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Cell Polarity in Yeast

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Abstract

A conserved molecular machinery centered on the Cdc42 GTPase regulates cell polarity in diverse organisms. Here we review findings from budding and fission yeasts that reveal both a conserved core polarity circuit and several adaptations that each organism exploits to fulfill the needs of its lifestyle. The core circuit involves positive feedback by local activation of Cdc42 to generate a cluster of concentrated GTP-Cdc42 at the membrane. Species-specific pathways regulate the timing of polarization during the cell cycle, as well as the location and number of polarity sites.

Keywords

cell polarity; Cdc42; GEF; GAP; positive feedback; yeast

INTRODUCTION

Cell polarity is essential for effective cell motility and other directional functions like neuronal signaling, transport across epithelia, and specification of body axes in developing embryos. A polarized cell has a clear axis, with a defined polarity site, or front. Different cells display an enormous variety of polarized morphologies, but the Rho-family GTPase Cdc42 and its relatives (Rac in animals, Rop in plants) appear to control polarization in most eukaryotes (Bi & Park 2012, Etienne-Manneville 2004, Wu & Lew 2013). Cdc42 is among the most highly conserved GTPases: Yeast Cdc42 and human Cdc42 are 80% identical, much more conserved than yeast and human Rho (66% identity) or Ras (34% identity). Signaling pathways that induce polarization act through Cdc42 regulators (Figure 1) to trigger the localized accumulation of membrane-bound GTP-Cdc42 at the site destined to become the cell's front. Local GTP-Cdc42 then organizes cytoskeletal elements through a variety of effectors to yield the polarized morphology appropriate to the cell type.

Here we review insights into the mechanism and regulation of cell polarity stemming from studies in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In these

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tractable model systems, the single Cdc42 is essential for polarization, and Rac GTPases are absent. *S. cerevisiae* cells are ovoid and proliferate by budding, whereas *S. pombe* cells are rod shaped and proliferate by elongation and medial fission (Figure 2). Fundamental features of polarity control are conserved in these very distantly related fungi. However, they exhibit differences in the number and size of polarity sites, in the regulation of polarity by the cell cycle, and in the linkages between the polarity and cytokinesis machinery.

MECHANISM OF POLARIZATION

Polarization often occurs toward cell type–specific directional cues, but many cells exhibit the ability to break symmetry and polarize spontaneously in a random direction when spatial cues are absent. In yeasts, germinating spores lack obvious positional cues and appear to break symmetry to initiate polar growth. This suggests that cells have a core polarity program that can be set off at a random site, yielding a concentrated cortical cluster of GTP-Cdc42. In this program, polarity sites are assembled through positive feedback (Johnson et al. 2011, Wu & Lew 2013).

Positive Feedback by Local Activation of Cdc42

Feedback by local activation of Cdc42 appears to operate in both *S. cerevisiae* and *S. pombe* and is independent of the cytoskeleton. This mechanism involves binding interactions between polarity factors and exploits the fact that diffusion of proteins in the cytoplasm is much faster than that of proteins at the membrane (Bendezu et al. 2015, Woods & Lew 2017, Woods et al. 2016). Cdc42 effector proteins termed p21-activated kinases (PAKs) bind to a polarity scaffold protein (Bem1 in *S. cerevisiae* and Scd2 in *S. pombe*), which binds to a Cdc42-directed GDP/GTP exchange factor (GEF) (Cdc24 in *S. cerevisiae* and Scd1 in *S. pombe*) (for a guide to the gene names discussed in this review, see Table 1) (Bose et al. 2001, Butty et al. 2002, Endo et al. 2003, Irazoqui et al. 2003, Ito et al. 2001, Kozubowski et al. 2008). PAKs, scaffolds, and GEFs associate to form complexes that diffuse freely in the cytoplasm (Figure 3). PAKs also associate with GTP-Cdc42 at the membrane, resulting in local recruitment of GEF from the cytoplasm to sites that already have some GTP-Cdc42. This local GEF promotes activation of neighboring GDP-Cdc42, recruiting more GEF (Kozubowski et al. 2008). High mobility of cytoplasmic factors enables their rapid recruitment to the developing polarity site, whereas slow diffusion of membrane factors allows them to accumulate at the site. Dynamical modeling of this reaction-diffusion system showed that such feedback would suffice to allow stochastically arising clusters of polarity factors to recruit more polarity factors in a self-amplifying loop (Figure 3) (Goryachev & Pokhilko 2008, Johnson et al. 2011, Woods & Lew 2017).

Considerable evidence supports positive feedback by local activation in *S. cerevisiae*. Point mutations that weaken or abolish PAK-Bem1 or Bem1-GEF interactions severely compromise the cells' ability to break symmetry, whereas protein fusions that restore the missing linkage rescue symmetry breaking (Kozubowski et al. 2008). Indeed, fusion of GEF to PAK enabled symmetry breaking even in the absence of the intermediary Bem1. Blocking local enrichment of GEF or Bem1 by tethering them all over the membrane prevented polarization of Cdc42 (Woods et al. 2015). Optogenetic recruitment of a small amount of

either GEF or Bem1 to an illuminated region of the membrane promoted local activation of Cdc42, recruitment of endogenous GEF and Bem1, and bud emergence at that site (Witte et al. 2017). In aggregate, these studies demonstrate that positive feedback by local activation is necessary and sufficient for cells to break symmetry.

Contribution of Actin and Vesicle Delivery: Positive or Negative Feedback?

In *S. cerevisiae*, the Cdc42 effector Bni1 is a formin that nucleates actin polymerization, and Bni1-associated filaments form linear bundles (actin cables) tethered to the polarity site (Evangelista et al. 1997, 2002; Sagot et al. 2002). These cables enable the delivery of secretory vesicles to the polarity site by type V myosins (Donovan & Bretscher 2012, 2015; Schott et al. 1999, 2002). The vesicles carry a plasma membrane tethering complex (the exocyst) whose subunits include two other Cdc42 effectors (Sec 3 and Exo70) (Adamo et al. 2001, Baek et al. 2010, Wu et al. 2010). Thus, both delivery and fusion of secretory vesicles are enhanced at sites enriched for GTP-Cdc42 (Figure 4). As Cdc42 itself is present on vesicles, delivery of Cdc42 by vesicles was hypothesized to lead to local enrichment of Cdc42, promoting polarization via a parallel positive feedback loop (Wedlich-Soldner et al. 2003).

Although Cdc42 clearly promotes local secretion, there is little evidence that local delivery of Cdc42 makes a major contribution to polarization. Elimination of Bni1 did not detectably alter the dynamics of polarity establishment, and even complete actin depolymerization had limited effects that could be explained by associated stress responses (Woods et al. 2016). In fact, theoretical considerations suggest that delivery and fusion of secretory vesicles would perturb polarity, rather than reinforcing it (Layton et al. 2011, Savage et al. 2012), because most polarity factors (e.g., GEFs, Bem1) are not present on vesicles, and Cdc42 is less concentrated on vesicle membranes than it is at the polarity site (Watson et al. 2014). Thus, each time a vesicle fuses at the polarity site, it adds membrane that dilutes the local polarity factors, constituting a negative feedback (rather than a positive feedback) loop.

Modeling the effects of vesicle delivery in a system in which polarity is established by local activation led to two novel conclusions. First, the high vesicle delivery rates typical of tip-growing fungi or plant cells would be expected to provide such a strong dilution effect that polarity would collapse without additional support mechanisms (Savage et al. 2012). Second, even with the slower vesicle delivery typical of yeasts, stochastic off-center vesicle delivery could yield displacement of the polarity site along the cortex (Figure 4) (Dyer et al. 2013). Subsequent studies detected a mobile polarity site in mating *S. cerevisiae* cells (Dyer et al. 2013, Hegemann et al. 2015, McClure et al. 2015) as well as germinating spores of *S. pombe* (Bonazzi et al. 2014), suggesting that negative feedback by vesicle delivery may provide physiologically important functions.

