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Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking

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Rho GTPases are well known to regulate actin dynamics. They activate two types of actin nucleators, WASP/WAVE proteins and Diaphanous-related formins (DRFs), which induce different types of actin organization. Their ability to interact with membranes allows them to target actin polymerization to discrete sites on the plasma membrane and to intracellular membrane compartments and thereby induce membrane protrusions or regulate vesicle movement. Most studies have concentrated on just three of the 22 mammalian Rho proteins, RhoA, Rac1 and Cdc42. However, recent research indicates that several other members of the Rho family, including Rif, RhoD, TC10 and Wrch1, and also related Rho-of-plants proteins (ROPs) in plants, stimulate actin polymerization and affect plasma membrane protrusion and/or vesicular traffic.

Introduction

Rho GTPases are found in all eukaryotic organisms and regulate cell polarity and motility through their effects on the cytoskeleton, membrane trafficking and cell adhesion [1,2]. In mammals there are 22 Rho GTPases (Table 1), many of which affect cell morphology [1,3,4]. In yeasts and plants, several Rho GTPases contribute directly to polarized cell growth by affecting the actin cytoskeleton [5–7].

Most Rho GTPases act on membranes and affect the movement of these membranes by changing the membrane-associated actin cytoskeleton. The best-studied members of the family are RhoA, Rac1 and Cdc42, and in mammalian cells Rac1 and Cdc42 have long been known to induce plasma membrane protrusions known as lamellipodia and filopodia by stimulating actin polymerization. Other Rho GTPases, including RhoG, RhoD, TC10 and Rif (also called RhoF), can also induce actin-based protrusions at the plasma membrane [3,8,9]. On the other hand, several Rho GTPases, including RhoA, RhoB, RhoD and Cdc42, affect specific steps of vesicle trafficking between different intracellular compartments, for example exocytosis or Golgi-to-endoplasmic reticulum (ER) transport and, as with plasma membrane protrusion, their ability to induce actin polymerization is important for these activities [10,11].

Here, I discuss the regulation and localization of Rho GTPases, how they induce different types of actin filament organization and how this in turn affects both plasma membrane protrusion and vesicle trafficking.

Regulation and localization of Rho GTPases

Most Rho family proteins can bind to GTP and GDP and have intrinsic GTPase activity. In their GTP-bound conformation they interact with and activate downstream target proteins. Their activity is regulated by guanine nucleotide exchange factors (GEFs), which stimulate release of GDP, allowing GTP to bind. They are downregulated by GTPase-activated proteins (GAPs), which catalyse GTP hydrolysis, converting the proteins to the GDP-bound inactive conformation. Exceptions are Rnd1, Rnd2, Rnd3 (also called RhoE) and RhoH (also called TTF), which exhibit no or low GTPase activity because they lack the key amino acids required to catalyse this reaction [12]. They are therefore not regulated by classical GEFs or GAPs and might instead be regulated by expression levels and/or phosphorylation [12–14].

To affect membrane dynamics, Rho GTPases need to act on membranes, and indeed they are usually activated on membranes by GEFs [15]. Post-translational modifications are critical for their interaction with membranes. Most Rho family proteins are post-translationally modified by prenylation (farnesylation or geranylgeranylation) and in some cases palmitoylation at the C-terminus, enhancing their interaction with membranes and often defining their localization to specific membrane compartments [4] (Table 1, Box 1). For example, the different modifications on RhoB and RhoA are crucial for their distinct localizations (Table 1) [16]. Several family members also have a polybasic domain close to the C-terminus, which contributes to their membrane association [4,17].

Rho guanine nucleotide dissociation inhibitors (RhoGDIs) bind to some but not all Rho GTPases and prevent their interaction with membranes by masking the prenyl group as well as inhibiting them from binding to downstream targets [18,19]. Mammals have three RhoGDIs,

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Table 1. Localization and modification of mammalian Rho GTP-binding proteins[a]

