REVIEW ARTICLE

Cell polarization in budding and fission yeasts

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Abstract

Polarization is a fundamental cellular property, which is essential for the function of numerous cell types. Over the past three to four decades, research using the best-established yeast systems in cell biological research, *Saccharomyces cerevisiae* (or budding yeast) and *Schizosaccharomyces pombe* (or fission yeast), has brought to light fundamental principles governing the establishment and maintenance of a polarized, asymmetric state. These two organisms, though both ascomycetes, are evolutionarily very distant and exhibit distinct shapes and modes of growth. In this review, we compare and contrast the two systems. We first highlight common cell polarization pathways, detailing the contribution of Rho GTPases, the cytoskeleton, membrane trafficking, lipids, and protein scaffolds. We then contrast the major differences between the two organisms, describing their distinct strategies in growth site selection and growth zone dimensions and compartmentalization, which may be the basis for their distinct shapes.

Introduction

Cell polarization is a basic feature underlying the functionality of virtually all cell types. It is thus not surprising that the two most established yeast systems in cell biological research, *Saccharomyces cerevisiae* (also known as budding yeast) and *Schizosaccharomyces pombe* (or fission yeast), have for a long time attracted the attention of researchers as models to study this basic cell biological question that is how cell asymmetry is established and maintained. The stereotypical shapes of these yeasts have facilitated the identification of a large number of morphological mutants, which, thanks to the elaborate genetic tools developed in these organisms, have led to an ever-increasing understanding of cell polarization. Most critically, the unicellularity and simplicity of these yeast models have allowed experimental and modeling approaches to start dissecting the fundamental principles of symmetry breaking – the mechanisms by which cell polarization can be initiated *de novo*.

The aim of this review is to provide an up-to-date overview of our understanding of cell polarization, both in budding and fission yeast, focusing our attention on cell polarization during the mitotic growth cycle, that is, intrinsic cell polarization in the absence of an external cue. After a brief introduction into the two model systems, we describe in detail the common major polarization mechanisms used by both species, reviewing the role of the cytoskeleton, membrane composition and fluxes, small Rho-family G-proteins, scaffold proteins, and feedback loops in generating polarized states. In the final part of the review, we then focus our attention on the differences between the two yeasts, which may underlie the unique shape of each organism.

Saccharomyces cerevisiae and *S. pombe* – a primer

Saccharomyces cerevisiae and *S. pombe* belong to the largest fungal phylum, the ascomycetes, defined by the presence of an ascus, a sac within which spores develop. While the ascomycetes form a monophyletic group, *S. cerevisiae* and *S. pombe* are very divergent species within this group: The archiascomycete lineage, which includes

FEMS MICROBIOLOGY REVIEWS

S. pombe, is estimated to have diverged from the rest of the ascomycetes between 400 and 1200 million years ago (Heckman *et al.*, 2001; Taylor & Berbee, 2006), a divergence roughly as large as that between humans and nematodes (Heckman *et al.*, 2001; Fig. 1a). Accordingly, the two species diverged significantly in their shapes and physiologies. The study of both systems thus reveals commonalities tracing back to the origins of the ascomycetes and likely well beyond. It can also expose differences reflecting the inherent diversification of cell biological processes and guard against the danger of over-simplification of one-model-fits-all approaches.

In the wild, *S. cerevisiae* is believed to exist mostly as a diploid. Starvation induces meiosis and sporulation. Haploid cells of opposite mating types mate upon encounter, even as early as during spore germination. *Saccharomyces cerevisiae* is found in vineyards, a habitat that led to its major impact on human economy in brewing and wine-making. Yeasts, important for these human activities, were proposed to represent domesticated strains initially derived from natural habitats such as trees exudates (Fay & Benavides, 2005). In the laboratory, *S. cerevisiae* can be easily maintained as either haploids or diploids. Each cell, almost round at birth, grows by budding, placing its new bud either next to the previous division site or at the opposite cell pole, and dividing at the neck position between the mother cell and daughter bud. During the initial bud growth phase, the bud grows apically, extending in length (Fig. 1b). The cell then undergoes a growth transition, where growth switches to an isotropic mode, with growth being distributed throughout the bud, resulting in the spherical expansion of the bud.

By contrast, *S. pombe* is stable only as haploid. In this species, starvation triggers mating between haploid cells of opposite mating types, a process then directly coupled to meiosis and sporulation for the production of stress-resistant spores. The ecology of *S. pombe* remains largely unknown: it was initially isolated from millet beer, and more recently again from alcoholic beverages or cultivated fruits, but almost nothing is known about its natural niches, nor about its (good or bad) influence on the fermentation of human drinks. *Schizosaccharomyces pombe* cells are rod-shaped. They maintain a constant diameter, grow in length by tip extension, and divide by medial fission. Fission yeast cells exhibit several polarity transitions during their mitotic cycle: A new-born cell initially grows at a single pole – the 'old' pole, which pre-existed before cell division – and then initiates growth at the second 'new' cell pole in G2 phase (Fig. 1b). The process is referred to as NETO, for New End Take-Off. At the end

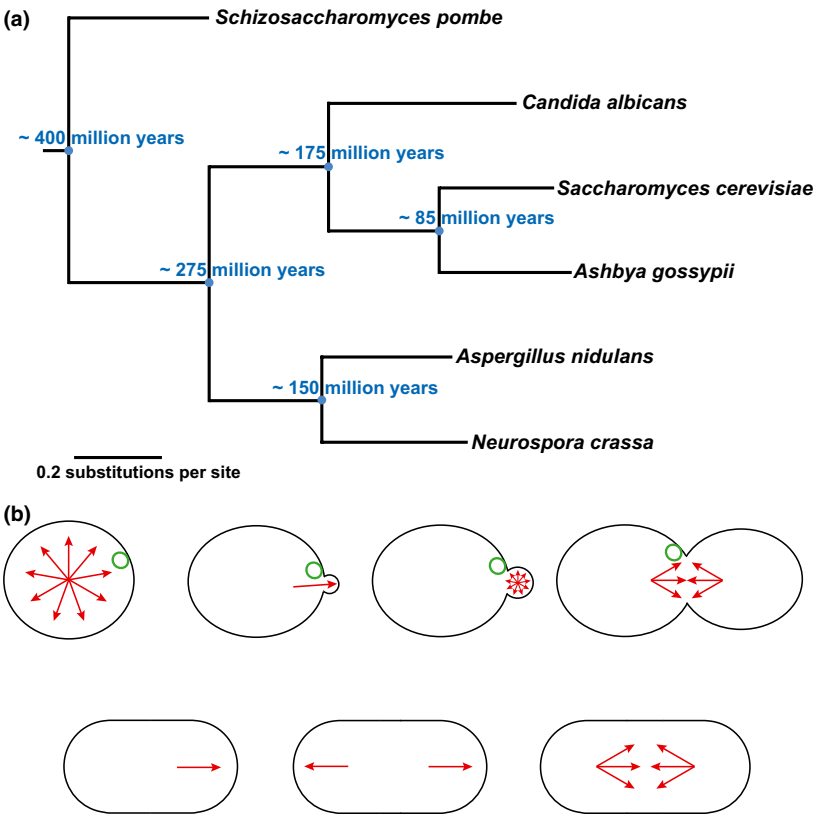

Fig. 1. Phylogenetic relationship between *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* and sites of growth. (a) Phylogenetic tree depicting the relationship between the different species indicated, adapted from Seiler and Justa-Schuch (2010). The branch lengths represent number of substitutions, and approximate divergence time is indicated in blue. Tree construction is described in Seiler and Justa-Schuch (2010). (b) Location of growth sites during mitotic cell division. Red arrows indicate the local addition of new plasma membrane material (proteins, lipids, and cell wall biosynthetic enzymes), which alternates between single and multiple sites in both yeasts. Green indicates bud landmark.

of G2, the cell stops growing, enters mitosis, and redirects its growth machinery toward building the septum at the cell equator.

Mechanisms of cell polarization – common principles

Small Rho G-proteins

Conserved Rho GTPases play a central role in cell polarization. In 1990, *S. cerevisiae cdc42* mutants were characterized, which were unable to bud at the restrictive temperature yet continued to grow (Adams *et al.*, 1990; Johnson & Pringle, 1990). The same year, yeast Cdc42 was shown to be a homolog of the mammalian GTP-binding protein G25K, which is now referred to as mammalian Cdc42 (Munemitsu *et al.*, 1990; Shinjo *et al.*, 1990). More recently, Cdc42 depletion was similarly shown to produce large round unbudded cells (Gladfelter *et al.*, 2001). By contrast, in *S. pombe*, depletion of Cdc42 results in small, round, and dense cells (Miller & Johnson, 1994). Thus, in the absence of Cdc42 function, *S. cerevisiae* cells continue to grow in an unpolarized fashion, whereas *S. pombe* cells do not appear to grow substantially. A second small GTPase, Rho1, is essential for viability in both *S. cerevisiae* and *S. pombe*. Inactivation of the Rho1 homolog in mammalian cells using *Clostridium botulinum* exoenzyme C3, which ADP-ribosylates RhoA, results in a loss of actin stress fibers and in cells rounding up (Rubin *et al.*, 1988; Chardin *et al.*, 1989; Paterson *et al.*, 1990). A temperature-sensitive *S. cerevisiae rho1* mutant arrests growth with small buds at the nonpermissive temperature (Yamochi *et al.*, 1994), and a fission yeast *rho1* deletion mutant displays rounded cells as terminal phenotype (Nakano *et al.*, 1997). In both budding and fission yeasts, Rho1 plays a critical role in activating 1,3-beta-glucan synthase, which is a major structural component of the cell wall (Arellano *et al.*, 1996; Drgonova *et al.*, 1996; Qadota *et al.*, 1996).

The regulation of these two Rho GTPases is critical for organization of the cytoskeleton, control of exo-/endocytosis, and cell wall remodeling in both *S. cerevisiae* and *S. pombe*. In addition, Cdc42 is required for breaking symmetry in spherical *S. cerevisiae* cells, via positive feedback mechanisms (Irazoqui *et al.*, 2003; Wedlich-Soldner *et al.*, 2003, 2004); and in both spherical *S. cerevisiae* cells and rod-shaped *S. pombe* cells, oscillations of Cdc42 have been observed, suggesting the existence of negative feedback loops (Das *et al.*, 2012b; Howell *et al.*, 2012). This dynamic behavior of Cdc42 may be a fundamental property of the polarization process, despite the dramatic differences in cell shape of these two fungi.

