Cell Polarization and Cytokinesis in Budding Yeast

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ABSTRACT Asymmetric cell division, which includes cell polarization and cytokinesis, is essential for generating cell diversity during development. The budding yeast *Saccharomyces cerevisiae* reproduces by asymmetric cell division, and has thus served as an attractive model for unraveling the general principles of eukaryotic cell polarization and cytokinesis. Polarity development requires G-protein signaling, cytoskeletal polarization, and exocytosis, whereas cytokinesis requires concerted actions of a contractile actomyosin ring and targeted membrane deposition. In this chapter, we discuss the mechanics and spatial control of polarity development and cytokinesis, emphasizing the key concepts, mechanisms, and emerging questions in the field.

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ASYMMETRIC cell division, which is composed of cell polarization and cytokinesis, plays important roles in generating cell diversity during development in plants and animals, as well as in the decision of stem cells to undergo self-renewal *vs*. differentiation (Knoblich 2010; Paciorek and Bergmann 2010; De Smet and Beeckman 2011). The budding yeast *Saccharomyces cerevisiae* reproduces by asymmetric cell division and has thus served as an excellent model for studying this fundamental process (Pruyne and Bretscher 2000b; Park and Bi 2007).

Cell polarization, or the formation of distinct cellular domains, is crucial for performing specific functions such as neuronal transmission (Witte and Bradke 2008), ion transport across epithelia (Drubin and Nelson 1996), and pollen tube growth in plants (Kost 2008). Budding yeast undergo pronounced polarized cell growth during three distinct phases: budding, mating, and filamentous growth (Pruyne and Bretscher 2000b; Park and Bi 2007). These polarization events all involve the conserved small GTPase Cdc42, cytoskeletal polarization, and exocytosis. They differ in the instructive cues and spatiotemporal controls. During budding, polarization is induced by the cell-cycle clock and oriented by the bud-site selection program (Pruyne and Bretscher 2000b; Park and Bi 2007). During mating, polarization is induced and oriented by an external gradient of pheromone (Arkowitz 2009; Saito 2010; Waltermann and Klipp 2010). During starvation, polarization is induced by specific nutrient environments (Dickinson 2008; Saito 2010; Waltermann and Klipp 2010) and oriented by a modified version of the bud-site selection program.

Cytokinesis is essential for increasing cell numbers and cell diversity during development (Balasubramanian *et al.* 2004; Barr and Gruneberg 2007; Pollard 2010). Cytokinesis can be viewed as a specialized form of polarized growth.

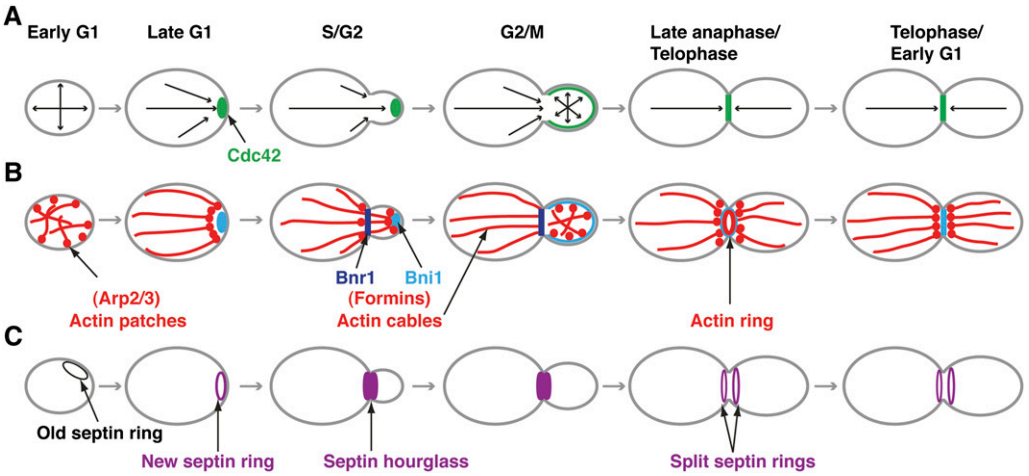

Figure 1 Cdc42, cell growth, and cytoskeletal polarizations during the cell cycle. (A) Cdc42 (green) localization and the direction of cell growth (arrows) are indicated. (B) Actin organization during the cell cycle. Branched actin filaments in actin patches, nucleated by the Arp2/3 complex, regulate endocytosis. Linear actin cables, nucleated by the formins Bni1 and Bnr1, guide polarized exocytosis. The actin ring, nucleated by the formins (mainly Bni1), is involved in cytokinesis. (C) Septin organization during the cell cycle. Polarized Cdc42 directs septin recruitment to the incipient bud site to form a cortical ring. Upon bud emergence, the septin ring is expanded into an hourglass spanning the entire mother-bud neck. At the onset of cytokinesis, the MEN triggers the splitting of the hourglass into two cortical rings. Modified from (Park and Bi 2007) with permission.

Both budding and division require polarized actin and exocytosis, but they differ in the temporal controls and the acquisition of an additional actomyosin system for the division process. During budding, the growth machine is directed toward the bud cortex to promote bud growth. Later in the cell cycle, the same growth machine is redirected to the mother-bud neck to promote cytokinesis. Cytokinesis in animal and fungal cells involves spatiotemporally coordinated functions of a contractile actomyosin ring (AMR) and targeted membrane deposition (Balasubramanian *et al.* 2004; Barr and Gruneberg 2007; Pollard 2010). The AMR is thought to generate a contractile force that powers the ingression of the plasma membrane (PM) and also guides membrane deposition. Targeted vesicle fusion at the division site increases surface area and also delivers enzymes for localized extracellular matrix (ECM) remodeling. Although the core principles and components of cytokinesis are largely conserved from yeast to human, different organisms use different means to specify the division site. In wild-type *S. cerevisiae*, bud-site selection specifies the site of budding and division. In this chapter, we will discuss the mechanisms of cell polarization during budding and cytokinesis, as well as their spatial control; the temporal control of these morphogenetic events is discussed in the chapter by Howell and Lew (2012) and the polarization mechanisms during mating and starvation are discussed elsewhere (Dickinson 2008; Arkowitz 2009; Saito 2010; Waltermann and Klipp 2010).

Establishment and maintenance of polarized cell growth

Polarization of growth and the cytoskeleton during the cell cycle

S. cerevisiae cells undergo cell-cycle–regulated polarized growth toward a single cortical site, leading to bud emergence and enlargement until telophase when the growth machinery is redirected to the bud neck to promote cytokinesis and cell separation (Figure 1A) (Hartwell 1971b; Pringle and Hartwell 1981). During this process, the mother cell displays little or no change in size. Pulse-chase labeling experiments using fluorophore-conjugated concanavalin A, which binds to cell surface glycoproteins, indicate that new growth is first targeted to the bud tip from late G1 to G2 phases of the cell cycle, an "apical growth" mode that drives cell lengthening, and then targeted to the entire bud upon the entry into mitosis, an "isotropic growth" mode that drives uniform bud expansion (Farkas *et al.* 1974; Lew and Reed 1993). The relative duration of the two growth modes determines the bud shape, which is normally ovoid. The apical-to-isotropic switch is controlled by Cdk1 (Cdc28)/cyclin complexes.

Three cytoskeletal systems are polarized during the yeast cell cycle: actin, septins, and cytoplasmic microtubules (Kaksonen *et al.* 2006; Moseley and Goode 2006; Moore *et al.* 2009; Oh and Bi 2011). Filamentous (F) actin structures in yeast include distinct actin cables, actin patches, and the cytokinetic actin ring (Figure 1B) (Pruyne and Bretscher 2000a; Park and Bi 2007). Polarized actin cables act as "tracks" to guide the delivery of secretory vesicles toward the site of growth (Adams and Pringle 1984; Pruyne *et al.* 1998). Actin patches regulate endocytosis (Kaksonen *et al.* 2003). The actin ring is involved in cytokinesis (Epp and Chant 1997; Bi *et al.* 1998; Lippincott and Li 1998a). Septins are assembled into a cortical ring at the incipient bud site, which is expanded into an hourglass structure upon bud emergence (Figure 1C). At the onset of cytokinesis, the septin hourglass is split into two cortical rings that sandwich the cytokinesis machine. The septin rings/hourglass function as a scaffold and/or diffusion barrier to effect bud morphogenesis, cytokinesis, and many other processes (Longtine *et al.* 1996; Gladfelter *et al.* 2001; Weirich *et al.* 2008; McMurray and Thorner 2009; Oh and Bi 2011). Cytoplasmic microtubules, which emanate from the spindle pole body (the

yeast counterpart of the animal centrosome), are also polarized during bud emergence and bud growth (Moore *et al.* 2009). However, disruption of microtubules does not affect polarized growth (Adams and Pringle 1984; Palmer *et al.* 1992; Sullivan and Huffaker 1992), suggesting that microtubules do not play a major role in this process. Thus, the actin cytoskeleton (especially cables) and the septins are the major determinants of cellular morphogenesis in budding yeast. Here, we discuss the key regulatory molecules and pathways that control the polarized assembly of actin cables and the septin ring.

Cdc42: the center of cell polarization

The small GTPase Cdc42 plays a central role in cell polarity from yeast to humans (Etienne-Manneville 2004; Park and Bi 2007). *CDC42* was initially identified as a temperature-sensitive mutant defective in polarized actin organization and cell growth in *S. cerevisiae* (Adams *et al.* 1990; Johnson and Pringle 1990). Homologs from other species, including humans, share 80–85% identity in amino acid sequence and functionally complement yeast *cdc42* mutants (Johnson and Pringle 1990; Munemitsu *et al.* 1990; Shinjo *et al.* 1990; Chen *et al.* 1993; Luo *et al.* 1994; Miller and Johnson 1994; Eaton *et al.* 1995). Thus, Cdc42 is a master regulator of cell polarity.

In response to temporal and spatial signals, Cdc42 in *S. cerevisiae* becomes polarized at a predetermined cortical site to drive bud growth (Park and Bi 2007). Remarkably, in the absence of any spatial cues, such as in *rsr1Δ* cells (Bender and Pringle 1989), Cdc42 can still polarize at a random site in the cell cortex and fulfill its essential role in polarized cell growth without evident defects (Irazoqui *et al.* 2003; Wedlich-Soldner *et al.* 2003). Thus, yeast cells possess intrinsic mechanisms for "symmetry breaking." Below, we will first describe the Cdc42 GTPase module and then discuss its functions and mechanisms. All polarity proteins relevant to our discussions are described in Table 1.