Rho GTPase	Official symbol (human)[b]	Localization	Post-translational modifications
RhoA	RHOA	PM and cytosol [16]	GG and PH [23,105]
RhoB	RHOB	PM and endosomes [16]	GG, F and P [105]
RhoC	RHOC	PM and cytosol [16]	GG [105]
RhoD	RHOD	PM and endosomes [97]	GG[c]
Rif	RHOF	PM [8]	GG[c]
Rnd1	RND1	PM [98]	F[c]
Rnd2	RND2	endosomes and cytosol? [99]	F[c]
Rnd3/RhoE	RND3	PM, Golgi and cytosol [13]	F and PH [14,106]
RhoH/TTF	RHOH	?	GG[c]
Rac1	RAC1	PM [18]	GG [107]
Rac2	RAC2	PM and cytosol (Knaus, 1991)	GG [107]
Rac3	RAC3	PM and endomembranes [100]	GG [100]
RhoG	RHOG	PM and endosomes [101]	GG[c]
Cdc42	CDC42	PM and Golgi [63]	GG[c]
TC10	RhoQ	PM and perinuclear [9]	F and P[c]
TCL	RHOJ	PM and endosomes [76]	F and P[c]
Wrch1	RHOV	PM and endomembranes [102]	P [102]
Chp/Wrch2	RHOU	PM and endomembranes [103]	P [103]
RhoBTB1	RHOBTB1	Vesicular[d] [3]	None known
RhoBTB2	RHOBTB2	Vesicular[d] [3]	None known
Miro1	RHOT1	Mitochondria [104]	None known
Miro2	RHOT2	Mitochondria [104]	None known

[a]Abbreviations: PM, plasma membrane; GG, geranylgeranylation; F, farnesylation; P, palmitoylation; PH, phosphorylation.
[b]Official symbol provided by HUGO gene nomenclature committee.
[c]Predicted.
[d]Localization not defined.

RhoGDIα, β and γ [20]. RhoGDIs could also target Rho GTPases to specific membrane compartments and/or protein complexes. For example, RhoGDIα is important for localization of Cdc42 to the plasma membrane [21]. RhoGDIγ (also known as RhoGDI-3) is localized to the Golgi and appears to target RhoG to this site [22], although what RhoG does at the Golgi is not known. RhoGDIα is itself phosphorylated by multiple kinases, either enhancing or inhibiting its interaction with Rho GTPases depending on the phosphorylation site [20]

The activity and localization of some Rho family proteins is regulated by phosphorylation. For example, RhoA is phosphorylated near the C-terminus by protein kinase A, and this prevents its association with the plasma membrane, enhances its affinity for RhoGDI, and protects it from degradation [23–25]. Rnd3/RhoE is phosphorylated by ROCK-I, which shifts its localization from membranes to the cytosol [14].

Box 1. Schematic of Rho GTPases

The core structure of Rho GTPases is shown in Figure I. Note that some Rho GTPases have an N-terminal extension beyond the core structure, and/or the length of the C-terminal hypervariable region is longer. RhoBTB and Miro proteins have additional domains. The effector domain changes conformation between GTP- and GDP-bound Rho proteins and is usually required for binding to downstream targets. The hypervariable region is very different between the three Rho or Rac isoforms and can include a polybasic region and/or sites for palmitoylation. The CAAX box contains a cysteine residue that becomes prenylated (farnesyl or geranylgeranyl group), and subsequently the remaining three amino acids (two aliphatic amino acids and a variable C-terminal amino acid) are removed and replaced with a methyl group.

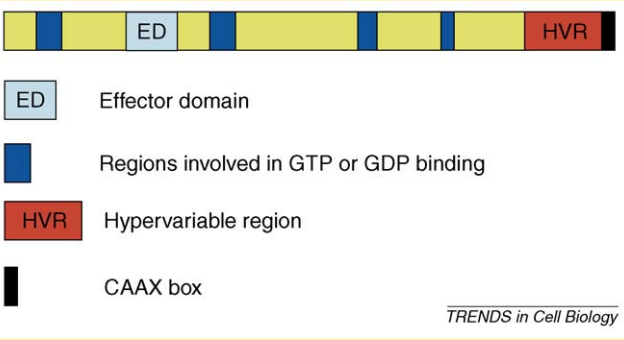

Figure I. The primary structure of Rho GTPases.