Contribution of Microtubules and Tip Factors

In *S. pombe* cells undergoing polar growth in interphase, antiparallel microtubule bundles are assembled with plus ends directed toward the cell tips (Figure 5). As microtubules grow, encounters with the cell sides cause bundle reorientation to yield longitudinal alignment, simply due to the tubular cell shape and the stiffness of the microtubules (Carazo-Salas &

Nurse 2006, Daga et al. 2006, Tran et al. 2001). Aligned microtubules deliver tip factors to the cell ends; such factors include Tea1 and its binding partner Tea4, which is a targeting subunit for protein phosphatase 1 (PP1) (Alvarez-Tabares et al. 2007, Martin et al. 2005, Mata & Nurse 1997, Tatebe et al. 2005). In turn, local PP1 promotes activation of Cdc42 by affecting the localization of its regulators Gef1 (a GEF) and Rga4 [a GTPase-activating protein (GAP)] (Kokkoris et al. 2014). PP1 may promote local association of Gef1 at cell tips by reversing the Gef1 phosphorylation catalyzed by Orb6 (a NDR/LATS-family kinase). Orb6-mediated Gef1 phosphorylation promotes binding to 14-3-3, which displaces Gef1 from the cortex (Das et al. 2009, 2015). In contrast to Gef1, the GAP Rga4 is excluded from cell tips through an unresolved mechanism (Kokkoris et al. 2014, Tatebe et al. 2008). Thus, cylindrical cell shape leads to aligned microtubules, which promote activation of Cdc42 at cell ends. In turn, Cdc42 promotes polar growth, maintaining the cylindrical cell shape (Figure 5) (Martin 2009).

Cells lacking Gef1 or microtubules display highly polarized GTP-Cdc42, indicating that this pathway is dispensable for generating well-polarized cells (Coll et al. 2003). Instead, the primary role of the microtubule pathway appears to be in positioning the polarity site (see below). In contrast, cells lacking the GEF Scd1 or the scaffold Scd2 (the Bem1 homolog required for positive feedback via local activation; see Figure 3) display a much more severe phenotype, with round cells and poorly polarized GTP-Cdc42 (Chang et al. 1994, Kelly & Nurse 2011). These findings suggest that positive feedback by local activation of Cdc42 is the dominant mechanism of polarization in *S. pombe* as well as in *S. cerevisiae*.

NUMBER AND SIZE OF POLARITY SITES

Positive feedback via local activation provides an intuitively appealing and experimentally well-supported mechanism to grow a concentrated cluster of GTP-Cdc42 at the membrane. It is less clear how feedback might be harnessed to create the desired number and size of polarity sites.

Making a Single Polarity Site: Competition Between Polarity Clusters in *Saccharomyces cerevisiae*

S. cerevisiae cells make one and only one bud at a time. However, imaging of polarity establishment revealed that cells frequently begin by growing more than one cluster of Cdc42 (Figure 6*a*) (Howell et al. 2012, Wu et al. 2015). Such multicluster intermediates were short lived, and within approximately 2 min only a single cluster survived. Similarly, optogenetic recruitment of the GEF Cdc24 to two sites could yield two clusters of GTP-Cdc42, but even with continued illumination, one of the clusters disappeared, leaving only a single polarity site (Witte et al. 2017). Thus, these yeast cells possess a powerful mechanism to eliminate supernumerary clusters.

Remarkably, a similar phenomenon occurs in some computational models of positive feedback by local activation (Goryachev & Pokhilko 2008, Otsuji et al. 2007). This suggests that both the growth of clusters and their winnowing down to yield a single cluster might be intrinsic to this positive feedback mechanism. How do multiple clusters resolve to one? Analyses of the models suggest that clusters compete for shared components present in the

cytoplasm, yielding a single winner (Figure 6*b*) (Wu et al. 2015). Growth of polarity clusters depletes components from the cytoplasm, reducing the shared pool of factors available. Because yeast cells are small (~5 μm diameter) and cytoplasmic diffusion of proteins is fast (~10 $\mu m^2/s$), all clusters have access to essentially the same shared pool. Due to nonlinear positive feedback, the more concentrated a Cdc42 cluster is, the better it can recruit cytoplasmic factors. As the largest cluster grows, it becomes more effective at recruiting cytoplasmic factors, so it continues to grow even with lower levels of available cytoplasmic components. However, smaller clusters cannot recruit as effectively, and they begin to experience a net loss of components to the cytoplasm. As these clusters shrink, they become ever less effective at recruiting cytoplasmic factors, making them shrink further in a vicious cycle until they disappear.

The modeling studies suggest that competition between polarity clusters may simply be an intrinsic feature of the positive feedback mechanism that generates polarity sites. Alternatively, some control system extrinsic to the feedback mechanism may ensure that cells make only a single bud. In the simplest case, cells would contain a single-copy entity necessary for budding, and the polarity cluster that managed to recruit that entity would be destined to make the one and only bud.

If the restriction to make a single bud stems from competition between polarity sites, then perturbations that weaken competition should allow cells to make more than one bud. The efficiency of competition is linked to the timescale in which polarity factors exchange in and out of the polarity site. Genetic manipulations that slowed such exchange also slowed competition, and up to 40% of cells made two, three, or even four buds simultaneously (Figure 6*c*) (Wu et al. 2015). The finding that weakening the competition mechanism allowed cells to make multiple buds argues against an extrinsic pathway to limit the number of buds. Thus, it seems likely that cells normally make only one bud because nascent polarity clusters compete for a shared cytoplasmic pool of proteins.

From One Site to Two: New-End Takeoff in *Schizosaccharomyces pombe*

As with *S. cerevisiae*, germinating spores of *S. pombe* always make one and only one polarity site. However, vegetative cells undergo a cell cycle–regulated switch termed new-end takeoff (NETO), after which they have two polarity sites (Figure 2*b*) (Mitchison & Nurse 1985a). How do these cells maintain two polarity sites?

Genetic analyses identified numerous factors that affect NETO (Arellano et al. 2002, Martin et al. 2005, Verde et al. 1995). Cells that lack Tea1 or Tea4 fail to develop a second polarity site, indicating that the microtubule-delivered tip factors are essential for NETO. However, these factors are clearly not sufficient, as tip factors are present at both cell ends even before NETO, when only one pole has Cdc42. What triggers the switch from one to two sites? A cell cycle pathway has been implicated (see below), but genetic analyses suggest that this is not the whole story.

Mutants lacking the GAP Rga4 or the formin For3 exhibit a remarkable phenotype in which the two daughter cells from a single cell division behave differently: One daughter makes a single Cdc42 polarity site, whereas the other makes two (Das et al. 2007, Feierbach &

Chang 2001). This monopolar or bipolar condition persists throughout interphase, so in these mutants a cell cycle signal is neither necessary nor sufficient to promote bipolarity. As the daughters are genotypically identical, the difference between one site and two sites must be determined by nongenetic differences between cells. For example, one cell may inherit an asymmetry between the two poles (leading to a single winning site), whereas its sister does not (leading to two symmetric sites nucleated by tip factors). The nature of this asymmetry remains mysterious.

Controlling the Size of the Polarity Cluster

In *S. pombe*, a large number of direct and indirect Cdc42 regulators can affect the size of the Cdc42 clusters (Das et al. 2015, Kelly & Nurse 2011). Mutants that reduced positive feedback (*scd1* or *scd2*) exhibited very wide clusters, which could be narrowed by targeting Scd1 to cell tips by artificial fusion to a tip factor (Kelly & Nurse 2011). Thus, positive feedback by local activation (Figure 3) helps to set cluster size, presumably by ensuring maximal Cdc42 activation at the center of the cluster, where GTP-Cdc42 is most concentrated. However, without some constraint, positive feedback could keep going until GTP-Cdc42 covered the entire plasma membrane. In computational models, the growth of polarity clusters becomes stalled due to depletion of the cytoplasmic pools of polarity proteins (Gierer & Meinhardt 1972, Goryachev & Pokhilko 2008, Mori et al. 2008). Consistent with the importance of limiting the abundance of polarity factors, co-overexpression of Cdc42 and its GEF in *S. cerevisiae* is lethal (Ziman & Johnson 1994).

