore-LIJ Research Institute, 350 Community Dr., Manhasset, New York 11030, USA. [2]Department of Surgery, North Shore-LIJ Research Institute, Manhasset, New York 11030, USA. [3]Departments of Surgery and Anatomy & Structural Biology, Albert Einstein College of Medicine, Bronx, New York 10461, USA. E-mail: msymons@nshs.edu

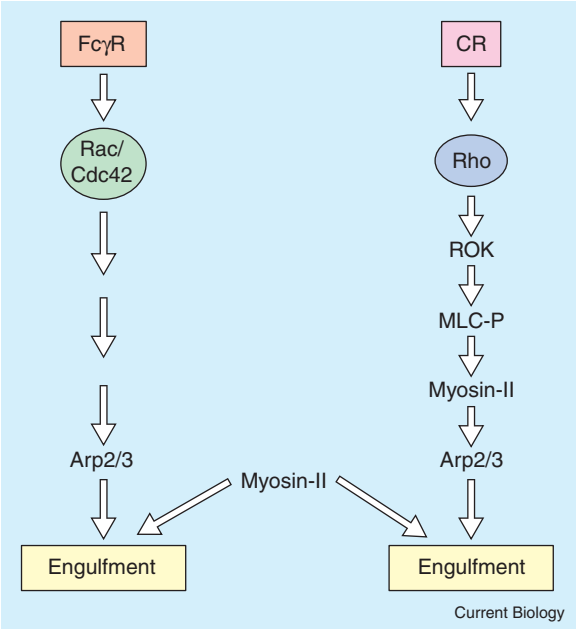

Figure 1. Differential involvement of Rho GTPases in FcγR- and CR-mediated phagocytosis.

Signaling by both FcγR and CR converges on the Arp2/3 complex to promote actin cup formation. CR-induced localization of the Arp2/3 complex to the phagocytic cup is mediated by the Rho → ROK → myosin-II pathway. Myosin-II also is likely to be involved in phagosome internalization in both FcγR- and CR-mediated phagocytosis.

constitutively active mutant of Pak stimulates pinocytosis, whereas the autoinhibitory domain of Pak inhibits Rac-induced pinocytosis. Interestingly, activated Pak also enhances the recycling of pinosomes to the plasma membrane. Macropinocytosis is an actin-dependent process, and the Lim-domain-containing protein kinase (LIMK) is a Pak substrate that may mediate Pak-regulated actin dynamics in macropinosome formation. Pak can directly phosphorylate LIMK [9], which in turn phosphorylates and inactivates cofilin [10,11], a protein involved in actin depolymerization. Pak also is an important signaling element in the control of cell motility [12,13], suggesting that Pak-regulated turnover of the plasma membrane might play a role in cell migration. We will return to the potential role of vesicular trafficking in cell migration in the last section of this review.

Macropinocytosis is also used by dendritic cells to capture antigens for subsequent presentation to CD4+ T cells. In immature dendritic cells, macropinocytosis is constitutive. Upon encountering an inflammatory stimulus dendritic cells start to mature; during this process, pinocytosis is downregulated and processing of the acquired antigens is initiated. Selected peptides are then loaded onto major histocompatibility complex (MHC) class II molecules for displaying to CD4+ cells [14]. In immature dendritic cells derived from bone marrow, macropinocytosis can be blocked by microinjection of either dominant-negative Cdc42 or Rac, while both constitutively active Cdc42 and Rac restore pinocytosis in mature dendritic cells [15]. However, in comparison to mature dendritic cells, the immature cells display increased levels of activated Cdc42, but not Rac, suggesting that Cdc42 rather than Rac is the physiological regulator of macropinocytosis in these cells.

A different scenario is played out in spleen-derived dendritic cells, where dominant negative Rac, but not Cdc42, inhibits macropinocytosis. Microinjection of constitutively active Rac, however, fails to reactivate macropinocytosis in mature spleen-derived dendritic cells [16]. At face value, these results would suggest that the signaling events that regulate macropinocytosis in dendritic cells vary depending on the source of the cells. It also is interesting to note that Rac activity is not essential for membrane ruffling in spleen-derived dendritic cells [16]. This suggests that Rac may be required for pinocytosis at a point downstream of lamellipodia formation, while other Rho family members may control membrane ruffling in these cells.

Phagocytosis

Phagocytosis plays an important role in several biological processes (for a recent review, see [17]). In higher organisms, it is required for host defense by allowing for the uptake and subsequent degradation of pathogenic agents and contributes to immune and inflammatory responses [18,19]. In addition, the phagocytosis of apoptotic cells is essential for tissue homeostasis and remodeling [20]. Professional phagocytosis is carried out by specialized cells such as macrophages and neutrophils, although many cell types have retained the ability to phagocytose through evolution and are known as ‘non-professional’ phagocytes. Phagocytosis is initiated by the binding of particle-attached ligands to specific receptors, including the Fcγ (FcγRs) and complement (CRs) receptors. Which mode of protrusive activity and which signaling pathways become activated depends on the type of the receptor being stimulated [21,22]. In the case of FcγR-mediated phagocytosis, the cell extends pseudopods that engulf the particle and subsequently fuse to form a phagosome. The phagosome is taken up by the cell and fuses with the lysosomal compartment where the content of the phagosome is digested. In contrast to antibody-coated particles, particles that are opsonized with complement fragments sink into a phagocytic cup and are taken up without pseudopod formation. CR activation requires additional stimuli such as chemokines and integrin ligation to form the phagocytic cup.

Because of the pivotal role of actin dynamics in phagocytosis, it comes as no surprise that Rho family members have been implicated in the regulation of this process (reviewed in depth in [19,22,23]). Both Cdc42 and Rac act at distinct stages of FcγR-mediated phagocytosis [24–26]: Cdc42 functions during pseudopod extension, whereas Rac acts in pseudopod fusion and phagosome closure [25]. Also Rho is recruited to the site of particle attachment, but its precise role in FcγR-mediated phagocytosis remains to be determined [27]. In contrast, CR-dependent phagocytosis is controlled by Rho, but not Cdc42 or Rac [26].

Currently, little is known about the molecular mechanisms that regulate recruitment and activation of Rho proteins in phagocytosis. Recent work, however, has implicated the Vav GEF in the activation of Rac during FcγR-mediated phagocytosis [28]. Vav translocates to nascent phagosomes where it activates Rac, but not Cdc42. Notably, Vav translocation and subsequent Rac activation occur independently of Cdc42, further demonstarting that Cdc42 and Rac deliver distinct contributions to the phagocytic process.

The precise orchestration of actin dynamics during phagosome formation and internalization remain largely elusive [19,22,23]. It is clear, however, that some of the signaling pathways that are activated during both FcγR- and CR-mediated phagocytosis converge on the Arp2/3 complex [29], an evolutionary conserved protein complex that nucleates actin filaments [30] (Figure 1). In addition, the Rho effector Rho-kinase (ROK) has been implicated in this process [31], which is in keeping with the specific involvement of Rho in CR-mediated phagocytosis. Notably, inhibitors of either ROK or myosin-II interfere with the accumulation of Arp2/3 and F-actin around bound particles, indicating that the Rho → ROK → myosin-II pathway is necessary for actin cup formation. This situation differs from FcγR-mediated phagocytosis, where myosin-II is only involved in phagosome internalization.

Another specialized phagocytic process is the rapid clearing of apoptotic cells, a critical and final step in programmed cell death, that prevents the induction of an inflammatory response [32]. The signaling events mediating this process have been elucidated by genetic studies in *Caenorhabditis elegans* (reviewed in [20,22,23]). Sequential steps in the engulfment of cell corpses involve the formation of a ternary complex composed of CED-2/CrkII, CED-5/DOCK180 and CED-12/ELMO and the subsequent activation of CED-10/Rac [33–38] (Figure 2). The signaling mechanisms that initiate the formation of this complex and regulate its recruitment to the plasma membrane are still elusive in *C. elegans* [39,40]. In human kidney epithelial cells, however, this is accomplished by αvβ5 integrin engagement and subsequent phosphorylation of the adaptor protein $p130^{Cas}$ [34].