Rho GTPases and actin polymerization

Rho GTPases activate two different kinds of molecules that directly stimulate actin polymerization, WASP/WAVE proteins and Diaphanous-related formins (DRFs; Box 2). Although RhoA, Rac1 and Cdc42 have been most studied for their effects on these proteins, other Rho GTPases interact with DRFs (Table 2). WASP/WAVE proteins induce actin polymerization via the Arp2/3 complex, which stimulates the formation of a new actin filament branching off an existing filament (Box 2) [26]. Cdc42 binds directly to WASP and N-WASP and stimulates their activation of the Arp2/3 complex [27]. The ability of WASP proteins to stimulate actin polymerization is also regulated by phosphorylation and by protein–protein interactions, for example they bind via their proline-rich region to the Src homology 3 (SH3) domain of cortactin, a well known regulator of both actin dynamics and endocytosis [27,28]. Rac indirectly activates WAVE proteins through the multi-protein WAVE complex (Box 2; Table 2) [29].

The DRFs Dia1, Dia2 and Dia3 stimulate the nucleation and extension of non-branching actin filaments. They bind as dimers at the barbed (or plus) end of actin filaments, preventing the binding of capping proteins, which normally terminate actin polymerization [30]. Each DRF is

Box 2. Actin nucleators activated by Rho GTPases

An actin nucleator can initiate the assembly of a new actin filament from actin monomers.

The Arp2/3 complex

This is a stable complex of seven proteins, including two actin-related proteins (Arp2 and Arp3). It binds to the sides of existing actin filaments and induces polymerization of actin to form a branching filament network. The Arp2/3 complex has a very low actin nucleating activity in the absence of an activator.

WASP/WAVE family

The Wiskott–Aldrich syndrome (WASP) proteins and WASP family Verprolin-homologous (WAVE) proteins activate the Arp2/3 complex. They have a C-terminal verprolin–cofilin–acidic (VCA) domain that binds directly to the Arp2/3 complex. The N-terminal region includes several protein–protein interaction domains.

WASP and N-WASP

These have 'CRIB' domains that bind directly and quite specifically to Cdc42. Cdc42 binding unfolds the proteins so that the VCA domain is accessible to Arp2/3 complex. WASP is expressed only in haematopoietic cells, whereas N-WASP is ubiquitously expressed. WASP proteins bind to a variety of proteins that regulate their ability to stimulate actin polymerization and/or link them to endocytic or exocytic pathways.

WAVE1–3 (also known as SCAR1–3)

These proteins are part of a multi-protein WAVE complex that includes Abi1, Sra1/PIR121, Nap/Kette and HSPC300. Rac binds to Sra1 and this leads to activation of the Arp2/3 complex-dependent nucleating activity of WAVE1–3. WAVE proteins are also regulated by IRSp53, which binds to Rac and Cdc42 and can associate with the WAVE complex.

Diaphanous-related formins Dia1–3 (also known as DIAPH1–3 or DRF1–3)

These proteins have an actin-nucleating region known as the FH2 domain, which nucleates parallel, unbranched actin filaments. Rho GTPases bind near the N-terminus, unfolding the proteins and stimulating their nucleating activity.

activated by a distinct subset of Rho GTPases (Box 2, Table 2) [31,32].

Rho proteins can also affect actin polymerization by regulating cofilin, an actin filament-severing and actin-depolymerizing factor. LIM kinases (LIMKs) directly phosphorylate cofilin and thereby inactivate it, leading to an increase in polymerized actin [33,34]. The Rho target ROCK and Rac/Cdc42-activated PAKs in turn phosphorylate and activate LIMKs (Figure 1, Table 2). PAK1–3 are somewhat promiscuous targets for Rho GTPases: most of the closest relatives of Rac1 and Cdc42 [4] have been reported to activate at least one of these kinases (Table 2), implying that they should all act via LIMKs to inactivate cofilin.