In both yeasts, Cdc42 activity is tightly regulated by activators (guanine nucleotide exchange factors, or GEFs) and inactivators (GTPase activating proteins, or GAPs, and Rho-GDP dissociation inhibitors, or RDIs), which dictate when, where, and how long this GTPase is active (Fig. 2). In budding yeast, the Ras-like Rsr1 (Bud1) GTPase, the scaffold protein Bem1, the GEF Cdc24, and the RDI Rdi1 are all important for the correct localization of activated Cdc42 (Park *et al.*, 1997; Butty *et al.*, 1998; Bose *et al.*, 2001; Butty *et al.*, 2002; Park *et al.*, 2002; Irazoqui *et al.*, 2003; Kozminski *et al.*, 2003; Richman *et al.*, 2004; Wedlich-Soldner *et al.*, 2004; Slaughter *et al.*, 2009; Kang *et al.*, 2010). In the fission yeast, while the Ras1 GTPase binds Scd1, a GEF for Cdc42, it has not been demonstrated that this GTPase directly binds Cdc42 (Chang *et al.*, 1994), as is the case in *S. cerevisiae*

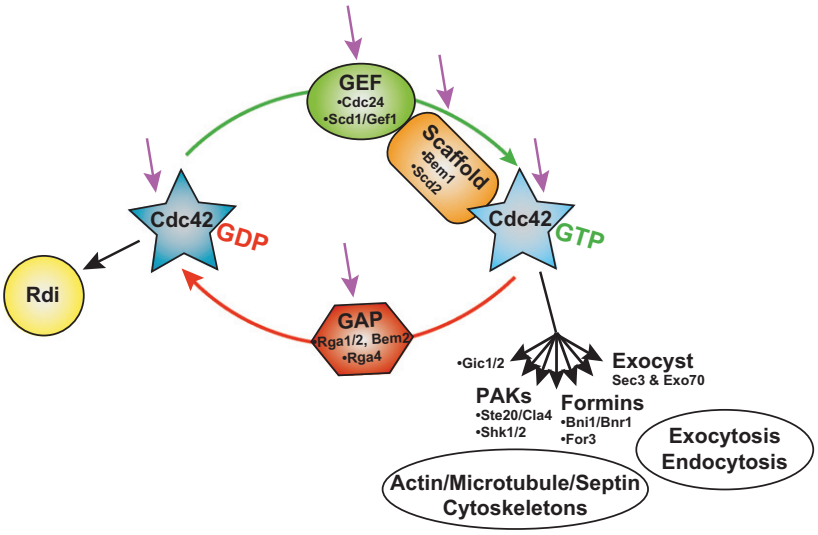

Fig. 2. The Cdc42 GTPase – its main interactions and cellular roles. Schematic representation of Cdc42 GDP/GTP cycle and interactions with downstream effectors. In each case, the top name is the *Saccharomyces cerevisiae* protein and the bottom one the *Schizosaccharomyces pombe* protein. Purple arrows represents interactions or inputs from upstream regulators, such as Ras-like GTPases (Rsr1 and/or Ras1), Boi1/2, or Pob1 proteins, the GTPase-binding protein Gps1, DYRK-family protein kinase Pom1 and the SH3 domain containing Tea4 protein. Note that all proteins represented interact with phosphatidylinositol phosphates and/or phosphatidylserine, except for Rdi and formins.

(Kozminski *et al.*, 2003; Kang *et al.*, 2010). Cdc42 localization and activation further depend on the scaffold protein Scd2 (Bem1 homolog; Chang *et al.*, 1994) and on a second GEF Gef1 (Coll *et al.*, 2003). Fission yeast also encodes a predicted Cdc42 RDI. In both these yeasts, several GAPs promote Cdc42 GTP hydrolysis, of which Bem2, Bem3, Rga1 and Rga2 in *S. cerevisiae* (Knaus *et al.*, 2007; Sopko *et al.*, 2007; Tong *et al.*, 2007; Lo *et al.*, 2013), and Rga4 in *S. pombe* (Das *et al.*, 2007; Tatebe *et al.*, 2008; Kelly & Nurse, 2011a) have been shown to restrict the distribution of active Cdc42. Rho1 activity is similarly controlled by a range of regulators. Several GEFs (Rom1, Rom2, and Tus1 in *S. cerevisiae* and Rgf1, Rgf2, and Rgf3 in *S. pombe*) activate Rho1, which then binds 1,3-beta-glucan synthase (Ozaki *et al.*, 1996; Manning *et al.*, 1997; Schmidt *et al.*, 1997; Schmelzle *et al.*, 2002; Tajadura *et al.*, 2004; Mutoh *et al.*, 2005; Garcia *et al.*, 2006, 2009; Krause *et al.*, 2012). There are four Rho1 GAPs in *S. cerevisiae* and three in *S. pombe*, which are involved in cell wall integrity and actin organization (Peterson *et al.*, 1994; Wang & Bretscher, 1995; Nakano *et al.*, 2001; Watanabe *et al.*, 2001; Schmidt *et al.*, 2002; Calonge *et al.*, 2003; Yang *et al.*, 2003).

In their GTP-bound form, Cdc42 and Rho1 activate a series of effectors to organize cell polarization (Fig. 2): GTP-Cdc42 specifically binds CRIB-containing proteins such as the PAK kinases (Ste20 and Cla4 in *S. cerevisiae* and Shk1/Pak1 and Shk2/Pak2 in *S. pombe*) and Gic1/2, which are only present in *S. cerevisiae*. In both yeasts, active Rho1 specifically binds protein kinase C (Pkc1/2 and Pck1/2 in *S. cerevisiae* and *S. pombe*, respectively). In addition, Cdc42, Rho1, and other Rho GTPases activate actin nucleators of the formin family to promote the formation of actin cables: Bni1 in *S. cerevisiae* is bound by Rho1, Rho3, and Cdc42, with Rho1 and Cdc42 regulating Bni1 indirectly through Pkc1 and Gic2, respectively (Kohno *et al.*, 1996; Evangelista *et al.*, 1997; Robinson *et al.*, 1999; Dong *et al.*, 2003; Chen *et al.*, 2012); and For3 in *S. pombe* is bound by Rho3 and Cdc42 (Nakano *et al.*, 2002; Martin *et al.*, 2007). These Rho-family GTPases also bind the exocyst components Sec3 and Exo70 to promote exocytosis (Guo *et al.*, 2001; Zhang *et al.*, 2001, 2008; Wu *et al.*, 2010a; Bendezu *et al.*, 2012). These interactions of activated Cdc42 and Rho1 GTPases with effectors are critical for cell polarity via reorganization of the actin cytoskeleton, secretion, and cell wall integrity and remodeling.

These different interactions of Rho GTPases with their activators and effectors constitute feedback loops, which activate and concentrate polarity proteins to a specific site that will become the site of new growth. For example, active Cdc42 binds the PAK kinase Ste20, which binds Bem1 (Scd2), itself in a complex with the GEF Cdc24 (Scd1), thus resulting in further local Cdc42 activation. Thus, a stochastic increase in active Cdc42 can lead to a dramatic increase in the active GTPase (Kozubowski *et al.*, 2008). In addition, actin-dependent vesicle-mediated trafficking of Cdc42 has been proposed to play a role in polarity establishment (Wedlich-Soldner *et al.*, 2003, 2004), through Cdc42-activating formins, leading to the nucleation and polymerization of additional actin cables, thus reinforcing the delivery of Cdc42 and other membrane components to this specific site. Both scaffold-dependent and actin-dependent feedback mechanisms have been proposed to be important for symmetry breaking, that is, the initiation of a new axis of polarity in the absence of previous spatial cues. Together, these feedback loops may be used to establish a robust polarization axis (Freisinger *et al.*, 2013).

However, the initial recruitment of Cdc42, Cdc24, and Bem1 to the site of polarized growth does not require an intact actin cytoskeleton (Ayscough *et al.*, 1997; Nern & Arkowitz, 1999; Irazoqui *et al.*, 2003; Wedlich-Soldner *et al.*, 2004; Irazoqui *et al.*, 2005; Yamamoto *et al.*, 2010), and modeling work taking into account vesicle membranes suggested vesicle trafficking may perturb, rather than reinforce, Cdc42 polarization (Layton *et al.*, 2011). By contrast, actin cables are important for maintaining these proteins to established polarity sites (Wedlich-Soldner *et al.*, 2004; Irazoqui *et al.*, 2005). To maintain a localized zone of active Cdc42, retrieval of Cdc42 through endocytosis and via Rdi-mediated membrane dissociation may also prevent spreading of Cdc42 through lateral diffusion (Irazoqui *et al.*, 2005; Slaughter *et al.*, 2009). Observations in fission yeast cells suggest a possible similar role of the actin cytoskeleton for Cdc42 localization as in *S. cerevisiae*, with active Cdc42 localizing correctly to sites of polarized growth at cell poles in the absence of actin cables, and complete disruption of the actin cytoskeleton promoting progressive displacement of active Cdc42 from cell tip to cell sides within 30 min (Bendezu & Martin, 2011). The actin cytoskeleton may however be important for the localization of the Cdc42 GEF Scd1 (Kelly & Nurse, 2011a).

The cytoskeleton

In general, microtubule and actin cytoskeletons function in delivery and removal of plasma membrane components, whereas the septin cytoskeleton has a more passive, barrier function, which may nonetheless recruit endocytic sites (Stimpson *et al.*, 2009; Fig. 3). In yeasts, the actin cytoskeleton marks the locations of exocytosis and endocytosis. By contrast, the microtubule cytoskeleton is not used as major vesicular transport route, but is crucial for nuclear functions including nuclear migration

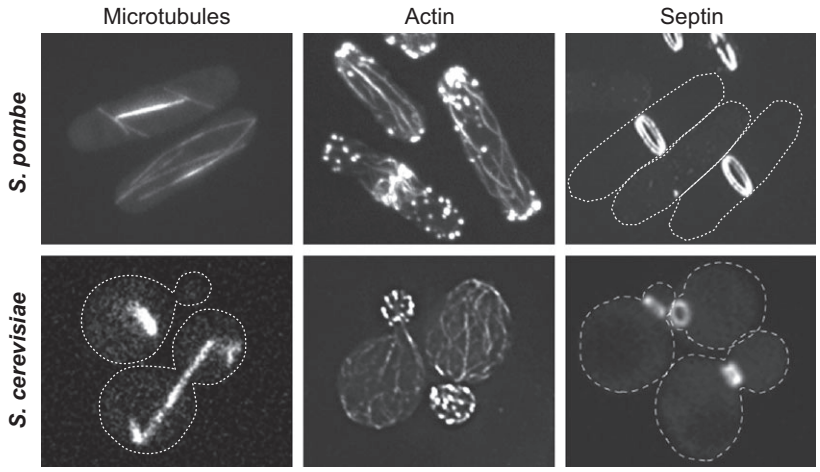

Fig. 3. The yeast cytoskeleton. Representative fluorescence images of the microtubule, actin, and septin cytoskeleton in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Microtubules are labeled by incorporation of GFP-tagged alpha-tubulin monomer. F-actin is stained with fluorescent phalloidin. Septins are tagged with GFP (Spn1 in *S. pombe*, Cdc3 in *S. cerevisiae*). The Spn1-GFP image is modified and reproduced from Wu *et al.* (2010b), with permission. The *S. cerevisiae* microtubule image is a kind gift from Yu Haochen, ETH.

and division in *S. pombe* and *S. cerevisiae*. In both organisms, microtubules are important for nuclear positioning, either via opposing pushing forces that center the nucleus in fission yeast or via microtubule capture-shrinkage and dynein motor movement, which generate pulling forces in budding yeast (Tran *et al.*, 2001; Pearson & Bloom, 2004; Piel & Tran, 2009; Winey & Bloom, 2012). However, a major difference between *S. cerevisiae* and *S. pombe* lies in the relative importance of the microtubule cytoskeleton in polarized growth. In *S. pombe*, microtubules play critical roles in marking cell poles for growth by depositing landmarks, the Tea1 and Tea4 proteins, at these locations (described in more details below). By contrast, microtubules are dispensable for cell polarization in *S. cerevisiae*.