Recent studies have shed light on how the DOCK180–ELMO complex activates Rac. Members of the DOCK180 family contain a domain, termed Docker/DHR-2, which is sufficient to stimulate Rac nucleotide exchange activity *in vitro* [41,42]. Apparently, ELMO can stimulate the GEF activity of DOCK180, but exactly how this is accomplished will require clarification [37,38,41]. Also Cdc42 is necessary for phagocytosis of apoptotic cells by macrophages, but the signaling events that mediate the activation of Cdc42 in this process are still unclear [35]. It is important to note that the phagocytic machinery is subverted by a large number of viral and bacterial pathogens in order to gain access to the cell interior. Not surprisingly, Rho family members are targets of numerous bacterial toxins. A discussion of these events falls outside of the scope of this review, however, and the reader is referred to two comprehensive recent reviews [43,44].

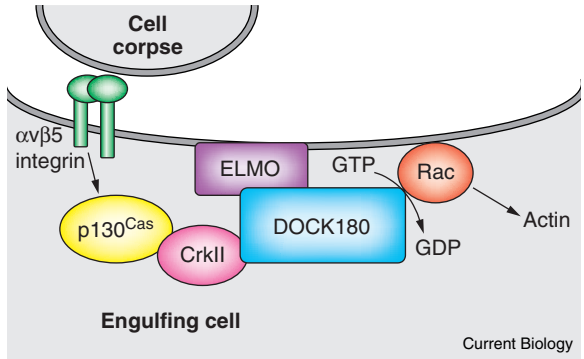

Figure 2. Model for Rac activation by apoptotic cells.

This scheme combines experimental evidence obtained in mammals and *C. elegans*. Integrins are one example of membrane receptors that detect signals from apoptotic cells. Integrin ligation triggers the recruitment of the $p130^{Cas}$–CrkII–DOCK180–ELMO complex to the plasma membrane. Localization of this complex may be aided by the PH domain of ELMO. Rac activation subsequently promotes reorganization of the actin cytoskeleton in a process that is probably mediated by a number of different Rac effectors.

Clathrin-Mediated Endocytosis

The first evidence for an involvement of Rho family members in clathrin-mediated endocytosis came from studies in HeLa cells, showing that overexpression of constitutively active forms of either Rac1 or RhoA causes inhibition of transferrin and EGF receptor internalization [45]. One mechanism by which these GTPases might participate in clathrin-mediated endocytosis is the modulation of phosphatidylinositol (PI) lipid metabolism, which plays a key role in this process [46]. Rac has been shown to interact with a number of enzymes that regulate PI metabolism, including type I PI 3 -kinase, members of the phosphatidylinositol 4-phosphate 5-kinase family (also termed type I PIPkinases) and synaptojanin 2, a PI lipid phosphatase [47,48]: also Rho associates with a type I PIPkinase [47]. Synaptojanin 2 is a particularly attractive candidate for a downstream effector of Rac in clathrin-mediated endocytosis, as other members of the synaptojanin family already have been shown to be essential for the timely progression of this process [49,50].

Rho proteins could also affect clathrin-mediated endocytosis via their action on the actin cytoskeleton. In principle, actin dynamics and actomyosin contractility could operate in many of the steps of the endocytic process, such as the invagination of the plasma membrane, dissolution of the cortical actin barrier and coated vesicle fission and movement away from the plasma membrane [51]. Although in the budding yeast, *Saccharomyces cerevisiae*, the essential function of the actin cytoskeleton in clathrin-mediated endocytosis has long been established [52,53], the role of actin dynamics during this process is less clear in mammalian cells. For instance, a comprehensive study using three different chemical inhibitors of actin polymerization showed that perturbation of actin dynamics has a variable effect on the internalization of the transferrin receptor that strongly depends on the cell line and assay conditions [54]. In recent years,

however, a number of regulators of the actin cytoskeleton have been shown to bind to endocytic proteins, such as clathrin, AP-2 and dynamin [55]. It is therefore tempting to speculate that Rho family GTPases could modulate clathrin-mediated endocytosis, at least in part, by acting on the actin cytoskeleton. Attempts to test this hypothesis, however, have thus far yielded negative results. For example, the inhibitory effects of constitutively active Rac and Rho on transferrin uptake were not affected by cytochalasin D, a drug that prevents actin filament assembly [45]. Nevertheless, these experiments could not exclude that post-internalization events might be modulated by actin dynamics.

Interestingly, different members of the Rho subfamily affect distinct stages of clathrin-mediated endocytosis. For instance, expression of constitutively active RhoA inhibits receptor uptake at an early step of the internalization process [45,56,57], whereas wild-type or constitutively active RhoB prevents intracellular trafficking of the receptor from early to late endosomes [58,59]. Notably, the three members of the Rho subfamily, RhoA, B and C, share about 83% amino acid identity and differ mainly in their carboxy-terminal hypervariable region, a critical determinant of membrane targeting [60]. Thus, overexpressed RhoA is largely cytosolic, while RhoB targets to both the plasma membrane and internal membranes [58,60–62]. Therefore, the intracellular localization of RhoA and RhoB may be instrumental for their differential roles in receptor trafficking. One effector of RhoB in membrane trafficking might be protein kinase C related kinase 1 (PRK1/PKN). Indeed, RhoB targets PRK1 to endosomal membranes and overexpression of kinase-inactive PRK1 releases the inhibitory effect of RhoB on the trafficking of internalized receptors [63].

It is interesting to note that although constitutively active RhoA inhibits clathrin-mediated endocytosis in non-polarized cells [45], it stimulates this process in MDCK cells [64], a model system for epithelial cell polarity. The reason for this cell-type dependency is not clear. Rac1, however, exerts an inhibitory role on receptor internalization in polarized and non-polarized cells [45,65].

Evidence supporting a role for Cdc42 in clathrin-mediated endocytosis has come from studies showing that an effector of Cdc42, activated Cdc42-associated kinase (Ack), interacts with the clathrin heavy chain [66,67]. Whereas Ack1 expressed at low levels enhances transferrin receptor internalization, high levels of Ack1 have the opposite effect. Notably, both effects are independent of the kinase activity of Ack1 [66]. The interaction of Ack with clathrin competes with the binding of clathrin to AP-2, suggesting that the inhibitory effect of overexpressed Ack on receptor internalization might be a consequence of preventing clathrin recruitment to coated pits [67]. A stimulatory role for Ack in clathrin-mediated endocytosis is also in accordance with studies in *C. elegans*, where ARK-1, a tyrosine kinase closely related to Ack, negatively regulates epidermal growth factor (EGF) signalling during vulva development [68], possibly by promoting internalization of the EGF receptor.

Recently, two groups, using *Drosophila* and mammalian systems, respectively, showed that Ack binds to and phosphorylates SH3PX1/SNX9, a member of the sorting nexin family [69,70]. The sorting nexins constitute a large and diverse group of proteins that are characterized by the presence of a PX motif, which binds PI lipids [71]. Most of the known functions of mammalian sorting nexins involve the regulation of receptor turnover at the plasma membrane. Although overexpression of SNX9 on its own does not affect EGF receptor degradation, it greatly stimulates EGF receptor degradation when co-expressed with wild-type, but not kinase-inactive Ack [70], suggesting a novel role for Ack in receptor turnover. How does this function relate to the binding of Ack to clathrin? Interestingly, Ack forms a ternary complex with clathrin and SNX9. Moreover, expression of an amino-terminal truncation mutant of Ack enhances binding of SNX9 to clathrin, whereas expression of a mutant of Ack that cannot bind clathrin inhibits the association of SNX9 with clathrin, suggesting that Ack could act as a scaffold protein to recruit SNX9 to clathrin-coated pits and/or vesicles [70]. Precisely how SNX9 promotes EGF receptor degradation and whether Cdc42 acts upstream of Ack in this context remains to be determined.