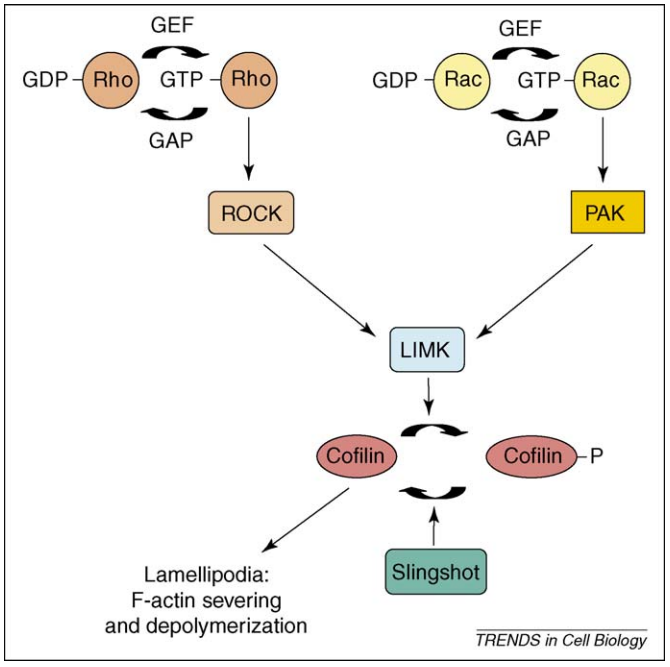

Figure 1. Regulation of cofilin by Rho and Rac. Rho and Rac are activated by exchange of GDP for GTP, catalysed by guanine nucleotide exchange factors (GEFs). They are inactivated by GTP hydrolysis, catalysed by GTPase-activating proteins (GAPs). In their active, GTP-bound conformation, Rho isoforms (RhoA, RhoB and RhoC) bind to and activate ROCK serine/threonine kinases (ROCK-1 and ROCK-2), whereas Rac isoforms (Rac1, Rac2 and Rac3) bind to and activate PAK serine/threonine kinases (PAK1, PAK2 and PAK3). Other Rho GTPases also activate PAKs (Table 2). ROCKs and PAKs can then phosphorylate and activate LIMKs (LIMK1 and LIMK2), which in turn phosphorylate and inactivate cofilin. Cofilin is dephosphorylated by the phosphatase Slingshot. Unphosphorylated cofilin stimulates severing and depolymerization of filamentous actin (F-actin) in lamellipodia.

Other downstream targets of Rho proteins also have the potential to affect actin polymerization, albeit indirectly. For example, Rho and Rac associate with PtdIns(4)*P* 5-kinase isoforms, which catalyse the formation of phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P_2). PtdIns(4,5)P_2 affects actin dynamics at the plasma membrane by altering the activity of PtdIns(4,5)P_2-regulated actin-binding proteins, such as cofilin and gelsolin [35]. Given that there is a large amount of PtdIns(4,5)P_2 in the plasma membrane, localized production or hydrolysis of PtdIns(4,5)P_2 in specialized membrane microdomains is

Table 2. Some Rho GTPase targets that regulate actin polymerization

Rho GTPase target	Function	Rho GTPase
WASP and N-WASP	Activator of Arp2/3 complex	Cdc42 (TCL, TC10[a])
Sra1	Part of WAVE protein complex: regulates Arp2/3 complex	Rac1[b]
IRSp53	Binds WAVE proteins; actin filament bundling	Rac1[b], Cdc42
Dia1	Actin nucleator	RhoA, RhoB and RhoC[b]
Dia2	Actin nucleator	RhoA, Cdc42 and Rif
Dia3	Actin nucleator	Cdc42 and RhoD
PAK (1–3)	Phosphorylation of multiple cytoskeletal proteins, including LIMK1 and LIMK2[c]	Rac1, Rac2, Rac3, Wrch1, Chp/Wrch2, Cdc42, TC10 and TCL
ROCK (1 and 2)	Phosphorylation of multiple cytoskeletal proteins, including LIMK1 and LIMK2[c] and MYPT[d]	RhoA, RhoB, RhoC and Rnd3/RhoE

[a]The affinity of TC10 for WASP is nearly 1000-fold weaker than that of Cdc42 [108].
[b]Only the mammalian isoforms of Rac and Rho that have been shown to interact with the actin regulator are listed.
[c]PAKs and ROCKs act via LIMKs to phosphorylate cofilin, which severs actin filaments.
[d]MYPT is a component of myosin light chain phosphatase.

