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REVIEW ARTICLE

Rho GTPases: regulation of cell polarity and growth in yeasts

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Eukaryotic cells display a wide range of morphologies important for cellular function and development. A particular cell shape is made via the generation of asymmetry in the organization of cytoskeletal elements, usually leading to actin localization at sites of growth. The Rho family of GTPases is present in all eukaryotic cells, from yeast to mammals, and their role as key regulators in the signalling pathways that control actin organization and morphogenetic processes is well known. In the present review we will discuss the role of Rho GTPases as regulators of yeasts' polarized growth, their mechanism of activation and signalling pathways in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. These two model yeasts have been very useful in the study of the molecular mechanisms responsible for cell polarity. As in other organisms with cell walls, yeast's polarized growth is closely related to cell-wall biosynthesis, and Rho GTPases are critical modulators of this process. They provide the co-ordinated regulation of cell-wall biosynthetic enzymes and actin organization required to maintain cell integrity during vegetative growth.

Key words: Cdc42, cell integrity, cytoskeleton, GTPase-activating protein (GAP), guanine-nucleotide-exchange factor (GEF), morphogenesis, Rho.

INTRODUCTION

Cellular shape is consequence of cellular growth, which is, in many cases, highly asymmetric. Polarized cell growth is fundamental to morphogenesis and the development of both unicellular and multicellular organisms [1]. In general, cell polarization is a response to specific internal or external cues that generate cellular asymmetry. Understanding how molecular interactions and cellular components can generate asymmetry remains one of the fundamental problems in biology. Unicellular organisms, such as the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, provide excellent models to study polarized growth because their morphology, size and division habits are extremely reproducible. The advantages of simplicity, being unicellular organisms, and straightforward genetics make yeasts potent systems for addressing important questions in cell morphogenesis.

Polarized growth requires a perfect co-ordination among major cellular processes such as cytoskeletal organization, secretion and endocytosis that have to be controlled by the cell-integrity pathway and regulated by the cell cycle. First of all, cells need to select growth sites and then reorganize their cytoskeleton and growth machinery accordingly [2]. The major players involved in the establishment of sites for polarized growth, such as the Rho GTPases or PAK (p21-activated kinase) proteins, are conserved from yeast to mammals, suggesting that the basic mechanisms involved may be conserved throughout evolution. *S. cerevisiae* has six Rho GTPases, named Rho1–5 and Cdc42 (cell division cycle 42) [3]. *S. pombe* also has six genes coding for Rho GTPases, *rho1*$^+$ to *rho5*$^+$ and *cdc42*$^+$. In both yeasts only Rho1 and Cdc42 are essential [4,5].

RHO GTPases

Rho proteins belong to the Ras superfamily of small G-proteins that is highly conserved in all eukaryotic organisms. They are GTP-binding proteins with biochemical similarity to the heterotrimeric G-proteins α subunit, whose molecular conformation changes depend on the nucleotide they are bound to [6]. They are active only when they are bound to GTP and can interact with effector proteins. Meanwhile, when they are bound to GDP, they cannot interact with their targets, so the signal is no longer transmitted. That is why they are known as 'molecular switches' [7]; however, Rho proteins do not necessarily need to undergo switching. For example, the ability of Rho1 to activate the enzyme glucan synthase does not require GTP hydrolysis [8].

Rho GTPases display a high affinity for both GDP and GTP. However, only when they are bound to GTP are their switch regions exposed, allowing them to interact with a variety of target proteins to carry out their molecular functions [9]. On the other hand, Rho proteins have an intrinsic GTPase activity; therefore they can hydrolyse GTP to GDP, which leads to a change in the conformation and inactivation of the protein. The transition from GDP- to GTP-bound Rho proteins is controlled by GEFs (guanine-nucleotide-exchange factors) that interact with Rho proteins and alter the nucleotide-binding site, thus facilitating the release of the nucleotide [10] (Figure 1). Since the cytoplasmic concentration of GTP is higher than that of GDP, it is more likely that the GTPase binds to GTP than to GDP. Alternatively, GAPs (GTPase-activating proteins) promote the hydrolysis of GTP bound to Rho proteins by correctly orienting the water molecule necessary for the hydrolysis and by stabilizing the transient state of the reaction [10]. The number of RhoGEFs

Abbreviations used: Arp2/3, actin-related protein 2/3; BAR, Bin/Amphiphysin/Rvs; CAR, contractile actomyosin ring; Cdc, cell division cycle; CRIB, Cdc42/Rac-interacting binding; GAP, GTPase-activating protein; GDI, guanine-nucleotide-dissociation inhibitor; GEF, guanine-nucleotide-exchange factor; MAPK, mitogen-activated protein kinase; MAPKKK, MAPK kinase kinase; MT, microtubule; NETO, new end take off; PAK, p21-activated kinase; PDK1, phosphoinositide-dependent kinase 1; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PRK, PKC-related kinase.

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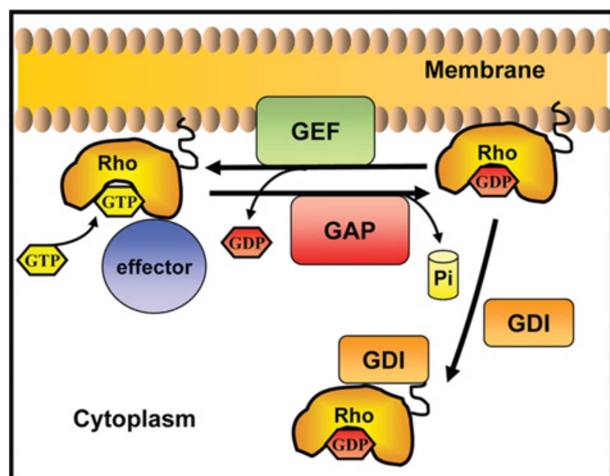

Figure 1 The GTPase cycle

Inactive GDP-bound small GTPases are activated by GEFs, which promote the release of GDP and allow its replacement by GTP. Active GTP-bound small GTPases can then promote the activation of different effectors until they return to their GDP-bound inactive state upon hydrolysis of the GTP into GDP, which is catalysed by GAPs. GDIs extract Rho GTPases from the membranes. An animated version of this Figure is available at http://www.BiochemJ.org/bj/426/0243/bj4260243add.htm.

and RhoGAPs surpasses that of Rho proteins [10]. A single Rho GTPase is regulated by more than one RhoGEF or RhoGAP [11]. At the same time, it has been shown that RhoGEFs and RhoGAPs regulate more than one Rho protein. This promiscuity led to the hypothesis that a Rho protein might be regulated by different RhoGEFs and RhoGAPs for specific processes. For instance, *S. pombe* Cdc42 has two GEFs, Scd1 and Gef1, the first mainly regulating apical growth and the latter mainly regulating cytokinesis [12,13]. RhoGEFs and RhoGAPs are multidomain proteins with the ability to bind to other proteins or to membranes. This enables them not only to change the activation state of the Rho GTPase, but also to serve as scaffold proteins in order to couple the upstream signal with downstream effectors and to directly localize the Rho GTPase [10].