In contrast to the roles of microtubules, the actin cytoskeleton is critical for polarity in both budding and fission yeast. With respect to cell polarity, there are two major forms of F-actin, cables, and patches. Actin patches assemble at sites of endocytosis, at the cell tips in *S. pombe* and the bud tip in *S. cerevisiae*, whereas cables run along the length of the cell serving as polarized tracks for delivery of secretory vesicles and organelles. Both of these actin structures are highly dynamic in these yeasts with patches moving at a velocity of about 1 $\mu m\ s^{-1}$ in *S. cerevisiae* (Yu *et al.*, 2011) and about 0.3 $\mu m\ s^{-1}$ in *S. pombe* (Pelham & Chang, 2001; Sirotkin *et al.*, 2010) and cables moving with a velocity of 0.3–0.4 $\mu m\ s^{-1}$ in both species (Huckaba *et al.*, 2004; Martin & Chang, 2006). The lifetime of actin patches can be divided into three phases: assembly at the cortex with limited mobility, slow inward movement via Arp2/3-dependent actin polymerization, and vesicles pinching off the membrane associated with rapid movements and loss of actin via depolymerization (Galletta & Cooper, 2009; Berro *et al.*, 2010; Lin *et al.*, 2010; Sirotkin *et al.*, 2010). In unpolarized *S. cerevisiae* cells, actin cable dynamics are dramatically increased (Yu *et al.*, 2011). In these two yeasts, the formins Bni1 and Bnr1 in *S. cerevisiae*, and For3 in *S. pombe*, are required for actin cable assembly. Type V myosins are the motors which mediate the movement of vesicles and organelles on the actin cable tracks, and these motors are also responsible for the translational actin cable motility along the cell cortex in unpolarized *S. cerevisiae* cells (Yu *et al.*, 2011) and for the organization of actin cables along cell length in *S. pombe* cells (Lo Presti *et al.*, 2012).

The contribution of each actin structure to polarized growth varies between organisms and conditions. Overall, in *S. cerevisiae*, actin cables are critical for polarized growth, whereas polarized actin patches are less important (Pruyne *et al.*, 1998; Karpova *et al.*, 2000). However, budding yeast cells that lack formin or tropomyosin function, and thus lack actin cables, can still grow in a polarized fashion to form small buds, which are unable to enlarge further (Yamamoto *et al.*, 2010). This formin-independent polarized growth is then dependent on actin patch components (Yamamoto *et al.*, 2010). Indeed, complete disruption of the actin cytoskeleton results in isotropic growth in actively growing cells (Ayscough *et al.*, 1997; Pruyne *et al.*, 1998; Yamamoto *et al.*, 2010); however, this is not the case for the initiation of polarized growth and bud emergence in quiescent cells, which do not require polymerized actin (Sahin *et al.*, 2008). In *S. pombe* cells that lack the formin For3, actin cables are absent and actin patches partly depolarized, yet these cells exhibit only modest defect in polarized growth (Feierbach & Chang, 2001). Even in a triple formin mutant or a tropomyosin mutant, *S. pombe* cells can still undergo polarized growth (Balasubramanian *et al.*, 1992; Bendezu & Martin, 2011).

Septins are GTP-binding proteins that polymerize to form filaments and rings, which can function as protein

scaffolds and also as barriers to diffusion, thereby defining cellular compartments. Septins function primarily in cytokinesis. In *S. cerevisiae,* septins are critical for maintaining organelles, actin patches, proteins required for exocytosis, and proteins required for cell polarity including Cdc42 to the bud (Barral *et al.*, 2000; Luedeke *et al.*, 2005; Orlando *et al.*, 2011). This barrier function of septins is not required for initial polarization of these components, but rather to counterbalance lateral diffusion of membrane-associated proteins (Orlando *et al.*, 2011). Septin ring assembly requires Cdc42 and specifically its GTP/GDP cycling (Gladfelter *et al.*, 2002; Caviston *et al.*, 2003). Formin and PAK are also required for initial septin ring assembly (Kadota *et al.*, 2004). Presently there is no indication that septins are important for cell polarity in *S. pombe*, where septins are instead important for sporulation and cell division (Onishi *et al.*, 2010; Wu *et al.*, 2010b). Of these three cytoskeletal elements, it is the actin cytoskeleton that plays a central role in the delivery and uptake of membrane material, critical for polarized growth.

Exo-/Endo-cytosis

The flux of membrane material, integral membrane proteins, and secreted proteins to and from the plasma membrane is required for cell growth: The targeting and maintenance of these processes to a specific site underlies polarized growth. A number of mechanisms restrict the regions of exocytosis to the incipient bud or cell tip and position endocytosis to prevent dispersal of the exocytosis site. Exocytosis is important for the delivery of membrane-associated proteins necessary for polarized growth, such as Rho G-proteins as well as the subsequent delivery of cell wall-remodeling enzymes which are required for bud or new end growth. It is important to note that in walled organisms, such as yeasts, cell growth is driven by turgor pressure, which provides the force for cell expansion (Slaughter & Li, 2006; Minc *et al.*, 2009a). Turgor pressure is counterbalanced by the rigidity of the cell wall, which is locally remodeled by enzymes delivered via exocytosis allowing growth at polarized sites.

Vesicle-based transport of Cdc42 has been suggested to be important for symmetry breaking in *S. cerevisiae* (Wedlich-Soldner *et al.*, 2003, 2004). How trafficking contributes to Cdc42 polarization remains unclear, however, as modeling efforts have indicated that directed membrane traffic, that is exocytosis, should result in polarization of integral membrane proteins, but not Cdc42, which diffuses more rapidly (Layton *et al.*, 2011). In this yeast, there are two exocytic pathways: one that originates from the trans-Golgi network (TGN; characterized by Bgl2-containing vesicles) and the other that comes from the endosome (characterized by invertase-containing vesicles); either one alone is sufficient, and at least one required, for delivery of Cdc42 to the bud (Orlando *et al.*, 2011). The endocytic recycling pathway may be sufficient for polarized growth both in the early stages of bud formation in *S. cerevisiae* and at all stages in *S. pombe* (Feierbach & Chang, 2001; Yamamoto *et al.*, 2010; Bendezu & Martin, 2011; Orlando *et al.*, 2011). Specifically in these studies, formin and tropomyosin mutants with no observable actin cables, yet with actin patches, were still able to grow in a polarized fashion. Even upon disruption of both actin cable and patches, Cdc42 and other polarity regulators were still observed in a tight cluster, which was, however, dependent on myosin V (Yamamoto *et al.*, 2010). Collectively these studies point to the existence of actin cable-independent transport, which requires the exocyst (Yamamoto *et al.*, 2010; Bendezu & Martin, 2011). It remains to be seen whether these actin cable-independent transport pathways are identical in the two yeasts.

When delivery of Cdc42 to the bud is somewhat defective, endocytosis plays a negative role depolarizing this GTPase (Irazoqui *et al.*, 2005; Orlando *et al.*, 2011). It has been further suggested that the rate of Cdc42 recycling, through endocytosis or through Rdi-mediated extraction from the membrane, is critical to maintain the polarized distribution of Cdc42 and can be tuned to define the size of the Cdc42 domain (Marco *et al.*, 2007; Slaughter *et al.*, 2009). This suggests that a balance between exo- and endocytosis is required for maintenance of a polarized site. A key question has been whether endocytosis and exocytosis occur at the same site on the plasma membrane or whether these two processes are segregated. Two recent studies reveal that indeed sites of endocytosis and exocytosis are distinct with exocytic sites being surrounded or corralled by a region of endocytosis (Jose *et al.*, 2013; Slaughter *et al.*, 2013). Such a mechanism of fencing or corralling would not only restrict and focus exocytosis, but also create microdomains at the site of polarization with different physical characteristics (Slaughter *et al.*, 2013). In *S. pombe*, endocytosis and exocytosis are localized to the cell ends, and it remains to be seen whether these processes are physically segregated and whether their spatial organization plays a role in polarized growth.

Defining the specific locations of exocytosis and endocytosis is fundamentally important for polarized growth. In addition, restricting the location of where new membrane material is delivered and existing membrane material is recovered is critical for growth of defined geometries. A major challenge is to overcome diffusion in the plane of the membrane, which is in part dictated by the physical characteristics of the plasma membrane.

Membrane lipids

The major lipids in *S. cerevisiae* and *S. pombe* are glycerophospholipids, sphingolipids, and sterols. In these yeasts, glycerophospholipids are comprised of phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol (PI) and its phosphorylated derivatives, phosphatidic acid (PA), and phosphatidylcholine (van der Rest *et al.*, 1995; Shui *et al.*, 2010). In *S. cerevisiae,* there are three main complex sphingolipids – inositol phosphate ceramide, mannosylinositolphosphate-ceramide (MIPC), and mannosyldiinositolphosphate-ceramide ($M[IP]_2C$) – whereas in *S. pombe,* this latter complex sphingolipid and the enzyme necessary for its synthesis has not been detected (Nakase *et al.*, 2010). Ergosterol is the major sterol in these two yeasts.