Cdc42 GTPase module: Like all members of the Ras superfamily, Cdc42 cycles between its inactive guanosine diphosphate (GDP)-bound and active guanosine triphosphate (GTP)-bound states (Figure 2). Cdc42 activation is catalyzed by the guanine nucleotide-exchange factor (GEF), Cdc24 (Zheng *et al.* 1994; Tcheperegine *et al.* 2005). Inactivation by GTP hydrolysis is stimulated by the GTPase-activating proteins (GAPs), Bem2, Bem3, Rga1, and Rga2 (Zheng *et al.* 1993, 1994; Marquitz *et al.* 2002; Tong *et al.* 2007). Bem2 is a GAP for both Cdc42 and Rho1 (Zheng *et al.* 1993; Marquitz *et al.* 2002), whereas the others are thought to be Cdc42 specific. Cdc42 is also regulated by the Rho GDP-dissociation inhibitor (GDI), Rdi1 (Masuda *et al.* 1994; Eitzen *et al.* 2001; Richman *et al.* 2004; Tcheperegine *et al.* 2005).

Cdc24, the only known GEF for Cdc42 in budding yeast (Zheng *et al.* 1994), localizes to the sites of polarized growth (Nern and Arkowitz 1999; Toenjes *et al.* 1999; Shimada *et al.* 2000) and plays an essential role in the establishment and maintenance of polarized cell growth (Hartwell 1971b; Sloat and Pringle 1978; Sloat *et al.* 1981; Adams *et al.* 1990; Gladfelter *et al.* 2002).

The GAPs for Cdc42 regulate different aspects of cell polarization. In contrast to the essential GEF, none of the individual GAPs are essential for cell viability. However, *rga1Δ* and *bem2Δ* are synthetically lethal (Chen *et al.* 1996), suggesting that GAPs are collectively required for cell viability. At the beginning of the cell cycle, three GAPs (Rga1, Rga2, and Bem3) regulate septin ring assembly at the incipient bud site, presumably facilitating the cycling of Cdc42 between its two states (Gladfelter *et al.* 2002; Smith *et al.* 2002; Caviston *et al.* 2003). In addition, three GAPs (Rga2, Bem2, and Bem3) are phosphorylated by Cdk1/G1 cyclins, which is thought to inhibit their GAP activity and thus contribute to the timely activation of Cdc42 during bud emergence (Knaus *et al.* 2007; Sopko *et al.* 2007). Rga1 is uniquely required to prevent Cdc42 activation (and hence budding) at the cytokinesis site (Tong *et al.* 2007). All GAPs localize to the sites of polarized growth at one point of the cell cycle (Caviston *et al.* 2003; Knaus *et al.* 2007; Sopko *et al.* 2007), with Bem2 and Rga1 showing additional localization at the mother-bud neck before cytokinesis (Caviston *et al.* 2003; Huh *et al.* 2003; Knaus *et al.* 2007). It remains unclear how the localization and/or activity of the GAPs are regulated during the cell cycle and how this regulation contributes to polarity establishment and maintenance.

Rho GDI proteins display three biochemical activities on their target GTPases: they inhibit dissociation of GDP (Fukumoto *et al.* 1990; Leonard *et al.* 1992; Chuang *et al.* 1993), inhibit both intrinsic and GAP-stimulated GTPase activity (Hart *et al.* 1992; Chuang *et al.* 1993; Hancock and Hall 1993), and extract the GTPase from membranes into the cytosol (Hori *et al.* 1991; Nomanbhoy and Cerione 1996; Johnson *et al.* 2009). The major function of the GDI appears to cycle GDP-bound Rho GTPases between the cytosol and the PM, but the underlying mechanism remains unclear. Rdi1, the only known and nonessential Rho GDI in *S. cerevisiae* (Masuda *et al.* 1994), extracts Cdc42, Rho1, and Rho4 from membranes effectively (Eitzen *et al.* 2001; Richman *et al.* 2004; Tcheperegine *et al.* 2005; Tiedje *et al.* 2008). Overexpression of Rdi1 in a *cdc24*-Ts mutant causes cell lethality and loss of cell polarity, indicating a negative role of Rdi1 in cell polarization (Tcheperegine *et al.* 2005). On the other hand, Rdi1 is thought to promote polarization by facilitating the cycling of Cdc42 between the PM and the cytosol (Slaughter *et al.* 2009). Indeed, deletion of *RDI1* reduces polarized growth in *Candida albicans* (Court and Sudbery 2007) and causes decreased pseudohyphal growth in *S. cerevisiae* (Tiedje *et al.* 2008).

Singularity in budding and Cdc42 polarization

S. cerevisiae cells bud once and only once per cell cycle (Hartwell 1971b). This singularity in budding appears to be controlled by Cdc42, as the hyperactive $cdc42^{G60D}$ mutant can drive polarization at multiple random sites in the complete

Table 1 Proteins involved in polarized cell growth

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Cdc42 and its regulators and effectors			
Cdc42	Rho-like GTPase	Essential for polarity establishment and maintenance ("Master regulator" of eukaryotic cell polarity) Mutants are defective in polarized actin organization, septin ring assembly, and bud emergence	Adams *et al.* 1990; Johnson and Pringle 1990; Iwase *et al.* 2006
Cdc24	GEF for Cdc42	Essential for polarity establishment and maintenance Mutants are defective in polarized actin organization, septin ring assembly, bud emergence, and shmoo formation	Sloat and Pringle 1978; Chenevert *et al.* 1994; Zheng *et al.* 1994; Shimada *et al.* 2004; Wiget *et al.* 2004
Bem2	GAP for Cdc42 and Rho1	Important for polarity establishment and maintenance	Bender and Pringle 1991; Kim *et al.* 1994; Peterson *et al.* 1994; Marquitz *et al.* 2002; Knaus *et al.* 2007
Bem3	GAP for Cdc42	Important for polarity regulation and septin ring assembly	Bender and Pringle 1991; Zheng *et al.* 1993; Zheng *et al.* 1994; Gladfelter *et al.* 2002; Smith *et al.* 2002; Caviston *et al.* 2003; Knaus *et al.* 2007
Rga1	GAP for Cdc42	Essential for preventing budding or cell polarization within old division sites Important for suppressing basal signaling during mating, and septin ring assembly	Stevenson *et al.* 1995; Chen *et al.* 1996; Gladfelter *et al.* 2002; Smith *et al.* 2002; Caviston *et al.* 2003; Tong *et al.* 2007
Rga2	GAP for Cdc42	Important for polarity regulation and septin ring assembly	Gladfelter *et al.* 2002; Smith *et al.* 2002; Caviston *et al.* 2003; Sopko *et al.* 2007
Rdi1	GDI for Cdc42, Rho1, and Rho4	Important for cycling of Rho GTPases between the cytosol and the PM during cell polarization	Masuda *et al.* 1994; Koch *et al.* 1997; Richman *et al.* 2004; Tcheperegine *et al.* 2005; Tiedje *et al.* 2008; Slaughter *et al.* 2009; Logan *et al.* 2011
Bem1	Scaffold for GEF-based amplification of Cdc42 activation	Important for polarity establishment during budding and mating Forms complex with Cdc24, Cla4, Rsr1 during budding	Bender and Pringle 1989; Bender and Pringle 1991; Chenevert *et al.* 1992; Leeuw *et al.* 1995; Park *et al.* 1997; Gulli *et al.* 2000; Bose *et al.* 2001; Irazoqui *et al.* 2003; Shimada *et al.* 2004
Cla4	PAK (p21-activated kinase) Effector of Cdc42	Important for septin ring assembly, phosphorylates septins Cdc3 and Cdc10 Important for polarity regulation Down-regulation of sterol uptake	Cvrckova *et al.* 1995; Benton *et al.* 1997; Weiss *et al.* 2000; Caviston *et al.* 2003; Versele and Thorner 2004; Wild *et al.* 2004; Kozubowski *et al.* 2008; Lin *et al.* 2009

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Table 1, *continued*

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Ste20	PAK Effector of Cdc42	Important for MAPK signaling during pheromone response, pseudohyphal growth, and invasive growth Down-regulation of sterol uptake	Leberer *et al.* 1992; Ramer and Davis 1993; Peter *et al.* 1996; Leeuw *et al.* 1998; Kozubowski *et al.* 2008; Lin *et al.* 2009
Skm1	PAK Effector of Cdc42	Down-regulation of sterol uptake Regulate morphogenesis	Martin *et al.* 1997; Lin *et al.* 2009
Bni1	Formin, nucleates the assembly of linear actin filaments Effector of Cdc42 and Rho1 Polarisome component	Plays important roles in exocytosis, cytokinesis, spindle orientation, and mating by promoting the assembly of actin cables and actin rings	Kohno *et al.* 1996; Evangelista *et al.* 1997; Lee *et al.* 1999; Miller *et al.* 1999; Pruyne *et al.* 2002; Sagot *et al.* 2002b; Moseley *et al.* 2004; Moseley and Goode 2005; Moseley and Goode 2006
Gic1	Effector of Cdc42	Shares a role with Gic2 in cytoskeletal polarization and polarized cell growth *gic1Δ gic2Δ* cells are temperature sensitive for growth Interacts with septins	Brown *et al.* 1997; Chen *et al.* 1997; Iwase *et al.* 2006
Gic2	Effector of Cdc42	Shares a role with Gic1 in cytoskeletal polarization and polarized cell growth *gic1Δ gic2Δ* cells are temperature sensitive for growth Interacts with septins Undergoes ubiquitin-mediated degradation by SCF^{GRR1}	Brown *et al.* 1997; Chen *et al.* 1997; Iwase *et al.* 2006; Jaquenoud *et al.* 1998
Actin cables, exocytosis, and key regulators			
Act1	Actin	Involved in exocytosis, endocytosis, cytokinesis, and many other functions	Engqvist-Goldstein and Drubin 2003; Moseley and Goode 2006; Park and Bi 2007
Bni1	Formin (See above)		
Bnr1	Formin, nucleates the assembly of linear actin filaments	Plays an important role in exocytosis by promoting actin cable assembly	Imamura *et al.* 1997; Pruyne *et al.* 2002; Sagot *et al.* 2002b; Moseley *et al.* 2004; Moseley and Goode 2005; Moseley and Goode 2006
Pfy1	Profilin	Plays a role in actin organization, endocytosis, and exocytosis Binds to monomeric actin, phosphatidylinositol 4,5-bisphosphate, and polyproline regions Catalyzes ADP/ATP exchange on actin monomers	Haarer *et al.* 1990; Haarer *et al.* 1996; Moseley and Goode 2006; Sagot *et al.* 2002b
Bud6	Actin-binding protein Formin-binding protein Polarisome component	Involved in bipolar bud-site selection, actin cable assembly, and polarized cell growth Binds to monomeric actin and Bni1	Amberg *et al.* 1997; Evangelista *et al.* 1997; Moseley *et al.* 2004; Moseley and Goode 2005
Tpm1	Major isoform of tropomyosin	Stabilizes actin filaments in actin cables and actin rings Involved in polarized cell growth and organelle inheritance Binds actin more efficiently upon Tpm1 acetylation by the NatB complex	Liu and Bretscher 1989a; Pruyne *et al.* 1998; Bretscher 2003; Singer and Shaw 2003