Rho proteins are also regulated by RhoGDIs (guanine-nucleotide-dissociation inhibitors). These proteins act as negative regulators of Rho GTPases at three levels: (i) inhibiting the dissociation of GDP from Rho proteins; (ii) blocking intrinsic and GAP-stimulated GTPase activity; and (iii) extracting Rho GTPases from membranes by masking their isoprenoid group, thus preventing their activation by RhoGEFs [14] (Figure 1).

RHO PROTEINS IN YEAST POLARIZED GROWTH

Understanding how cellular components can generate asymmetry and establish polarized growth is essential for the understanding of morphogenesis. External or internal spatial cues determine the selection of the specific site where the growth machinery will be activated. During vegetative growth, the cell uses internal cues to activate growth in a co-ordinated fashion with cell-cycle progression [15]. The two model yeasts, *S. cerevisiae* and *S. pombe*, have very different cell-growth patterns and molecular pathways linking growth site selection and growth activation, as will be described below.

Polarized growth can be divided into three key steps: (i) marking of the polarization site in response to internal or external signals to the cell; (ii) establishment of polarity by recognition of the polarization site and signalling to the cytoskeleton to allow its asymmetric organization; and (iii) asymmetric distribution of the cellular components and polarized secretion, leading to polarized cell growth. Rho GTPases are activated specifically in the vicinity of the 'landmark proteins' that mark the site and are required in the establishment of polarity and in the polarized growth.

Establishment of polarized growth: the master regulator Cdc42

S. cerevisiae

S. cerevisiae cells have a slightly elongated round shape and divide by the formation of a single bud per cell cycle, which grows in an apical manner in early G_1 to reach a critical size. Then, the bud growth shifts to isotropic and the bud becomes round. After mitosis, when the nuclei have separated, a septum is formed between mother and daughter cells, physically separating them. This septum is eventually degraded, allowing the release of both cells. The budding site is selected according to the positional information of the former budding cycle. In haploid cells, buds are assembled adjacent to the place where the former bud emerged (axial pattern). In contrast, in diploid cells buds are formed at the distal pole where the last bud was made (bipolar pattern). The GTPase Bud1/Rsr1, its GEF, Bud5, and its GAP, Bud2, are the core elements connecting the cortical cues to Cdc42 [16].

The key step in the establishment of polarized growth is the accumulation of active Cdc42 to the presumptive bud site. Cdc24 is the only GEF controlling Cdc42 activity and cell polarization [15]. Loss-of-function *cdc24* mutant strains display a phenotype similar to *cdc42* mutants. The Cdc24 PH (pleckstrin homology) domain, which binds phosphoinositides, and Cdc24 interaction with several proteins are necessary for a tight localization of this GEF to the bud site [16]. During early G_1-phase Cdc24 remains sequestered by the protein Far1 in the nucleus, avoiding ectopic activation of Cdc42. G_1 cyclin–Cdc28 phosphorylation of Far1 in late G_1 triggers its degradation and the release of Cdc24 from the nucleus [17,18]. G_1 cyclin–Cdc28 phosphorylates Cdc24 *in vitro*. However, the relevance of such phosphorylation is controversial; while some authors have found that a point mutation in a consensus G_1 cyclin–Cdc28 phosphorylation site results in Cdc24 localization defects [19], others have not detected such defects in Cdc24 with all consensus phosphorylation sites mutated, concluding that phosphorylation may not be a major regulation process for Cdc24 [20]. G_1 cyclin–Cdc28 might be required for phosphorylating other Cdc24 complex proteins such as Rga2, Boi1 or Boi2 [21]. Cdc24 localization is also regulated by oligomerization. Induction of Cdc24 oligomerization results in reduction of active Cdc42 due to a delay in Cdc24 nuclear export [22].

Bud1/Rsr1 interaction with Cdc24 triggers the correct localization of this GEF to the selected site (Figure 2A). This results in a local activation of Cdc42, which dictates the polymerization of the actin cytoskeleton and targets secretion to the selected bud site. Even in the absence of a functional Bud1/Rsr1 module, Cdc24 and active Cdc42 are able to cluster at a single cortical site in a random fashion. This suggests that Cdc24 accumulation may be controlled by a parallel pathway. One possibility is that Bem1, a scaffold protein able to bind Cdc24, Cdc42 and the kinase Cla4, facilitates the interaction of Cdc42 with other proteins, allowing its cortical clustering. The double mutant *bud1Δ bem1Δ* is not able to activate bud emergence and it does not show polarized Cdc42. It has recently been shown that the expression of the fusion protein Cdc24–Cla4 can bypass the requirement for Bem1 in the absence of Rsr1 [23]. This observation led to the hypothesis that the role of Bem1 is to link Cdc24 and Cla4, and the interaction of Cdc24

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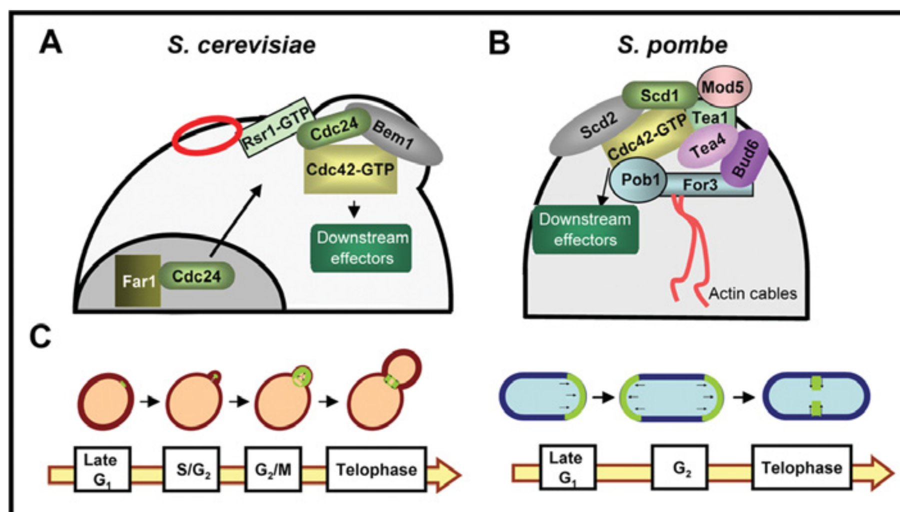