Interestingly, in budding yeast, phosphatidylinositols and phosphatidylserine levels peak upon bud emergence (Cottrell *et al.*, 1981), suggesting that these two phospholipids function in polarized growth. *Schizosaccharomyces pombe* cells which lack phosphatidylserine synthase are morphologically abnormal with bent, bulbous branched and ovoid morphologies, suggesting that phosphatidylserine is required for polarized growth (Matsuo *et al.*, 2007). In addition, these cells have cytokinesis defects and the actin cytoskeleton is severely perturbed. In *S. cerevisiae,* phosphatidylserine is required for the endocytosis of the integral membrane protein Arn1 (Guo *et al.*, 2010) and the polarized distribution of endocytic actin patches to small buds (Sun & Drubin, 2012). Phosphatidylserine is primarily found on the inner leaflet of the plasma membrane, and its distribution is highly polarized in *S. cerevisiae* with an accumulation at the bud and bud neck plasma membrane (Fairn *et al.*, 2011). Furthermore, phosphatidylserine colocalizes with Cdc42 (Slaughter *et al.*, 2013) and phosphatidylserine is required for the polarized localization of Cdc42 and Bem1 to the small buds (Fairn *et al.*, 2011). It is thought that the increased negative charge of phosphatidylserine-containing membrane domains stabilizes Cdc42 at the membrane via its polybasic C-terminal regions (Das *et al.*, 2012a). It will be interesting to know whether modulation of the cell tip plasma membrane negative charges in *S. pombe* is critical for polarized growth.

Phosphatidylethanolamine is thought to be localized to the inner leaflet of the plasma membrane; however, this lipid has also been shown to be exposed on the outer leaflet of the plasma membrane localizing specifically to the sites of polarized growth that is the presumptive bud site, the cortex of the emerging small bud cortex and the bud neck large-budded cells in *S. cerevisiae,* and one end or both ends as well as at the division plane in *S. pombe* (Iwamoto *et al.*, 2004). Furthermore, *S. pombe* cells that are mutant in phosphatidylethanolamine synthesis have dramatic morphological defects (Luo *et al.*, 2009), suggesting that this lipid is critical for polarized growth. In *S. cerevisiae,* phospholipid flippase mutants (Lem3, which flips phosphatidylethanolamine from the outer leaflet to the inner leaflet), active Cdc42, remain at the growth site, resulting in hyperpolarized growth (Saito *et al.*, 2007). If the flipping of phosphatidylethanolamine from the outer leaflet to the inner leaflet is blocked, this disrupts guanine nucleotide dissociation inhibitor-dependent dissociation of Cdc42 from the polarized growth site (Das *et al.*, 2012a). It has been proposed that the flipping of phosphatidylethanolamine to the inner leaflet may reduce the overall negative charge of the bud plasma membrane (predominantly from phosphatidylserine at the growth site), which is likely to reduce the interaction of the C-terminal polybasic region of Cdc42 with inner leaflet. Homlogs of these flippases exist in *S. pombe*; however, their roles in polarized growth have not been examined.

In both *S. cerevisiae* and *S. pombe,* $PI(4,5)P_2$ is required for cell growth and actin cytoskeleton organization, as well cell division (Desrivieres *et al.*, 1998; Homma *et al.*, 1998; Zhang *et al.*, 2000). $PI(4,5)P_2$ is important for both endocytosis and exocytosis (He *et al.*, 2007; Sun *et al.*, 2007; Zhang *et al.*, 2008; Yakir-Tamang & Gerst, 2009; Bendezu & Martin, 2011; Sun & Drubin, 2012). This lipid has been shown to bind two exocyst subunits and septins as well as recruit a Rho1 GEF, PAK, and a Cdc42 effector (Audhya & Emr, 2002; He *et al.*, 2007; Orlando *et al.*, 2008; Takahashi & Pryciak, 2008; Zhang *et al.*, 2008; Bertin *et al.*, 2010). In budding yeast, $PI(4,5)P_2$ is enriched at sites of polarized growth including the bud, bud neck, and at the site of cell division during cytokinesis (Garrenton *et al.*, 2010; Guillas *et al.*, 2013) and at the site of cell division in fission yeast (Zhang *et al.*, 2000). It has been proposed that $PI(4,5)P_2$ is generated by the exocytic delivery of phosphatidylinositol to the plasma membrane and that this results in the activation of Cdc42 and regulation of the actin cytoskeleton (Yakir-Tamang & Gerst, 2009).

Sterols and sphingolipids have structural roles in the membrane, being important for permeability and fluidity (Hannich *et al.*, 2011). In *S. cerevisiae,* sterol and sphingolipid mutants exhibit defects in endocytosis (Munn *et al.*, 1999; Zanolari *et al.*, 2000; Friant *et al.*, 2001; Heese-Peck *et al.*, 2002). Furthermore, two proteins involved in ergosterol biosynthesis, Erg4 and Ncp1, are important for cell polarity as *erg4* and *ncp1* deletion mutants have defects in bud morphology and bud site selection (Tiedje *et al.*, 2007). Interestingly, *S. pombe* ergosterol mutants appear to not be affected in endocytosis and have normal morphologies (Iwaki *et al.*, 2008); however, the sphingolipid component MIPC is required for normal cell morphology, with mutants exhibiting pear or round shapes and defects in endocytosis (Nakase *et al.*, 2010). In these

two yeasts, sterol distribution has been investigated using the fluorescent sterol-binding compound filipin, yet sterol binding by filipin is dependent on the membrane composition, and hence, caution is required when using this sterol reporter (Jin *et al.*, 2008; Hannich *et al.*, 2011). In *S. cerevisiae,* filipin distribution is essentially uniform at the plasma membrane in budding cells (Bagnat & Simons, 2002; Beh & Rine, 2004; Malathi *et al.*, 2004). In fission yeast, filipin is enriched at the cell tip plasma membrane and the site of cytokinesis (Wachtler *et al.*, 2003; Takeda *et al.*, 2004). In yeasts, sphingolipids and ergosterol are enriched in specific membrane domains, which have been referred to as rafts or distinct domains (Takeda & Chang, 2005; Mollinedo, 2012; Spira *et al.*, 2012). The role of such domains in polarized growth, however, remains to be established. Very recently polarity factors including Tea proteins and Mod5 in *S. pombe* and Kel1 and the scaffold protein Spa2 in *S. cerevisiae* have been observed in distinct 50- to 100-nm clusters at the cell cortex (Dodgson *et al.*, 2013) yet the connection between these structures and plasma membrane lipids is an open question.

In budding yeast, while the septin ring functions as a diffusion barrier dramatically reducing diffusion into the mother cell, endocytic cycling is also important for efficient polarization of membrane-associated proteins (Valdez-Taubas & Pelham, 2003). In such a mechanism, membrane-associated proteins are polarized as long as they are endocytosed before they diffuse to equilibrium. This mechanism, which depends on slow membrane diffusion coupled with endocytosis, may function in cells without 'neck' diffusion barriers such as in *S. pombe*. Ergosterol appears to be required for slow diffusion in the *S. cerevisiae* plasma membrane (Valdez-Taubas & Pelham, 2003).

Scaffold proteins

Scaffold proteins are modular domain proteins that bring together two or more additional proteins, which facilitate the coordination and integration of different processes or signals as well as increase local concentrations of signaling proteins. Bem1 (Scd2) is the quintessential scaffold protein as it has been shown to bind the Rho GTPase Cdc42, PAK which binds activated Cdc42, the GEF Cdc24 (Scd1) as well phosphoinositides (Chang *et al.*, 1994; Leeuw *et al.*, 1995; Bose *et al.*, 2001; Endo *et al.*, 2003; Irazoqui *et al.*, 2003; Wheatley & Rittinger, 2005; Winters & Pryciak, 2005). In addition, Bem1 can bind Boi1/2 (Pob1), which is itself a scaffold protein (Bender *et al.*, 1996; Matsui *et al.*, 1996). In *S. cerevisiae*, Cdc42-dependent symmetry breaking does not require the cytoskeleton; however, Bem1 is essential (Irazoqui *et al.*, 2003). It is thought that local fluctuations in the levels of activated Cdc42 result in the recruitment of Bem1 via interaction with the bound PAK. This also brings the Cdc42 activator Cdc24, which facilitates the activation of inactive Cdc42, in the proximity, resulting in a feedback amplification loop. Hence, the critical function of Bem1 is to bring the PAK and GEF together. Indeed, it was shown that the requirement for Bem1 during symmetry breaking can be bypassed by fusing the GEF and PAK (Kozubowski *et al.*, 2008). Importantly, these experiments were carried out in cells lacking the Ras-like GTPase Rsr1, which is normally present and also binds Bem1, Cdc42, and Cdc24 (Park *et al.*, 1997; Kang *et al.*, 2010) and, as we discuss below, likely also contributes to Cdc42 activation. In fission yeast, Ras1 has a clear role in Cdc42 activation, but no direct interaction has been observed between Ras1 and Scd2. It is also less clear in fission yeast whether Bem1/Scd2 has a strict scaffold function: In *scd2*Δ cells, which display a very wide growth zone, Scd1 fails to localize to cell tips, but active Cdc42 is nonetheless observed at the cell tip, however, over a wider zone (Kelly & Nurse, 2011a). The width of *scd2*Δ cells can be restored to wild-type dimensions by targeting Scd1 to the cell tips using N-terminal tip targeting domain of the formin For3, suggesting that the GEF–PAK interaction *per se* is not critical for polarized growth in this yeast. One possibility is that the function of Bem1 in *S. cerevisiae* to bring together the PAK and GEF may be critical only for symmetry breaking, but not for maintenance of a polarized growth state. The function of scaffold proteins in cell polarity is likely to require their role in feedback loops, which serve to increase small differences in protein distribution or activity.

A second example of a scaffold protein is the Boi1/2 or Pob1 protein, which contains an amino-terminal SH3 domain, a SAM (sterile alpha motif) protein interaction domain, a central proline-rich region, and a carboxy-terminal PH domain (Bender *et al.*, 1996; Matsui *et al.*, 1996; Toya *et al.*, 1999). Boi1/2 or Pob1 proteins have been shown to bind Bem1 (via their proline-rich region), the GTP-bound form of Cdc42 (via their PH domain), and a formin (via their SAM domain; Bender *et al.*, 1996; Matsui *et al.*, 1996; McCusker *et al.*, 2007; Rincon *et al.*, 2009). The PH domain of this protein also binds the acidic phospholipids $PI(4,5)P_2$ and phosphatidylserine (Hallett *et al.*, 2002). In *S. cerevisiae,* Boi1/2 are phosphorylated by Cdk and associate with the Cdc42 GEF Cdc24 (McCusker *et al.*, 2007). While interaction with formins has not been observed in *S. cerevisiae,* in fission yeast, Pob1 is critical for localization of For3 as well as the exocyst (Rincon *et al.*, 2009; Nakano *et al.*, 2011). Whether Boi1/2 or Pob1 binds these different proteins simultaneously or sequentially remains to be addressed. A major function of this protein may be to increase the local concentration of the Bem1 scaffold protein thereby promoting signal amplification.