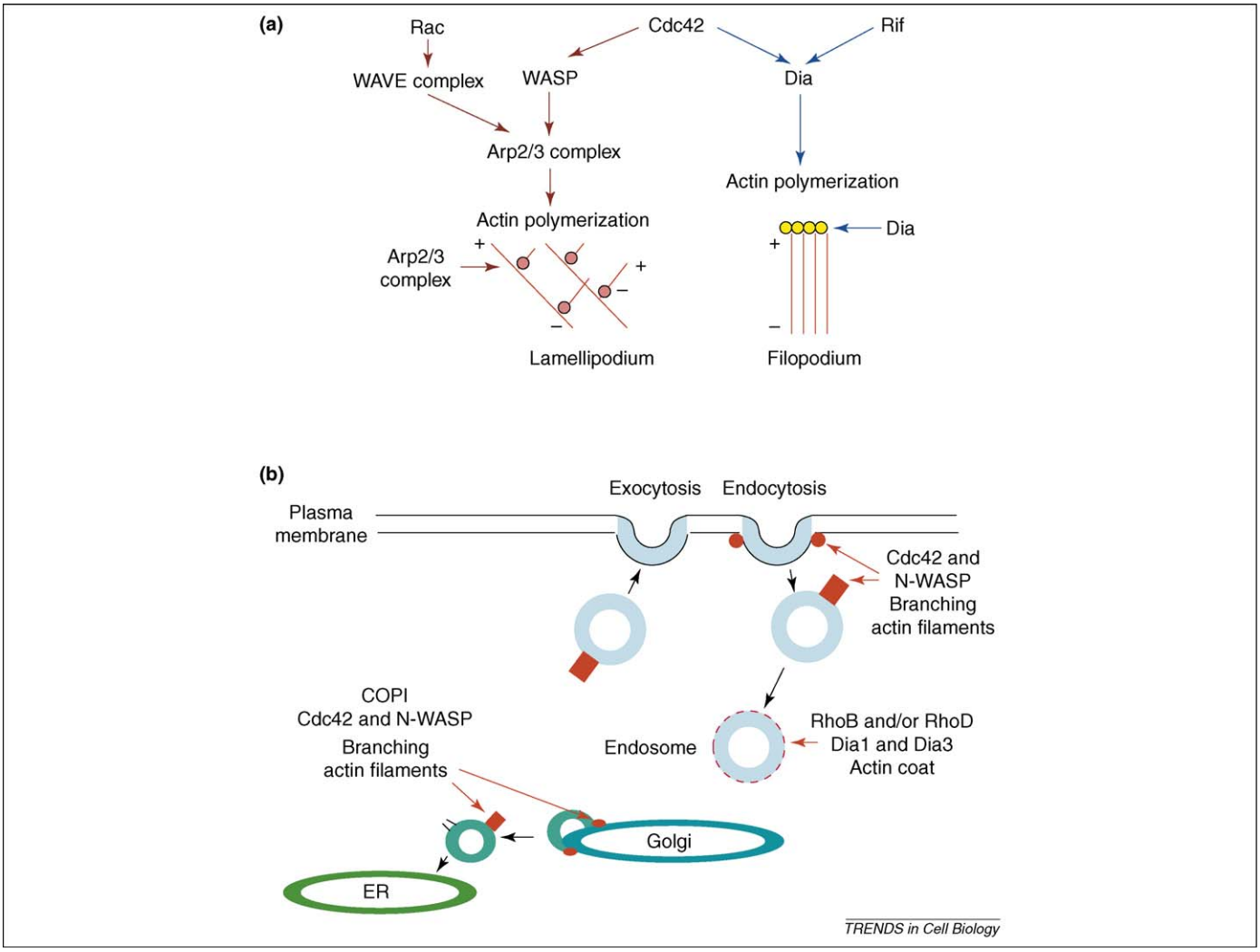

Figure 2. Rho family proteins, actin filaments and membrane dynamics. **(a)** At the plasma membrane Rho GTPases stimulate membrane protrusions through actin polymerization. Rac activates the WAVE protein complex, leading to Arp2/3 complex-mediated actin polymerization to form a branching actin filament network in lamellipodia, where the Arp2/3 complex induces a new filament to polymerize from the side of an existing filament. Cdc42 might also contribute to lamellipodial extension through WASP proteins, which activate the Arp2/3 complex. Cdc42 and Rif activate the DRFs Dia1 and/or Dia2, which bind to the barbed (+) ends of filaments and induce actin polymerization in parallel bundles at the plasma membrane, forming filopodia. + indicates barbed ends, – indicates pointed ends of filaments. **(b)** On endomembranes, Cdc42 stimulates formation of a branching actin filament network through N-WASP and WASP during endocytosis and exocytosis of vesicles at the plasma membrane, and traffic of vesicles from the Golgi to the endoplasmic reticulum (ER). This actin polymerization might assist the process in which a vesicle pinches off the source membrane, and/or might help drive the vesicle towards the target membrane, acting in concert with myosin motors. RhoB and/or RhoD are hypothesized to stimulate polymerization of an actin coat on endosomes via Dia1 and Dia3, thereby slowing endosomal dynamics.

presumably needed to regulate the activity of these proteins.