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Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Tpm2	Minor isoform of tropomyosin	Stabilizes actin filaments in actin cables and actin rings Shares an essential role with Tpm1 in polarized cell growth and organelle inheritance Plays a role in morphogenesis that is distinct from Tpm1	Liu and Bretscher 1989a; Drees *et al.* 1995; Pruyne *et al.* 1998; Bretscher 2003; Singer and Shaw 2003; Yoshida *et al.* 2006
Sac6	Fimbrin, an actin-bundling protein	Acts in concert with Scp1 (transgelin-like protein) for the organization and maintenance of the actin cytoskeleton	Adams *et al.* 1989; Goodman *et al.* 2003; Winder *et al.* 2003
Abp140	AdoMet-dependent tRNA methyltransferase and actin-binding protein	Binds actin filaments in actin patches and cables Modifies the anti-codon loop of tRNA-Thr and tRNA-Ser	Asakura *et al.* 1998; D'Silva *et al.* 2011; Noma *et al.* 2011
Ypt31, Ypt32	Rab GTPases	Play important roles in intra-Golgi traffic or vesicle budding from the *trans*-Golgi Share an essential role in the processes mentioned above	Benli *et al.* 1996; Jedd *et al.* 1997;Grosshans *et al.* 2006
Sec4	Rab GTPase	Essential for post-Golgi vesicle transport and tethering Interacts with the exocyst subunit Sec15 to promote exocyst assembly at the PM	Salminen and Novick 1987; Goud *et al.* 1988; Guo *et al.* 1999b
Sec2	Rab GEF	Essential for post-Golgi vesicle transport and tethering GEF for Sec4	Walch-Solimena *et al.* 1997
Msb3, Msb4	Rab GAPs Polarisome components	Regulate post-Golgi exocytosis by acting as a GAP for Sec4 Localize to the sites of polarized growth and share a role in exocytosis Multi-copy suppressor of *cdc42* and *cdc24* mutants Interact with Spa2	Albert and Gallwitz 1999; Bi *et al.* 2000; Gao *et al.* 2003; Tcheperegine *et al.* 2005
Myo2	Myosin-V	Essential for actin-based transport of cargoes (secretory vesicles, vacuoles, late Golgi elements, peroxisomes, and the mitotic spindle)	Johnston *et al.* 1991; Lillie and Brown 1994; Govindan *et al.* 1995; Bretscher 2003
Myo4	Myosin-V	Involved in actin-based transport of cargoes (Ash1 mRNA) Cortical ER inheritance	Bobola *et al.* 1996; Estrada *et al.* 2003
Spa2	Scaffold protein Polarisome component	Interacts with Bud6, Bni1, Pea2, Msb3, and Msb4 Scaffold for the MAPK cell wall integrity pathway Mutants are defective in polarized growth and bipolar budding	Snyder 1989; Sheu *et al.* 1998; Sheu *et al.* 2000; van Drogen and Peter 2002; Tcheperegine *et al.* 2005
Pea2	Polarisome component	Required for efficient mating and bipolar budding Interacts with Spa2, and regulates polarized growth	Chenevert *et al.* 1994; Valtz and Herskowitz 1996; Sheu *et al.* 1998
Sec3	Exocyst subunit	Required for the tethering of post-Golgi vesicles to the PM Localizes to the sites of polarized growth and acts a spatial landmark for exocytosis Binds to PIP2 Interacts with Rho1 and Cdc42	TerBush *et al.* 1996; Finger *et al.* 1998; Guo *et al.* 1999a; Guo *et al.* 2000; Guo *et al.* 2001; Boyd *et al.* 2004; Zhang *et al.* 2001; Zhang *et al.* 2008; Yamashita *et al.* 2010
Sec5, Sec6, Sec8, Sec10, Exo84	Exocyst subunits	Required for the tethering of post-Golgi vesicles to the PM	Guo *et al.* 1999a; TerBush *et al.* 1996; Guo *et al.* 2000; Boyd *et al.* 2004

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Table 1, *continued*

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Sec15	Exocyst subunit	Required for the tethering of post-Golgi vesicles to the PM Interacts with Sec4-GTP and Bem1	Boyd *et al.* 2004; France *et al.* 2006; Guo *et al.* 1999a; Guo *et al.* 1999b; Guo *et al.* 2000; TerBush *et al.* 1996
Exo70	Exocyst subunit	Required for the tethering of post-Golgi vesicles to the PM Binds to PIP2 Interacts with Cdc42, and Rho3	TerBush *et al.* 1996; Guo *et al.* 1999a; Robinson *et al.* 1999; Guo *et al.* 2000; Boyd *et al.* 2004; He *et al.* 2007; Wu *et al.* 2010
Sec1	Fusion factor	Involved in docking and fusion of post-Golgi vesicles with the PM Interacts with the exocyst and binds to assembled SNARE complexes Localizes to the sites of polarized growth	Novick and Schekman 1979; Carr *et al.* 1999; Scott *et al.* 2004; Wiederkehr *et al.* 2004
Actin patches, endocytosis, and key regulators			
Act1	Actin (see above)		
"Arp2/3 complex"	Nucleates the assembly of branched actin filaments	Plays an essential role in endocytosis	Winter *et al.* 1999b; Moseley and Goode 2006
Ent1, Ent2	Epsin-like protein	Involved in endocytosis and actin patch assembly Contain a clathrin-binding motif at the C terminus Functionally redundant Interacts with Cdc42-GAPs	Wendland *et al.* 1999; Aguilar *et al.* 2006; Mukherjee *et al.* 2009
Las17	Activator of the Arp2/3 complex (WASP homolog)	Promotes Arp2/3-mediated actin assembly	Li 1997; Madania *et al.* 1999; Winter *et al.* 1999a; Lechler *et al.* 2001
Vrp1	WIP (WASP-interacting protein)-like protein	Proline-rich protein involved in actin-patch organization, endocytosis, and cytokiensis	Donnelly *et al.* 1993; Munn *et al.* 1995; Thanabalu and Munn 2001
Myo3, Myo5	Myosin-Is	Localize to actin patches and share important roles in endocytosis and polarized actin organization	Geli and Riezman 1996; Goodson *et al.* 1996; Anderson *et al.* 1998; Geli *et al.* 2000
Sac6	Fimbrin (see above)		
Septins and key regulators			
Cdc3, Cdc10, Cdc11, Cdc12, Shs1	Septins	Components of the septin ring/hourglass that acts as scaffold and/or diffusion barrier to effect cytokinesis, bud morphogenesis, etc. Vegetatively expressed, form heterooligomeric complex	Hartwell 1971b; Byers and Goetsch 1976b; Haarer and Pringle 1987; Sanders and Field 1994; Longtine *et al.* 1996; Frazier *et al.* 1998; Gladfelter *et al.* 2001; Mortensen *et al.* 2002; Vrabioiu *et al.* 2004; Bertin *et al.* 2008; McMurray and Thorner 2009; Oh and Bi 2011;
Bni5	Septin-associated protein	Required for Myo1 targeting to the bud neck before cytokinesis Regulates septin organization	Lee *et al.* 2002; Fang *et al.* 2010

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Table 1, *continued*

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Elm1	Septin-associated kinase	Regulates septin organization, bud morphogenesis, cytokinesis, and mitotic exit Regulates other septin-associated kinases	Blacketer *et al.* 1993; Bouquin *et al.* 2000; Mortensen *et al.* 2002; Woods *et al.* 2003; Asano *et al.* 2006; Szkotnicki *et al.* 2008; Caydasi *et al.* 2010; Moore *et al.* 2010
Gin4	Septin-associated kinase	Regulates septin organization and bud growth	Altman and Kellogg 1997; Okuzaki *et al.* 1997; Longtine *et al.* 1998; Mortensen *et al.* 2002
Nap1	Nucleosome assembly protein	Regulates septin organization and bud growth Interacts with mitotic cyclin Clb2 Involved in the transport of H2A and H2B histones to the nucleus	Ishimi and Kikuchi 1991; Kellogg *et al.* 1995; Iwase and Toh-E 2001; Longtine *et al.* 2000; Mosammaparast *et al.* 2002
Other Rho GTPases			
Rho2	Rho GTPase	May have overlapping role with Rho1 in polarized cell growth May regulate microtubule assembly Nonessential for viability No specific GEFs, GAPs, and GDI have been identified	Madaule *et al.* 1987; Kim *et al.* 1994; Ozaki *et al.* 1996; Manning *et al.* 1997; Perez and Rincon 2010
Rho3	Rho GTPase	Regulates exocytosis and interacts with Myo2 and Exo70 Involved in formin activation Nonessential for viability Shares a role with Rho4 in regulating polarized cell growth Rgd1 is a GAP for Rho3	Matsui and Toh-e 1992; Adamo *et al.* 1999; Doignon *et al.* 1999; Robinson *et al.* 1999; Dong *et al.* 2003; Wu and Brennwald 2010; Wu *et al.* 2010
Rho4	Rho GTPase	Involved in formin activation Nonessential for viability Shares a role with Rho3 in regulating polarized cell growth Regulates interaction between Bnr1 and Hof1 Is regulated by the GAP (Rgd1) and the GDI (Rdi1)	Matsui and Toh-e 1992; Kamei *et al.* 1998; Doignon *et al.* 1999; Dong *et al.* 2003; Tiedje *et al.* 2008
Rho5	Rho GTPase	Null mutation confers resistance to various stresses (cell wall stress, osmotic stress, and oxidative stress) Nonessential for viability Rgd2 acts as a GAP for Rho5	Roumanie *et al.* 2001; Schmitz *et al.* 2002; Annan *et al.* 2008; Singh *et al.* 2008
Rho1 and its regulators and effectors			
Rho1	Rho GTPase	Essential GTPase that is required for cell wall remodeling and resistance to oxidative stress Functions as a regulatory subunit for the glucan synthases Fks1 and Fks2 and also regulates Pkc1 and Mpk1-mediated cell wall integrity pathway Regulates formin activation and exocytosis Regulates actin ring assembly and cytokinesis	Yamochi *et al.* 1994; Drgonova *et al.* 1996; Kohno *et al.* 1996; Qadota *et al.* 1996; Drgonova *et al.* 1999; Guo *et al.* 2001; Tolliday *et al.* 2002; Dong *et al.* 2003; Yoshida *et al.* 2006; Yoshida *et al.* 2009; Lee *et al.* 2011