Initially, *Drosophila* SNX9 was identified as a binding partner of Dock/Nck, a SH3/SH2-containing adaptor protein that provides a link between membrane receptors and the actin cytoskeleton [72,73]. More recently, DSNX9 has also been shown to interact with Wiskott-Aldrich Syndrome protein (WASP), a scaffold protein involved in the regulation of actin polymerization [72,74], thus implicating actin dynamics in endosome trafficking.

Another intriguing connection between Cdc42-modulated actin dynamics and endocytic trafficking is provided by the endocytic scaffold protein intersectin. The neuronal isoform of intersectin, intersectin-long (intersectin-l), has a Dbl homology (DH) in tandem with a Pleckstrin homology (PH) domain at its carboxyl-teminus, conferring Cdc42-specific nucleotide exchange activity. Interestingly, the exchange activity of intersectin-l is stimulated by binding to N-WASP, an effector of Cdc42 that mediates actin nucleation through activation of the Arp2/3 complex [75]. In principle, this stimulatory effect of N-WASP could drive a positive feedback loop that would promote localized actin polymerization at clathrin-coated pits, the compartment to which intersectin has been localized [76].

Support for this notion comes from a recent study on the EphB receptors in hippocampal neurons [77]. This tyrosine kinase receptor relays signals from B-ephrin ligands to regulate dendritic spine development, a process that is thought to be powered by actin polymerization [78]. EphB binds to and activates intersectin-l in a cooperative fashion with N-WASP and dominant negative versions of either intersectin-l, N-WASP or Cdc42 inhibit dendritic spine formation.

Clathrin-Independent Endocytic Pathways

Rho GTPases are also involved in clathrin-independent receptor internalization. For example, the interleukin-2 (IL-2) receptor localizes to detergent-resistant

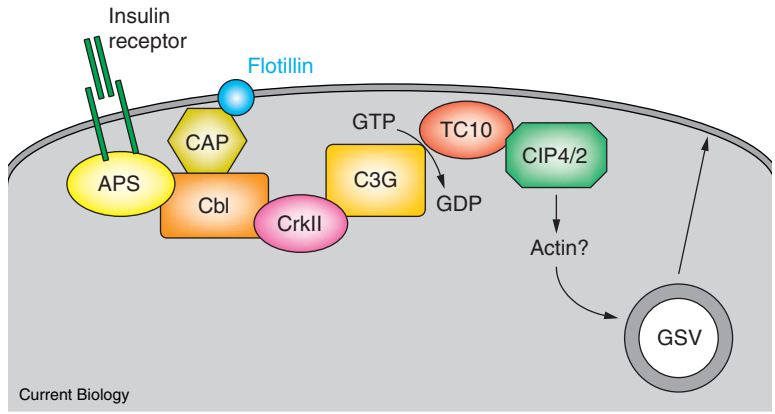

Figure 3. Control of Glut4 translocation by TC10.

Ligation of the insulin receptor leads to the recruitment of Cbl via the adaptor protein containing PH and SH2 domains (APS). Upon phosphorylation, Cbl relocalizes to lipid rafts via Cbl-associated protein (CAP) and flotillin. Subsequently, Cbl recruits the adaptor protein CrkII along with C3G which promotes nucleotide exchange on TC10. A TC10 effector that may mediate the translocation of Glut4 storage vesicles (GSVs) is Cdc42-interacting protein 4/2 (CIP 4/2), possibly by promoting a reorganization of the actin cytoskeleton.

membrane domains, also termed lipid rafts [79] and becomes internalized in a clathrin-independent but dynamin-dependent fashion [80]. In contrast to clathrin-mediated endocytosis, this process is inhibited by expression of dominant-negative RhoA or Rac, but is not affected by the constitutively active versions of these GTPases, indicating essential roles for both Rho and Rac in endocytosis of the IL-2 receptor.

Recently, a novel clathrin-independent pathway for the internalization of glycosylphosphatidylinositol-anchored proteins has been described [81]. This pathway is also responsible for a major fraction of fluid-phase uptake and is distinct from macropinocytosis that is stimulated by growth factors or developmentally regulated pinocytosis such as observed in dendritic cells. Notably, this pinocytic pathway is specifically mediated by Cdc42, as it is inhibited by expression of a dominant-negative Cdc42, but is unaffected by dominant-negative forms of either Rac or Rho.

Exocytosis

In secretory cells, material to be exported, such as neurotransmitters, peptides or ATP, is stored in secretory vesicles that are released to the cell exterior during exocytosis. Secretory vesicles are present in at least two compartments, including a 'rapidly releasable pool' (RRP) and a 'slowly releasable pool' (SRP). Stimulus-induced reorganization of the actin cytoskeleton is thought to play an important role in exocytosis [82–84]. On one hand, actin filaments could act as a diffusion barrier between the SRP and RRP pools. On the other hand, they could provide tracks aiding in vesicle transport to exocytic sites. In addition, secretory vesicle-associated actin polymerization could serve as a propelling force for vesicle transport.

Mast cell degranulation

Studies over the past several years have revealed the importance of Rho GTPases in stimulus-induced exocytosis in a number of different cell types [82,85–87]. A valuable paradigm for the study of signaling pathways that control exocytosis is the immunoglobulin E (IgE) induced degranulation of secretory granules in mast cells. Several reports have indicated a role for Rho proteins in regulated secretion in these cells [86,87]. Rac and Cdc42 regulate multiple steps of IgE-induced degranulation in an extensively characterized mast cell line. Constitutively active forms of Rac1 and Cdc42 stimulate the formation of inositol trisphosphate (IP_3), clustering of the IP_3 receptor and the subsequent release of intracellular calcium [88]. Also extracellular calcium influx is enhanced by activated Rac1 and Cdc42. Calcium regulates both the priming and triggering steps of exocytosis, although the identity of the relevant calcium sensors is still under debate [89]. The effect of Cdc42 and possibly Rac on IP_3 production might be mediated by phospholipase Cγ1 (PLCγ1), as *in vitro* studies showed direct binding of PLCγ1 to Cdc42 [88]. However, Rac1 and Cdc42 probably use additional signaling mechanisms to mobilize calcium, since these GTPases can also restore calcium mobilization and degranulation in cells that are defective in antigen-stimulated PLCγ activation [90].

The precise role of actin dynamics in IgE-induced degranulation remains to be clarified [91,92]. In permeabilized mast cells, activated Rho stimulates calcium-induced cortical F-actin disassembly and participates in antigen-induced degranulation at a point downstream of calcium influx, presumably at the level of the RRP pool. These two activities, however, are mediated via distinct signaling pathways [91], as Rho-stimulated actin reorganization is Rock-dependent, whereas Rho-induced secretion is not.