Figure 2 Schematic representation of *S. cerevisiae* and *S. pombe* polarized growth establishment and Cdc42 localization

(**A**) Cdc24 is recruited by GTP-Rsr1 and Bem1, and activated by Cdc24. Once activated, *S. cerevisiae* Cdc42 regulates polarized bud growth by regulating the organization of the actin cytoskeleton, the septins and the exocytic machinery. (**B**) The Tea1–Tea4 complex is deposited by MTs at cell ends, where it is anchored by Mod5. Tea1 binds to Bud6, which binds to For3. Tea4 also binds to the formin For3, which is recruited and activated by Cdc42–Pob1 and Bud6. (**C**) Active Cdc42 distribution in budding and fission yeasts through the cell cycle.

and Cla4 is sufficient for 'symmetry breaking' and polarization. This interaction would lead to a positive-feedback loop in which Cla4 phosphorylates and activates Cdc24, which would activate Cdc42, which would activate Cla4 and thus amplify the signal pathway [24]. By contrast, other authors believe that Cdc24 phosphorylation by Cla4 leads to the dissociation of Cdc24 and Bem1 and thereby serves as a negative-feedback loop that might help in the shifting from apical to isotropic growth [25]. However, the mutation of all Cdc24 phosphorylation sites leads to no apparent vegetative growth defects, raising the possibility that this Cla4 phosphorylation is not a major manner of Cdc24 regulation [20].

The initial clustering of Cdc42 to the presumptive bud site is independent of the actin cytoskeleton, but F-actin influences Cdc42 dynamic distribution through its effects on two antagonic processes: targeted secretion and endocytosis [26]. Although Cdc42 clustering may initiate at more than one site, it has recently been shown that rapid competition between foci determines the winning focus, giving rise to the growth of a single bud [27]. In this focus, the distribution of Cdc42 is stably maintained by two mechanisms of dynamic recycling that are coupled to the GTPase cycle: the Cdc42 GDI, Rdi1, mediates a fast recycling pathway, whereas endocytosis mediates a slower one [28].

S. cerevisiae has four Cdc42 GAPs: Rga1, Rga2 and Bem3 are Cdc42-specific GAPs, and Bem2 is also a GAP for Rho1 (Table 1). The strain lacking Bem2 is thermosensitive, with large round cells, either unbudded or with a small bud, when grown at restrictive temperatures. Surprisingly, this resembles a Cdc42 loss-of-function phenotype, not expected from the deletion of a Cdc42 GAP. Inactivation of Bem2 results in Cdc42 polarization toward multiple sites; therefore Bem2 might contribute to the restriction of Cdc42 activation at a single cortical site [29]. *bem3Δ* cells show an aberrant morphology, which is aggravated by the lack of *RGA1* and *RGA2*, suggesting that these GAPs have a secondary role in polarized growth regulation. Additionally, the strain lacking *BEM3*, *RGA1* and *RGA2* has a defect in septin ring assembly [30], which suggests a role of Cdc42 in the regulation of septin organization. Although these Cdc42 GAPs show somewhat overlapping functions, it is likely that their different localization pattern may account for differential regulation of Cdc42 at specific processes. Rga2, Bem2 and Bem3 are phosphorylated by G_1 cyclin–Cdc28 during bud emergence and this phosphorylation reduces their GAP activity [21,29,31].

Table 1 Rho GEFs and GAPs in *S. cerevisiae* and *S. pombe*

	S. cerevisiae		*S. pombe*	
GTPase	GEF	GAP	GEF	GAP
Cdc42	Cdc24	Rga1 Rga2 Bem2 Bem3	Scd1 Gef1	Rga4
Rho1	Rom1 Rom2 Tus1	Lrg1 Bem2 Sac7 Bag7	Rgf1 Rgf2 Rgf3	Rga1 Rga5 Rga8
Rho2	?	?	?	Rga2 Rga4 Rga7
Rho3	?	Rgd1	?	?
Rho4	?	Rgd1	?	Rga7
Rho5	?	Rgd2	?	?

S. cerevisiae has one GDI, Rdi1, which can extract Cdc42 from internal and plasma membranes. Rdi1 localizes to the cytoplasm, to the tip of small buds and to the mother-bud neck region [16]. Although the lack of *RDI1* does not affect vegetative cell growth in yeasts, diploid *rdi1Δ* cells display a defect in filamentous growth [32]. It has been shown that overexpression of the Cdc42 effector protein Cla4 abolishes the interaction of Rdi1 and Cdc42 by an unknown mechanism [32]. Thus Cla4 would participate in a positive-feedback loop disrupting the negative regulation of Rdi1 over Cdc42.

Once Cdc42 is polarized to the presumptive bud site, it organizes the actin cytoskeleton, the septins and the polarized secretion to carry out the polarized cell growth. To control all of these processes, Cdc42 interacts with a variety of effector proteins

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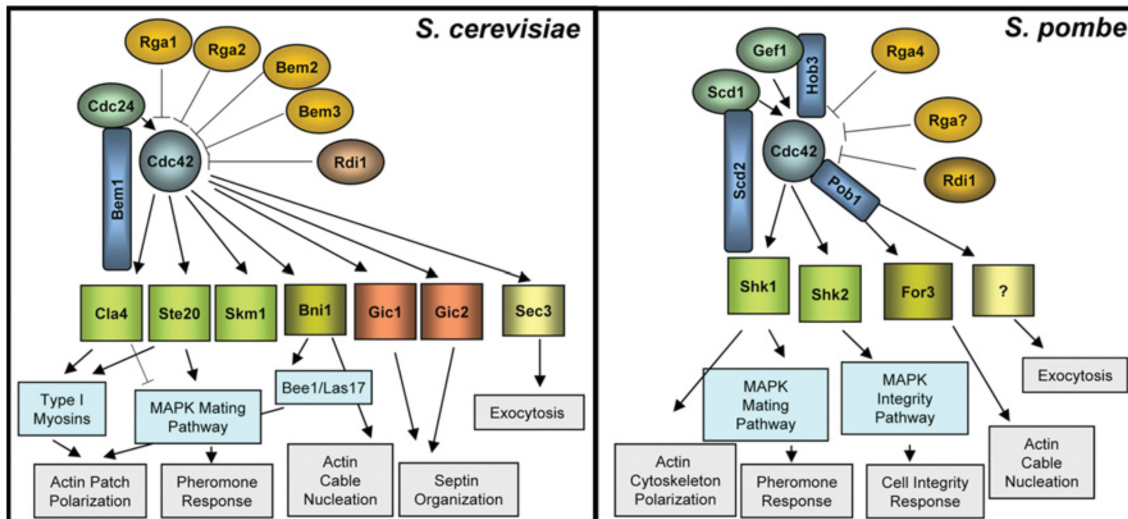