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Table 1, *continued*

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Rom1	GEF for Rho1	Nonessential for cell viability *rom1Δ* and *rom2Δ* are synthetically lethal	Ozaki *et al.* 1996; Manning *et al.* 1997; Schmidt *et al.* 1997
Rom2	GEF for Rho1	*rom2Δ* cells are temperature-sensitive for growth *rom1Δ* and *rom2Δ* are synthetically lethal	Ozaki *et al.* 1996; Manning *et al.* 1997; Schmidt *et al.* 1997
Tus1	GEF for Rho1	Nonessential for cell viability Multicopy suppressor of *tor2* mutation Regulates cytokinesis	Schmelzle *et al.* 2002; Yoshida *et al.* 2006; Yoshida *et al.* 2009
Lrg1	GAP for Rho1	Involved in Pkc1 and Mpk1-mediated cell wall integrity pathway Regulates glucan synthesis specifically *lrg1Δ* and *sac7Δ* are synthetically lethal	Muller *et al.* 1994; Lorberg *et al.* 2001; Watanabe *et al.* 2001
Bem2	GAP for Rho1 and Cdc42 (see above)		
Sac7	GAP for Rho1	Involved in actin organization Mutations in *SAC7* suppresses mutations in *TOR2* and *ACT1*	Dunn and Shortle 1990; Schmidt *et al.* 1997; Schmidt *et al.* 2002
Bag7	GAP for Rho1	Nonessential for cell viability Structurally and functionally related to Sac7	Roumanie *et al.* 2001; Schmidt *et al.* 2002
Rdi1	GDI for Rho1, Rho4, and Cdc42 (see above)		
Fks1	Catalytic subunit of 1,3-β-D-glucan synthase Effector of Rho1	Cell wall synthesis Binds to regulatory subunit Rho1 Localizes to sites of polarized growth *fks1Δ* and *fks2Δ* are synthetically lethal	Parent *et al.* 1993; Douglas *et al.* 1994; Inoue *et al.* 1995; Mazur *et al.* 1995; Drgonova *et al.* 1996; Qadota *et al.* 1996
Fks2	Catalytic subunit of 1,3-β-D-glucan synthase Effector of Rho1	Cell wall synthesis Binds to regulatory subunit Rho1 *fks1Δ* and *fks2Δ* are synthetically lethal Involved in spore wall formation	Douglas *et al.* 1994; Mazur *et al.* 1995; Drgonova *et al.* 1996; Qadota *et al.* 1996; Ishihara *et al.* 2007
Pkc1	Protein kinase C	Regulates cell wall remodeling through the cell-wall–integrity pathway Localizes to sites of polarized growth Involved in formin activation	Levin *et al.* 1990; Levin and Bartlett-Heubusch 1992; Levin *et al.* 1994; Andrews and Stark 2000; Dong *et al.* 2003; Denis and Cyert 2005
Bni1	Formin and effector of Rho1 and Cdc42 (see above)		
Sec3	Exocyst subunit and effector of Rho1 and Cdc42 (see above)		
Proteins involved in cell wall remodeling and CWI signaling			
Fks1	Glucan synthase and effector of Rho1 (see above)		
Fks2	Glucan synthase and effector of Rho1 (see above)		
Chs2	Chitin synthase II	Required for primary septum formation during cytokinesis Activity is stimulated by trypsin treatment *in vitro* Localization regulated by exocytosis and Cdk1	Sburlati and Cabib 1986; Shaw *et al.* 1991; Bi 2001; Schmidt *et al.* 2002; VerPlank and Li 2005; Zhang *et al.* 2006; Teh *et al.* 2009
Chs3	Catalytic subunit of chitin synthase III	Required for synthesis of majority of cellular chitin Required for synthesis of the chitin ring ("bud-scar" chitin) during bud emergence Activated by Chs4 Trafficking between chitosomes and the PM is subjected to cell-cycle–dependent complex regulation	Shaw *et al.* 1991; Bulawa 1992; Bulawa 1993; Chuang and Schekman 1996; Ziman *et al.* 1996; DeMarini *et al.* 1997; Ziman *et al.* 1998

(continued)

Table 1, *continued*

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Pkc1	Protein kinase C (see above)		
Bck1 (Slk1)	Mitogen-activated protein kinase kinase kinase (MAPKKK)	Involved in Pkc1-activated cell wall integrity pathway Activates downstream Mkk1 and Mkk2	Lee and Levin 1992; Levin 2005; Park and Bi 2007
Mkk1	MAPKK	Involved in Pkc1-activated cell wall integrity pathway Activates downstream Mpk1 Functionally redundant with Mkk2	Irie *et al.* 1993; Levin 2005; Park and Bi 2007
Mkk2	MAPKK	Involved in Pkc1-activated cell wall integrity pathway Activates downstream Mpk1 Functionally redundant with Mkk1	Irie *et al.* 1993; Levin 2005; Park and Bi 2007
Mpk1 (Slt2)	MAPK	Involved in Pkc1-activated cell wall integrity pathway Regulates cell-cycle progression	Torres *et al.* 1991; Lee *et al.* 1993; Levin 2005; Park and Bi 2007
Swi4-Swi6	Transcriptional activator	Regulates G1/S-specific transcription (G1 cyclins, etc.)	Andrews and Herskowitz 1989; Nasmyth and Dirick 1991; Andrews and Moore 1992; Sidorova and Breeden 1993; Dodou and Treisman 1997; Watanabe *et al.* 1997; Levin 2005; Levin 2005; Park and Bi 2007;
Rlm1	MADS-box transcription factor	Involved in Pkc1-activated cell wall integrity pathway Activated by Mpk1	Dodou and Treisman 1997; Watanabe *et al.* 1997; Levin 2005; Park and Bi 2007
Mss4	Phosphatidylinositol-4-phosphate 5-kinase (PI4P-5K)	Regulates actin and septin organizations, polarity establishment, exocytosis, cell wall remodeling and many other processes	Audhya and Emr 2002; Wild *et al.* 2004; He *et al.* 2007; Takahashi and Pryciak 2007; Orlando *et al.* 2008; Zhang *et al.* 2008; Bertin *et al.* 2010; Garrenton *et al.* 2010

[a] Information on individual proteins is partly based on *Saccharomyces* Genome Database (SGD: http://www.yeastgenome.org/).
[b] Only functions related to polarized cell growth and cytokinesis are indicated here.

absence of the GEF (Figure 3A) (Caviston *et al.* 2002). This G60D mutation falls in the GTP hydrolysis domain and presumably slows, but does not block, GTP hydrolysis, as Cdc42 variants locked in the GTP-bound state (*e.g.*, $cdc42^{G12V}$ and $cdc42^{Q61L}$) cannot support cell growth (Ziman *et al.* 1991; Irazoqui *et al.* 2003). Interestingly, a single copy of wild-type *CDC42* completely suppresses the multi-budded phenotype of $cdc42^{G60D}$, suggesting that efficient cycling of Cdc42 between GDP- and GTP-bound states enforces singularity in polarization (Caviston *et al.* 2002). Rewiring the Cdc42-GTP amplification loop by fusing the scaffold Bem1 to a transmembrane protein (Snc2) also leads to cell polarization at multiple sites with low frequency (Howell *et al.* 2009). Intriguingly, both experiments and mathematical modeling suggest that during cell polarization, multiple Cdc42-GTP clusters form transiently and compete with each other until one "wins" (Goryachev and Pokhilko 2008; Howell *et al.* 2009).

Two distinct mechanisms, both involving positive feedback loops, have been hypothesized to account for Cdc42 polarization at a single cortical site in the absence of any spatial cue such as in *rsr1Δ* cells (Figure 3B). The first is actin independent and involves the scaffold protein Bem1, which binds Cdc42-GTP, Cdc24, and Cla4 (Figure 3B, top) (Bose *et al.* 2001; Butty *et al.* 2002; Irazoqui *et al.* 2003; Goryachev and Pokhilko 2008; Kozubowski *et al.* 2008). The importance of Bem1 in this mechanism is highlighted by the synthetic lethality between *bem1Δ* and *rsr1Δ* (Irazoqui *et al.* 2003). Cla4, a p21-activated kinase (Pak), is an effector of Cdc42 (Cvrckova *et al.* 1995) and is chiefly responsible for phosphorylation of Cdc24, along with Cdk1 (Gulli *et al.* 2000; Bose *et al.* 2001; Wai *et al.* 2009). Yet the role of Cla4 in polarized growth remains controversial (Gulli *et al.* 2000; Bose *et al.* 2001; Hofken and Schiebel 2002; Irazoqui *et al.* 2003; Wild *et al.* 2004; Kozubowski *et al.* 2008; Wai *et al.* 2009). The consensus is that some Cdc42-GTP molecules are spontaneously clustered at a cortical site, which bind to the Cdc24-Bem1-Cla4 complex, leading to further activation of Cdc42 by Cdc24 in the initial cluster. Increased

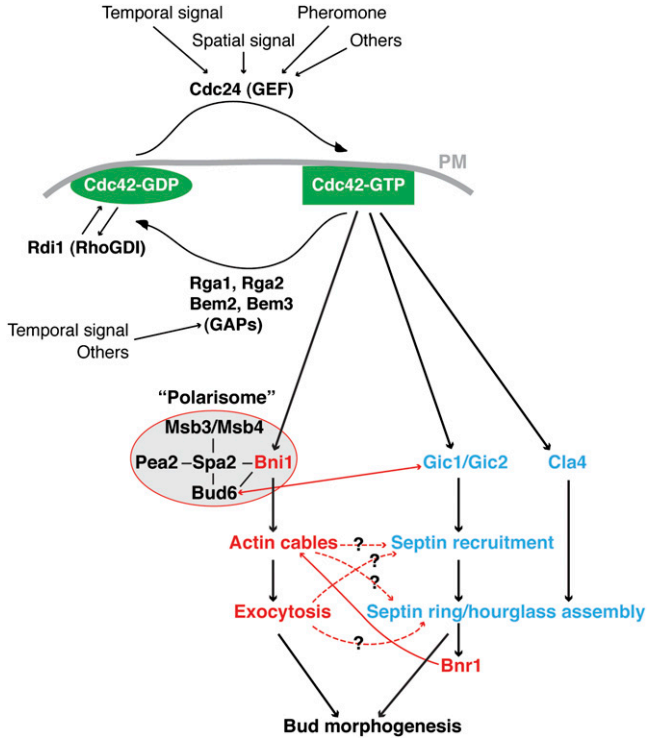

Figure 2 Regulation of Cdc42 and a model for Cdc42-controlled actin cable and septin ring assembly. Internal or external cues activate the GEF Cdc24, which converts Cdc42 to its active GTP-bound state. Activated Cdc42 binds to its effectors to promote actin cable and septin ring assembly (see text for details). Cdc42-GAPs (Rga1, Rga2, Bem2, and Bem3), which are also regulated by internal and external cues, inactivate Cdc42 by stimulating its intrinsic GTPase activity. Rdi1 (Rho GDI) cycles Cdc42-GDP between the PM and the cytosol. Modified from (Park and Bi 2007) with permission.