Feedback loops

A feedback loop is a circuit in which information passes from the output back to the input, that is, fed back into itself. Both positive and negative feedback loops have been shown to be involved in cell polarization. In positive feedback loops, signals are typically self-reinforcing, hence grow larger, and are amplified or enhanced over time. Negative feedback loops, in contrast, tend to dampen or buffer changes, frequently resulting in an oscillation around a particular value in a self-regulating fashion. Positive feedback loops in cell polarization can be divided into those that are cytoskeleton dependent and those that appear to function independently of the cytoskeleton. The classical cytoskeleton-dependent feedback loop involves targeting of cell polarity proteins via the cytoskeleton (such as Tea1/4 proteins via microtubules or Cdc42 via actin-mediated vesicle transport) to a particular location on the plasma membrane, which then leads to nucleation or stabilization of the cytoskeleton and increased delivery or targeting of the cell polarity proteins. In *S. cerevisiae*, such an actin-dependent feedback loop for Cdc42 polarization (Wedlich-Soldner *et al.*, 2003, 2004) is not required for symmetry breaking (which occurs in the absence of F-actin) (Irazoqui *et al.*, 2003), but given the importance of the actin cytoskeleton in polarized growth, it is likely to contribute to bud formation in wild-type cells. Similarly, in *S. pombe*, the delivery of the landmark Tea1 via microtubule ends may promote the targeting of microtubules to cell poles resulting in further Tea 1 delivery to the cell end (Mata & Nurse, 1997). In fission yeast, a further positive feedback loop between cell shape and the cytoskeleton is well established, in which cell shape leads to microtubule reorganization, which then results in repositioning the polarisome and ultimately new cell growth (Terenna *et al.*, 2008; Minc *et al.*, 2009a, b). These feedback loops in both budding and fission yeast rely on the polymeric and dynamic properties of the cytoskeleton, which facilitates targeting or delivery of critical components over relatively long distances.

Cytoskeleton-independent feedback loops act by a Turing reaction-diffusion mechanism. In these feedback loops, an activated protein recruits its activator further increasing the levels of the activated protein. This process is limited by diffusion, which results in substrate depletion, as this process occurs at membrane surface. Such feedback loops have been shown to underlie pattern formation in different organisms (Meinhardt & Gierer, 1974). This type of positive feedback loop functions with activated Cdc42, Bem1, and Cdc24 (Butty *et al.*, 2002) in *S. cerevisiae* symmetry breaking (Irazoqui *et al.*, 2003; Goryachev & Pokhilko, 2008; Kozubowski *et al.*, 2008). In *S. pombe,* an analogous feedback loop has been suggested between Tea1 and its prenylated tether Mod5, in which cell tip-localized Mod5 facilitates the anchoring of Tea1 (delivered via microtubules) and conversely Tea1 at the cell tips restricts the diffusion of Mod5 (Snaith & Sawin, 2003; Snaith *et al.*, 2005). The molecular details of these interactions, however, remain to be elucidated.

In contrast to positive feedback loops, which serve to amplify small signals, negative feedback loops lead to adaptive behavior and, in general, promote stability of the system. The molecular details of negative feedback loops in fission and budding yeast, however, are less clear. This type of feedback loop is characterized by oscillatory behavior such as the oscillation of Cdc42 in these yeasts (Das *et al.*, 2012b; Howell *et al.*, 2012). Negative feedback could be mediated via G-protein GAPs and may or may not require the cytoskeleton (Ozbudak *et al.*, 2005; Das *et al.*, 2012b; Howell *et al.*, 2012; Lo *et al.*, 2013). It is also possible that site-specific endocytosis or dilution of polarity factors by exocytosis in a Cdc42-dependent fashion may constitute part of a negative feedback loop. Modeling has revealed that negative feedback decreases the sensitivity of the system to the concentration of polarity factors (Howell *et al.*, 2012). This robustness conferred by negative feedback loops may be important in systems where the levels of polarity factors, such as Cdc42, are limiting and thus are likely to exhibit substantial variations from cell to cell.

Generating distinct cells shapes

The shapes and modes of division of *S. cerevisiae* and *S. pombe* are obviously very distinct. With the many commonalities between the major polarization mechanisms described above, how are these specific shapes generated? To answer this question, it is necessary to delineate the main differences between the shapes of *S. cerevisiae* and *S. pombe*. At least two major differences can be defined in (1) the positioning of growth sites and (2) the dimensions and compartmentalization of the growth zone. Below, we review the distinct strategies used by the two yeasts in generating these differences.

Positioning of growth sites

Saccharomyces cerevisiae and *S. pombe* cells place their growth sites at distinct locations (Fig. 4a): *S. pombe* marks its poles as sites of growth, using both the 'old' end (the one that existed before the last division) and the 'new' end (the one created at division). By contrast, *S. cerevisiae* avoids the previous division site and instead

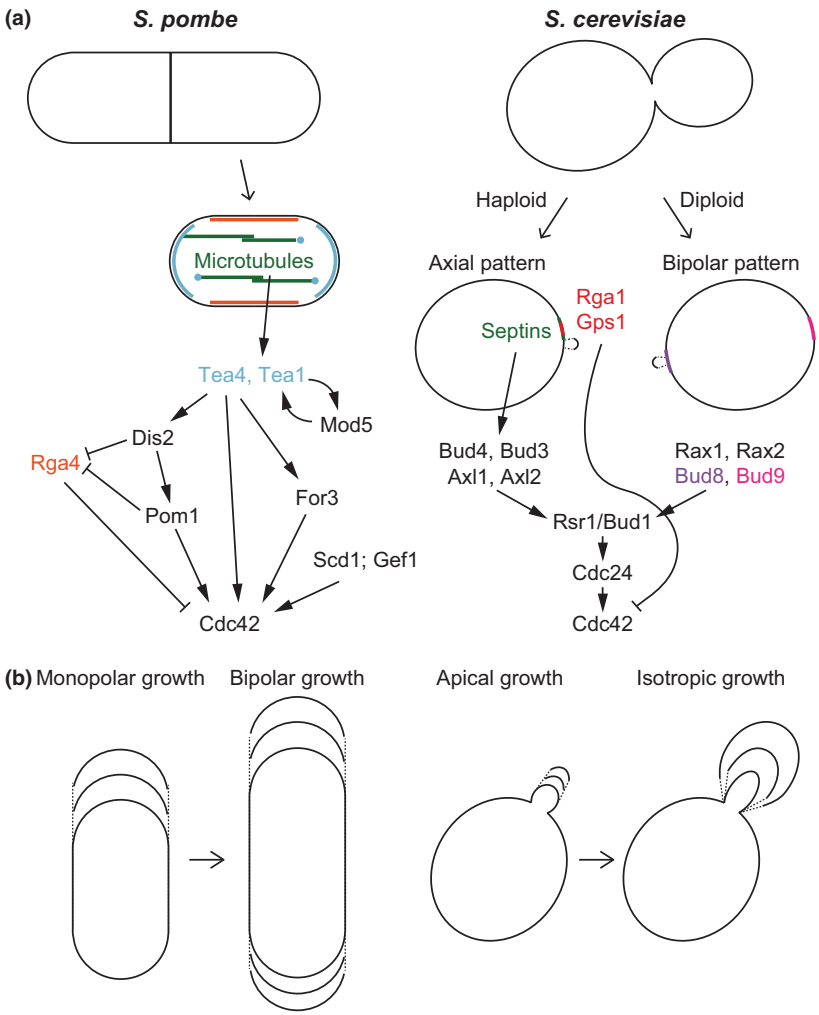

Fig. 4. Distinct placement and sizes of growth zones in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. (a) Schematic representation of the position of new growth after cell division, from both cell poles in *S. pombe*, including the new pole formed by division, adjacent to the bud scar in haploid *S. cerevisiae* and at the opposite pole in diploid *S. cerevisiae*. The main molecular players are indicated. Please see text for details. (b) Illustration of the dimensions of the growth zones, which remains constant at one and then both cell ends in *S. pombe*. In *S. cerevisiae*, the growth zone is initially constant at the bud tip and then enlarges at the isotropic switch.

places its incipient bud site adjacent to it, or in the case of diploid cells at either cell pole. In both organisms, this is achieved through the use of landmarks, which serve to harness the polarization apparatus and place it at appropriate location. In the absence of the landmark(s), the cell retains the ability to polarize growth, but does so at a random location.

One interesting difference between the two yeasts is that in one case, the division site is used as new growth site (*S. pombe*), while in the second (*S. cerevisiae*), active mechanisms prevent the use of that site. Indeed, the formation of the daughter bud at the site of division causes cell lethality. This is prevented in *S. cerevisiae* by spatially and temporally inhibiting the activation of Cdc42 at the bud neck thereby restricting active Cdc42 outside the bud neck region. This is accomplished by the additive actions of a Cdc42 GAP Rga1 and a GTPase-binding protein Gps1, which both localize within the bud neck (Tong *et al.*, 2007; Lo *et al.*, 2013; Meitinger *et al.*, 2013).

Growth site selection in fission yeast

The mode of growth of fission yeast cells ensures that newborn daughters inherit their rod shape from their mother. During vegetative growth, fission yeast cells thus maintain their shape and do not need to establish it *de novo*. Thus, although growth sites have to be re-established after division, this occurs at geometrically defined cellular regions, the cell poles. Symmetry breaking can, however, occur under artificial situations, for instance upon cell wall re-assembly from spheroplasts (cells from which the cell wall has been digested), or in other life stages, such as during mating when cells position a new site of growth toward a partner cell, or during spore germination when a growth pole is created from a spherical spore. Where this has been studied, the Cdc42 machinery has been shown to play a central role (Kelly & Nurse, 2011b; Bendezu & Martin, 2013), but mechanisms for growth site selection are unknown. We thus focus below on the mechanisms in place during vegetative growth.