Remarkably, yeast cells tolerate overexpression of either Cdc42 or its GEF (although not both) without marked changes in the polarity site (Howell et al. 2012). A clue to the basis for the robustness of the polarity site to overexpression came with the finding that the polarity circuit contains not only positive feedback but also negative feedback loops (Das et al. 2012, Howell et al. 2012, Wu & Lew 2013). Negative feedback has the capacity to buffer polarity sites by down-regulating some polarity component. In *S. cerevisiae*, a major negative feedback pathway operates via inhibitory phosphorylation of Cdc24 by PAKs (Figure 7*a*) (Kuo et al. 2014, Wai et al. 2009). When such phosphorylation is blocked, clusters accumulate more GTP-Cdc42 (Kuo et al. 2014).

A separate negative feedback mechanism was proposed to operate through Cdc42-directed GAPs in *S. cerevisiae* (Figure 7*b*). GTP-Cdc42 clusters lead to the assembly of septin filaments surrounding the polarity site (Oh & Bi 2011). Septins bind to the Cdc42-directed GAPs Rga1 and Bem2, limiting the spread of GTP-Cdc42 across the septin zone (Caviston et al. 2003). Polarity clusters shrank in a septin-dependent manner at the time of bud emergence, consistent with a role for septins in tuning down the size of polarity clusters (Okada et al. 2013).

The studies discussed above suggest that a limited abundance or (because of negative feedback) activity of polarity factors prevents Cdc42 clusters from accumulating more Cdc42. Given a certain Cdc42 content, the lateral spread of GTP-Cdc42 may depend primarily on Cdc42-directed GAPs. GAPs set the expected lifetime of GTP-bound Cdc42, which in turn controls the extent of diffusional spread of GTP-Cdc42 (Figure 7*c*). Consistent with an important role for GAPs, cells lacking certain GAPs exhibit larger polarity clusters

than do wild-type cells, whereas cells overexpressing GAPs exhibit smaller clusters (Das et al. 2007, Freisinger et al. 2013, Revilla-Guarinos et al. 2016, Woods et al. 2016).

Why is the size of the polarity cluster important? Across a large number of mutants, there is an excellent correlation between the size of Cdc42 clusters and the width of *S. pombe* cells (Das et al. 2015, Kelly & Nurse 2011), supporting the idea that cluster size controls the size of the polar growth zone. However, a mutant lacking Rga2 (a GAP for Rho2 instead of Cdc42) exhibited slightly wider clusters of Cdc42 but narrower cell width (Abenza et al. 2015). Careful comparison of many polarized protein markers in these mutants suggested that the pattern of exocytosis downstream of Cdc42 may be the proximal determinant of cell wall expansion and hence the width of the cell (Abenza et al. 2015).

A correlation between the size of Cdc42 clusters and the width of the growth zone suggests a causal relationship, but cause and effect could in principle go either way. A recent study on germinating spores of *S. pombe* suggested that the size of a Cdc42 cluster depends primarily on the micrometer-scale curvature of the membrane (Figure 7*d*) (Bonazzi et al. 2015). In this system, Cdc42 clusters migrated around the cell before stabilizing to promote polarized growth (Bonazzi et al. 2014). The size of the cluster changed significantly as it moved around the cell, with wider clusters observed at positions with flatter (lower-curvature) cell shape. Manipulating cell shape by forcing spores into prefabricated chambers strengthened the correlation. Moreover, during cell outgrowth, Cdc42 clusters shrank to accommodate the increased curvature (Figure 7*d*). These findings suggest that the initial outgrowth zone is determined by mechanical criteria reflecting the biophysics of the cell wall, with the Cdc42 cluster size adjusting in response to the local cell shape (Bonazzi et al. 2015).

How might cell shape control Cdc42 cluster size? Curvature scaling was dependent on actin and exocytosis, leading to a model in which dilution of polarity factors by vesicle fusion regulates the size of a polarity cluster (Bonazzi et al. 2015). According to this hypothesis, cell curvature affects the catchment area from which actin cables can recruit secretory vesicles: Flatter regions enable more vesicle recruitment, diluting and hence broadening the polarity site. This idea may be worth revisiting in light of new findings showing that the effects of perturbing actin on the distribution of Cdc42 result primarily from induction of a stress-activated pathway involving the MAPK Sty1 (Mutavchiev et al. 2016). In *sty1* mutants lacking this pathway, actin depolymerization had little or no effect on Cdc42 (or even, surprisingly, on polar growth) (Mutavchiev et al. 2016). It would be interesting to know whether curvature scaling of Cdc42 clusters in germinating spores is still observed in *sty1* mutants and, if so, whether that scaling is still actin dependent.

POSITIONING THE POLARITY SITES

The core polarity program discussed thus far can generate a polarized cell without preexisting positional cues. This suffices to explain symmetry breaking in germinating yeast spores, but vegetatively growing cells of both *S. cerevisiae* and *S. pombe* select specific, nonrandom sites at which to establish polarity. Moreover, once established, polarity sites must be retained at the appropriate locations to achieve proper cell morphology.

Bud Site Selection in *Saccharomyces cerevisiae*: Heritable Landmarks Influence Future Bud Site Positioning

In *S. cerevisiae*, buds emerge in specific patterns that vary according to cell type (Chant & Pringle 1995) and nutrient conditions (Mosch & Fink 1997). These patterns are due to a set of landmarks that are deposited at specific positions during the growth of the bud and that remain to mark those positions in newborn cells. The three best-understood landmarks are depicted in Figure 8*a*. Axl2 is localized to a ring at the previous division site of mother and daughter cells (Roemer et al. 1996). Bud8 and Bud9 are localized to the distal and proximal poles of newborn daughter cells, respectively (Harkins et al. 2001). All three landmarks are transmembrane proteins trafficked through the secretory pathway. Concentration of the landmarks at their designated sites is a complex process that has been reviewed recently (Bi & Park 2012).

Once localized, the landmarks communicate with the Cdc42 polarity machinery via a GTPase termed Rsr1 (Figure 8*a*) (Bender & Pringle 1989, Chant & Herskowitz 1991). Landmarks can bind and thereby localize the Rsr1-directed GEF Bud5 to the designated sites, leading to local activation of Rsr1 (Kang et al. 2001). GTP-Rsr1 interacts with the Cdc42-directed GEF Cdc24, presumably recruiting it to those sites (Zheng et al. 1995). In the absence of Rsr1, or in cells carrying a mutation that disrupts the interaction between Rsr1 and Cdc24, cells bud in random patterns (Bender & Pringle 1989, Shimada et al. 2004, Sloat et al. 1981).

The landmark/Rsr1 system clearly influences the location of future bud sites, but it does not specify a unique site. For example, Axl2 delineates a ring, yet somehow a specific position on the outside of that ring gets picked to become the next polarity site (Figure 8*a*). Similarly, bipolar landmarks specify two broad poles within which a single polarity site develops. How does that position get picked from all the possible locations? As during symmetry-breaking polarization, cells often generate more than one initial cluster of Cdc42 at the poles and then eliminate supernumerary clusters (Wu et al. 2013). Thus, the GEF recruited by Rsr1 may provide a small stimulus that biases where positive feedback takes off, with the final polarity site growing and competing as it does during symmetry breaking.

As the landmark/Rsr1 system favors the site of cell division, why don't yeast cells bud from the middle of the division site? Genetic analyses identified two mechanisms to block such rebudding. First, Cdc42-directed GAPs are concentrated at the neck during cytokinesis, and one GAP (Rga1) remains at the division site after cell separation. Rga1 provides a central inhibitory zone within the Axl2 ring (Figure 8*b*), ensuring that the polarity site forms outside, and not within, the ring (Tong et al. 2007).