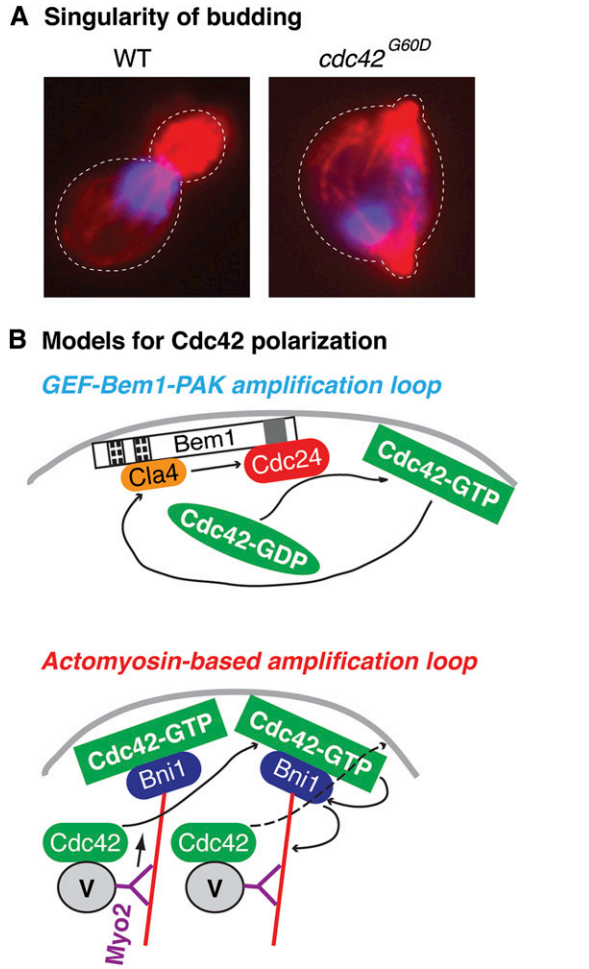

Figure 3 Singularity of polarization. (A) During each cell cycle, wild-type (WT) cells undergo a single round of budding. (Blue, DNA; red, actin.) In contrast, cells with the hyperactive *cdc42*^{G60D} allele can bud more than once per cell cycle. (B) Models for Cdc42 polarization. (Top) The scaffold protein Bem1 binds Cdc24 and Cla4 through distinct domains. Cdc24 increases Cdc42-GTP concentration at the polarization site. Increased Cdc42-GTP binds Cla4, which phosphorylates and may activate Cdc24, creating a positive feedback loop. (Bottom) Cdc42-GTP binds the formin Bni1, causing polarization of actin cables toward the growth site. The cables mediate myosin-V (Myo2)-dependent transport of vesicles (V) carrying Cdc42 as a "cargo." The released Cdc42 will be converted to its active form, which binds to Bni1, leading to more actin cables and more Cdc42 transport, generating a positive feedback loop.

Cdc42-GTP at the cortical site, in turn, recruits more Cdc24-Bem1-Cla4 complexes and the positive cycle continues.

The second positive feedback loop for Cdc42 polarization involves an actomyosin-based transport system (Figure 3B, bottom) (Wedlich-Soldner *et al.* 2003). Here, a spontaneously formed cluster of Cdc42-GTP orients actin cables toward the cluster. The cables guide Myo2 (myosin-V)-powered delivery of more Cdc42 on secretory vesicles to the cluster, leading to an increased local concentration of Cdc42. The increased Cdc42, in turn, directs more actin cables toward the cluster and the positive cycle continues. The major finding supporting this hypothesis is that wild-type or GTP-locked Cdc42^{Q61L}, and other polarity factors such as Bem1, fail to establish a polarization state in several mutants defective in actin cable-mediated transport (Wedlich-Soldner *et al.* 2003; Zajac *et al.* 2005). In addition, Cdc42 is associated with two different populations of secretory vesicles (Orlando *et al.* 2011). In contrast, other studies found that Cdc42 and Bem1 could polarize successfully in several mutants with defects in the same actin transport pathway (Yamamoto *et al.* 2010). More importantly, endogenous Cdc42 polarizes at a single cortical site with nearly normal kinetics in cells treated with latrunculin A (LatA), which disrupts all F-actin structures (Ayscough *et al.* 1997; Moseley and Goode 2006). Furthermore, recent modeling work suggests that actomyosin-based transport would perturb, rather than enforce, Cdc42 polarization (Layton *et al.* 2011). Thus, it remains unclear to what degree the actomyosin-based feedback system contributes to Cdc42 polarization.

The actomyosin-based transport system and the Bem1-based amplification loop may act in concert to ensure robust cell polarization, as treatment of *bem1Δ* cells with LatA prevents Cdc42 polarization completely (Wedlich-Soldner *et al.* 2004). A caveat with this view is that LatA treatment may prevent Rsr1 localization to the sites of polarized growth, thereby mimicking *rsr1Δ bem1Δ* cells (Kozubowski *et al.* 2008). On the other hand, endogenous Cdc42 is indeed

associated with secretory vesicles (Orlando *et al.* 2011). In addition, rewired cells with only the actin-dependent positive feedback mechanism are capable of Cdc42 polarization and bud growth (Howell *et al.* 2009). Thus, the directed transport system may play a fine-tuning role. Fluorescence recovery after photobleaching (FRAP) analysis indicates that Cdc42 at the polarization site undergoes rapid cycling between the PM and the cytosol, suggesting that its polarization is dynamically maintained (Wedlich-Soldner *et al.* 2004). Indeed, actin cable-mediated delivery (exocytosis) and actin patch-mediated dispersal (endocytosis) of Cdc42 are proposed to counteract lateral diffusion of Cdc42 on the PM, resulting in dynamic maintenance of Cdc42 at the polarization site (Irazoqui *et al.* 2005). Further studies suggest that internalization of Cdc42 occurs by two recycling pathways, the fast-acting Rdi1 pathway and the slow-acting endocytic pathway (Slaughter *et al.* 2009).

Key questions in Cdc42 polarization: Despite recent progress, there are many outstanding questions regarding the mechanism of Cdc42 polarization. For example, what is the relative contribution of the GEF-Bem1-Pak amplification loop *vs.* the actomyosin-based transport system to the initial polarization of Cdc42 at a single cortical site? Is Bem1 a special Cdc42 effector dedicated to the amplification loop? What is the role of the Pak Cla4 in the initial Cdc42 polarization? What fraction of Cdc42 is delivered to the site of polarization via vesicle transport? What is the nucleotide-bound state of Cdc42 on the vesicles?

Cdc42-controlled actin and septin organization

Actin organization, dynamics, and functions: In both budding and fission yeasts, three F-actin structures—cables, patches, and ring—play critical roles in polarized exocytosis, endocytosis, and cytokinesis, respectively (Figures 1B) (Moseley and Goode 2006; Kovar *et al.* 2011). Here, we briefly discuss the structures and functions of actin cables and patches, with a focus on how they are polarized in response to Cdc42.

Actin cables and exocytosis: Actin cables consist of staggered linear actin filaments that are bundled together (Karpova *et al.* 1998). They are dynamic structures, as they disappear within ~15 sec of treatment with LatA (Ayscough *et al.* 1997; Karpova *et al.* 1998; Yang and Pon 2002). Actin cables are polarized toward the sites of cell growth (Figure 1, A and B) (Adams and Pringle 1984; Novick and Botstein 1985; Karpova *et al.* 1998). The immediate depolarization of vesicle markers upon cable disassembly in formin (*bni1*-Ts *bnr1Δ*) and tropomyosin (*tpm1-2 tpm2Δ*) mutants indicates that actin cables are chiefly responsible for polarized exocytosis (Pruyne *et al.* 1998; Evangelista *et al.* 2002; Sagot *et al.* 2002a).

Actin cable assembly involves nucleation by formins, stabilization by tropomyosins, and cross-linking or bundling by actin-binding proteins (ABPs) (Moseley and Goode 2006). There are two arrays of actin cables in budding yeast, one polarized toward the bud cortex and the other toward the mother-bud neck; these are nucleated by two formins, Bni1 and Bnr1, respectively (Kohno *et al.* 1996; Zahner *et al.* 1996; Evangelista *et al.* 1997, 2002; Imamura *et al.* 1997; Pruyne *et al.* 2002; Sagot *et al.* 2002a,b; Moseley *et al.* 2004; Pruyne *et al.* 2004). Bni1 localizes to the sites of polarized cell growth during the cell cycle (Evangelista *et al.* 1997; Ozaki-Kuroda *et al.* 2001; Pruyne *et al.* 2004; Buttery *et al.* 2007) and is dynamic at all locations (Buttery *et al.* 2007). Bni1-nucleated actin filaments form either linear cables to mediate bud growth or a ring to drive cytokinesis. In contrast, Bnr1 is stably localized to the mother side of the bud neck from bud emergence to the onset of cytokinesis (Pruyne *et al.* 2004; Buttery *et al.* 2007). Even though Bni1 and Bnr1 nucleate actin cables at distinct locations, *bni1Δ* and *bnr1Δ* are synthetically lethal (Kamei *et al.* 1998; Vallen *et al.* 2000) and redundantly required for polarized growth (Evangelista *et al.* 2002; Sagot *et al.* 2002a). *bni1Δ* cells show stronger defects in polarity and cytokinesis than do *bnr1Δ* cells (Imamura *et al.* 1997; Kamei *et al.* 1998; Vallen *et al.* 2000), suggesting that Bni1 is the major formin involved in these processes.

Formin-nucleated actin filaments are stabilized by tropomyosins, Tpm1 and Tpm2, which are the only proteins known to exclusively decorate actin cables (Liu and Bretscher 1989a,b; Drees *et al.* 1995; Pruyne *et al.* 1998). The major isoform Tpm1 and the minor isoform Tpm2 share an essential role in maintaining actin-cable structures (Drees *et al.* 1995; Pruyne *et al.* 1998). The tropomyosin-decorated actin filaments are presumably cross-linked into cables by ABPs such as Sac6 (yeast fimbrin) (Adams *et al.* 1991) and ABP140 (Asakura *et al.* 1998; Yang and Pon 2002; Riedl *et al.* 2008), but the precise architecture remains unknown.

Polarized actin cables guide exocytic vesicles from the Golgi to the incipient bud site to drive bud emergence and enlargement (Figure 4A) (Pruyne *et al.* 1998). Distinct steps of exocytosis, including vesicle budding, delivery, tethering, and fusion at specific membrane compartments, are regulated by distinct Rab GTPases (Guo *et al.* 2000; Hutagalung and Novick 2011). To enhance specificity and order of action, the Rab GTPases are organized into activation and inactivation cascades (Hutagalung and Novick 2011). Ypt32 promotes vesicle budding from the Golgi and interacts with both a GAP (Gyp1) for the earlier Rab Ypt1 and the GEF (Sec2) for the subsequent Rab Sec4 (Ortiz *et al.* 2002; Rivera-Molina and Novick 2009). The vesicles are transported by Myo2 (a myosin-V) along actin cables from the mother compartment to the bud (Johnston *et al.* 1991; Schott *et al.* 2002) in a manner dependent on Sec4 and Sec2 (Goud *et al.* 1988; Walch-Solimena *et al.* 1997). The vesicles are then tethered to the PM by an evolutionarily conserved eight-protein complex called the exocyst (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84) (TerBush *et al.* 1996), which is an effector of Sec4 (Guo *et al.* 1999b) thought to form dynamically during each round of exocytosis (Boyd *et al.* 2004). Sec4 and all

exocyst subunits except Sec3 can associate with vesicles (Figure 4A, right, Exocyst B′), but Sec3 and a fraction of Exo70 can also associate with the PM at the sites of polarized growth (Figure 4A, right, Exocyst A′) (Boyd *et al.* 2004). When a vesicle arrives, a functional exocyst is assembled, which brings the vesicle membrane and the PM into proximity for the fusion event that is mediated by the SNARE [soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptor] proteins (Rothman 1996; Boyd *et al.* 2004).