Glut4 trafficking

An important aspect of the anabolic action of insulin is the stimulation of glucose uptake via the Glut4 transporter [93]. In the resting state, most of the Glut4 resides in a specialized membrane compartment. Insulin triggers a signaling cascade that is mediated by the Rho family member TC10 and results in transport of Glut4 to the plasma membrane via targeted exocytosis (Figure 3). Insulin-stimulated signaling mechanisms that lead to the activation of TC10 have recently been elucidated [93]. Ligation of insulin receptors leads to the recruitment of Cbl via the adaptor protein containing PH and SH2 domains (APS). Cbl in turn recruits the adaptor protein CrkII along with the exchange factor C3G, which promotes nucleotide exchange on TC10 [94]. A critical feature of the assembly of this signaling complex is its localization to lipid rafts [95]. The recruitment of Cbl to lipid rafts is mediated via its

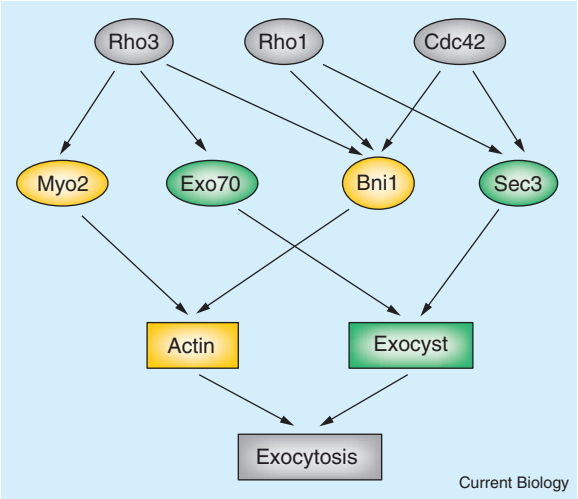

Figure 4. Control of the yeast exocyst complex by Rho GTPases.

At least three *S. cerevisiae* Rho GTPases have been implicated in polarized exocytosis, Rho1, Rho3 and Cdc42. Each of these GTPases directly interacts with a component of the exocyst, a multiprotein complex that functions in the correct targeting of secretory vesicles. Polarized exocytosis also requires a well-organized actin cytoskeleton for efficient transport of secretory vesicles to the bud site, and Rho GTPases appear to act in concert to accomplish this task.

binding partner Cbl-associated protein (CAP), which in turn associates with the integral membrane protein flotillin. Conversely, targeting of TC10 to lipid rafts is likely to be mediated via its carboxy-terminal hypervariable domain. As insulin-stimulated translocation of Glut4 is dependent on remodeling of the actin cytoskeleton [96,97], TC10-mediated regulation of actin dynamics probably contributes to this specialized exocytic process. A TC10 effector that may mediate Glut4 translocation is Cdc42-interacting protein 4/2 (CIP 4/2), an adaptor protein that also interacts with Cdc42 and has been implicated in the control of the actin cytoskeleton [98,99].

Secretory Vesicle Targeting in Yeast

Rho proteins also function in the targeting of secretory vesicles to discrete sites on the plasma membrane, a process that is mediated by a multiprotein complex termed the exocyst [100]. In *S. cerevisiae*, the exocyst is regulated by at least three Rho family proteins: Rho1p, Rho3p and Cdc42p (Figure 4). Rho1p and Cdc42p act on the Sec3p component of the exocyst, which appears to function as a landmark for exocytic targeting on the plasma membrane [101]. Sec3p is a direct effector of both Rho1p and Cdc42p and both of these GTPases are essential for targeting Sec3p to the tip of the emerging bud [102–104]. Rho3 directly interacts with Exo70p, another component of the exocyst, although the exact role of this interaction in the regulation of the exocyst is unclear [105]. Rho3 also binds to unconventional myosin Myo2p, through which it may regulate the delivery of exocytic vesicles [105]. In addition, Rho1p, Rho3p and Cdc42p bind to the formin homology protein Bni1, which promotes actin polymerization via binding to profilin. Given these interactions and the fact that proper assembly of the actin cytoskeleton is essential for polarized exocytosis, a picture emerges, in which these three Rho GTPases coordinate acto-myosin dynamics and vesicle targeting to promote polarized secretion (Figure 4).

An exocyst complex known as the Sec6/8 complex [106] has also been characterized in mammalian cells. This complex is found at sites of active exocytosis such as neuronal growth cones, synapse-assembly domains in axons and the apex of the basolateral membrane in epithelial cells [107,108]. The mechanisms that regulate the Sec6/8 complex may well differ from those that regulate the exocyst in yeast. Hence, of all the subunits of the Sec6/8 complex expressed as GFP fusion proteins, only Exo70 has been shown to be recruited to cell-cell contacts in MDCK cells [109]. While to date there is no evidence that the Sec6/8 complex is regulated by Rho GTPases, a role for the Ral GTPase in regulated secretion via direct interaction with the Sec5 component of the Sec6/8 complex has been recently identified by several laboratories [110,111].

Functional Implications of Vesicular Trafficking Regulated by Rho GTPases

Cell Migration

Rho GTPases play a critical role in the regulation of cell motility and invasion largely due to their direct action on actin dynamics and acto-myosin contractility [112,113]. Although the precise function of vesicular trafficking in cell migration has been debated for a long time [114,115], accumulating evidence reveals that facilitated recycling of cell adhesion molecules is important for membrane protrusion and cell migration. For example, recycling of the αvβ3 integrin from early endosomes mediated by the Rab4 GTPase has been shown to be necessary for the adhesion and spreading of fibroblasts on vitronectin [116], whereas the oriented recycling of αvβ1 has been implicated in the migration of neutrophils [117]. There is also evidence that L1, a member of the immunoglobulin superfamily of cell adhesion molecules, undergoes endocytosis preferentially at the central domain of growth cones and subsequently is recycled to the leading edge, thus establishing an adhesive gradient that contributes to neurite outgrowth [118]. It is, therefore, conceivable that Rho GTPases could regulate cell migration by coordinating both vesicular trafficking and actin dynamics. This notion is illustrated by the functional analysis of RhoD, a Rho protein that localizes both to the plasma membrane and early endosomes [119]. Expression of a constitutively active form of RhoD inhibits both the motility of early endosomes and cell migration [120,121]. A causal relationship between these two events remains to be established, however.

Matrix metalloproteinases (MMPs) play a critical role in breakdown and remodeling of the extracellular matrix. It is interesting to note that endocytosis is an important regulatory mechanism that controls the

activity of MMPs [122,123]. This suggests that Rho GTPase-regulated trafficking of MMPs may contribute to the role of these GTPases in cell motility and invasion.

Cell–Cell Junctions and Epithelial Cell Polarity

Rho GTPases also seem to play a role in the establishment of cadherin-based adherens junctions [124], during which E-cadherin is internalized, probably via clathrin-dependent endocytosis, and recycled to the basolateral membrane. As this process has been implicated in the formation and stability of adherens junctions [125], inhibition of E-cadherin cycling by Rho GTPases could stabilize adherens junctions. In support of this notion, expression of constitutively active versions of Rac1 or RhoA in MDCK cells blocks the disassembly of adherens junctions and endocytosis of E-cadherin caused by hepatocyte growth factor (HGF) or phorbol esters [126]. However, this picture is complicated by the finding that activated Rac1 induces the disassembly of adherens junctions in keratinocytes and that this is also accompanied by E-cadherin internalization [127]. Therefore, the role of Rac in E-cadherin cycling is probably cell type dependent.

In polarized epithelial cells, membrane proteins and lipids segregate into two distinct compartments, the apical and basolateral domains. This compartmentalization allows for vectorial transport along the apical–basal axis and is greatly aided by polarized membrane trafficking. Early studies had already demonstrated a role for Cdc42 in the polarization response of helper T cells toward antigen-presenting cells (APC) [128]. This process comprises the re-orientation of the microtubule-organizing center (MTOC) and the Golgi apparatus toward the APC contact site, facilitating the delivery of secretory granules. More recent studies also have implicated Cdc42-mediated secretory vesicle trafficking in the maintenance of epithelial cell polarity [129–131]. Despite these indications, the precise function of Cdc42 in polarized trafficking remains controversial (reviewed in [124]).

Cell Proliferation

Rho GTPases are also important for cell cycle progression and cell transformation. Some of the downstream pathways that contribute to these proliferative effects result in the induction of cyclin D1 and downregulation of cyclin-dependent kinase inhibitors [132]. As endocytic trafficking results in the removal of growth factor receptors from the cell surface, thereby promoting their downregulation, the inhibitory effects of Rac and Rho on clathrin-mediated endocytosis [45,58] also could contribute to cell proliferation.