Rho GTPases and plasma membrane dynamics

Rho GTPases are believed to stimulate plasma membrane protrusion by inducing actin filament nucleation and polymerization on or close to membranes. This process has been studied extensively *in vitro* using beads or membrane vesicles with portions of actin-nucleating proteins attached [26].

Lamellipodia

Lamellipodia are broad sheet-like protrusions containing a network of branching actin filaments and are found at the front of migrating cells. Cdc42 and Rac are both active at the leading edge of cells, and inhibition of each reduces lamellipodium extension [36,37]. Their target WAVE and N-WASP proteins are localized at the front of lamellipodia, but although the requirement of WAVEs for lamellipodium extension is clear, the involvement of N-WASP is controversial [38–40]. Given its role in exocytosis (see below), it is possible that N-WASP acts indirectly by stimulating delivery of proteins and membrane lipids required for membrane extension to the leading edge, rather than directly stimulating actin polymerization in lamellipodia (Figure 2).

Cofilin seems to act very early in the initiation of lamellipodium extension, severing existing filaments to generate new actin filament ends for the Arp2/3 complex to use. Rho GTPases probably act through LIMKs to turn down this response and limit cofilin severing of filaments [33,34,41] (Figure 1). On the other hand, cofilin is also thought to remove actin monomers from the pointed (minus) ends of actin filaments in lamellipodia, allowing recycling of actin monomers to the leading edge [42]. Phosphorylation of cofilin would reduce this process, and

thus LIMK activity should be restricted to a very limited region of the lamellipodium.

RhoG also has an important role in lamellipodia, perhaps through activation of Rac through a RacGEF, the ELMO–DOCK180 complex [43,44]. RhoG does not interact with PAKs or IRSp53, but whether it binds to Sra1 or regulates WAVEs has not been tested (Table 2). RhoG, Rac and Cd42 are also required for phagocytosis, in which the plasma membrane extends around a microorganism or apoptotic cell using a similar mechanism to lamellipodium extension [44,45].

RhoA has always been assumed to act at the back of migrating cells to induce tail detachment, but recently active RhoA has been found to localize to membrane ruffles and lamellipodia under some conditions, where Dia1 but not ROCK appears to be its major target [46,47]. Exactly what RhoA does in lamellipodia or membrane ruffles in unclear, but it could contribute via Dia1 to actin polymerization at integrin-based focal adhesions [48].

Filopodia

Filopodia are finger-like membrane protrusions that contain parallel bundles of actin filaments. They are believed to be important for sensing the extracellular environment, either for soluble signals or for other cells [49]. Cdc42 has always been thought to be the main mediator of filopodium extension, so it was surprising that Cdc42-null fibroblastoid cells can still form filopodia [50]. However, Rif/RhoF, RhoD and Wrch1 can all induce filopodium extension [3,8,32] and might therefore substitute for Cdc42 in Cdc42-null cells. Recent evidence indicates that DRFs are the major mediators of actin polymerization in filopodia, both in mammalian cells and *Dictyostelium* [51], and Cdc42 and Rif induce filopodia through Dia2 [32,52]. Consistent with this, cells lacking WASP proteins have no apparent defects in filopodium extension [53]. It has been suggested that filopodia can form from actin filaments in lamellipodia [54], but lamellipodia are not essential for filopodium extension, because they can still form in cells where WAVE proteins have been knocked down by RNAi [55].

Microvilli

Microvilli are membrane protrusions that contain parallel bundles of actin filaments and can be rapidly disassembled in response to specific stimuli [56,57]. They are found on the surface of many cell types, for example microvilli on the epithelial cells of the gut are important for nutrient absorption. Ezrin–radixin–moesin (ERM) proteins are involved in microvillus formation, and Rho and Rac act antagonistically to regulate ERM phosphorylation and activity [57,58]. What nucleates actin filaments in microvilli is still unclear: WASP was thought to play a role [59], but functional microvilli still form in the absence of WASP in *Drosophila* rhabdomeres [60] and lymphocytes [61].