Figure 3 Regulators and targets of Cdc42 in *S. cerevisiae* and *S. pombe*

such as: the PAKs Ste20, Cla4 and Skm1, the formin Bni1, the Gic proteins Gic1 and Gic2, and the exocyst subunit Sec3 (Figure 3).

PAKs are serine/threonine kinases that participate in a variety of cellular processes. Cdc42 interacts with the CRIB (Cdc42/Rac-interacting binding) domain of Ste20, relieving the intramolecular interaction responsible for its autoinhibited state [33]. Cdc42 also regulates the localization of Ste20 to the growing regions [34,35]. Ste20 deletion leads to a defect in pseudohyphal differentiation and mating response [35].

The other major PAK family member is Cla4. Deletion of the Cla4 CRIB domain results in increased kinase activity, suggesting that the CRIB domain down-regulates Cla4 kinase activity [36]. Deletion of both *STE20* and *CLA4* is lethal and the double mutant displays a phenotype of large unbudded cells similar to that seen in the *cdc42-1* mutant strain. Ste20 and Cla4 might regulate the conserved Arp2/3 (actin-related protein 2/3) complex that nucleates actin to form patches, highly dynamic actin structures involved in endocytosis [2]. Ste20 and Cla4 phosphorylate and activate type I myosins Myo3 and Myo5, known Arp2/3 activators [37–39]. However, Cdc42 might not play an essential role in actin-patch assembly, but rather in actin-patch polarization since *cdc42* thermosensitive mutant strains display a depolarized pattern of actin patches throughout the cell cortex.

In *S. cerevisiae*, there are two formins, Bni1 and Bnr1, which nucleate actin cables. Bni1 localizes to the site of bud emergence at the cortex of larger buds, and then redistributes to the mother-bud neck during septum formation. Bnr1 localizes to the mother-bud neck after bud emergence and remains there until cytokinesis. Bni1 nucleates actin cables from the bud tip, while Bnr1 assembles actin cables towards the bud neck [2]. Cells lacking Bni1 and Bnr1 are not viable. Besides their differential localization and physiological functions, they seem to have different dynamics and mechanics for anchoring to the cell cortex [40]. Bni1 interacts in a two-hybrid assay with Rho1, Rho3 and Cdc42, whereas Bnr1 specifically interacts with Rho4 [41]. Rho3 and Rho4 seem to be the main activators of formin activity [41]. *S. cerevisiae* Cdc42 seems to have a main role in polarizing actin cables and a secondary role activating formins in the absence of both Rho3 and Rho4. The Cdc42 effect in actin cable organization is probably due to its role in the localization of Bni1. However, depolarized formin activity can still produce a partially polarized actin cytoskeleton, in a mechanism dependent on the type II myosin Myo1 [42]. This suggests that Cdc42 might have additional effects on actin cable polarization beyond Bni1 localization. It is noteworthy that Cdc42 regulates Bni1 localization, which is in turn necessary for cortical targeting of the Arp2/3 activator Bee1 [39]. Therefore Cdc42 regulates Arp2/3 activation, not only through PAKs, but also through Bni1.

Cdc42 effectors Gic1 and Gic2 were discovered by sequence comparison when looking for proteins that contained a CRIB domain [43]. Gic1 and Gic2 localize to the presumptive bud site, to the tip of the buds, to the mother-bud neck region and to the tip of the mating projection [43,44]. They require the interaction with PIP_2 (phosphatidylinositol 4,5-bisphosphate) to properly localize to the bud tip, but not for plasma-membrane association [45]. Gic proteins have a role in septin recruitment to the bud site since the double mutant *gic1Δ gic2Δ* shows no septin localization at 37 °C [46]. This result indicates that another pathway might be functioning at lower temperatures. The kinase Cla4 also plays a role in septin organization since *cla4Δ* cells display a defect in septin ring assembly and later septin reorganization [47–49]. Cdc42 GAPs also play a role in septin organization that might be related to the elongated bud phenotype of these cells [30,46].

Another target of Cdc42 is the exocyst subunit Sec3 [50]. The characterization of the *S. cerevisiae cdc42-6* thermosentive strain that accumulated post-Golgi vesicles in small budded cells with no apparent actin defects, led to the hypothesis that Cdc42 regulates exocytosis independently of actin cable organization [51,52]. Cdc42, together with PIP_2, regulates Sec3 localization to the bud tip [50,53]. Rho1 also has a role in the localization of Sec3 [54]. This protein and Exo70 are the only exocyst subunits present at the cell cortex and not in the secretory vesicles, and they might act redundantly as a spatial landmark indicating where the exocyst complex should be assembled to permit the vesicle tethering [55]. However, the secretion defect seen in *cdc42-6* cells is not a problem of exocyst localization, therefore Cdc42 might have additional roles in activating polarized secretion [52]. The mutant *cdc42-6* is suppressed by increased dosage of *RHO3*, concordant with a role of Rho3 in exocytosis. Rho3 positively regulates late secretory function through physical interaction with the Exo70 component of the exocyst [56]. Interestingly, disruption of the interaction between Rho3 and Exo70 did not cause a phenotype [55]. This suggests that Rho3 has additional roles in secretion. All of these data led to the proposal of the 'localized activation'

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model, in which activated Cdc42 and Rho3 bind to a cortical exocyst subunit triggering a conformational change in the exocyst complex that results in the relief of its autoinhibitory state [57]. The increase in the exocyst function would result in the deposition of more polarity factors, including Cdc42 and the components of exocyst complex, eventually amplifying the signal [58].