An intriguing connection between Cdc42-regulated vesicle trafficking and cell transformation is revealed by the observation that Cdc42 binds to the γ-subunit of the COPI [133], a coatamer protein required for retrograde transport of Golgi components to the endoplasmic reticulum (ER) [134]. Notably, the binding of γCOPI to Cdc42 correlates with the transforming potential of Cdc42 [133]. Even though the precise function of the Cdc42–γCOPI interaction is not clear yet, inhibition of COPI function probably interferes indirectly with anterograde transport from ER to Golgi, a function that also is regulated by Cdc42 [135].

Regulated exocytosis also may contribute to cell transformation. It has recently been shown that BAIAP3, a member of the Munc13 family of exocytic proteins, is upregulated by the EWS-WT1 transcription factor, a chimeric fusion of the Ewing's sarcoma and Wilms' tumor genes [136]. Ectopic expression of BAIAP3 in tumor cells strongly enhances their transformed properties, possibly by stimulating exocytosis of autocrine factors. Rho proteins, therefore, also could promote tumor growth by their stimulatory effect on exocytosis.

Concluding Remarks

Advances made over the past few years have made us appreciate the pleiotropic effects of Rho GTPases on vesicular trafficking along both the endocytic and exocytic pathways. Membrane trafficking is intimately involved in many of the functions that are controlled by Rho family members, including the establishment of cell polarity and the control of cell proliferation and motility. It is, therefore, likely that the roles of Rho GTPases in these processes are mediated, at least in part, via their modulation of trafficking events, as discussed in this review. This notion, however, may also hold for other processes for which vesicular trafficking is essential, such as developmental pattern formation and synaptic plasticity [137,138]. A theme that was developed throughout this review is the specific involvement of several Rho proteins at particular stages of vesicle trafficking. This does not come as a surprise, because of the differential localization of Rho family members to distinct membrane compartments. We expect that future studies will identify additional trafficking functions for some of the less well-characterized members of the Rho family.

A considerable challenge we are now facing is the dissection of the downstream signaling events that mediate the roles of Rho proteins in membrane trafficking. Although the precise role of the actin cytoskeleton in membrane trafficking is still not understood, Rho GTPases are ideally placed to serve as coordinators of actin dynamics and vesicle trafficking events. The recent identification of a large number of components of the trafficking machinery acting as downstream effectors of Rho GTPases indicates that cytoskeletal reorganization is definitely not the entire story.

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References

1. Ridley, A.J. (2001). Rho family proteins: coordinating cell responses. Trends Cell Biol. *11*, 471–477.
2. Symons, M., and Settleman, J. (2000). Rho family GTPases: more than simple switches. Trends Cell Biol. *10*, 415–419.
3. Gulli, M.P. and Peter, M. (2001). Temporal and spatial regulation of Rho-type guanine-nucleotide exchange factors: the yeast perspective. Genes Dev. *15*, 365–379.
4. Higgins, M.K., and McMahon, H.T. (2002). Snap-shots of clathrin-mediated endocytosis. Trends Biochem. Sci. *27*, 257–263.