Role of Cdc42 in actin cable-mediated exocytosis: the "polarisome" platform: Cdc42 is thought to control actin cable-mediated exocytosis by regulating the localization and/or activity of the formins. Bni1 localizes to the sites of polarized growth throughout the cell cycle (Evangelista *et al.* 1997; Ozaki-Kuroda *et al.* 2001; Pruyne *et al.* 2004) and its localization depends on Cdc42 (Ozaki-Kuroda *et al.* 2001). Bni1 binds directly to Cdc42 and Rho1 through its N-terminal GTPase-binding domain (GBD) (Figure 4A) (Kohno *et al.* 1996; Evangelista *et al.* 1997). However, Bni1 lacking this domain appeared to localize normally (Ozaki-Kuroda *et al.* 2001). Thus, how Cdc42 controls Bni1 localization remains unknown.

The FH2 domain of Bni1 forms a dimer that nucleates nonbranched actin filaments (Pruyne *et al.* 2002; Sagot *et al.* 2002b) (Figure 4A). This dimer also functions as a "leaky capper" for the barbed end of an actin filament, which allows processive elongation and prevents capping by a "tight capper" (Pring *et al.* 2003; Zigmond *et al.* 2003). The nucleation activity of Bni1 is thought to be autoinhibited by the binding of its N-terminal diaphanous inhibitory domain (DID) to its C-terminal diaphanous autoregulatory domain (DAD), and activated by the binding of a small GTPase to its GBD, which presumably opens the autoinhibitory loop (Figure 4A) (Dong *et al.* 2003; Moseley and Goode 2006). Whether Cdc42, Rho1, or any other small GTPases activate Bni1 in such a manner remains to be tested.

Bni1 displays rapid dynamics at the sites of polarized growth. It localizes to the PM transiently and then undergoes retrograde movements along actin cables (Buttery *et al.* 2007). Bni1 is thought to be activated only at the PM where its potential activators (Cdc42, Rho1, Rho3, and Bud6) are localized (Dong *et al.* 2003; Moseley and Goode 2005; Buttery *et al.* 2007). How Cdc42 controls the localization-coupled activation of Bni1 and whether Cdc42 also controls the dissociation of Bni1 from the PM requires further investigation.

The polarisome (Figures 2 and 4A, right) consists of the scaffold protein Spa2 (Sheu *et al.* 1998, 2000; van Drogen and Peter 2002), Pea2, which interacts with Spa2 and regulates its localization (Valtz and Herskowitz 1996), Bni1 (Evangelista *et al.* 1997), Bud6, an actin monomer-binding protein and an activator of Bni1 nucleation activity (Amberg *et al.* 1997; Moseley *et al.* 2004; Moseley and Goode 2005), and Msb3 and Msb4, a pair of GAPs for the Rab GTPase Sec4 (Bi *et al.* 2000; Gao *et al.* 2003; Tcheperegine *et al.* 2005). Sec4-GTP is associated with secretory vesicles and promotes vesicle tethering through the exocyst (Guo *et al.* 2000). Msb3 and Msb4 located at the sites of polarized growth stimulate GTP hydrolysis by Sec4, facilitating the cycling of Sec4 between its nucleotide-bound states (Walworth *et al.* 1989; Bi *et al.* 2000; Gao *et al.* 2003). Spa2 interacts with Bni1 and Msb3/Msb4 via distinct domains (Fujiwara *et al.* 1998; Sheu *et al.* 1998; Tcheperegine *et al.* 2005). Thus, by physically linking Bni1 regulation to Sec4 regulation, the polarisome helps to coordinate actin cable formation and vesicle fusion (Tcheperegine *et al.* 2005).

Bnr1 contains all the domains present in Bni1 except the Spa2-binding domain (SBD) and Bud6-binding site (BBS) (Moseley and Goode 2005). Bnr1 activation is also thought to involve opening of an autoinhibitory loop by small GTPases (Moseley and Goode 2006). Rho4 is the only GTPase that binds to the GBD of Bnr1 (Imamura *et al.* 1997), but this binding is not required for Bnr1 activation *in vivo* (Dong *et al.* 2003). In addition, Bnr1 localizes to the mother-bud neck in a septin-dependent manner from bud emergence to the onset of cytokinesis (Pruyne *et al.* 2004); whereas Rho4 localizes to the sites of polarized growth (Tiedje *et al.* 2008). Thus, Rho4 is unlikely to activate Bnr1 directly. The Rho4-binding site overlaps with the putative septin-binding site in Bnr1 (Kikyo *et al.* 1999; Gao *et al.* 2010). This raises the possibility that Rho4 and septin binding to Bnr1 may be competitive, and that binding of Bnr1 to the septin ring may activate Bnr1, whereas Rho4 binding is somehow involved in the "priming" of Bnr1 for septin binding (or simply keeping a reservoir of Bnr1 at the PM). In these scenarios, Cdc42 would control Bnr1 activation indirectly via septin ring assembly.

Role of Cdc42 in actin cable-independent exocytosis: Cdc42 may have a direct role in exocytosis. A specific *cdc42* mutant displays exocytosis defects despite seemingly well-polarized actin cables (Adamo *et al.* 2001). In addition, formin and tropomyosin mutants deficient in actin cable assembly still form small buds (Yamamoto *et al.* 2010). Bud emergence and limited bud growth can even occur in the complete absence of F-actin when cells exit from quiescence (Sahin *et al.* 2008). These results suggest that Cdc42 can control polarized exocytosis independently of F-actin. Cdc42 interacts directly with Sec3 and Exo70 (Zhang *et al.* 2001; Wu and Brennwald 2010; Wu *et al.* 2010), two of the exocyst subunits that can localize to the sites of polarized growth even in the absence of F-actin (Finger *et al.* 1998; Boyd *et al.* 2004). In addition, Bem1, which interacts with Cdc42 and Cdc24, binds to the exocyst subunit Sec15 (Zajac *et al.* 2005; France *et al.* 2006). Hence, Cdc42 may regulate exocytosis directly by controlling exocyst localization and/or activation.

Actin patches and endocytosis: Actin patches are motile, short-lived, cortical foci with a diameter of ~200 nm (Rodal *et al.* 2005) and a lifespan of <20 sec (Doyle and Botstein 1996; Waddle *et al.* 1996; Karpova *et al.* 1998; Smith *et al.* 2001; Carlsson *et al.* 2002). The patches consist of short, branched actin filaments (Young *et al.* 2004; Rodal *et al.* 2005) nucleated by the evolutionarily conserved Arp2/3 complex (Winter *et al.* 1997, 1999b; Pollard and Borisy 2003; Moseley and Goode 2006). Actin patches play a critical

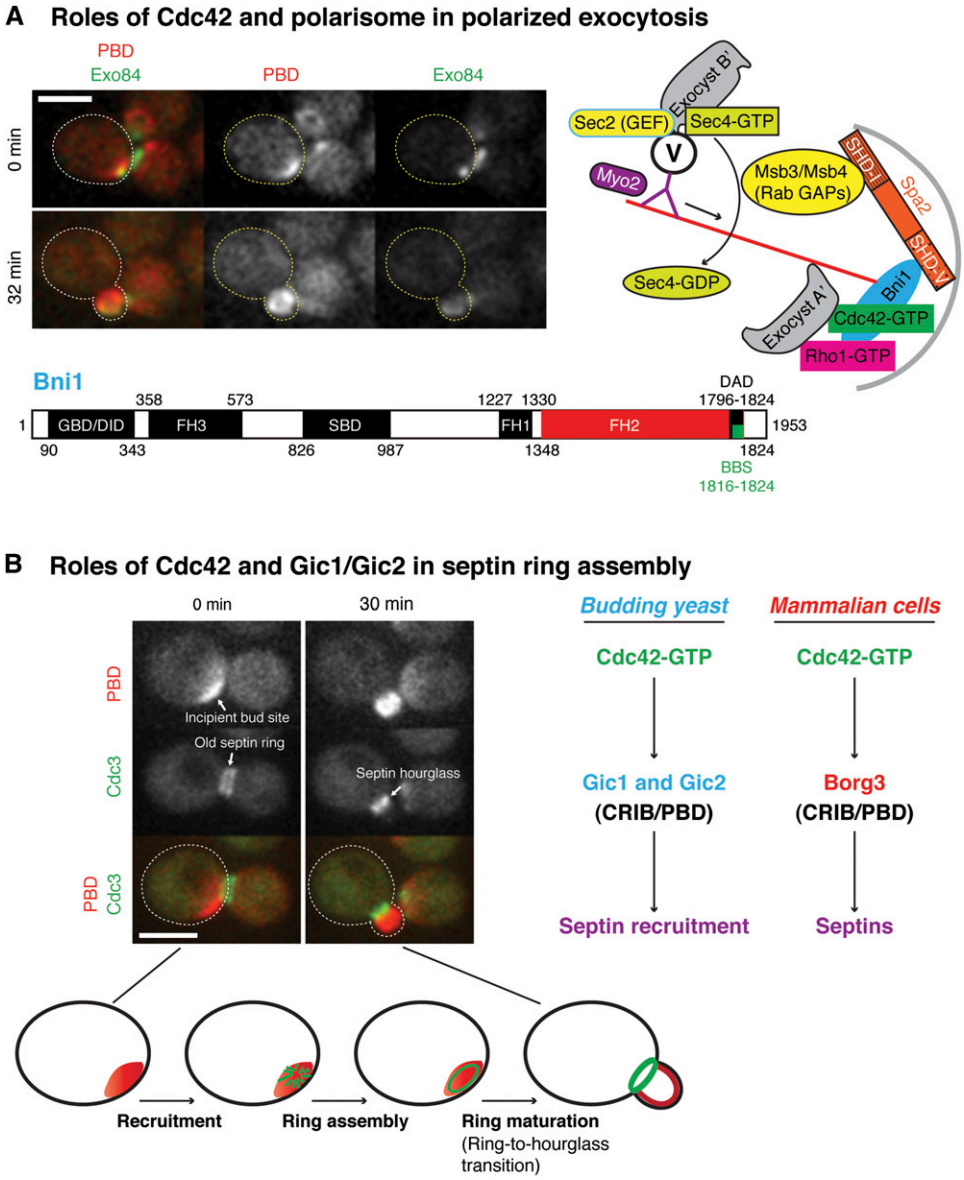

Figure 4 Roles of Cdc42 in polarized exocytosis and septin ring assembly. (A) Roles of Cdc42 and polarisome in polarized exocytosis. (Left) Spatial relationship between Cdc42-GTP and exocytosis. Gic2-PBD (p21-binding domain) fused to tdTomato is a reporter for Cdc42-GTP (PBD) and Exo84-GFP is a marker for polarized exocytosis. Localizations from a time-lapse series are shown. (Right) Spa2, the scaffold protein of the polarisome, binds Bni1 and the Rab GAPs Msb3/Msb4 through distinct domains. Cdc42 and Rho1 control the localization of the exocyst by interacting with the exocyst components Sec3 and Exo70 (Exocyst A'). Cdc42 and Rho1 also control the localization and activation of Bni1, which promotes actin cable assembly toward the site of cell growth. Vesicles (V) carrying the Rab Sec4, Sec2 (the GEF for Sec4), and part of the exocyst (Exocyst B', which consists of Sec5, Sec6, Sec8, Sec10, Sec15, and Exo70 and Exo84) are transported by myosin-V (Myo2) along the actin cables. Upon vesicle arrival, intact exocyst is assembled by the interaction of exocyst A' with exocyst B', which then promotes vesicle tethering to the PM and subsequent membrane fusion. GTP hydrolysis of Sec4 is stimulated by Msb3 and Msb4. (Bottom) Domains of Bni1 (Mosley and Goode 2005, 2006; Ozaki-Kuroda *et al.* 2001). GBD/DID, GTPase-binding domain/diaphanous inhibitory domain; FH 1-3, formin homology 1-3; SBD, Spa2-binding domain; DAD, diaphanous auto-regulatory domain; BBS, Bud6-binding site. (B) Roles of Cdc42 in septin ring assembly. (Left) Spatial relationship between Cdc42-GTP (PBD) and the septins at the incipient bud site (0 min) and after bud emergence (30 min). (Bottom) Stages of septin ring assembly. (Right) Comparison of yeast and mammalian pathways.