Rho GTPases and vesicle trafficking

Several Rho GTPases localize to specific intracellular membrane compartments in addition to or instead of the plasma membrane (Table 1), and for some of these there is good evidence that their principal site of action is likely to be on these compartments.

Cdc42 and Golgi-to-ER transport

Cdc42 but not RhoA or Rac1 localizes in part to the Golgi [62,63], together with its targets N-WASP, IQGAP and the Golgi vesicle coat protein, coatomer [10,64]. Cdc42 and N-WASP together regulate Golgi-to-ER transport [65]. Recently, secramine was identified as a small molecule inhibitor of Golgi-to-ER transport, and seems to act by inhibiting Cdc42 association with membranes [66]. Although it is not yet known whether secramine affects other Rho GTPases, it promises to be a useful reagent for future studies. By binding to coatomer, Cdc42 inhibits recruitment of the microtubule motor dynein to coatomer-associated Golgi vesicles, and hence microtubule-based transport of these vesicles [67]. Given that IQGAP binds directly to Cdc42 and actin filaments and is involved in microtubule capture [68], it will be interesting to know how it contributes to Golgi-to-ER transport.

Endocytosis and exocytosis

Cdc42 and N-WASP also regulate endocytosis and exocytosis [11,69] (Figure 2). The Tuba and intersectin families of Cdc42 GEFs, which also bind to N-WASP, could activate Cdc42 during the endocytic process [70,71]. Interestingly, one intersectin isoform, intersectin-1L, specifically couples to Cdc42 during exocytosis [72]. Cdc42, N-WASP and actin polymerization are thus central to multiple steps of vesicle trafficking, and Cdc42 might define a distinct phase of actin filament-dependent vesicle transport, possibly acting together with myosin motors, that needs to be functionally separated from microtubule-based transport.

The Cdc42 relatives TC10 and TCL seem to have very different functions in regulating protein trafficking. TC10 was found to regulate insulin-induced transport of the glucose transporter GLUT4 from intracellular compartments to the plasma membrane [73] and has also been implicated in targeting the cystic fibrosis transmembrane conductance regulator (CFTR) to the plasma membrane [74]. TC10 seems to act in lipid rafts and caveolae at the plasma membrane and on the secretory pathway and could regulate GLUT4 recruitment by affecting the actin cytoskeleton surrounding caveolae and/or by binding to the exocyst complex, which is involved in targeted exocytosis [75]. TCL, on the other hand, localizes to the plasma membrane and early endosomes and affects delivery of clathrin-dependent endocytosed receptors to early endosomes [76], although whether it acts through an actin nucleator such as N-WASP or a DRF (Box 1, Table 2) is not known.

Constitutively active RhoA and Rac1 mutants inhibit clathrin-mediated endocytosis [77], and in the case of Rac this could be via synaptojanin2, a polyphosphoinositide phosphatase that induces uncoating of clathrin vesicles, probably by removing its substrate PtdIns(4,5)P_2 [78]. For Rho, one possible mechanism is through ROCK-mediated phosphorylation of endophilin, a partner of synaptojanin also involved in vesicle uncoating [79].

Rho and Rac also affect clathrin-independent endocytosis, and this could take place through molecules that bridge them to ARF family GTPases, which are well known to regulate membrane dynamics [80]. These bridges include the GIT proteins, which are ARF GAPs and bind

to PIX, a GEF for Rac and Cdc42; and Arfaptins, which bind to Rac and ARFs [81]. RhoA and/or Cdc42 are connected to ARFs via ARAPs, which have both a RhoGAP and ArfGAP domain [82,83]. ARAP1 localizes to the Golgi, where it could potentially affect Cdc42 activity [82]. Another link between ARFs, Rho GTPases and membrane traffic is phospholipase D, which is required for regulated exocytosis, membrane delivery and constitutive membrane trafficking [84]. Phospholipase D generates the membrane lipid phosphatidic acid, which in turn activates PtdIns4*P* 5-kinase, leading to PtdIns(4,5)P_2 generation and thus potentially affecting actin dynamics (see earlier).