5. Johannes, L., and Lamaze, C. (2002). Clathrin-dependent or not: is it still the question? Traffic *3*, 443–451.
6. Hinshaw, J.E. (2000). Dynamin and its role in membrane fission. Annu. Rev. Cell Dev. Biol. *16*, 483–519.
7. Ridley, A.J., Paterson, H.F., Johnston, C.L., Diekmann, D., and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell *70*, 401–410.
8. Dharmawardhane, S., Schurmann, A., Sells, M.A., Chernoff, J., Schmid, S.L., and Bokoch, G.M. (2000). Regulation of macropinocytosis by p21-activated kinase-1. Mol. Biol. Cell *11*, 3341–3352.
9. Edwards, D.C., Sanders, L.C., Bokoch, G.M., and Gill, G.N. (1999). Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. Nat. Cell Biol. *1*, 253–259.
10. Arber, S., Barbayannis, F.A., Hanser, H., Schneider, C., Stanyon, C.A., Bernard, O., and Caroni, P. (1998). Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. Nature *393*, 805–809.
11. Yang, N., Higuchi, O., Ohashi, K., Nagata, K., Wada, A., Kangawa, K., Nishida.E. and Mizuno, K. (1998). Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. Nature. *393*, 809–812.
12. Sells, M.A., Boyd, J.T., and Chernoff, J. (1999). p21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. J. Cell Biol. *145*, 837–849.
13. Kiosses, W.B., Daniels, R.H., Otey, C., Bokoch, G.M., and Schwartz, M.A. (1999). A role for p21-activated kinase in endothelial cell migration. J. Cell Biol. *147*, 831–844.
14. Mellman, I., and Steinman, R.M. (2001). Dendritic cells: specialized and regulated antigen processing machines. Cell *106*, 255–258.
15. Garrett, W.S., Chen, L.M., Kroschewski, R., Ebersold, M., Turley, S., Trombetta, S., Galan, J.E. and Mellman, I. (2000). Developmental control of endocytosis in dendritic cells by Cdc42. Cell *102*, 325–334.
16. West, M.A., Prescott, A.R., Eskelinen, E.L., Ridley, A.J., and Watts, C. (2000). Rac is required for constitutive macropinocytosis by dendritic cells but does not control its downregulation. Curr. Biol. *10*, 839–848.
17. Aderem, A. (2002). How to eat something bigger than your head. Cell *110*, 5–8.
18. Underhill, D.M., and Ozinsky, A. (2002). Phagocytosis of microbes: complexity in action. Annu. Rev. Immunol. *20*, 825–852.
19. Greenberg, S., and Grinstein, S. (2002). Phagocytosis and innate immunity. Curr.Opin. Immunol. *14*, 136–145.
20. Fadok, V.A., and Chimini, G. (2001). The phagocytosis of apoptotic cells. Semin. Immunol. *13*, 65–72.
21. Aderem, A., and Underhill, D.M. (1999). Mechanisms of phagocytosis in macrophages. Annu. Rev. Immunol. *17*, 593–623.
22. Chimini, G., and Chavrier, P. (2000). Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. Nat. Cell Biol. *2*, E191–E196.
23. May, R.C., and Machesky, L.M. (2001). Phagocytosis and the actin cytoskeleton. J. Cell Sci. *114*, 1061–1077.
24. Cox, D., Chang, P., Zhang, Q., Reddy, P.G., Bokoch, G.M. and Greenberg, S. (1997). Requirements for both Rac1 and Cdc42 in membrane ruffling and phagocytosis in leukocytes. J. Exp. Med. *186*, 1487–1494.
25. Massol, P., Montcourrier, P., Guillemot, J.C., and Chavrier, P. (1998). Fc receptor-mediated phagocytosis requires CDC42 and Rac1. EMBO J. *17*, 6219–6229.
26. Caron, E., and Hall, A. (1998). Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. Science *282*, 1717–1721.
27. Hackam, D.J., Rotstein, O.D., Schreiber, A., Zhang, .W and Grinstein, S. (1997). Rho is required for the initiation of calcium signaling and phagocytosis by Fcgamma receptors in macrophages. J. Exp. Med. *186*, 955–966.
28. Patel, J.C., Hall, A., and Caron, E. (2002). Vav regulates activation of Rac but not Cdc42 during FcgammaR-mediated phagocytosis. Mol. Biol. Cell *13*, 1215–1226.
29. May, R.C., Caron, E., Hall, A., and Machesky, L.M. (2000). Involvement of the Arp2/3 complex in phagocytosis mediated by FcgammaR or CR3. Nat. Cell Biol. *2*, 246–248.
30. Welch, M.D., and Mullins, R.D. (2002). Cellular control of actin nucleation. Annu.Rev.Cell Dev Biol. *18*: 247-288.
31. Olazabal, I.M., Caron, E., May, R.C., Schilling, K., Knecht, D.A. and Machesky, L.M. (2002). Rho-kinase and myosin-II control phagocytic cup formation during CR, but not FcgammaR, phagocytosis. Curr.Biol. *12*, 1413–1418.
32. Savill, J., and Fadok, V. (2000). Corpse clearance defines the meaning of cell death. Nature *407*, 784–788.
33. Reddien, P.W., and Horvitz, H.R. (2000). CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in Caenorhabditis elegans. Nat. Cell Biol. *2*, 131–136.
34. Albert, M.L., Kim, J.I., and Birge, R.B. (2000). alphavbeta5 integrin recruits the CrkII-Dock180-rac1 complex for phagocytosis of apoptotic cells. Nat. Cell Biol. *2*, 899–905.
35. Leverrier, Y., and Ridley, A.J. (2001). Requirement for Rho GTPases and PI 3-kinases during apoptotic cell phagocytosis by macrophages. Curr. Biol. *11*, 195–199.
36. Wu, Y.C., Tsai, M.C., Cheng, L.C., Chou.C.J. and Weng, N.Y. (2001). C. elegans CED-12 acts in the conserved crkII/DOCK180/Rac pathway to control cell migration and cell corpse engulfment. Dev Cell. *1*, 491–502.
37. Zhou, Z., Caron, E., Hartwieg, E., Hall, A., and Horvitz, H.R. (2001). The C. elegans PH domain protein CED-12 regulates cytoskeletal reorganization via a Rho/Rac GTPase signaling pathway. Dev. Cell *1*, 477–489.
38. Gumienny, T.L., Brugnera, E., Tosello-Trampont, A.C., Kinchen, J.M., Haney, L.B., Nishiwaki, K., Walk, S.F., Nemergut, M.E., Macara, I.G., Francis, R. *et al*. (2001). CED-12/ELMO, a novel member of the CrkII/Dock180/Rac pathway, is required for phagocytosis and cell migration. Cell *107*, 27–41.
39. Hengartner, M.O. (2001). Apoptosis: corralling the corpses. Cell *104*, 325–328.
40. Henson, P.M., Bratton, D.L., and Fadok, V.A. (2001). Apoptotic cell removal. Curr. Biol. *11*, R795–R805.
41. Brugnera, E., Haney, L., Grimsley, C., Lu, M., Walk, S.F., Tosello-Trampont, A.C., Macara, I.G., Madhani, H., Fink, G.R., and Ravichandran, K.S. (2002). Unconventional Rac-GEF activity is mediated through the Dock180-ELMO complex. Nat. Cell Biol. *4*, 574–582.
42. Cote, J.F., and Vuori, K. (2002). Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. J. Cell Sci. *115*, 4901–4913.
43. Steele-Mortimer, O., Knodler, L.A., and Finlay, B.B. (2000). Poisons, ruffles and rockets: bacterial pathogens and the host cell cytoskeleton. Traffic *1*, 107–118.
44. Sansonetti, P. (2002). Host-pathogen interactions: the seduction of molecular cross talk. Gut *50* (*Suppl 3*), III2–III8.
45. Lamaze, C., Chuang, T.H., Terlecky, L.J., Bokoch, G.M., and Schmid, S.L. (1996). Regulation of receptor-mediated endocytosis by Rho and Rac. Nature *382*, 177–179.
46. Cremona, O., and De Camilli, P. (2001). Phosphoinositides in membrane traffic at the synapse. J. Cell Sci. *114*, 1041–1052.
47. Ren, X.D., and Schwartz, M.A. (1998). Regulation of inositol lipid kinases by Rho and Rac. Curr. Opin. Genet. Dev. *8*, 63–67.
48. Malecz, N., McCabe, P.C., Spaargaren, C., Qiu, R., Chuang, Y., and Symons, M. (2000). Synaptojanin 2, a novel rac1 effector that regulates clathrin-mediated endocytosis. Curr. Biol. *10*, 1383–1386.
49. Cremona, O., Di Paolo, G., Wenk, M.R., Luthi, A., Kim, W.T., Takei, K., Daniell, L., Nemoto, Y., Shears, S.B., Flavell, R.A., et al. (1999). Essential role of phosphoinositide metabolism in synaptic vesicle recycling. Cell *99*, 179–188.
50. Harris, T.W., Hartwieg, E., Horvitz, H.R., and Jorgensen, E.M. (2000). Mutations in synaptojanin disrupt synaptic vesicle recycling. J. Cell Biol. *150*, 589–600.
51. Qualmann, B., Kessels, M.M., and Kelly, R.B. (2000). Molecular links between endocytosis and the actin cytoskeleton. J. Cell Biol. *150*, F111–F116.
52. Geli, M.I. and Riezman, H. (1998). Endocytic internalization in yeast and animal cells: similar and different. J. Cell Sci. *111*, 1031–1037.
53. Munn, A.L. (2001). Molecular requirements for the internalisation step of endocytosis: insights from yeast. Biochim. Biophys. Acta *1535*, 236–257.
54. Fujimoto, L.M., Roth, R., Heuser, J.E., and Schmid, S.L. (2000). Actin assembly plays a variable, but not obligatory role in receptor-mediated endocytosis in mammalian cells. Traffic *1*, 161–171.
55. Schafer, D.A. (2002). Coupling actin dynamics and membrane dynamics during endocytosis. Curr. Opin. Cell Biol. *14*, 76–81.
56. Vogler, O., Krummenerl, P., Schmidt, M., Jakobs,, K.H. and Van Koppen, C.J. (1999). RhoA-sensitive trafficking of muscarinic acetylcholine receptors. J Pharmacol. Exp. Ther. *288*, 36–42.
57. Hrboticky, N., Feldmeer, T., Essler, M., Wiedemann, A., and Aepfelbacher, M. (2002). Involvement of the GTPase Rho in the cellular uptake of low density lipoprotein by human skin fibroblasts. Biochim. Biophys. Acta *1580*, 123–132.
58. Gampel, A., Parker, P.J., and Mellor, H. (1999). Regulation of epidermal growth factor receptor traffic by the small GTPase rhoB. Curr. Biol. *9*, R955–R958.
59. Qualmann, B., and Mellor, H. (2003). Regulation of endocytic traffic by Rho GTPases. Biochem. J., in press.

60. Michaelson, D., Silletti, J., Murphy, G., D'Eustachio, P., Rush, M., and Philips, M.R. (2001). Differential localization of Rho GTPases in live cells: regulation by hypervariable regions and RhoGDI binding. J. Cell Biol. *152*, 111–126.

61. Adamson, P., Paterson, H.F., and Hall, A. (1992). Intracellular localization of the P21rho proteins. J. Cell Biol. *119*, 617–627.

62. Robertson, D., Paterson, H.F., Adamson, P., Hall, A., and Monaghan, P. (1995). Ultrastructural localization of ras-related proteins using epitope-tagged plasmids. J. Histochem. Cytochem. *43*, 471–480.

63. Mellor, H., Flynn, P., Nobes, C.D., Hall, A., and Parker, P.J. (1998). PRK1 is targeted to endosomes by the small GTPase, RhoB. J. Biol. Chem. *273*, 4811–4814.

64. Leung, S.M., Rojas, R., Maples, C., Flynn, C., Ruiz, W.G., Jou, T.S., and Apodaca, G. (1999). Modulation of endocytic traffic in polarized Madin-Darby canine kidney cells by the small GTPase RhoA. Mol. Biol. Cell *10*, 4369–4384.

65. Jou, T.S., Leung, S.M., Fung, L.M., Ruiz, W.G., Nelson, W.J., and Apodaca, G. (2000). Selective alterations in biosynthetic and endocytic protein traffic in Madin-Darby canine kidney epithelial cells expressing mutants of the small GTPase Rac1. Mol. Biol. Cell *11*, 287–304.