Rga1 acts only at the immediately completed division site, but the exclusion of rebudding from a used site extends to all previous bud sites. A pair of interacting landmark proteins termed Rax1 and Rax2 mark all so-called cytokinesis remnants (Figure 8*b*) (Chen et al. 2000), and Rax1/2 anchors a memory complex that blocks budding at such remnants (Meitinger et al. 2014). In the absence of Rga1 or the memory complex, cells can bud repeatedly from the same location. However, the cytoplasmic channel through the mother-bud neck becomes thinner with each new bud so that eventually the cells cannot successfully

complete nuclear division (Meitinger et al. 2014). In summary, budding yeast cells inherit prelocalized landmarks that either promote or inhibit subsequent polarization at their locations.

Polarizing at Cell Tips in *Schizosaccharomyces pombe*: Guidance from Microtubules and Cell Shape

After cell division, fission yeast cells polarize first to the old end (away from the cytokinesis site) and later to the new end created during cytokinesis. How are these positions established? A dominant mechanism involves microtubule plus ends. In normally shaped cylindrical cells, microtubule bundles align along the long axis, with plus ends pointed toward the cell ends, and polarity is established at the ends. However, when microfluidic approaches were used to create bent fission yeast cells, microtubule ends often contacted the convex cell sides, and local deposition of tip factors like Tea1 and Tea4 at these contact sites promoted local enrichment of Cdc42 (Minc et al. 2009, Terenna et al. 2008). Thus, rather than inheriting marked poles, fission yeast cells polarize wherever microtubule ends make contact with the cortex.

But why do the cells first polarize to the old end instead of to the new end? In several cytokinesis mutants, new end growth can be delayed due to the persistence of remnants of the cell division apparatus (Bohnert & Gould 2012), suggesting that cytokinetic remnants suppress initial polarization at the new end.

Although microtubules clearly play a major role in selecting the cell ends as sites of polarization, polarization at cell ends can still occur when microtubules are depolymerized, so other pathways must exist. A recent study suggested that, in cells recovering from starvation, polarization can be targeted to cell ends by sterol-rich membrane domains (SRMs) rather than by microtubules (Makushok et al. 2016). In *S. pombe*, SRMs localize to the cell growth zones and to the cell division site (Takeda et al. 2004, Wachtler et al. 2003). Upon release from starvation, SRM clustering at cell ends preceded detectable polarization of other factors and did not require microtubules (Makushok et al. 2016). Exactly how polarity sites are chosen in cells lacking microtubules remains a fertile area for exploration.

Keeping the Polarity Site on Track

As discussed above, a polarity site consists of a dynamic cluster of polarity factors maintained by positive feedback. However, delivery of vesicles that insert fresh membrane at the polarity site can lead to perturbations of polarity factor concentration that result in displacement of the polarity site. This raises the question of how cells can prevent the polarity site from moving around to promote bud emergence (in *S. cerevisiae*) or cylindrical extension (in *S. pombe*).

In germinating spores of *S. pombe*, polarity site movement ceases upon breakage of the rigid outer spore wall (Bonazzi et al. 2014). This observation suggested that mechanical features of the cell wall control movement of the polarity site. In particular, successful remodeling of the cell wall to allow polar growth may feed back on the polarity machinery to stall the movement. How the proposed mechanical feedback is converted to biochemical changes that stabilize the polarity site remains a fascinating question.

In mating cells of *S. cerevisiae*, a high dose of pheromone can stall polarity site movement (Dyer et al. 2013, McClure et al. 2015). Two genetically separable pathways can independently constrain polarity site mobility. The first involves recruitment of Cdc24 to sites of pheromone receptor activation (McClure et al. 2015, Nern & Arkowitz 2000). Vesicles carry newly synthesized pheromone receptors, and vesicle fusion allows these receptors to interact with extracellular pheromone. The activated receptors then recruit GEF to the sites where vesicle fusion occurred, counteracting the vesicular dilution effect (McClure et al. 2015).

The second pathway requires the bud site selection protein Rsr1 (Figure 8*a*) (Dyer et al. 2013, Nern & Arkowitz 2000) and may function even in the absence of pheromone (Kozminski et al. 2003, Ozbudak et al. 2005). Indeed, in filamentous fungi that make long hyphae, Rsr1 homologs are critical for keeping the polarity site consistently oriented and for preventing it from wandering or transiently disappearing (Bauer et al. 2004, Brand et al. 2008, Hausauer et al. 2005, Pulver et al. 2013). Following polarity establishment, Rsr1 and its GEF Bud5 relocate from landmark sites to the polarity site (Kang et al. 2001, Park et al. 2002), suggesting that Rsr1 may participate in a positive feedback loop that maintains the polarity site at a consistent location.

Generating a regular cylindrical shape by polar growth requires that polarity sites in *S. pombe* remain pointed in the same direction during most of the cell cycle. This directional constancy requires the longitudinally oriented microtubule bundles as well as the tip factors (including Tea1) discussed above. Cells lacking Tea1 form bent shapes, suggesting that the polarity site has a propensity to move laterally unless it is stabilized through the action of tip factors. As there are only a few microtubule bundles and their tips contact different parts of the cell ends at any given time, maintaining a precisely centered polarity site would likely require a mechanism that somehow averaged the positions of the microtubule tips over time. An elegant mechanism to achieve this outcome involves oligomerization of Tea1 into a stable scaffold at cell ends (Bicho et al. 2010).

Packets of Tea1 are deposited at cell ends from microtubule tips and are then incorporated into a stable scaffold that displays 100-fold-slower turnover relative to that of the polarity factors. Structural and mutagenesis findings suggest that Tea1 can trimerize, allowing it to polymerize into a two-dimensional lattice (Bicho et al. 2010). Super-resolution microscopy indicated that the scaffold had denser and less dense regions, where gaps in the lattice could allow for insertion of new Tea1 packets (Dodgson et al. 2013). Tea1 polymerization requires transient interaction with a membrane-anchored protein termed Mod5, which is concentrated at cell tips through interaction with Tea1 (Bicho et al. 2010, Snaith et al. 2005, Snaith & Sawin 2003). Modeling of this system suggested that the combination of Mod5-catalyzed Tea1 incorporation into the lattice and Tea1-mediated concentration of Mod5 at cell ends would create a focusing of the lattice such that it remained centered at cell ends during polar growth (Bicho et al. 2010). By anchoring other factors that impact Cdc42 (see above), this Tea1 scaffold has the potential to keep the polarity sites on track.

In addition to the Tea1 system, another pathway mediated by the AAA+ ATPase Knk1 is required to keep the polarity site from making excursions in *S. pombe. knk1* mutants form

kinks indicative of occasional lateral sliding of the polarity site (Scheffler et al. 2014). Knk1 is localized to cell ends in a Cdc42-dependent manner, but its mechanism of action remains a mystery.

COORDINATING POLARITY WITH THE CELL CYCLE

Budding Yeast Polarize in G1 and Depolarize in G2

Budding yeast cells switch between periods of isotropic growth and polar growth in a manner coordinated with the cell cycle (Figure 2*a*) (Howell & Lew 2012). During early G1, before cell cycle commitment at what is termed start, cells grow isotropically, and Cdc42 is not polarized (Gulli et al. 2000). Activation of G1 cyclin/cyclin-dependent kinase (CDK) triggers passage through start, after which Cdc42 GTP loading increases (Atkins et al. 2013) and Cdc42 becomes polarized (Gulli et al. 2000, Richman et al. 2002) to begin growing a bud. Cdc42-directed GAPs are substrates of G1 CDKs, and mutagenesis of CDK target sites suggested that phosphorylation inhibits GAP activity, removing a block to the accumulation of GTP-Cdc42 (Knaus et al. 2007, Sopko et al. 2007). More recently, another link emerged from studies using optogenetic approaches to activate small amounts of Cdc42 at illuminated sites of the cell cortex (Witte et al. 2017). After start, illumination provoked a strong accumulation of Bem1 as well as GTP-Cdc42, leading to bud emergence. But before start, Bem1 was not recruited to the illuminated site, and GTP-Cdc42 accumulation was weak. Moreover, whereas Bem1 and the GEF Cdc24 were colocalized after start, they could be observed in separate puncta before start. These findings suggested that association between Bem1 and Cdc24, and therefore Bem1-mediated positive feedback, is dependent on CDK activation at start (Witte et al. 2017).