S. pombe

S. pombe cells are rod-shaped; they grow through tip extension and divide by the formation of a medial septum. These morphogenetic events are tightly coupled to cell-cycle progression. Thus, during G_1, cells grow through the old end in a monopolar manner. At the beginning of G_2, when cells reach a critical size, growth is activated in the new end in a process known as NETO (new end take off) producing bipolar growing cells [59]. When cells enter mitosis, tip growth stops and, upon nuclear division, an actomyosin ring forms and then contracts. Concomitantly, new membrane and cell-wall material is deposited in an ordered fashion to separate the daughter cells. The controlled digestion of the primary septum will lead to the release of the daughter cells, which will begin growing through their old ends.

Contrary to budding yeast, the *S. pombe* MT (microtubule) cytoskeleton plays a crucial role in defining the site of polarized growth [60,61]. The use of microfluidic techniques to change the normal shape of fission yeast cells and make them grow in a bent fashion has allowed confirmation that the sites of MT contact in the cell cortex accumulate polarity proteins and eventually give rise to an ectopic growing tip [62,63]. Tea1 was the first protein discovered important for growth site selection. The lack of Tea1 leads to monopolar growing cells and to the activation of ectopic growth sites, especially in cells re-entering exponential growth [64]. Tea1 is transported in the plus end MTs and anchored to the cortex through the interaction with the membrane-associated protein Mod5 [65] (Figure 2B). MT-transported Tea4 and Tea3 also play a role in tethering of Tea1 to the cell tips, the first to the growing end and the latter to the non-growing end [66,67]. Tea4 is an important link between Tea1 and For3, the formin required for actin cable assembly during interphase [67,68].

The establishment of polarized actin cables relies on the Cdc42 activation of the interphase formin For3 [69]. For3 localizes to the growing ends and the division region through a complex regulation that involves N-terminal and C-terminal domains [69]. It depends on two major proteins: the GTPase Cdc42 and Bud6 [69,70]. The latter requires Tea1 for tip localization and, subsequently, contributes to For3 localization by anchoring its C-terminal region to the cell cortex [69,70]. In the absence of Bud6, For3 is partially delocalized and cells display a less robust pattern of actin cable array [69]. Cdc42 relieves the autoinhibited state of For3 [69]. Moreover, *cdc42* mutants show a severe defect in actin cables and For3 localization [69,71]. These defects are partially suppressed by the expression of the Boi family member Pob1 [71]. The interaction of Pob1 with For3 is necessary for tethering the For3 N-terminal domain and for Cdc42-mediated For3 activation [71]. Surprisingly, cells lacking For3 are viable despite the lack of detectable actin cables. It is possible that other molecules, such as the exocyst complex, regulate polarized secretion and growth in the absence of actin cables.

S. pombe Cdc42 localizes to the growing ends, the division site and the endomembranes [72]. The lack of Cdc42 activity leads to small dense round cells, suggesting that although macromolecular synthesis continues, incorporation of new material to the membranes is inhibited [4]. These cells are also unable to mate [4]. There are two specific Cdc42 GEFs in *S. pombe*, Scd1 [73] and Gef1 [12,13] (Figure 3). The double deletion of these genes is not viable, suggesting that Scd1 and Gef1 share the essential role of Cdc42 activation. Scd1 localizes to the nucleus, the mitotic spindle, the growing cell ends and the septum [74], which suggests a variety of functions in different processes.

S. pombe establishment of cell polarity differs strongly from the budding yeast model in which Bud1/Rsr1 connects the landmark signal to Cdc42. *S. pombe* contains a Bud1/Rsr1 orthologue, called Ras1, which has been shown to play an important role both in mating and in vegetative polarized growth [75,76]. In contrast with *S. cerevisiae*, lack of Ras1 activity leads to a polarized growth defect rather than to a defect in the selection of the polarized growth site, and cells become round. Nevertheless, the Ras1–Cdc42 activation module is conserved in both yeasts. Thus Scd1, the fission yeast Cdc42 GEF homologue to Cdc24, is a major Ras1 target [73]. Cells lacking Scd1 show a spherical phenotype, are incapable of mating [73] and have defective endocytosis [77]. Scd1 also plays a role in proper mitotic spindle formation [74]. Moreover, Scd1, together with Cdc42, has been shown to interact with the presumptive kinesins Klp5 and Klp6 to regulate cell separation [78]; however, the mechanism of this regulation remains to be clarified.

The homologue protein of *S. cerevisiae* Bem1 in *S. pombe* is Scd2, a scaffold protein that stimulates the Scd1 interaction with Ras1 and Cdc42 [73]. Scd2 binds to GTP–Cdc42 and facilitates its interaction with the downstream effector Shk1 [79].

Gef1, the other Cdc42 GEF, localizes to the cell equator during actomyosin ring contraction, and to the growing cell poles. Gef1 deletion leads to a delay in cytokinesis and defects in NETO transition [12,13]. It has recently been described that activated Cdc42 localization is regulated by the NDR (nuclear Dbf2-related) kinase Orb6 through the proper localization of Gef1 to the poles. Defects in Orb6 signalling lead to Gef1 mislocalization to the cell sides, thereby ectopically activating Cdc42 [80].

The only Cdc42 GAP described in *S. pombe* is Rga4 [81]. This GAP localizes to the cell sides and to the division site, and is excluded from the cell tips [81,82]. Rga4 was found as an interacting partner of the DYRK (dual-specificity YAK-1-related) kinase Pom1 [81]. This kinase is localized to both cell ends and is required for NETO and actomyosin ring positioning [83]. Pom1 regulates Rga4 localization and therefore Cdc42 activation [81]. Cells lacking Rga4 are shorter and wider than wild-type cells [82].

As in budding yeast, PAKs (Shk1 and Shk2) are Cdc42 effectors in *S. pombe*. Shk1 was identified in a screening for *S. cerevisiae* Ste20 homologues [84,85]. Shk1 is essential, and the null-mutant gives rise to round small cells reminiscent of the *cdc42*-null cells. The expression of Ste20 suppresses the lethality of *shk1* deletion, suggesting that the cellular functions of these proteins are highly conserved. It has been shown that Shk1 phosphorylates Tea1 *in vitro* and that both proteins might co-operate for proper cell division [86]. Moreover, it has been demonstrated that Shk1 has a negative effect on cytokinesis completion by phosphorylating myosin light chains [87]. The PAK Shk2 is not essential and cells lacking Shk2 do not show an apparent phenotype [88,89]. Shk2 interacts with Cdc42 and, when overexpressed, can suppress the lethality of *shk1* deletion [88,89]. Shk2 has been shown to interact with Mkh1, the MAPKKK [MAPK (mitogen-activated protein kinase) kinase kinase] of the cell integrity pathway. Moreover, whereas Shk2 overexpression is lethal in a wild-type background, it is not lethal in cells lacking Mkh1 or the MAPK Pmk1 [90]. However, Shk2 does not seem to play a relevant role in the activation of that MAPK pathway [91].