role in endocytosis (Engqvist-Goldstein and Drubin 2003), which regulate the levels of membrane lipids and proteins including cell wall synthetic enzymes (Ziman *et al.* 1996; Valdivia *et al.* 2002). Both receptor-mediated and fluid-phase endocytosis occur in *S. cerevisiae* (Engqvist-Goldstein and Drubin 2003). The receptor-mediated endocytosis is carried out by the sequential actions of distinct protein modules that regulate distinct processes such as coat formation, membrane invagination, and vesicle scission (Kaksonen *et al.* 2005).

Role of Cdc42 in actin patch polarization: In wild-type cells, actin patches are concentrated at the sites of polarized growth (Adams and Pringle 1984). In *cdc42* and *cdc24* mutants, actin patches are assembled but distributed randomly at the cell cortex (Adams and Pringle 1984; Adams *et al.* 1990; Johnson and Pringle 1990), suggesting that Cdc42 is required for actin patch polarization, not assembly.

How does Cdc42 control actin patch polarization? One possibility is that Cdc42 may subtly enhance patch assembly at sites of polarized growth by stimulating p21-activated kinases (PAKs, which are Cdc42 effectors) to phosphorylate and activate myosin-I (Lechler *et al.* 2001). Another possible contributor is that Cdc42 interacts directly with some early-acting components of the endocytic machinery. Indeed, Cdc42-GAPs (Rga1, Rga2, and Bem3) interact with Ent1 and Ent2, a functionally redundant pair of epsin-like proteins with essential roles in endocytosis (Aguilar *et al.* 2006; Mukherjee *et al.* 2009). These interactions are independent of the endocytic function of Ent1 and Ent2 and are believed to couple polarity to endocytosis. In addition, Cdc42 appears to control polarized localization of Vrp1 [verprolin, yeast equivalent of Wiskott-Aldrich syndrome protein (WASP)-interacting protein (WIP)] and Las17 via the formins independently of F-actin (Lechler *et al.* 2001). Furthermore, Ent2 exhibits

a two-hybrid interaction with Cdc24 and affects its localization (Drees *et al.* 2001; Cole *et al.* 2009). Thus, Cdc42 interactions may influence the sites of endocytic internalization, and endocytic proteins may modulate Cdc42 activity. Cdc42 may also influence actin patch location by controlling polarized delivery of endocytosis-promoting factors on vesicles (Gao *et al.* 2003). This hypothesis explains why a defect in secretion or actin cable assembly can cause defects in actin patch polarization (Pruyne *et al.* 1998; Gao *et al.* 2003) and why endocytosis is always spatially coupled to exocytosis.

Septin organization and dynamics: Septins, a conserved family of GTP-binding proteins, assemble into heterooligomeric complexes and higher-order structures such as filaments, rings, and gauzes (Byers and Goetsch 1976a; Gladfelter *et al.* 2001; Longtine and Bi 2003; Hall *et al.* 2008; Weirich *et al.* 2008; McMurray and Thorner 2009; Oh and Bi 2011). Here, we briefly discuss septin organization and dynamics in the cell, with a focus on the role of Cdc42 in septin ring assembly.

Plasticity in septin complex and filament assembly: In *S. cerevisiae*, five of the seven septins (Cdc3, Cdc10, Cdc11, Cdc12, and Shs1/Sep7) are expressed vegetatively and the other two (Spr3 and Spr28) are expressed exclusively during sporulation (Longtine *et al.* 1996). *CDC3* and *CDC12* are essential under all tested conditions (Frazier *et al.* 1998; McMurray *et al.* 2011). *cdc10Δ* and *cdc11Δ* cells are temperature sensitive for growth and display severe defects in septin organization, morphogenesis, and cytokinesis (Frazier *et al.* 1998; McMurray *et al.* 2011). Deletion of *SHS1* has the least effect in most genetic backgrounds (Mino *et al.* 1998; Lee *et al.* 2002; Dobbelaere *et al.* 2003; Bertin *et al.* 2008), but it enhances *cdc10Δ* mutants and suppresses *cdc11Δ* mutants (Iwase *et al.* 2007; McMurray *et al.* 2011). In the W303 genetic background, *shs1Δ* causes cold sensitivity that is suppressed by increased dosage of Cdc11 but not of other septins (Iwase *et al.* 2007). *In vitro* reconstitution experiments indicate that four septins, Cdc3, Cdc10, Cdc11, and Cdc12, form rod-shaped, nonpolar octameric septin complexes (Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11). It is not clear how Shs1 fits, but there might be two types of septin complexes, with Shs1 replacing Cdc11 in the second type (Bertin *et al.* 2008), consistent with the genetic interactions observed between *SHS1* and *CDC11* (Iwase *et al.* 2007; McMurray *et al.* 2011).

Septin localization and filament assembly appear to be essential for cell survival. All vegetatively expressed septins display an identical pattern of localization at the mother-bud neck during the cell cycle (Longtine *et al.* 1996). Their localizations are interdependent, at least at high temperatures (Longtine *et al.* 1996; Iwase *et al.* 2007). The only exception is Shs1, as *shs1Δ* cells fail to form colonies at low temperatures but the other septins are still localized (Iwase *et al.* 2007). This lethality may be related to a specific role of Shs1 in cytokinesis. A recent study indicates that the survival of cells deleted for individual septin genes such as *cdc10Δ* and *cdc11Δ* is due to plasticity in septin complex and filament assembly; *i.e.*, the remaining septin subunits are still capable of forming rod-shaped complexes that can assemble into filaments and rings, but they do so with reduced efficiency (Frazier *et al.* 1998; McMurray *et al.* 2011). Structure-based mutations that have little or no effect on septin complex formation but disrupt filament assembly invariably cause cell death, suggesting that filament assembly is essential (McMurray *et al.* 2011).

Septin dynamics and regulation during the cell cycle: In budding yeast, septins undergo cell-cycle–regulated organizational changes (Figure 1C) (Weirich *et al.* 2008; McMurray and Thorner 2009; Oh and Bi 2011). After the launch of a new cell cycle, a nascent septin ring is assembled at the incipient bud site. This ring is dynamic as indicated by FRAP analysis (Caviston *et al.* 2003; Dobbelaere *et al.* 2003). Upon bud emergence, the septin ring is converted into a stable hourglass that covers a wider region of the mother-bud neck. Around the onset of cytokinesis, the septin hourglass is split into two dynamic rings that sandwich the cytokinesis machinery. The transition from the dynamic ring to the stable hourglass is likely caused by the arrangement or cross-linking of septin filaments into ordered arrays; whereas the hourglass splitting is triggered by the mitotic exit network (MEN) (Cid *et al.* 2001; Lippincott *et al.* 2001). Septins also undergo cell-cycle–dependent modifications including phosphorylation (Longtine *et al.* 1998; Mortensen *et al.* 2002; Egelhofer *et al.* 2008) and SUMOylation (Johnson and Blobel 1999; Johnson and Gupta 2001). How these modifications affect septin organization and/or dynamics requires further investigation.

Role of Cdc42 in polarized septin ring assembly: In *S. cerevisiae*, Cdc42 controls polarized septin ring assembly at the incipient bud site (Figure 4B) (Pringle *et al.* 1995; Cid *et al.* 2001; Iwase *et al.* 2006). In the first step, septin complexes are recruited to the incipient bud site to increase local concentration for filament and ring assembly. Newly recruited septins are usually present in disorganized "clouds" or "patches" (Iwase *et al.* 2006). Septin recruitment completely depends on Cdc42 (Cid *et al.* 2001; Iwase *et al.* 2006) and partly on Gic1 and Gic2, a pair of Cdc42 effectors (Iwase *et al.* 2006). Both Gic1 and Gic2 contain a Cdc42/Rac interactive binding (CRIB) motif, which interacts specifically with Cdc42-GTP (Burbelo *et al.* 1995; Brown *et al.* 1997; Chen *et al.* 1997), and both proteins interact with septins (Iwase *et al.* 2006). Besides a few highly related yeast species, Gic1 and Gic2 do not have apparent homologs in other organisms, although Borg3 (also called CDC42EP5), a CRIB motif-containing effector of Cdc42 in mammalian cells, also interacts with septins (Figure 4B) (Joberty *et al.* 2001). As *gic1Δ gic2Δ* cells are able to assemble septin rings at low but not high temperatures (Brown *et al.* 1997; Chen *et al.* 1997; Iwase *et al.* 2006), Cdc42 must also control septin recruitment through other pathways.

Once recruited to the incipient bud site, septin complexes associate with the PM via interactions between septin polybasic motifs and plasma-membrane phospholipids (Zhang

et al. 1999; Casamayor and Snyder 2003; Rodriguez-Escudero *et al.* 2005; Tanaka-Takiguchi *et al.* 2009; Bertin *et al.* 2010). These septins then undergo an organizational change from clouds to a smooth ring of ~1.0 μm in diameter within minutes (Figure 4B) (Iwase *et al.* 2006). This step requires Cdc42 to cycle between GTP- and GDP-bounds states (Gladfelter *et al.* 2002), plus the GAPs for Cdc42 (Gladfelter *et al.* 2002; Smith *et al.* 2002; Caviston *et al.* 2003; Kadota *et al.* 2004). The GAPs may facilitate the "unloading" of septin complexes from the recruitment pathways (Gladfelter *et al.* 2002; Smith *et al.* 2002; Caviston *et al.* 2003; Kadota *et al.* 2004). The PAK Cla4 also regulates septin ring assembly by directly phosphorylating a subset of septins (Versele and Thorner 2004).