66. Teo, M., Tan, L., Lim, L., and Manser, E. (2001). The tyrosine kinase ACK1 associates with clathrin-coated vesicles through a binding motif shared by arrestin and other adaptors. J. Biol. Chem. *276*, 18392–18398.

67. Yang, W., Lo, C.G., Dispenza, T., and Cerione, R.A. (2001). The Cdc42 target ACK2 directly interacts with clathrin and influences clathrin assembly. J. Biol. Chem. *276*, 17468–17473.

68. Hopper, N.A., Lee, J., and Sternberg, P.W. (2000). ARK-1 inhibits EGFR signaling in C. elegans. Mol. Cell *6*, 65–75.

69. Worby, C.A., Simonson-Leff, N., Clemens, J.C., Huddler, D., Jr., Muda, M., and Dixon, J.E. (2002). Drosophila Ack targets its substrate, the sorting nexin DSH3PX1, to a protein complex involved in axonal guidance. J. Biol. Chem. *277*, 9422–9428.

70. Lin, Q., Lo, C.G., Cerione, R.A. and Yang.W. (2002). The Cdc42 target ACK2 interacts with sorting nexin 9 (SH3PX1) to regulate epidermal growth factor receptor degradation. J. Biol. Chem. *277*, 10134–10138.

71. Worby, C.A., and Dixon, J.E. (2002). Sorting out the cellular functions of sorting nexins. Nat. Rev. Mol. Cell Biol. *3*, 919–931.

72. Worby, C.A., Simonson-Leff, N., Clemens, J.C., Kruger, R.P., Muda, M., and Dixon, J.E. (2001). The sorting nexin, DSH3PX1, connects the axonal guidance receptor, Dscam, to the actin cytoskeleton. J. Biol. Chem. *276*, 41782–41789.

73. Buday, L., Wunderlich, L., and Tamas, P. (2002). The Nck family of adapter proteins: regulators of actin cytoskeleton. Cell. Signal. *14*, 723–731.

74. Suetsugu, S., Miki, H., and Takenawa, T. (2002). Spatial and temporal regulation of actin polymerization for cytoskeleton formation through Arp2/3 complex and WASP/WAVE proteins. Cell Motil. Cytoskeleton *51*, 113–122.

75. Hussain, N.K., Jenna, S., Glogauer, M., Quinn, C.C., Wasiak, S., Guipponi, M., Antonarakis, S.E., Kay, B.K., Stossel, T.P., and Lamarche-Vane, N. (2001). Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP. Nat. Cell Biol. *3*, 927–932.

76. Hussain, N.K., Yamabhai, M., Ramjaun, A.R., Guy, A.M., Baranes, D., O'Bryan, J.P., Der, C.J., Kay, B.K., and McPherson, P.S. (1999). Splice variants of intersectin are components of the endocytic machinery in neurons and nonneuronal cells. J. Biol. Chem. *274*, 15671–15677.

77. Irie, F., and Yamaguchi, Y. (2002). EphB receptors regulate dendritic spine development via intersectin, Cdc42 and N-WASP. Nat. Neurosci. *5*, 1117–1118.

78. Matus, A. (2000). Actin-based plasticity in dendritic spines. Science *290*, 754–758.

79. Simons, K., and Toomre, D. (2000). Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol. *1*, 31–39.

80. Lamaze, C., Dujeancourt, A., Baba, T., Lo, C.G., Benmerah, A., and Dautry-Varsat, A. (2001). Interleukin 2 receptors and detergent-resistant membrane domains define a clathrin-independent endocytic pathway. Mol. Cell *7*, 661–671.

81. Sabharanjak, S., Sharma, P., Parton, R.G., and Mayor, S. (2002). GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. Dev. Cell *2*, 411–423.

82. Doussau, F., and Augustine, G.J. (2000). The actin cytoskeleton and neurotransmitter release: an overview. Biochimie *82*, 353–363.

83. Oheim, M., and Stuhmer, W. (2000). Interaction of secretory organelles with the membrane. J. Membr. Biol. *178*, 163–173.

84. Bader, M.F., Holz, R.W., Kumakura, K., and Vitale, N. (2002). Exocytosis: the chromaffin cell as a model system. Ann. N. Y. Acad. Sci. *971*, 178–183.

85. Vitale, N., Gasman, S., Caumont, A.S., Gensse, M., Galas, M.C., Chasserot-Golaz, S and Bader, M.F. (2000). Insight in the exocytotic process in chromaffin cells: regulation by trimeric and monomeric G proteins. Biochimie *82*, 365–373.

86. Pinxteren, J.A., O'Sullivan, A.J., Larbi, K.Y., Tatham, P.E., and Gomperts, B.D. (2000). Thirty years of stimulus-secretion coupling: from Ca(2+) toGTP in the regulation of exocytosis. Biochimie *82*, 385–393.

87. Ridley, A.J. (2001). Rho proteins: linking signaling with membrane trafficking. Traffic *2*, 303–310.

88. Hong-Geller, E., and Cerione, R.A. (2000). Cdc42 and Rac stimulate exocytosis of secretory granules by activating the IP(3)/calcium pathway in RBL-2H3 mast cells. J. Cell Biol. *148*, 481–494.

89. Rettig, J., and Neher, E. (2002). Emerging roles of presynaptic proteins in Ca++-triggered exocytosis. Science *298*, 781–785.

90. Hong-Geller, E., Holowka, D., Siraganian, R.P., Baird, B., and Cerione, R.A. (2001). Activated Cdc42/Rac reconstitutes Fcepsilon RI-mediated Ca2+ mobilization and degranulation in mutant RBL mast cells. Proc. Natl. Acad. Sci. USA *98*, 1154–1159.

91. Sullivan, R., Price, L.S., and Koffer, A. (1999). Rho controls cortical F-actin disassembly in addition to, but independently of, secretion in mast cells. J. Biol. Chem. *274*, 38140–38146.

92. Holowka, D., Sheets, E.D., and Baird, B. (2000). Interactions between Fc(epsilon)RI and lipid raft components are regulated by the actin cytoskeleton. J. Cell Sci. *113*, 1009–1019.

93. Saltiel, A.R., and Pessin, J.E. (2002). Insulin signaling pathways in time and space. Trends Cell Biol. *12*, 65–71.

94. Chiang, S.H., Baumann, C.A., Kanzaki, M., Thurmond, D.C., Watson, R.T., Neudauer, C.L., Macara, I.G., Pessin, J.E., and Saltiel, A.R. (2001). Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. Nature *410*, 944–948.

95. Watson, R.T., Shigematsu, S., Chiang, S.H., Mora, S., Kanzaki, M., Macara, I.G., Saltiel, A.R., and Pessin, J.E. (2001). Lipid raft microdomain compartmentalization of TC10 is required for insulin signaling and GLUT4 translocation. J. Cell Biol. *154*, 829–840.

96. Kanzaki, M., Watson, R.T., Khan, A.H., and Pessin, J.E. (2001). Insulin stimulates actin comet tails on intracellular GLUT4-containing compartments in differentiated 3T3L1 adipocytes. J. Biol. Chem. *276*, 49331–49336.

97. Jiang, Z.Y., Chawla, A., Bose, A., Way, M., and Czech, M.P. (2002). A phosphatidylinositol 3-kinase-independent insulin signaling pathway to N-WASP/Arp2/3/F-actin required for GLUT4 glucose transporter recycling. J. Biol. Chem. *277*, 509–515.

98. Tian, L., Nelson, D.L., and Stewart, D.M. (2000). Cdc42-interacting protein 4 mediates binding of the Wiskott-Aldrich syndrome protein to microtubules. J. Biol. Chem. *275*, 7854–7861.

99. Chang, L., Adams, R.D., and Saltiel, A.R. (2002). The TC10-interacting protein CIP4/2 is required for insulin-stimulated Glut4 translocation in 3T3L1 adipocytes. Proc. Natl. Acad. Sci. USA *99*, 12835–12840.

100. 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.

101. 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.