Although there is a conserved Arp2/3 complex in *S. pombe*, so far it has not been reported to be regulated by Rho GTPases. *cdc42* conditional mutants at restrictive temperatures [69,71] or cells lacking Rho3 at high temperature [92] show delocalized actin

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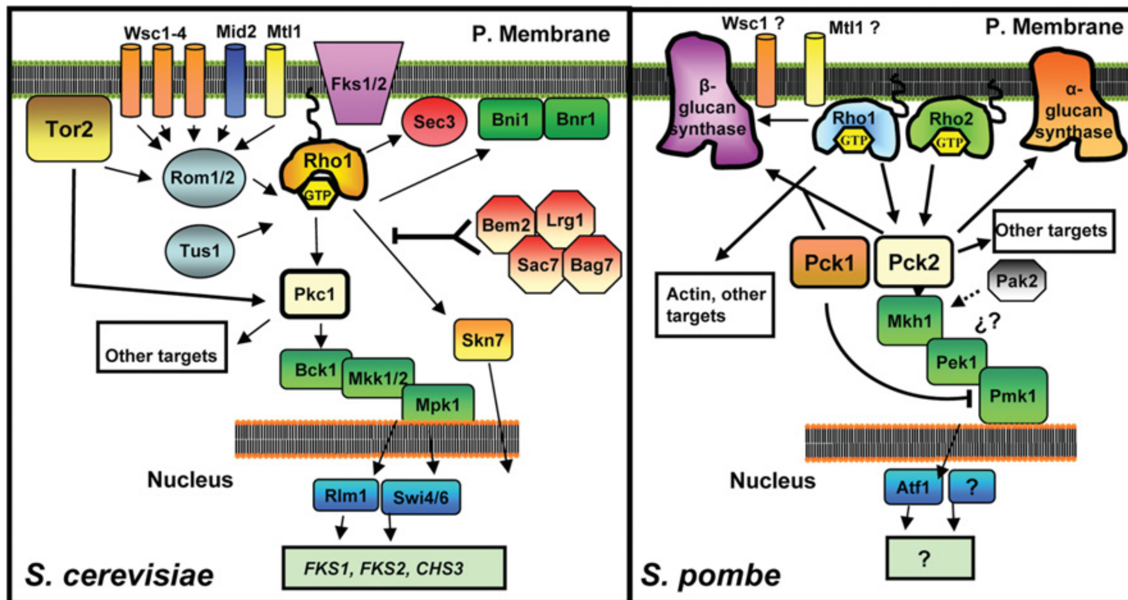

Figure 4 Schematic representation of *S. cerevisiae* and *S. pombe* MAPK cell-integrity pathways

S. cerevisiae Rho1 plays a central role in integrating multiple signals from the cell-wall sensors and the actin cytoskeleton through GAPs and GEFs to its effectors including Pkc1, which activates the MAPK cell-integrity pathway. *S. pombe* Rho1 and Rho2 activate Pck1 and Pck2. The latter regulates cell-wall biosynthetic enzymes and activates the MAPK cell-integrity pathway. P. membrane, plasma membrane.

patches, but it is not known whether this reflects the regulation of Arp2/3 by these proteins. Switching off *rho1*$^+$ expression causes disappearance of the actin cytoskeleton [93], suggesting a main role of this protein in maintaining actin polymerization. However, the signalling molecules that participate in this process are not known.

Not much is known about polarized secretion in *S. pombe*. The *rho3*$^+$ gene was identified as a multicopy suppressor of *sec8-1*, a temperature-sensitive exocyst mutant [94]. *rho3* also shows genetic interaction with *exo70*. Vesicles accumulate in *rho3*Δ cells growing at 36 °C and secretion is impaired [94]. Interestingly, *rho3*$^+$ is essential only at higher temperatures, whereas the exocyst complex is essential at all temperatures [95]. Rho3 function might be redundant with other proteins such as Cdc42. Indeed, the thermosensitivity of the *cdc42-879* strain [71], which displays secretion defects, can be partially suppressed by increased dosage of *rho3*$^+$ (S. Rincón and P. Perez, unpublished work). *S. pombe* Rho4 also regulates secretion in a non-redundant manner with Rho3 [96]. This GTPase is involved in secretion only during fission yeast cytokinesis [97], and it might be required for the exocyst localization at the division area (B. Santos and P. Perez, unpublished work).

RHO GTPases AND CELL INTEGRITY

Polarized growth requires remodelling of the cell wall at the growing site. Growth in yeast is always limited by the existing cell wall. The cells must organize regions where the wall will be weakened to allow the incorporation of new material [8]. Therefore a co-ordinated regulation of cell-wall biosynthetic enzymes and actin organization is necessary to keep cell viability. Rho GTPases might be the key molecules in this co-ordination. The main structural component responsible for the rigidity and strength of the cell wall is the linear polymer (1,3)-β-D-glucan. Direct activation by Rho1 of the enzymes responsible for the biosynthesis of this polymer is a general mechanism in fungal-wall biosynthesis [3,8].

S. cerevisiae Rho1 is an essential protein that localizes to the area of polarized growth independently of the actin cytoskeleton [98]. Rho1 is activated by the GEFs Rom1, Rom2 and Tus1 [99,100]. Rom1 and Rom2 have redundant functions in the activation of Rho1, and probably Rho2. Cells lacking both GEFs are not viable. Tus1 is also necessary for the cell-wall integrity pathway [100], and its phosphorylation by cyclin2–Cdc28 is required for the efficient activation of Rho1 at the G_1/S transition [101]. Tus1 and Rom2 participate in Rho1 localization and activation during cytokinesis [102,103]. The regulation of both GEFs is mediated by Polo kinase phosphorylation.