In the case of fission yeast, the microtubule cytoskeleton plays a fundamental role in positioning landmarks and sites of growth (Fig. 4a). Microtubules are organized in a few antiparallel bundles, nucleated around the nucleus in the middle of the cell, and extending dynamic plus ends toward both cell poles. This microtubule distribution is achieved through self-organization, relying both on a limited number of microtubule-associated proteins (Sawin & Tran, 2006) and on the shape of the cell, which serves as a guide to promote the alignment of microtubules along the long cell axis (Terenna *et al.*, 2008; Minc *et al.*, 2009b). That microtubules are necessary and instructive for polarized growth has been demonstrated by loss-of-function experiments and physical manipulation of cell shape. Studies using pharmacological treatment or mutations to depolymerize microtubules have shown that cells lacking microtubules do not precisely position their growth site at cell poles and upon growth re-initiation often grow from ectopic sites (Umesono *et al.*, 1983; Sawin & Nurse, 1998; Sawin & Snaith, 2004). Furthermore, physical bending of wild-type cells in micro-chambers resulted in microtubules touching cell sides, which was sufficient to promote polarized growth at this location (Terenna *et al.*, 2008; Minc *et al.*, 2009b). As microtubules use the existing cell shape to target cell poles and are instructive to mark these as sites of growth, they form part of a positive feedback system ensuring the robustness of the fission yeast rod shape.

Microtubules transport on their plus ends a complex of two landmarks, Tea1 and Tea4, which are deposited at the cell poles upon microtubule contact (Mata & Nurse, 1997; Feierbach *et al.*, 2004; Martin *et al.*, 2005; Tatebe *et al.*, 2005). Both landmark proteins are thought to act primarily as a protein–protein interaction platform (Fig. 4a): Tea1 consists of kelch repeats and coiled coils, which mediate interaction with the microtubule plus end and the plasma membrane (Martin *et al.*, 2005; Snaith *et al.*, 2005); Tea4 contains an SH3 domain and an RVxF motif for PP1 (Protein Phosphatase 1) binding (Alvarez-Tabares *et al.*, 2007) and is thought to link with downstream effectors. Microtubule plus end binding occurs through interaction of Tea1 with the CLIP-170 Tip1 (Martin *et al.*, 2005), itself localized to the microtubule plus end through the actions of the kinesin Tea2 and the EB1 protein Mal3 (Busch & Brunner, 2004; Busch *et al.*, 2004; Bieling *et al.*, 2007). How the transfer of Tea1–Tea4 from the microtubule end to the cell pole occurs remains unclear, but Mod5, a prenylated Tea1-binding partner, is an important player. Mod5 is required for the accumulation of Tea1 at the cell pole, especially in the absence of microtubule delivery (Snaith & Sawin, 2003). Recent modeling work proposed that Mod5 plays not simply a stoichiometric anchor function, but rather a catalytic role to promote the formation of a stable Tea1 network at the cell poles (Bicho *et al.*, 2010). Bipolar Tea1 localization also depends on Tea3, a Tea1-like protein, and Tea4 itself, both proteins playing a more modest (and poorly understood) role (Arellano *et al.*, 2002; Snaith & Sawin, 2003; Martin *et al.*, 2005). As Mod5 enrichment at the cell poles likewise depends on Tea1 (Snaith & Sawin, 2003), these proteins are also part of a self-amplifying loop to focus cell polarization.

Similar to microtubule disruption, *tea1*Δ or *tea4*Δ cells are curved and, upon growth re-initiation, form T-shapes (Mata & Nurse, 1997; Martin *et al.*, 2005; Tatebe *et al.*, 2005). They also fail to undergo NETO, growing instead in a monopolar manner throughout interphase. How these landmarks may recruit the growth machinery, and in particular the Cdc42 module, remains a mystery, but Tea4 is sufficient to promote Cdc42 activation when ectopically localized to cell sides (K. Kokkoris and S.G.M., unpublished data). Several connections between the landmark and the polarization machinery have been proposed. First, Tea4 directly binds the formin For3, which assembles an array of actin cables from cell poles (Martin *et al.*, 2005). This interaction was proposed to be central for the initiation of growth at the second cell pole, by allowing the recruitment of the formin to the second cell end for NETO. In support of this idea, forced bipolar localization of For3 promotes bipolar cell growth. However, as For3 is strictly required neither for polarized growth, nor for bipolar growth, nor for the Tea4-mediated growth at ectopic sites (Feierbach & Chang, 2001; K. Kokkoris and S.G.M., unpublished data), it is likely that the Tea4–For3 connection is only one part of the story.

Second, Tea4 acts as a type 1 phosphatase co-factor: It is required for the localization at cell poles of the PP1 Dis2, which it binds via both its SH3 domain and RVxF motif (Alvarez-Tabares *et al.*, 2007; Hachet *et al.*, 2011). Mutation of this motif abolishes PP1 binding and localization, leading to cell polarization defects (Alvarez-Tabares *et al.*, 2007). One critical role of the Tea4-Dis2 phosphatase complex is to localize at cell poles the DYRK-family protein kinase Pom1 (Hachet *et al.*, 2011). Tea4 binds Pom1 directly and brings it in proximity of the PP1 Dis2, which dephosphorylates the kinase, thereby revealing a region with phospholipid-binding affinity that allows Pom1 to bind the plasma membrane. As it diffuses at the plasma membrane, Pom1 autophosphorylates, thus reducing its lipid-binding affinity. Therefore, at the plasma membrane, Pom1 forms concentration gradients from cell poles (Padte *et al.*, 2006; Hachet *et al.*, 2011). These provide temporal and positional information for cell division: (1) Pom1 delays CDK1 activation and mitotic entry through negative regulation of Cdr2, a medially placed mitotic inducer. This mechanism was proposed to couple cell length with the cell

cycle, delaying division until a sufficient cell length has been achieved (Martin & Berthelot-Grosjean, 2009; Moseley *et al.*, 2009). However, recent data failed to reveal a defect in size homeostasis in cells lacking *pom1* (Wood & Nurse, 2013), and measurements of Pom1 cortical levels failed to reveal important changes in short versus long cells (Bhatia *et al.*, 2014). (2) Pom1 ensures that division occurs at the geometric middle of the cell by modulating the localization of this same Cdr2 kinase, as well as that of the anillin-like protein Mid1 (Celton-Morizur *et al.*, 2006; Padte *et al.*, 2006; Almonacid *et al.*, 2009), and by preventing septum formation at cell poles (Huang *et al.*, 2007). Deletion of *pom1* also produces phenotypes akin to those of *tea1*Δ or *tea4*Δ in terms of growth positioning – curved, T-shaped, and monopolar cells – suggesting Pom1 represents an important downstream effector of the landmark (Bahler & Pringle, 1998).

Tea1, Tea4, and Pom1 may promote Cdc42 activation by regulating Cdc42 GEFs and GAPs. Both Pom1 and Tea4 associate with Rga4 and somehow (but not via direct phosphorylation) prevent its localization at cell poles (Tatebe *et al.*, 2008; K. Kokkoris and S.G.M., unpublished data). Of note, the role of Tea4 in locally excluding Rga4 can only in part be explained by Pom1, suggesting that the Tea4-Dis2 phosphatase has other important substrates that remain to be defined. These interactions may serve to create at cell poles zones locally devoid of Rga4 GAP, but surrounded by this GAP, which serve to limit Cdc42 activation to a defined zone. However, the exact mechanisms by which Rga4 is controlled remain unclear, and furthermore, the relatively weak phenotype of *rga4*Δ cells suggests that this, again, is only part of the story.

While the Tea1–Tea4 complex marks cell poles for growth and are required for NETO, this complex is unlikely to provide the signal for the transition to bipolar growth. NETO requires correct completion of the last stages of cytokinesis to render the new cell pole growth-competent (Bohnert & Gould, 2012). NETO is also known to depend on progression of the cell cycle to the G2 phase and is modulated by the DNA replication checkpoint (Mitchison & Nurse, 1985; Kume *et al.*, 2011). This growth transition is triggered by the activation of CDK1 on spindle poles bodies at mid-G2 phase (Grallert *et al.*, 2013). How this signal is transmitted to induce growth initiation at the second cell pole is unknown. Aside from Tea1, Tea4, and Pom1, numerous mutants have been described with at least partial NETO defect (Martin & Chang, 2005), suggesting that CDK1 may have multiple targets for this growth transition.

In summary, in fission yeast, a landmark complex, consisting of Tea1 and Tea4 proteins, is deposited at cell poles by microtubules, which themselves align along the length of the cell. This complex recruits the Cdc42 machinery, in part by excluding the Cdc42 GAP Rga4, thereby creating a local environment favorable for Cdc42 activation. It likely also more directly recruits Cdc42 activators, although the mechanisms are presently unknown. Finally, it binds a Cdc42 effector, the formin For3, which, as itself binds Cdc42 (Martin *et al.*, 2007), may also contribute through feedback mechanisms to Cdc42 activation at cell poles.

Bud site selection in budding yeast

In the case of budding yeast, the microtubule cytoskeleton is completely dispensable for cell polarization (Huffaker *et al.*, 1988; Jacobs *et al.*, 1988). Budding yeast prevents growth at the previous division site and instead selects a new site for growth with a defined pattern depending on cell type (Fig. 4a). Normally, haploid cells place their new bud in an axial pattern, adjacent to the previous division site. Diploid cells place their new bud in a bipolar pattern, at the opposite end from the previous division site. Budding patterns are dependent on cell type rather than ploidy, with axial budding observed in a or α cells and bipolar budding observed in cells expressing both a and α information. Three distinct groups of '*BUD*' genes important for the positioning of the bud site – in haploids, diploids, or both – were identified through genetic screens for mutants exhibiting altered budding patterns (reviewed in Bi & Park, 2012).

Three genes are required for bud site selection in both haploids and diploids: the small Ras-like GTPase Rsr1, its GEF Bud5 and its GAP Bud2 (Chant *et al.*, 1991; Chant & Herskowitz, 1991; Park *et al.*, 1993). All three proteins localize to the incipient bud site with Bud2 localizing to the bud neck after bud emergence (Park *et al.*, 1999); thus, G-protein, GAP, and GEF localize with somewhat distinct timings, and all are required for the correct budding pattern, suggesting Rsr1 needs to cycle between its GTP- and GDP-bound forms. Bud5 localization is crucial for the establishment of the correct budding pattern (Kang *et al.*, 2001; Marston *et al.*, 2001), suggesting it serves to activate Rsr1 at the correct location. This tripartite module is thought to connect directly to the Cdc42 module: First, Rsr1-GTP interacts with itself, as well as with Cdc42-GDP through a polybasic region necessary for bud site selection (Kozminski *et al.*, 2003; Kang *et al.*, 2010). Second, Rsr1-GTP binds the Cdc42 GEF Cdc24, and Rsr1-GDP binds the scaffold Bem1 (Park *et al.*, 1997). Rsr1 is also necessary for Cdc24 recruitment to the correct incipient bud site. A model has been proposed for how the Rsr1 module directs polarity establishment to a specific site: Cycling of Rsr1 between its GTP and GDP-bound states may promote the local recruitment of Cdc24, Bem1, and Cdc42, leading to a critical level of

active Cdc42 at the incipient bud site (Bi & Park, 2012). Interestingly, Rsr1 may not function only in bud site selection, but may also have a role in polarity establishment *per se*: *rsr1* deletion or mutation of its polybasic region prevents budding in strains lacking the Cdc42 effectors Gic1 and Gic2 (Kozminski *et al.*, 2003; Kang *et al.*, 2010). In fact, the specialization of Rsr1 to bud site selection and its relatively minor role in polarity establishment may be unique to *S. cerevisiae*. Indeed, Ras-like GTPases in close relatives of budding yeast (such as *Ashbya gossypii*) or in distant basidiomycetes (such as *Ustilago maydis*), like in *S. pombe* (see below), are more important in polarity establishment and maintenance (Lee & Kronstad, 2002; Bauer *et al.*, 2004).