In G2, activation of mitotic cyclin/CDK leads to a switch from apical growth (directed toward the tip of the bud) to isotropic growth within the bud (Figure 2*a*) (Lew & Reed 1993). After the switch, tight clustering of Cdc42 at the bud tip no longer occurs. How G2 CDK activity triggers this switch is not clear but may involve increasing GAP activity (Saito et al. 2007) or inhibiting Bem1-mediated positive feedback (Howell & Lew 2012, Kozubowski et al. 2008).

During septum formation, Cdc42 is concentrated at the mother-bud neck along with Cdc24 and Bem1 (Figure 2*a*). However, this Cdc42 is inactive, and septum formation does not require Cdc42 (indeed, it can be impeded by active Cdc42) (Atkins et al. 2013, Onishi et al. 2013). As neck-localized Cdc42 does not appear to be used for cytokinesis, it is unclear why cells concentrate these polarity proteins at the neck. One reason may be to facilitate equal partitioning of Cdc42 and its regulators during cell division: If this relocalization did not occur, the daughter cell (which had recently accumulated polarity factors) might inherit many more of those factors than the mother.

Fission Yeast Initiate a New Site in G2 and Depolarize During Mitosis

Fission yeast cells are born at a length of ~7 μm and grow in a unipolar manner until they reach a length of ~9.0–9.5 μm, when they initiate NETO and grow in a bipolar fashion until they reach ~14 μm (Figure 2*b*) (Mitchison & Nurse 1985b). Growth alone is insufficient to

trigger NETO, as mutants defective in DNA replication elongate but do not undergo NETO. In contrast, cell cycle mutants arrested in G2 grow in a bipolar fashion, suggesting that completion of S phase is required for NETO (Mitchison & Nurse 1985b).

Genetic screens have identified genes that delay NETO in response to inhibition of DNA synthesis (Koyano et al. 2010, Kume et al. 2011). In particular, Cds1 (an S phase checkpoint kinase) phosphorylates Ppb1 (a calcineurin-family phosphatase), which dephosphorylates Tip1 (a plus TIP protein related to Clip170) and Cki3 (casein kinase 1γ). Tip1 dephosphorylation reduces microtubule dynamics, which in turn reduces delivery of Tea1 to the new end (Kume et al. 2011). Cki3 dephosphorylation leads to membrane association of Cki3; at the membrane, Cki3 phosphorylates Tea1, reducing its affinity for Tea4 (Koyano et al. 2010). Reduced Tea1 delivery combined with reduced Tea4 interaction delivers much less Tea4 to the new end, delaying NETO.

In contrast to the pathway above, CDK and the Polo kinase Plo1 stimulate NETO. G2 levels of CDK activity activate a spindle pole body (SPB)-bound fraction of Plo1, which promotes NETO (Grallert et al. 2013). How SPB-bound active CDK and Plo1 drive NETO is unknown, but SPB localization is key, as activated Plo1 localized elsewhere in the cell cannot drive NETO. It will be interesting to investigate links between the inhibitory Cds1-Ppb1-Cki3 pathway and the stimulatory CDK-Plo1 pathway. Interestingly, regulators of Orb6 (which regulates polarity through Gef1 and Rga4) form a morphogenesis network (MOR) with some components localized to the SPB, so CDK-Plo1 may regulate NETO via the MOR (Hirata et al. 2002, Hou et al. 2003, Kanai et al. 2005, Mendoza et al. 2005, Verde et al. 1998).

Polarized growth ceases during mitosis, when the fission yeast cell prepares to undergo cytokinesis (Figure 2*b*). As in budding yeast, Cdc42 and its regulators Gef1 and Scd1/Scd2 become concentrated at the division site (Wei et al. 2016). However, in contrast to the case in budding yeast, this Cdc42 is active and important for normal cytokinesis (Wei et al. 2016). Thus, it may be that polar growth ceases because its components are relocated and repurposed for cytokinesis. Genetic and biochemical studies indicate that activation of SIN (septation initiation network) during cytokinesis leads to dispersal of MOR components from the SPB, contributing to the inhibition of polarized growth (Gupta et al. 2013, Ray et al. 2010).

COORDINATING POLARITY WITH CYTOKINESIS

Cell polarity plays both direct and indirect roles in the choice of the correct division site. In budding yeast, the polarity site assembles a septin ring that remains at the bud neck and directly positions the cytokinesis machinery later in the cell cycle. In fission yeast, cell polarity also plays at least three roles in division site determination.

Medial division in fission yeast is tightly correlated with the position of the interphase nucleus (Chang et al. 1996). The nucleus is attached to microtubule bundles and is dynamically centered in the cell by a balance of microtubule pushing forces originating from the two cell ends (Brunner & Nurse 2000, Tran et al. 2001). Experimental misplacement of

the interphase nucleus leads to cell division at nonmedial locations (Daga & Chang 2005, Tolic-Norrelykke et al. 2005). The nucleus promotes medial placement of the actomyosin ring via the anillin-like protein Mid1 (Bahler et al. 1998, Paoletti & Chang 2000, Rincon et al. 2017, Sohrmann et al. 1996). Mid1 localizes to the nucleus and the overlying cell membranes in nodes during interphase. The nuclear pool of Mid1 is released into mature nodes upon mitosis-specific phosphorylation by Plo1 (Bahler et al. 1998, Huang et al. 2008, Vavylonis et al. 2008, Wu et al. 2006).

Tip factors also participate in division site placement. Tea1 and Tea4 promote localization of Pom1 kinase at cell ends (Bahler & Pringle 1998, Tatebe et al. 2005), inhibiting actomyosin ring assembly in at least two ways. Pom1 prevents assembly of Mid1 at the nongrowing end (Celton-Morizur et al. 2006, Padte et al. 2006) and acts in a Mid1-independent manner as well. Pom1 may phosphorylate and inactivate the fraction of the F-BAR protein Cdc15 that is localized at the cell ends (Huang et al. 2007), a role conserved in *Schizosaccharomyces japonicus* (Gu et al. 2015), in which division site placement is regulated predominantly by the polarity factors, rather than by Mid1.

Proper cylindrical geometry also plays an important role in the maintenance of the actomyosin ring at the site of its assembly. In spherical or conically shaped fission yeast cells, the contracting actomyosin ring slides along the cell perimeter, from the region of higher diameter toward the region of lower diameter (Mishra et al. 2012). This sliding causes division septum placement at nonmedial/equatorial locations, revealing a third way in which cylindrical cell morphology and correct polarity influence division site placement.

CONCLUSIONS

Studies on budding and fission yeasts highlight both a conserved core polarity circuit and several species-specific features that adapt the core circuit to serve the very different needs of the two yeasts. The core circuit consists of a positive feedback loop in which GTP-Cdc42 at the membrane recruits a GEF-containing complex from the cytoplasm to promote activation of neighboring Cdc42. This core feedback loop allows Cdc42 to break symmetry and to form clusters at random locations. However, with the exception of germinating spores, yeast cells have upstream pathways that influence the locations at which Cdc42 clusters form. In budding yeast, cells inherit prelocalized landmarks that either promote or inhibit polarization in their vicinity. In fission yeast, microtubules deliver polarity-promoting tip factors to the cell ends. Polarity is regulated by the cell cycle so that polarized growth is switched on and off, but at different cell cycle stages in each yeast. One of the most striking differences between the two yeasts concerns the number of polarity sites. Budding yeast always make a single mature site through a process in which nascent sites compete with each other for shared cytoplasmic factors. Fission yeast transition from one to two sites as they progress through the cell cycle. How the common core pathway is adapted to produce these different features remains poorly understood.