The Rho1 GDP/GTP cycle is regulated by the GAPs Bem2, Sac7, Bag7 and Lrg1 [104] (Table 1). Bem2 and Sac7 negatively regulate the MAPK cell-integrity pathway [105,106]. Sac7 and Bag7 have some redundant functions in the Rho1 regulation of actin organization [106]. Lrg1 is the main regulator of Rho1 as activator of the (1,3)-β-D-glucan [107]. The compensatory effect of GEFs and GAPs in Rho1 activity regulation is known, because a *S. cerevisiae* strain lacking all of the GEFs is viable in the absence of the GAPs Lrg1 and Sac7 [103]. GTP-bound Rho1 interacts with six effector proteins: (1,3)-β-D-glucan synthase components, Fks1 and Fks2, Pkc1, the formin Bni1, the Skn7 transcription factor, and the Sec3 exocyst subunit [3] (Figure 4).

S. pombe Rho1 has 73 % and 67 % identity with *S. cerevisiae* Rho1 and *Homo sapiens* RhoA respectively [108]. Rho1 localizes to places of active cell growth [93] and plays an essential role in cell-wall biosynthesis and actin cytoskeleton regulation [5,109]. This GTPase is activated by three GEFs called Rgf1, Rgf2 and Rgf3 [110–113]. All of these GEFs have similar molecular structures to the budding yeast Rho1GEFs Rom1 and Rom2 [113]. Rgf1 specifically regulates Rho1 during polarized growth [113] and Rgf3 during cytokinesis [110]. Rgf2 performs an

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essential function during the sporulation process, and a secondary function redundant to Rgf1 during polarized growth [114].

Rho1 is negatively regulated by three GAPs, Rga1, Rga5 and Rga8 [115–117] and, probably, by the GDI Rdi1, which also binds Rho4 and Cdc42 [118]. Rga1 is a major regulator of Rho1 and cells lacking Rga1 are sick and have a thick cell wall [115]. Rga5 regulates cell integrity and cytokinesis [116]. Rga8 is a Rho1 GAP regulated by Shk1, therefore it might participate in the cross-talk between Rho1 and Cdc42 [117].

S. pombe rho2^{+} was isolated in a screening of genes causing aberrant morphology when overexpressed. *rho2*$^{+}$ overexpression causes actin depolarization, and an increase in the amount of cell-wall α-glucan [119,120]. Like Rho1, Rho2 localizes to the growth areas [119] and interacts with Pck1 and Pck2 [121]. However, only Pck2 seems to be part of the Rho2 signalling pathway [120]. *rho2*$^{+}$ is not essential, but cells lacking Rho2 have some cell-wall defects [119,120]. No GEF-activating Rho2 has been described. The GAP Rga2, structurally similar to *S. cerevisiae* Bem3, is a specific Rho2 GAP that also participates in the regulation of cell dimension, probably through the interaction with Cdc42 [122].

The PKC (protein kinase C)–MAPK cell-integrity pathway

Rho1 is a direct activator of the (1,3)-β-D-glucan synthase, which is the enzyme responsible for the synthesis of the main cell-wall polymer in both budding and fission yeast. Additionally, in *S. cerevisiae* it also activates the kinase from the PKC family that triggers the MAPK cell-integrity pathway. Any cell-wall defect is detected by a family of membrane receptors, Wsc1-4 and Mid2-Mlt1 [3] (Figure 4). *MID2*, *MTL1* and the *WSC* genes show distinct expression patterns, indicating that these sensors mediate signalling under different conditions [123]. Wsc1 and Mid2 act during cell-wall remodelling required for vegetative growth. Additionally, Mid2 acts during the mating process to ensure cell-wall integrity during projection formation. Wsc1 and Mid2 interact and activate Rho1 through the GEF Rom2 [124]. Although Rom1 and Tus1 are also Rho1GEFs, the upstream signalling is not clear. Interestingly, Tor2, an essential phosphatidylinositol kinase involved in the co-ordination between nutritional signals and cell growth, also regulates actin organization by activating Rom2 and Pck1 [3,125,126]. Thus Tor2 might act as a nutrient sensor that connects protein synthesis, required for active growth, with cell-wall biosynthesis and cell integrity. It has been demonstrated previously that the Pkc1 pathway is required for viability in quiescence acting downstream of TOR (target of rapamycin) signalling inhibition to maintain cell integrity [127].

Yeast PKCs are structurally and functionally related to the Rho-associated kinases [PRKs (PKC-related kinases)] of mammalian cells. They contain Rho1-binding domains that are responsible for their stabilization and targeting to the bud tip [121,128]. The binding of GTP-RhoA leads to a conformational change in PRKs that allows PDK1 (phosphoinositide-dependent kinase 1) binding and phosphorylation of the PRK activation loop [129]. A similar mechanism may cause yeast PKC activation. *S. cerevisiae* has two homologues of PDK1, Pkh1 and Pkh2, which share an essential function as activators of Pkc1 [130]. Pkc1 is localized and stabilized by active Rho1 to the growth areas where it is activated by Pkh1 or Pkh2 and triggers the MAPK cell-integrity pathway. This pathway activates the transcription factor Rlm1, which regulates the expression of at least 25 genes related to cell-wall biosynthetic processes, including the (1,3)-β-D-glucan synthase encoding genes *FKS1* and *FKS2* [3]. Interestingly, the function of the MAPK pathway is only indispensable in conditions of high temperature (37 °C), since the mutants lacking this route lyse at high temperatures. Both Rho1 and Pkc1 have additional effects in the control of integrity, since *pkc1* mutants require osmotic stabilization at all temperatures and *rho1* mutant lysis occurs even in the presence of osmotic stabilizer [3].

In *S. pombe*, the signalling mechanism from the cell surface to the Rho GTPases is not known. Proteins similar to the Wsc family of receptors exist, but their relationship with Rho proteins is not clear. GTP-bound Rho1 and Rho2 interact with the two fission yeast PKC homologues Pck1 and Pck2 [121,122]. Both bind to Rho1 and Rho2 [121] (Figure 4). Pck1 and Pck2 are unstable and the interaction with GTP-Rho1 or GTP-Rho2 increases their stability [121,122]. *S. pombe* Ksg1 kinase is a homologue of human *PDK1* [131]. It is possible that this kinase activates Pck1 and Pck2, although it has not been studied. *pck1*$^{+}$ and *pck2*$^{+}$ share overlapping roles in cell viability, but only Pck2 is able to activate the integrity pathway downstream of Rho2 [132], whereas Pck1 has a negative effect [133]. The role of Rho1 in the integrity pathway is not clear. It has been recently described that the Rho1GEF Rgf1 activates this pathway [114].