Bipolar landmarks include four transmembrane proteins: Bud8, Bud9, Rax1 and Rax2 (Zahner *et al.*, 1996; Chen *et al.*, 2000; Fujita *et al.*, 2004). Bud8 and Bud9 form landmarks for the distal and proximal poles of the cell, respectively, localizing and promoting bud formation at these locations (Zahner *et al.*, 1996). All four proteins are somewhat co-dependent for localization, with Bud9 being dependent on the three others (Kang *et al.*, 2004). Bud8 and Bud9 require the actin cytoskeleton for localization, whereas only Bud9 requires the septin cytoskeleton (Harkins *et al.*, 2001; Schenkman *et al.*, 2002). Rax1 and Rax2 localize very stably to both proximal and distal poles, forming persistent rings marking the previous sites of division. This localization is consistent with a role as a stable mark, over several generations (Chen *et al.*, 2000; Fujita *et al.*, 2004). However, how these landmarks couple to the Rsr1 module remains unclear, as regions of Bud8 and Bud9 necessary for interaction with the Rsr1 GEF Bud5 have been mapped to the extracellular domains of these membrane proteins, suggesting that these interactions are indirect (Krappmann *et al.*, 2007).

The major axial landmarks include four proteins, Axl1, Axl2 (Bud10), Bud3, and Bud4, of which only Axl1 is expressed specifically only in haploid cells (Chant & Herskowitz, 1991; Fujita *et al.*, 1994; Roemer *et al.*, 1996). Bud3 has a region with homology to a guanine nucleotide exchange factor domain; the anillin-like Bud4 is a GTP-binding protein; Axl1 is an endoprotease necessary for the processing of the a-factor pheromone, whose enzymatic activity is dispensable for its landmark function; Axl2 is a transmembrane protein. These factors localize to the division site, with Bud4 functioning as a platform for the assembly of the axial landmark (Kang *et al.*, 2012). Bud4 localizes to the division site in a septin-dependent manner (Sanders & Herskowitz, 1996; Kang *et al.*, 2013). In contrast to the bipolar landmarks, this localization is transient, providing only a short-term memory of the previous division site. Bud4 is then required for the ordered recruitment of Bud3, Axl1, and Axl2 (Kang *et al.*, 2012). Bud4, Axl1, and Axl2 then depend on each other to associate with the Rsr1 GEF Bud5 (Kang *et al.*, 2012). Here again, it is unclear whether these interactions are direct. How exactly the new growth site is placed adjacent to the previous site remains unclear, although proteins required for cytokinesis play an important role in determining the new site of polarized growth.

The axial and bipolar landmarks provide spatial constraints, dictating where polarized growth will occur. It is intriguing that a number of these axial and bipolar landmarks are transmembrane proteins, raising the possibility that they may be anchored to the cell wall thereby increasing their positional stability. In addition, polarized growth also occurs at a specific cell cycle stage, that is, it is initiated by the cyclin-dependent kinase (Cdk) Cdc28 during G1. This occurs via Cdk-dependent activation of Cdc42 (Lew & Reed, 1993; Gulli *et al.*, 2000; Butty *et al.*, 2002; Moffat & Andrews, 2004), leading to polarization of the actin cytoskeleton and subsequent bud emergence. The activation of Rho1 and Cdc42 to a defined region on the cell cortex is Cdk dependent (Evangelista *et al.*, 1997; Nern & Arkowitz, 2000; Shimada *et al.*, 2000). Specifically, Cdk phosphorylates a number of Rho G-protein regulators including GAPs and scaffold proteins (Knaus *et al.*, 2007; McCusker *et al.*, 2007; Sopko *et al.*, 2007; Kono *et al.*, 2008). Cdk also controls the dynamics of membrane trafficking, in particular endocytic and exocytic domains at the growth site (McCusker *et al.*, 2012). In addition to initiating polarized growth, which leads to bud emergence, Cdk is also required for subsequent bud growth (McCusker *et al.*, 2007). Hence, the strict spatial control of polarized growth is accompanied by a precise regulation of its timing.

In summary, budding yeast cells use distinct landmark proteins for haploid and diploid cells to position the incipient bud site at axial or bipolar positions. Although entirely distinct from the landmark system used by fission yeast cells, one commonality is that the localization of all the landmarks proteins examined thus far relies on the cytoskeleton (actin and/or septin, rather than microtubules in fission yeast). In budding yeast, these landmarks recruit the Ras-like protein Rsr1, through interactions that remain to be defined. In turn, Rsr1 cycles between active and inactive states to promote the local activation and recruitment of Cdc42.

Homologs of landmarks proteins in the other yeast

While both yeast species use very distinct strategies to position their growth sites, some of the factors used as landmarks in one species also exist in the other. For instance, *S. pombe* encodes homologous genes to two bipolar *BUD* genes Rax1 (SPAC23G3.05c) and Rax2

(Rax2), with Rax2 playing some role in defining the old end (Choi *et al.*, 2006). It also encodes proteins related to three axial *BUD* genes Bud4 (Mid2), Axl1 (Mug138), and Axl2 (SPAC11G7.01). As Bud4, Mid2 interacts with septins, but in agreement with the divergent role of septins in the two species, plays distinct roles in cell division (see below; Berlin *et al.*, 2003). *Schizosaccharomyces pombe* also has a single Ras-like protein, Ras1, which, at the sequence level, is more closely related to the *S. cerevisiae* Ras proteins Ras1 and Ras2, implicated in cAMP signaling, than to Rsr1. Ras1 has important roles in cell polarization, as mutant cells are nearly round, but none reported in growth site positioning (Fukui *et al.*, 1986). Instead, it may be involved in polarity establishment, by interacting (like Rsr1) with the Cdc42 GEF Scd1 and likely activating it (Fukui & Yamamoto, 1988; Chang *et al.*, 1994). However, any possible role in linking the landmark with Cdc42 may have been masked by its predominant function in polarity establishment.

Conversely, *S. cerevisiae* has homologs for Tea1 and Tea3 (Kel1 and Kel2), and for Tea4 (Bud14). These proteins all localize to the bud tip, but in an actin-dependent manner, and are all involved in cell polarization (Philips & Herskowitz, 1998; Cullen & Sprague, 2002). Kel1 also associates with and is required for the localization to the bud cortex of Lte1, an activator of mitotic exit (Hofken & Schiebel, 2002; Seshan *et al.*, 2002). Bud14 displays many similarities to Tea4: Bud14 localization depends on Kel1 and Kel2 and as Tea4, depends on Tea1. As Tea4, Bud14 acts as PP1 regulatory subunit, associating with the sole PP1 catalytic subunit Glc7 and targeting it to the bud tip (Cullen & Sprague, 2002; Knaus *et al.*, 2005). Its main function appears to be in regulating microtubule interaction with the cell cortex in a dynein-dependent manner for spindle orientation (Knaus *et al.*, 2005). Finally, as Tea4, Bud14 interacts with a formin, Bnr1. It functions as an inhibitor of Bnr1, displacing it from the actin filament end, thereby promoting the formation of short actin filaments (Chesarone *et al.*, 2009). Thus, Bud14 and Tea4 share many physical interactors, which are used in distinct manner in the two species.

Dimensions and compartmentalization of the growth zone

The size and shape of the active Cdc42 region are thought to define the final dimensions of the growth zone. In trying to define the differences between the dimensions of the growth zones in *S. cerevisiae* and *S. pombe*, two obvious differences come to mind (Fig. 4b).

The first one lies in the size of the growth zone. In *S. cerevisiae*, the size of the active Cdc42 zone is small prior to bud emergence, on the order of 0.6–0.7 μm in diameter (Tong *et al.*, 2007; I. Guillas & R.A.A. unpublished data). Upon bud emergence, the zone of active Cdc42 covers more or less uniformly the bud plasma membrane (Tong *et al.*, 2007; I. Guillas & R.A.A. unpublished data), expanding as the bud enlarges. By contrast, the *S. pombe* growth zone is significantly wider, covering the entire hemispherical pole of the cell, roughly 4 μm in width or 12 μm^2 in surface (F. Bendezú and S.G.M., unpublished data), and remains constant over time, with cell sides inert for growth, resulting in rods of constant width. This tubular growth mode is widespread among many fungi forming hyphae. Besides the size of the growth zone, it is possible that differences in the composition or physical resistance of the cell wall contribute to establishing these distinct modes of growth, as the more spheroidal shape of *S. cerevisiae* would be predicted to distribute pressure more evenly and thus reduce the effects of turgor pressure, while the tubular shape of *S. pombe* may require higher wall resistance. However, physical measurements of the overall elastic moduli of the cell walls of *S. pombe* and *S. cerevisiae* showed very similar values of 101 ± 30 and 112 ± 6 MPa, respectively (Smith *et al.*, 2000; Minc *et al.*, 2009a).

The second difference is the presence of a constriction at the base of the bud, the bud neck, delimiting in *S. cerevisiae* the bud growth compartment from the nongrowing, or very slowly growing (Woldringh *et al.*, 1993) mother cell. The bud neck forms a geometrical barrier that compartmentalizes the bud from the mother cell. Such geometrical barrier does not exist in *S. pombe*.

Dimension and compartmentalization in budding yeast

In *S. cerevisiae*, the septin cytoskeleton plays a key role in the formation of the bud. Septins were first discovered in the budding yeast, which encodes seven distinct septin genes, as cytoskeletal filaments encircling the mother-bud neck (Byers & Goetsch, 1976). These contain a variable N-terminal domain, a polybasic region, a GTP-binding domain, and a C-terminal coiled coil and form rod-shaped octameric complexes *in vitro* (Frazier *et al.*, 1998; Bertin *et al.*, 2008). Phospholipids are important for the assembly of septins into filaments: Their basic region binds to phosphoinositides (Casamayor & Snyder, 2003) and PIP_2 monolayers promote the assembly of septin filaments *in vitro* (Bertin *et al.*, 2010).