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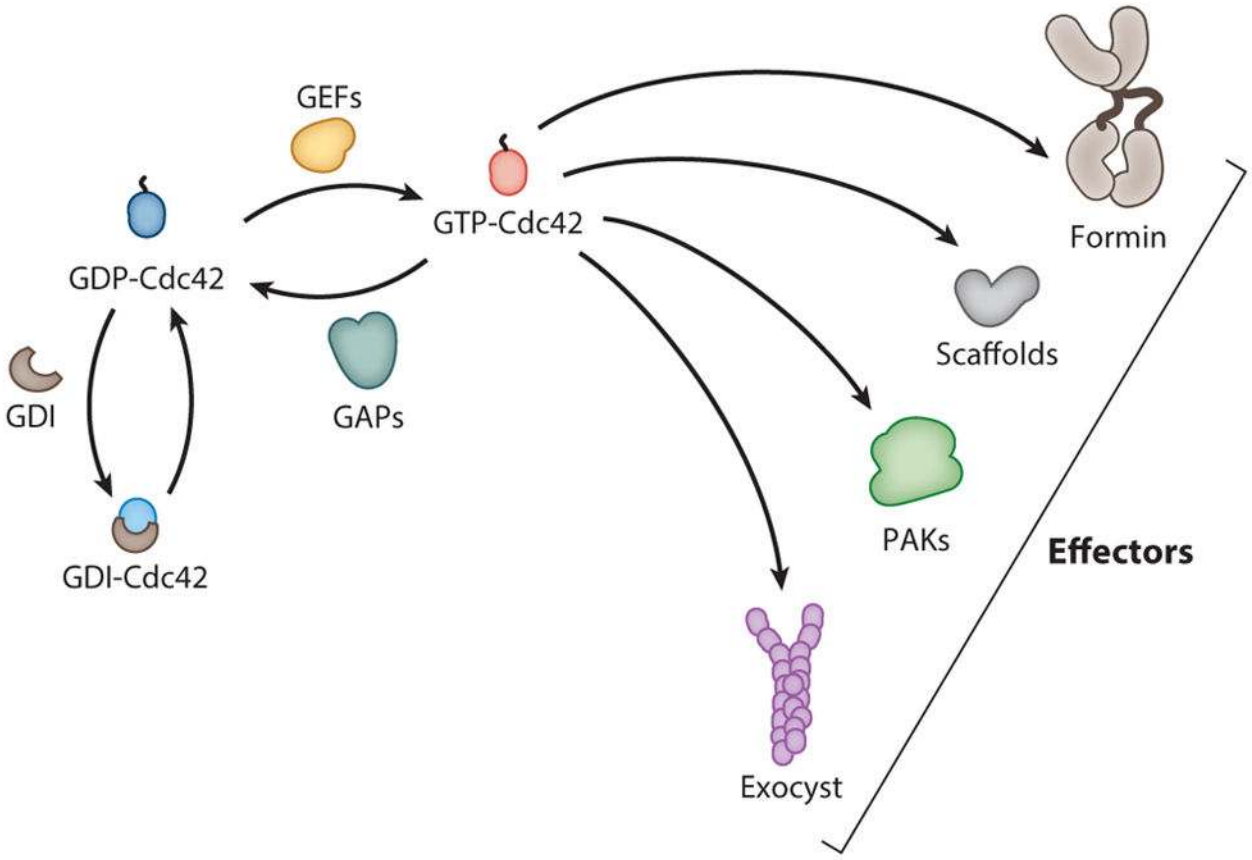

Figure 1.

Cdc42 regulators and effectors. Like other G proteins, Cdc42 exhibits slow intrinsic rates of GDP/GTP exchange and GTP hydrolysis, which are accelerated by GDP/GTP exchange factors (GEFs) or GTPase-activating proteins (GAPs). Cdc42 is prenylated at its C terminus, and much of the cell's Cdc42 is found on the cytoplasmic face of cellular membranes, including the plasma membrane (Erickson et al. 1996, Richman et al. 2002, Ziman et al. 1993). A soluble pool of Cdc42 is also present and bound to GDIs (guanine nucleotide dissociation inhibitors) that can extract it from the membrane (Koch et al. 1997, Leonard & Cerione 1995, Leonard et al. 1992, Masuda et al. 1994, Tiedje et al. 2008). Effectors bind specifically to GTP-Cdc42, and not to GDP-Cdc42.

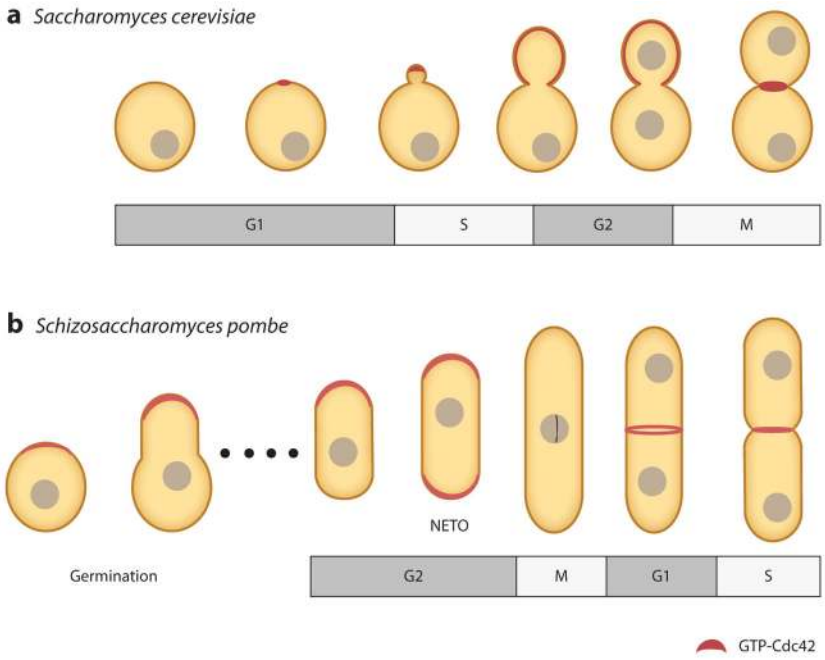

Figure 2.

Polarity in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. (*a*) In *S. cerevisiae*, cells polarize Cdc42 in late G1 at a single site and grow a bud starting in S phase. Cdc42 becomes depolarized within the bud in G2 and concentrates (in the inactive form) at the mother-bud neck during cytokinesis. (*b*) In *S. pombe*, germinating spores break symmetry to polarize at a single site to generate a cylindrical cell. In rich media, cytokinesis and cell separation take a significant fraction of the cell cycle, and rapid G1 and S phases occur during this time, so cells are born in G2. Initially, cells polarize Cdc42 to the old end (opposite the cytokinesis site). After further growth, a second polarity site is established at the new end, and cells grow at both ends until mitosis, when Cdc42 relocates from the ends to the middle of the cells.

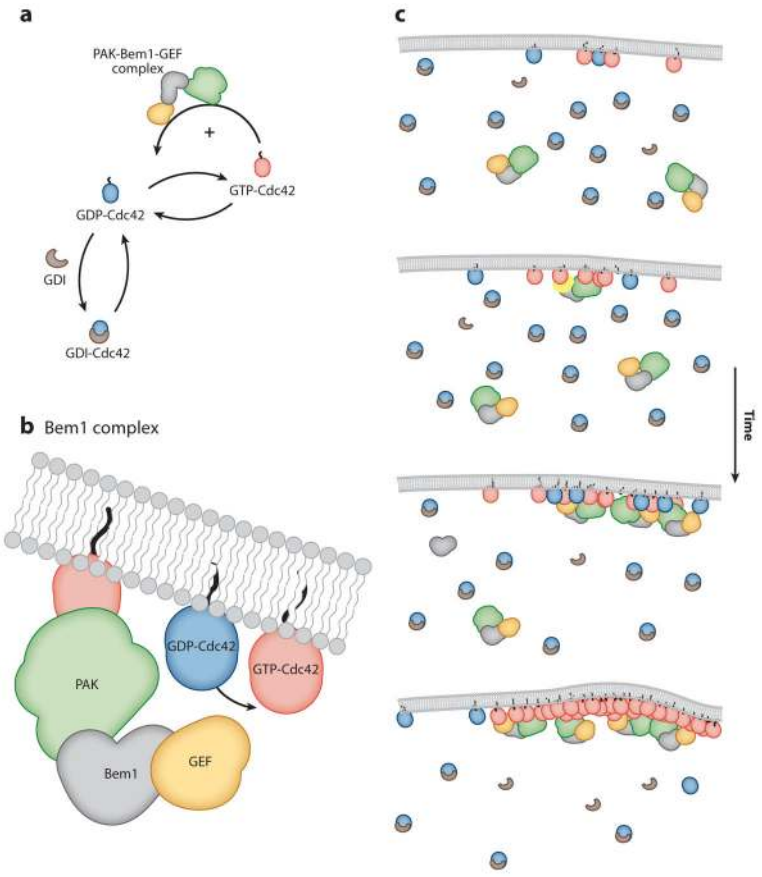

Figure 3.