The *S. pombe* MAPK integrity module includes Mkh1 (MAPKKK), Pek1/Shk1 (MAPKK) and Pmk1/Spm1 (MAPK) [134–138] (Figure 4). Deletion of any gene in this module causes cytokinesis defects, hypersensitivity to potassium ions and β-glucanase treatment, and vacuole fusion defects [134,136–139]. It has been described that under cell-wall damage conditions, Pmk1 can phosphorylate the transcription factor Atf1 [140], which is also phosphorylated by Sty1 [141]. Takada et al. [140] proposed that the cell integrity and stress pathways converged at Atf1 in order to activate genes required to repair cell damage. However, no activation of any gene related to cell-wall biosynthesis has yet been proven.

RHO PROTEINS IN CYTOKINESIS

Cytokinesis is required for the separation of daughter cells after mitosis. Animal and fungal cells organize an actomyosin contractile ring necessary for cell cleavage [142]. Besides actin and myosin, which form intercrossed filaments, at least 20 other proteins are required to form the ring, most of them conserved among eukaryotes (see [143–145] for reviews). In yeast cells, ring constriction and septum formation are co-ordinated and several regulatory proteins form a signalling pathway, named MEN (mitosis exit network) in *S. cerevisiae* and SIN (septation initiation network) in *S. pombe*. These pathways trigger the contraction of the F-actin ring and co-ordinate cytokinesis with the nuclear cycle (see [114,145–147] for reviews).

In animal cells RhoA is essential in the assembly and contraction of the CAR (contractile actomyosin ring). Similarly, in budding yeast Rho1 is required for Bni/Bnr1 assembly of the actomyosin ring [148]. Rho1 is localized and activated at the division area by Tus1 and Rom2 during CAR assembly [102], and the regulation of both GEFs is mediated by Polo kinase phosphorylation. Rho1 is also localized later by interaction with the membrane microdomain formed between the septin rings, which is rich in PIP_2 [103]. Rho1 seems to be necessary late in cytokinesis for secondary septum formation [103].

In *S. pombe*, the role of Rho GTPases in ring assembly or function is poorly understood. We identified the paxillin homologue, Pxl1, which is a Rho1 negative modulator and collaborates in the CAR formation and constriction [149]. How is Rho1 negatively regulated by Pxl1? It is likely that Pxl1 modulates a GAP or GEF protein that mediates this regulation. *S. pombe* Cdc42 might also be involved in cytokinesis since the BAR (Bin/Amphiphysin/Rvs) domain-containing protein Hob3, required for cytokinesis, interacts with the Cdc42GEF, Gef1 [150]. Deletion of *hob3*$^{+}$ causes cell elongation and multiseptation

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[151]. Hob3 recruits Cdc42 to the division site via the BAR domain. It is not known how the Gef1–Hob3–Cdc42 complex and the spatial restriction of Cdc42 activity works in the CAR constriction. This is a novel mechanism for spatially regulating Cdc42 signalling.

S. pombe Rho3 and Rho4 are also required for cytokinesis. They regulate the polarized secretion of the hydrolytic enzymes that digest the primary septum [94,97]. Rho3 regulates the exocyst [94] and might play a general role in secretion, not only during cytokinesis. *S. pombe rho3*$^{+}$ can be substituted for budding yeast *RHO3*, but not for other GTPases, suggesting that they have a similar function [92]. Rho4 is the only Rho GTPase localized exclusively to the division area [96] and it regulates the secretion of lytic enzymes, such as Agn1 or Eng1, required for septum degradation [97]. Preliminary results from our laboratory suggest that Rga7 might be a Rho4GAP that participates in cytokinesis (B. Santos and P. Perez, unpublished work).

RHO PROTEINS AND MATING

The process of mating is an example of polarized growth in response to an external cue. Haploid yeasts of opposite sexual type are able to mate and generate a diploid zygote which will subsequently undergo meiosis, giving rise to four ascospores. GPCRs (G-protein-coupled receptors) detect the presence of the opposite mating type pheromone and activate heterotrimeric G-proteins which, in turn, activate the mating MAPK pathway. This pathway activates the transcription of a variety of genes necessary for the completion of mating and eventually meiosis. The overall process of mating is quite similar in *S. cerevisiae* and *S. pombe*, although there are some differences in the signalling molecules. The formation of a tip projection, called a 'shmoo', is necessary for mating to take place. The actin and MT cytoskeletons are remodelled during this process and allow the 'shmoo' growth and proper nuclear orientation for karyogamy. The importance of the cytoskeleton in mating suggests a requirement for Rho GTPases function [16]. In fact, Cdc42 plays a critical role in this process. In *S. cerevisiae*, both Cdc24 and Cdc42 are necessary for actin polarization and for the activation of the mating MAPK cascade [16]. Bem1 and Ste20 also play a role in mating signalling, and it has been suggested that the correct interaction between Cdc42, Bem1 and Ste20 allows efficient pheromone signalling [152]. Cla4 might play a role in mating signalling as a negative regulator of the MAPK pathway, since *cdc42* mutants resistant to pheromone treatment are suppressed by *cla4* deletion [153]. In *S. pombe*, several *cdc42* loss-of-function mutants are sterile (S. Rincón and P. Perez, unpublished work), and both Scd1 and Scd2 deletions lead to conjugation defects. Nevertheless MAPK signalling is not impaired, suggesting that the mating defect in these strains is due to the inability to perform polarized cell growth [73]. Additionally, the Cdc42 effector Shk1 mediates the transition of the MAPK Byr2 to an activated state [154].

Cdc42 might also be involved in later events during mating, such as localization of the polarisome and cell fusion ([155–157] and S. Rincón and P. Perez, unpublished work).

Little is known about the role of other Rho GTPases in the yeast mating process. However, Rho1 might have a role in cell-wall biogenesis regulation during cell fusion.

CONCLUDING REMARKS

In the present review we have tried to summarize the relevance of Rho proteins in polarized cell growth. In the last few years, an important understanding of the mechanisms underlying the regulation of polarized growth in yeasts has developed. The role of Rho proteins in the actin cytoskeleton organization has been emphasized as the main determinant in the generation and maintenance of the polarized phenotype. However, Rho proteins also participate in other key pathways for polarized growth, independent of the actin cytoskeleton, such as the exocytic machinery, the maintenance of cell integrity and endocytosis. These different pathways may be more complicated; they receive inputs from the cell cycle, the stress pathways or the nutrient situation, and need to be studied further.

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