In vivo, the septins form higher-order structures, first as a patch, which quickly matures into a ring around the incipient bud site, then as an hourglass structure around the neck of the growing bud, finally as two split rings on either side of the division site (Lippincott *et al.*, 2001; Rodal *et al.*, 2005; Iwase *et al.*, 2006). Septin filament

formation is critical for their function, and septin mutants that prevent filament formation are inviable (McMurray *et al.*, 2011). The ring structures are dynamic, exhibiting substantial turnover, whereas the hourglass structure is highly stable (Caviston *et al.*, 2003; Dobbelaere *et al.*, 2003). It is likely that the transition between these states involves disassembly and reassembly of septin filaments along different growth axes (DeMay *et al.*, 2011; Bertin *et al.*, 2012). The maturation of the septin cytoskeleton into its diverse forms is regulated by multiple factors, including post-translational modifications. For instance, the Nim1-like kinase Gin4 phosphorylates the septin Shs1 and promotes the formation of the hourglass structure (Mortensen *et al.*, 2002; Gladfelter *et al.*, 2004), and septins are also sumoylated; however, the function of this modification remains to be established (Johnson & Blobel, 1999; Johnson & Gupta, 2001).

The initial recruitment of septins at the incipient bud site depends on Cdc42 (Cid *et al.*, 2001; Gladfelter *et al.*, 2002; Iwase *et al.*, 2006). Cdc42 may control septin ring formation by first recruiting septins to the plasma membrane for subsequent ring assembly and then promoting the formation of a polarized membrane domain within the septin ring by activating the assembly of actin cables for vesicle delivery, contributing to the maturation of the septins into a ring (Oh & Bi, 2011). Recent data have indeed shown that polarized exocytosis sculpts a hole in the initial septin density, which is critical for maturation of the septin ring (Okada *et al.*, 2013). Septins were further proposed to inhibit Cdc42 activity, acting in a negative feedback loop (Okada *et al.*, 2013). Together, these activities lead to the creation of spatially segregated Cdc42/growth zone from the septin/bud neck collar.

The septin cytoskeleton is critical for cell morphogenesis in *S. cerevisiae*. Mutants with misorganized septin arrest with elongated buds that have abnormally wide necks, although these mutants remain highly polarized. Septins are thought to act at the bud neck both as a scaffold for polarization and cytokinesis factors, as well as a diffusion barrier. Their scaffold function is well illustrated by the fact that a mislocalized septin mutant remains competent to recruit several cytokinesis components to the mislocalized sites (Roh *et al.*, 2002). Septins are required for the recruitment of a large number of factors at the bud neck for cytokinesis and are involved in chitin deposition, which maintains the neck dimensions during polarized growth (Schmidt *et al.*, 2003). They also play an important role for bud site selection in haploid cells, anchoring the axial landmarks at sites adjacent to the former division site (Chant & Pringle, 1995; Kang *et al.*, 2013).

Septins also form a diffusion barrier at the bud neck at the plasma membrane, the endoplasmic reticulum, and the nuclear envelope (Barral *et al.*, 2000; Shcheprova *et al.*, 2008). This restricts the diffusion of plasma membrane proteins and morphogenesis factors such as exocyst or polarisome components across the neck during polarized growth (Barral *et al.*, 2000; Takizawa *et al.*, 2000) and the diffusion of cytokinetic factors away from the neck during cytokinesis (Dobbelaere & Barral, 2004). Given that septins bind phosphoinositide phosphates, septin rings may also restrict the mobility of lipids (Garrenton *et al.*, 2010; Vernay *et al.*, 2012). The septins are similarly required for the polarization of Cdc42 to the bud during bud growth, but not at the incipient bud site (Orlando *et al.*, 2011). The septins thus contribute to define the limit of new growth by spatially segregating membrane domains.

Growth zone dimensions in fission yeast

Schizosaccharomyces pombe cells have no reported diffusion barrier between the growth zone and the cell sides, which instead appear as a continuum. In addition, septins do not appear to play a role during vegetative polarized growth and only exhibit defects in primary septum degradation for cell separation (Berlin *et al.*, 2003; Tasto *et al.*, 2003; Martin-Cuadrado *et al.*, 2005) and in conjugation and sporulation (Onishi *et al.*, 2010). How then are the limits of the growth zone defined? Despite the absence of a clear barrier, the plasma membrane at cell poles is distinct from the sides, as it is rich in ergosterols, detected by filipin staining (Wachtler *et al.*, 2003). This suggests that distinct membrane domains exist and are maintained. One possibility is that there may be a kinetic barrier defined by differences in diffusion rates at cell tips and cell sides.

The width of the cell, and thus the size of the growth zone, is modulated by regulators of Cdc42 activity. Deletion of the main Cdc42 GEF Scd1 or of the scaffold Scd2 results in a substantial widening of the zone of growth, producing almost round cells (Chang *et al.*, 1994; Kelly & Nurse, 2011a, b). Similarly, deletion of the Cdc42 GAP Rga4 results in wider cells, which is additive to that observed in *scd1*Δ cells (Das *et al.*, 2007; Tatebe *et al.*, 2008; Kelly & Nurse, 2011a). As Rga4 localizes to cell sides and Scd1-Scd2 to cell poles, this suggests that these proteins function additively to restrict and respectively concentrate active Cdc42 to the cell poles, thereby defining the proper growth dimension.

The width of the cell, and thus presumably the growth zone, is also altered under distinct conditions. For instance, disruption of actin cable-mediated transport, in *for3*Δ or *myo52*Δ cells (Feierbach & Chang, 2001; Motegi *et al.*, 2001; Win *et al.*, 2001), or partial disruption of the actin cytoskeleton with low-dose LatA treatments (Kelly & Nurse, 2011a), leads to wider cells, although the

specific effects on the distribution of active Cdc42 are unclear. Thus far, only a single mutant, deletion of *rga2*, has been reported to yield thinner cells, and thus smaller growth zones (Villar-Tajadura *et al.*, 2008). Rga2 encodes a GAP for Rho2, but also associates with Cdc42 and somehow indirectly promotes its activation. The fluctuations, or even regular oscillations, in Cdc42 activity between the two cell poles may also contribute to defining the width of the growth zone, but their precise role remain unclear (Bendezu & Martin, 2012; Das *et al.*, 2012a).

Advantages and adaptations of molecular processes to distinct cell shapes

The very distinct shapes of *S. cerevisiae* and *S. pombe* cells have important consequences on how these cells have solved some fundamental cellular problems, such as cell fate specification, aging, equal segregation of the genetic material, or polarized exocytosis.

The compartmentalization of the *S. cerevisiae* bud confers the ability to generate diversity between the mother and daughter cells. For instance, the transport and tethering in the bud of the *ash1* mRNA, which codes for a repressor of the HO endonuclease, prevents mating-type switching in the daughter cell, thus generating a cell fate difference (Cosma, 2004). By contrast, fission yeast cells evolved a distinct mechanism to promote mating-type switching in only one daughter cell. This does not rely on a cytoplasmic polarity, but on the inherent asymmetry in DNA replication, where the lagging, but not the leading, strand at the Mat1 locus imposes an imprint on the DNA, marking it for transposition (Klar, 2007). In *S. cerevisiae*, the barrier at the bud neck was also proposed to contribute to the retention of aging factors in the mother cell, thus allowing the rejuvenation of the daughter cell (Shcheprova *et al.*, 2008), although the mechanism remains debated (Khmelinskii *et al.*, 2011) and other factors, such as nuclear geometry, may also contribute (Gehlen *et al.*, 2011). Whether fission yeast cells asymmetrically distribute aging factors between daughter cells remains unclear, especially as these cells were recently proposed not to age in the absence of stress (Coelho *et al.*, 2013).

Conversely, the bud neck imposes a geometrical constraint on the cell, which has developed mechanisms to overcome it. For instance, as mentioned earlier, *S. cerevisiae* is strictly dependent on vesicular transport across the bud neck to grow a large bud (Johnston *et al.*, 1991; Imamura *et al.*, 1997; Yamamoto *et al.*, 2010). Maybe for this reason, budding yeast cells have developed robust strategies to polarize actin cables from the bud tip into the mother cell, both by localizing formins to the bud tips through multiple redundant domains (Evangelista *et al.*, 1997; Fujiwara *et al.*, 1998; Sheu *et al.*, 1998; Chen *et al.*, 2012) and by promoting alignment of actin cables through the bud neck through myosin II-dependent capture mechanisms, even in absence of localized cable assembly (Gao & Bretscher, 2009). By contrast, the fission yeast cell does not strictly need active long-range cytoskeletal transport of vesicles for polarized exocytosis and polarized growth: Cells lacking both actin cables and microtubules still polarize growth to opposite cell poles, suggesting that vesicles carrying cell wall enzymes can freely diffuse and be trapped at cell poles (Bendezu & Martin, 2011).

Finally, the bud neck also represents an impediment to equal segregation of the genetic material. In *S. cerevisiae* cells, robust, redundant mechanisms promote and monitor the correct alignment of the spindle across the mother-bud axis (Pereira & Yamashita, 2011). Astral microtubules are captured at the bud neck and transported along actin cables in a Kar9-dependent manner and pulled toward the bud tip by dynein motors. This process is closely monitored and the cell cycle halted by the spindle orientation checkpoint in case of spindle misalignment. By contrast, fission yeast cells may align their spindle along the length of the cell primarily through the physical properties of microtubules. Despite early reports of a spindle orientation checkpoint (Gachet *et al.*, 2001; Oliferenko & Balasubramanian, 2002), more recent work found no evidence for such a checkpoint (Vogel *et al.*, 2007; Meadows & Millar, 2008). The spindles may be already prealigned at mitotic entry along the long cell axis through the action of interphase microtubules, which align along the long cell axis by sliding along the cell sides (Vogel *et al.*, 2007; Daga & Nurse, 2008). Intranuclear astral microtubules may then contribute to spindle orientation by exerting pushing, rather than pulling forces (Tolic-Norrelykke *et al.*, 2004; Zimmerman *et al.*, 2004). Thus, in the absence of a neck, the fission yeast cell may rely more on simple physical properties, such as diffusion or microtubule sliding.

In summary, studies of fundamental properties as well as cell-type-specific adaptions of the polarization machineries in budding and fission yeast are starting to reveal how a cell may be adapting common principles to its own specific needs to generate diverse cell shapes from very similar toolkits. It will be interesting to investigate whether manipulation of the size of the growth zone along with the addition or removal of a septin diffusion barrier can alter the modes of growth of these two yeasts.

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