Positive feedback by local activation of Cdc42. (*a*) GTP-Cdc42 can bind effectors [p21-activated kinases (PAKs)] that in turn bind the scaffold Bem1, which binds the GDP/GTP exchange factor (GEF) Cdc24. (*b*) The PAK-Bem1-GEF complex allows one GTP-Cdc42 to promote activation of neighboring GDP-Cdc42 in a positive feedback loop. (*c*) Over time, positive feedback promotes local accumulation of GTP-Cdc42 and PAK-Bem1-GEF complexes at the membrane, depleting cytoplasmic pools of these components.

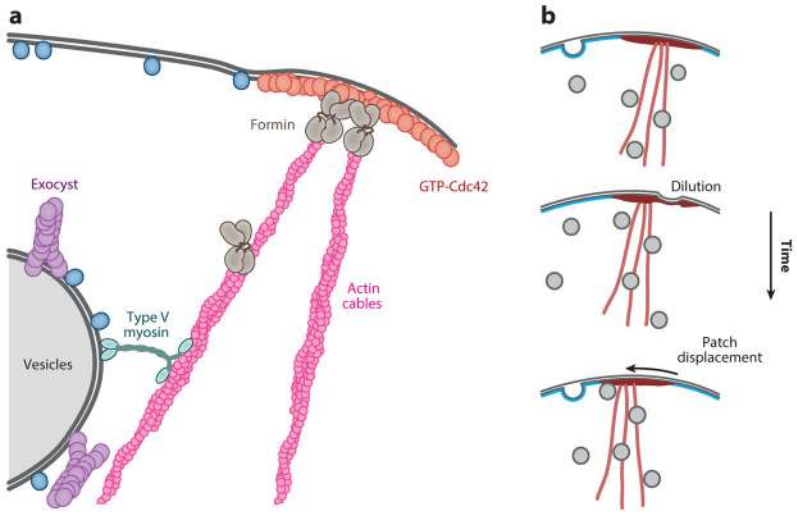

Figure 4.

Effect of actin-mediated vesicle delivery on Cdc42. (*a*) In *Saccharomyces cerevisiae*, actin cables become oriented toward the polarity site by formins, and secretory vesicles are delivered to the polarity site by type V myosin. Exocyst complexes bind Cdc42 and promote vesicle fusion (exocytosis). (*b*) Vesicles lack most polarity factors, so vesicle fusion transiently dilutes polarity factor concentration, and off-center fusion of vesicles can lead to displacement of the centroid of the polarity site away from the vesicle fusion site.

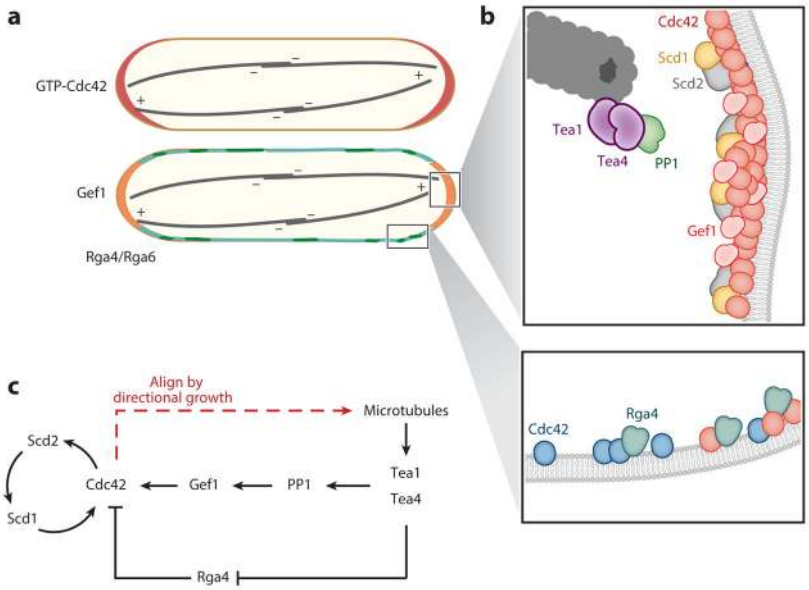

Figure 5.

Effect of microtubules and cell shape on Cdc42 in *Schizosaccharomyces pombe*. (*a*) Microtubule bundles align longitudinally within the cell, with plus ends pointing toward cell tips and minus ends in the cell middle. (*b*) Tip factors (Tea1, Tea4) are delivered to cell ends and bring binding partners, including protein phosphatase 1 (PP1), which promotes localization of the GDP/GTP exchange factor (GEF) Gef1 and exclusion of the GTPase-activating protein (GAP) Rga4 from cell ends. The spatial separation of GEF and GAP promotes Cdc42 activation at cell ends and inactivation at cell sides. (*c*) Cdc42 is further concentrated by the action of a positive feedback loop similar to that in *Saccharomyces cerevisiae*, with Scd1 = Cdc24 and Scd2 = Bem1.

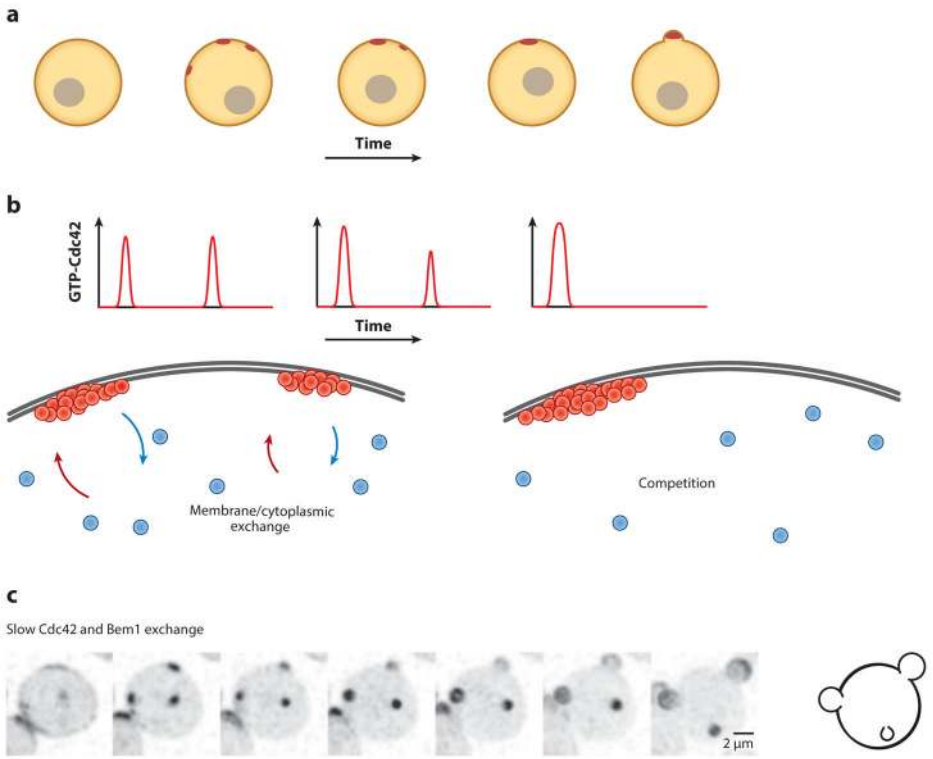

Figure 6.