Endosomal dynamics

RhoB was the first member of the Rho family found to localize to intracellular membrane vesicles. It localizes to endosomes and delays the trafficking of membrane receptors, such as the EGF receptor, to late endosomes [85]. Two targets of Rho GTPases co-localize with RhoB: Dia1 and the protein kinase PRK1. Dia1 is required downstream of RhoB for recruitment of an actin coat to endosomes [86] (Figure 1), which could inhibit subsequent movement away from the cell periphery to the late endosomal compartment. Src is also found on RhoB-positive endosomes and could regulate actin polymerization on endosomes [87], but whether it does this via Dia1 is not known. PRK1 also acts downstream of RhoB to regulate EGF receptor trafficking [85], although its targets have not been identified.

Similar to RhoB, RhoD affects endosomal dynamics, and also acts through a DRF, a Dia3 splice variant, and Src [88]. Dia proteins bind to Src via its SH3 domain [89], potentially bringing Src together with its substrates and thereby affecting actin recruitment and endosomal dynamics. RhoG has also been reported to localize to caveolar endocytic vesicles [90], although its function there is unknown.

RhoBTB1 and RhoBTB2 are little-characterized members of the Rho family that both localize to vesicular structures (Table 1) [3]. RhoBTB proteins are present in *Dictyostelium* (called RacA), *Drosophila* and mammals, and contain BTB domains C-terminal to the GTPase domain [3]. BTB domains are protein–protein interaction modules found in a variety of proteins, including some transcription factors. Whether RhoBTBs affect membrane trafficking is not known, but RhoBTB2 is a candidate tumour suppressor (DBC2) and regulates the expression of genes involved in the actin cytoskeleton and membrane trafficking [91].

Rho GTPases and polarized membrane growth in plants

Studies on Rho homologues in plants have provided new insight into the links between the actin cytoskeleton and membrane dynamics. Plants have Rho-related proteins known as ROPs (Rho-of-plants) or RACs, several of which affect membrane trafficking [5,6]. Plants have homologues of mammalian Rho targets including WAVEs, formins, phospholipase D and NADPH oxidase, although it is not clear whether plant formins interact with ROPs [92]. In *Arabidopsis*, constitutively active AtRAC10 induces deformation of root and leaf cells by disrupting the actin cytoskeleton and membrane cycling [93]. Elongation of the pollen tube tip and growing root hairs depends on extremely rapid delivery of membrane and wall material and requires the actin cytoskeleton [6]. ROPs are proposed to stimulate growth and exocytosis through a combination of actin dynamics, Ca^{2+} influx and PtdIns(4)*P* 5-kinase stimulation to induce PtdIns(4,5)P_2 synthesis. ROPs also activate the NADPH oxidase and this might contribute to growth via Ca^{2+} influx [5,94]. It would therefore be interesting to know whether NADPH oxidases contribute to exocytosis in animals. Further research on plant ROPs is likely to provide novel insights into Rho GTPase function in multicellular eukaryotes.

Conclusions and future perspectives

By stimulating actin dynamics, Rho GTPases induce plasma membrane protrusion and regulate vesicle trafficking. The two types of Rho-regulated actin nucleators, WASPs/WAVEs and DRFs, induce a branching actin filament network or parallel filaments, respectively, and thus contribute to different types of membrane protrusion: WAVEs (but probably not WASPs) to lamellipodia and DRFs to filopodia. WASPs and DRFs also seem to have distinct roles in vesicle trafficking: WASPs facilitate membrane trafficking at the plasma membrane, contributing to endocytosis and exocytosis, and also stimulate Golgi-to-ER transport, whereas DRFs have so far been implicated in slowing endosomal dynamics. Further studies on individual actin nucleators should reveal whether DRFs affect other steps of membrane trafficking, and whether WAVEs play a role in any aspect of endomembrane movement.

Some Rho GTPases affect both vesicle trafficking and membrane protrusion, but for most it is not yet known whether they use the same or a different actin nucleator at each membrane site, or indeed whether they act via a combination of downstream targets. Most studies on Rho GTPases and membrane dynamics have focused on RhoA, Rac1 and Cdc42, but recent investigations of other Rho family members, both in mammals and plants, have yielded important and often unexpected insight into how these proteins affect membrane protrusion and/or vesicle trafficking. Finally, although this review has concentrated on the actin cytoskeleton, Rho GTPases might also affect membrane protrusion and traffic through their effects on microtubule dynamics and lipid-modifying enzymes [95,96]. In the future, more detailed studies on little-characterized family members should reveal new aspects of Rho GTPase function and regulation on endomembranes and the plasma membrane.

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