102. Guo, W., Tamanoi, F., and Novick, P. (2001). Spatial regulation of the exocyst complex by Rho1 GTPase. Nat. Cell Biol. *3*, 353–360.

103. Adamo, J.E., Moskow, J.J., Gladfelter, A.S., Viterbo, D., Lew, D.J., and Brennwald, P.J. (2001). Yeast Cdc42 functions at a late step in exocytosis, specifically during polarized growth of the emerging bud. J. Cell Biol. *155*, 581–592.

104. Zhang, X., Bi, E., Novick, P., Du, L., Kozminski, K.G., Lipschutz, J.H., and Guo, W. (2001). Cdc42 interacts with the exocyst and regulates polarized secretion. J. Biol. Chem. *276*, 46745–46750.

105. Robinson, N.G., Guo, L., Imai, J., Toh, E., 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.

106. Hsu, S.C., Ting, A.E., Hazuka, C.D., Davanger, S., Kenny, J.W., Kee, Y., and Scheller, R.H. (1996). The mammalian brain rsec6/8 complex. Neuron *17*, 1209–1219.

107. Hazuka, C.D., Foletti, D.L., Hsu, S.C., Kee, Y., Hopf, F.W., and Scheller, R.H. (1999). The sec6/8 complex is located at neurite outgrowth and axonal synapse-assembly domains. J. Neurosci. *19*, 1324–1334.

108. 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.

109. Matern, H.T., Yeaman, C., Nelson, W.J., and Scheller, R.H. (2001). The Sec6/8 complex in mammalian cells: characterization of mammalian Sec3, subunit interactions, and expression of subunits in polarized cells. Proc. Natl. Acad. Sci. USA *98*, 9648–9653.

110. Lipschutz, J.H. and Mostov, K.E. (2002). Exocytosis: the many masters of the exocyst.Curr.Biol 2002.Mar. *12*: R212-R214.

111. Novick, P., and Guo, W. (2002). Ras family therapy: Rab, Rho and Ral talk to the exocyst. Trends Cell Biol. *12*, 247–249.

112. Schmitz, A.A., Govek, E.E., Bottner, B. and Van Aelst, L. (2000). Rho GTPases: signaling, migration, and invasion. Exp. Cell Res. *261*, 1–12.

113. Etienne-Manneville, S., and Hall, A. (2002). Rho GTPases in cell biology. Nature *420*, 629–635.

114. Heath, J.P., and Holifield, B.F. (1991). Cell locomotion: new research tests old ideas on membrane and cytoskeletal flow. Cell Motil. Cytoskeleton *18*, 245–257.

115. Bretscher, M.S., and Aguado-Velasco, C. (1998). Membrane traffic during cell locomotion. Curr. Opin. Cell Biol. *10*, 537–541.

116. Roberts, M., Barry, S., Woods, A. van der Sluijs, S.P. and Norman, J. (2001). PDGF-regulated rab4-dependent recycling of alphavbeta3 integrin from early endosomes is necessary for cell adhesion and spreading. Curr.Biol. *11*, 1392–1402.

117. Pierini, L.M., Lawson, M.A., Eddy, R.J., Hendey, B., and Maxfield, F.R. (2000). Oriented endocytic recycling of alpha5beta1 in motile neutrophils. Blood *95*, 2471–2480.

118. Kamiguchi, H., and Yoshihara, F. (2001). The role of endocytic l1 trafficking in polarized adhesion and migration of nerve growth cones. J. Neurosci. *21*, 9194–9203.

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

120. Tsubakimoto, K., Matsumoto, K., Abe, H., Ishii, J., Amano, M., Kaibuchi, K., and Endo, T. (1999). Small GTPase RhoD suppresses cell migration and cytokinesis. Oncogene *18*, 2431–2440.

121. Murphy, C., Saffrich, R., Olivo-Marin, J.C., Giner, A., Ansorge, W., Fotsis, T., and Zerial, M. (2001). Dual function of rhoD in vesicular movement and cell motility. Eur. J. Cell Biol. *80*, 391–398.

122. Jiang, A., Lehti, K., Wang, X., Weiss, S.J., Keski-Oja, J., and Pei, D. (2001). Regulation of membrane-type matrix metalloproteinase 1 activity by dynamin-mediated endocytosis. Proc. Natl. Acad. Sci. USA *98*, 13693–13698.

123. Hornebeck, W., Emonard, H., Monboisse, J.C., and Bellon, G. (2002). Matrix-directed regulation of pericellular proteolysis and tumor progression. Semin. Cancer Biol. *12*, 231–241.

124. Van Aelst, L., and Symons, M. (2002). Role of Rho family GTPases in epithelial morphogenesis. Genes Dev. *16*, 1032–1054.

125. Le, T.L., Yap, A.S., and Stow, J.L. (1999). Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. J. Cell Biol. *146*, 219–232.

126. Kamei, T., Matozaki, T., Sakisaka, T., Kodama, A., Yokoyama, S., Peng, Y.F., Nakano, K., Takaishi, K., and Takai, Y. (1999). Coendocytosis of cadherin and c-Met coupled to disruption of cell-cell adhesion in MDCK cells—regulation by Rho, Rac and Rab small G proteins. Oncogene *18*, 6776–6784.

127. Akhtar, N., and Hotchin, N.A. (2001). RAC1 regulates adherens junctions through endocytosis of E-cadherin. Mol. Biol. Cell *12*, 847–862.

128. Stowers, L., Yelon, D., Berg, L.J., and Chant, J. (1995). Regulation of the polarization of T cells toward antigen-presenting cells by Ras-related GTPase CDC42. Proc. Natl. Acad. Sci. USA *92*, 5027–5031.

129. Kroschewski, R., Hall, A., and Mellman, I. (1999). Cdc42 controls secretory and endocytic transport to the basolateral plasma membrane of MDCK cells. Nat. Cell Biol. *1*, 8–13.

130. Musch, A., Cohen, D., Kreitzer, G., and Rodriguez-Boulan, E. (2001). cdc42 regulates the exit of apical and basolateral proteins from the trans-Golgi network. EMBO J. *20*, 2171–2179.

131. Rojas, R., Ruiz, W.G., Leung, S.M., Jou, T.S., and Apodaca, G. (2001). Cdc42-dependent modulation of tight junctions and membrane protein traffic in polarized madin-darby canine kidney cells. Mol. Biol. Cell *12*, 2257–2274.

132. Sahai, E., and Marshall, C.J. (2002). Rho GTPases and cancer. Nat. Rev. Cancer. *2*, 133–142.

133. Wu, W.J., Erickson, J.W., Lin, R., and Cerione, R.A. (2000). The gamma-subunit of the coatomer complex binds Cdc42 to mediate transformation. Nature *405*, 800–804.

134. Allan, B.B., and Balch, W.E. (1999). Protein sorting by directed maturation of Golgi compartments. Science *285*, 63–66.

135. Luna, A., Matas, O.B., Martinez-Menarguez, J.A., Mato, E., Duran, J.M., Ballesta, J., Way, M. and Egea, G. (2002). Regulation of protein transport from the Golgi complex to the endoplasmic reticulum by CDC42 and N-WASP Mol. Biol. Cell *13*, 866–879.

136. Palmer, R.E., Lee, S.B., Wong, J.C., Reynolds, P.A., Zhang, H., Truong, V., Oliner, J.D., Gerald, W.L., and Haber, D.A. (2002). Induction of BAIAP3 by the EWS-WT1 chimeric fusion implicates regulated exocytosis in tumorigenesis. Cancer Cell *2*, 497–505.

137. Narayanan, R., and Ramaswami, M. (2001). Endocytosis in Drosophila: progress, possibilities, prognostications. Exp. Cell Res. *271*, 28–35.

138. Deitcher, D. (2002). Exocytosis, endocytosis, and development. Semin. Cell Dev Biol. *13*, 71–76.