Competition between polarity sites in *Saccharomyces cerevisiae*. (*a*) Cells often initially make >1 cluster of Cdc42 and other polarity factors but then eliminate all but one cluster, which goes on to form the bud. (*b*) Computational models of local activation initiated with two peaks of Cdc42 concentration show competition such that one peak grows and the other shrinks. Polarity factors exchange dynamically between membrane clusters and the shared cytoplasm, and in the computational model this exchange allows a larger peak to outcompete a smaller peak. (*c*) In cells genetically manipulated to slow the exchange of polarity factors between membrane and cytoplasm, competition slows, and cells can make >1 bud simultaneously. Adapted from Wu et al. (2015).

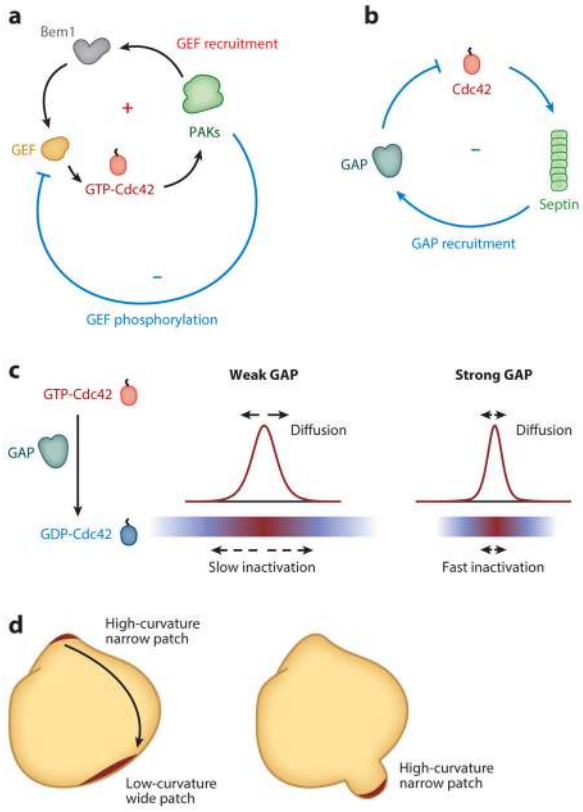

Figure 7.

Size of polarity sites: GTPase-activating proteins (GAPs), feedback, and cell curvature. (*a*) GDP/GTP exchange factor (GEF) phosphorylation by p21-activated kinase (PAK) constitutes a negative feedback loop that limits GEF activity and hence the amount of GTP-Cdc42 in a polarity cluster. (*b*) A second negative feedback loop operates through septins and GAPs. (*c*) Cdc42-directed GAPs set a timescale for GTP hydrolysis, and the diffusion of GTP-Cdc42 at the membrane converts that timescale to a length scale that determines the lateral spread of GTP-Cdc42. Stronger GAP activity translates to a shorter lifetime and hence less lateral spread of active Cdc42. (*d*) Curvature of the cell membrane affects the size of the Cdc42 cluster in germinating spores of *Schizosaccharomyces pombe*, with higher curvature translating to a smaller cluster.

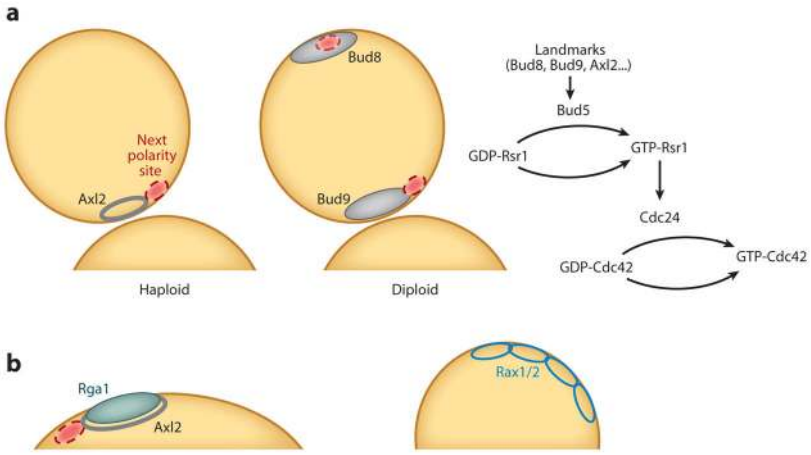

Figure 8.

Landmarks and bud site selection in *Saccharomyces cerevisiae*. (*a*) Axl2, Bud8, and Bud9 are transmembrane proteins that are inherited at the indicated locations in newborn daughter cells and influence the location of polarity sites (*red*) in the next cell cycle. Haploid cells prefer Axl2-marked sites, and diploid cells prefer Bud8/Bud9-marked sites. These landmarks act by recruiting Bud5, the GDP/GTP exchange factor (GEF) for Rsr1, to locally generate GTP-Rsr1. GTP-Rsr1 in turn recruits Cdc24, the Cdc42-directed GEF. (*b*) The Cdc42-directed GTPase-activating protein (GAP) Rga1 blocks rebudding at the cytokinesis site, and the landmarks Rax1 and Rax2 anchor a memory complex at cytokinesis remnants that blocks polarization at all previous bud sites.

Table 1

Genes referred to in this review

Regulators	*Saccharomyces cerevisiae*	*Schizosaccharomyces pombe*	Comments
GEFs	Cdc24	Scd1	Critical for polarity establishment
	Bud3	Gef1	Helps to position polarity site
GAPs	Bem2		Major Cdc42 GAP in the budding yeast; also works on Rho1
	Bem3		
	Rga1 Rga2	Rga4 Rga6	Rga1 blocks rebudding at division site; Rga4 and -6 block spreading of GTP-Cdc42 from cell ends
GDI	Rdi1	Rdi1	Enhances mobility of GDP-Cdc42
Effectors	Bni1	For3	Formin: promotes actin nucleation at the polarity site
	Cla4 Ste20	Shk2 Shk1	PAKs: participate in both positive feedback and negative feedback
	Bem1	Scd2	Scaffold: participates in positive feedback (links PAKs to GEF)
	Gic1 Gic2		Scaffold
	Boi1 Boi2	Pob1	Scaffold: promotes exocytosis
	Exo70 Sec3		Exocyst subunit: promotes exocytosis
Positioning the polarity patch		Tea1 Tea4 Mod5	Tip factors; target PP1, For3, and Pom1 to cell ends. Tea1 polymerizes into stable scaffold with help from Mod5
		Orb6	NDR/LATS kinase: phosphorylates Gef1
		Knk1	AAA ATPase: stabilizes Cdc42 at tips
	Axl2		Landmark at previous division site
	Bud8 Bud9		Landmarks at distal (Bud8) and proximal (Bud9) poles of cells
	Rax1 Rax2		Landmarks at cytokinesis remnants
	Bud5		GEF for Rsr1
	Rsr1		Ras-family GTPase: recruits Cdc24
Cell cycle control		Cds1	S phase checkpoint kinase
		Ppb1	Phosphatase: acts on Tip1 and Cki3
		Tip1	Microtubule binding Clip170 family
		Cki3	Casein kinase
		Plo1	Polo family kinase: promotes NETO
		Pom1	Kinase: inhibits actomyosin ring assembly at poles
		Mid1	Anillin homolog
		Cdc15	F-BAR protein
Other		Sty1	Stress-activated MAPK
		Rga2	GAP for Rho2

A blank cell denotes one of the following: (*a*) no obvious homolog exists (e.g., Tea1 in *S. cerevisiae*); (*b*) a clear homolog exists but has not been studied with regard to the point of interest for this review (e.g., exocyst subunits as Cdc42 effectors in *S. pombe*); or (*c*) proteins in the same family are present, but we do not know whether they share similar functions (e.g., Bem2 compared to other GAPs in *S. pombe*). Abbreviations: GAP, GTPase-activating protein; PAK, p21-activated kinase; GEF, GDP/GTP exchange factor; GDI, guanine nucleotide dissociation inhibitor; MAPK, mitogen-activated protein kinase; NETO, new-end takeoff.