Following bud emergence, the dynamic septin ring at the incipient bud site is converted into a stable septin hourglass at the mother-bud neck. The mechanism underlying this transformation remains unknown, but it involves the Lkb1-related kinase Elm1 (Bouquin *et al.* 2000), the Gin4 kinase (also a substrate of Elm1) (Asano *et al.* 2006), and the septin-associated proteins Bni5 and Nap1 (Altman and Kellogg 1997; Lee *et al.* 2002), as cells lacking any of these proteins can assemble a seemingly normal septin ring but not the hourglass (Gladfelter *et al.* 2004).

An integrated model for Cdc42-controlled actin cable and septin ring assembly: We hypothesize that in *S. cerevisiae*, Cdc42 controls actin cable and septin ring assembly at the incipient bud site through two genetically separable, biochemically cross-talking pathways, the polarisome and the Gic1/Gic2 pathways (Figure 2). In this model, the polarisome assists Cdc42 in polarized actin cable assembly and cable-dependent exocytosis. This pathway also contributes to septin ring assembly by an unknown mechanism (Goehring *et al.* 2003; Kadota *et al.* 2004). The actin cytoskeleton is involved in fine tuning septin ring assembly (Kadota *et al.* 2004; Kozubowski *et al.* 2005; Iwase *et al.* 2006). Thus, the polarisome could regulate septin ring assembly through Bni1-nucleated actin filaments and/or through actin cable-mediated delivery of transmembrane cargoes such as Axl2, which is known to play a role in septin organization (Gao *et al.* 2007). In contrast, the Gic1/Gic2 pathway mainly regulates septin ring assembly through direct interactions with septin complexes. This pathway also regulates actin cable organization by affecting septin-dependent Bnr1 localization (Pruyne *et al.* 2004). The polarisome and the Gic1/Gic2 pathways act in parallel, as inactivation of both pathways results in synthetic lethality (Bi *et al.* 2000; Jaquenoud and Peter 2000). The two pathways also cross-talk, as Bud6 and Pea2 interact with the Gic proteins by two-hybrid analysis (Jaquenoud and Peter 2000). In this view, Cdc42 controls polarized assembly of actin cables and the septin ring at the same time and same location through molecular pathways that are integrated in a way that permits independent but also cooperative assembly of the two cytoskeletal structures.

Key questions in Cdc42-controlled actin and septin organization: A number of basic questions regarding the roles of Cdc42 in actin and septin organization remain unanswered. For example, does Cdc42 control Bni1 localization directly or indirectly through other polarity proteins? How is Bni1 activated? How does Cdc42 control exocytosis? How does Cdc42 control polarized actin patch organization or endocytosis? How does Cdc42 control septin recruitment to the incipient bud site in addition to the Gic1/Gic2 pathway? How do Cdc42 effectors interact with septin complexes and/or filaments at the molecular level? Answers to these questions will clarify the roles of Cdc42 in cytoskeletal polarization and exocytosis.

Rho1 in polarized growth

RHO1 is essential and its role in polarized growth has been analyzed extensively, whereas the functions of the other four Rhos (Rho2–Rho5) remain poorly understood. Here, we briefly review the Rho1 GTPase module and its roles in cell wall remodeling, actin organization, and exocytosis during polarized bud growth.

Rho1 GTPase module: Rho1 is regulated by its GEFs (Rom1, Rom2, and Tus1) (Ozaki *et al.* 1996; Manning *et al.* 1997; Schmelzle *et al.* 2002), GAPs (Bem2, Lrg1, Sac7, and Bag7) (Dunn and Shortle 1990; Bender and Pringle 1991; Zheng *et al.* 1993; Kim *et al.* 1994; Peterson *et al.* 1994; Schmidt *et al.* 1997, 2002; Martin *et al.* 2000; Roumanie *et al.* 2001; Watanabe *et al.* 2001; Fitch *et al.* 2004), and GDI (Rdi1) (Masuda *et al.* 1994; Tiedje *et al.* 2008) (Figure 5). Inactive Rho1 is associated with post-Golgi vesicles and is activated by its GEFs at the PM upon vesicle arrival (McCaffrey *et al.* 1991; Abe *et al.* 2003).

Rho1 in cell wall remodeling: Rho1 plays a major role in localized cell wall remodeling (Levin 2005; Park and Bi 2007). Cell walls provide the rigidity to withstand turgor, protect against sudden changes in osmolarity and other environmental stresses, and also function in cell–cell communication during mating (Klis *et al.* 2006; Lesage and Bussey 2006). Cell walls consist of an electron-transparent inner layer, which contains glucan (*D*-glucose polymers) and chitin (*N*-acetylglucosamine polymers), and an electron-dense outer layer, which contains heavily glycosylated mannoproteins. The inner layer is responsible for mechanical protection, whereas the outer layer is responsible for cell–cell or cell–environment communication.

Glucan and chitin, the two major cell wall polymers, are synthesized by glucan synthases and chitin synthases, respectively. Fks1 and Fks2 are the catalytic subunits of β-1,3-glucan synthases, which function during normal and stressed growth conditions, respectively (Douglas *et al.* 1994; Inoue *et al.* 1995; Mazur *et al.* 1995). There are three chitin synthases, CS I–III, in *S. cerevisiae* with Chs1, Chs2, and Chs3 as their catalytic subunits, respectively (Cabib *et al.* 2001; Klis *et al.* 2006; Lesage and Bussey 2006). The expression, localization, activity, and function of all three

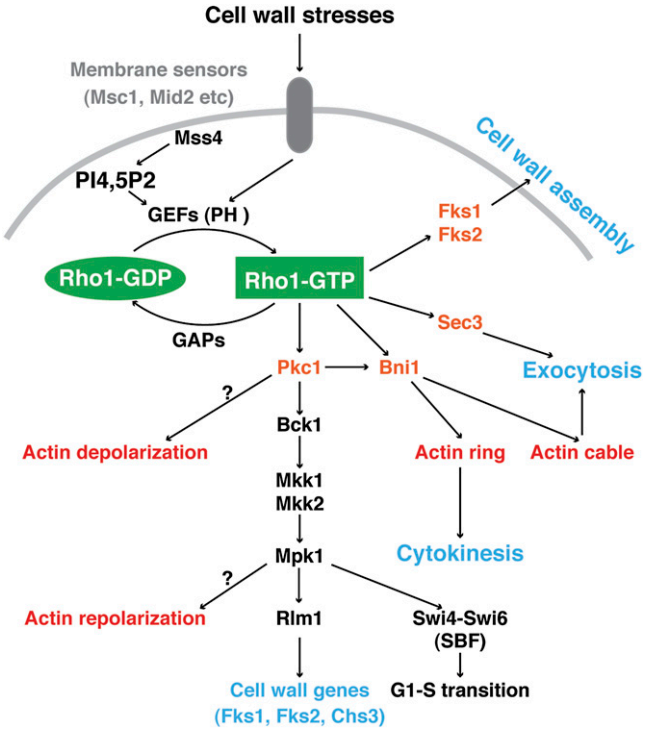

Figure 5 Rho1 in cell wall remodeling, exocytosis, and cytokinesis. Cell wall stresses are sensed by membrane proteins (gray), which activate Rho1-GEFs. Mss4 synthesizes PI(4,5)P2 at the PM (gray line), which recruits GEFs via their PH domains. Activated Rho1 controls cell wall assembly by directly activating glucan synthases (Fks1 and Fks2) and by activating the Pkc1-MAPK–mediated CWI pathway, which induces expression of cell-wall synthetic enzymes. Rho1 also regulates exocytosis and cytokinesis via Sec3 and Bni1. In response to heat stress, Rho1 and Pkc1 cause transient actin depolarization, and the MAPK pathway promotes subsequent actin repolarization. Modified from (Park and Bi 2007) with permission.

chitin synthases are subjected to complex and distinct regulations during the cell cycle.

Rho1 regulates cell wall synthesis by two different mechanisms (Figure 5). Under normal growth conditions, Rho1 promotes cell wall synthesis by functioning as the regulatory subunit of the glucan synthases (Drgonova *et al.* 1996; Mazur and Baginsky 1996; Qadota *et al.* 1996). Under stressed conditions, Rho1 also regulates the expression of cell wall-synthetic genes through the cell wall integrity (CWI) pathway (Heinisch *et al.* 1999; Cabib *et al.* 2001; Levin 2005; Park and Bi 2007). The CWI pathway is a mitogen-activated protein kinase (MAPK) pathway, which is activated by Rho1 and its effector Pkc1, the sole protein kinase C in *S. cerevisiae* (Figure 5) (Kamada *et al.* 1996). Cell wall stresses are sensed by transmembrane "receptors" (Wsc1, Mid2, etc.), which activate the Rho1 GEFs (Philip and Levin 2001; Audhya and Emr 2002). The MAPK cascade activates the transcriptional factors Swi4–Swi6 (SBF complex) and Rlm1, which induce the expression of genes required for G1–S transition and cell wall synthesis, respectively (Levin 2005; Park and Bi 2007).

Rho1 in actin organization: Depletion of Rho1 at 30° does not cause clear defects in polarized actin organization (Yamochi *et al.* 1994), but specific temperature-sensitive mutations in *RHO1* disrupt actin organization at 37° (Helliwell *et al.* 1998; Guo *et al.* 2001). Rho1 regulates actin organization through Pkc1-mediated activation of the formin Bni1 (Dong *et al.* 2003), and also through the CWI pathway at 37° (Mazzoni *et al.* 1993) (Figure 5). Upon shift to 37°, wild-type cells depolarize actin patches and cables transiently, reaching the peak of depolarization within ~30–45 min, and then repolarize at 60–120 min (Lillie and Brown 1994; Desrivieres *et al.* 1998). The heat stress is thought to weaken the cell wall, which activates the CWI pathway to repair damage (Kamada *et al.* 1995). CWI pathway activation first depolarizes actin through Pkc1 and then repolarizes actin through MAPK activation (Delley and Hall 1999).

Rho1 in exocytosis: Rho1 regulates polarized exocytosis by regulating actin cable assembly through the formin Bni1 (see above) and also by controlling the localization of the exocyst through an interaction with Sec3 (Guo *et al.* 2001). Rho1 also regulates the trafficking of Chs3 from the "chitosomes" to the PM (Valdivia and Schekman 2003).

Key questions on the roles of Rho1 in cell polarization: The function of Rho1 in cell wall remodeling is well understood. In contrast, it remains unclear how Rho1 regulates actin organization. For example, how Rho1 regulates Bni1 localization and/or activation has not been clearly established. It is also unclear how Rho1 regulates actin depolarization or repolarization through the CWI pathway. Another outstanding question is how Rho1 is spatiotemporally coordinated with Cdc42 and other small GTPases during polarized growth. For example, does Cdc42 simply specify the location for Rho1-mediated glucan synthesis or also regulate Rho1 activation and/or its effector pathways?

Mechanism of Cytokinesis

Cytokinesis in *S. cerevisiae* is carried out by the concerted actions of the contractile actomyosin ring (AMR), targeted membrane deposition, and primary septum (PS) formation (Figure 6, A and B) (Bi 2001). AMR contraction is followed closely by the centripetal growth of the PS, a specialized chitinous cell wall (Lesage and Bussey 2006). At the end of PS formation, two secondary septa (SS) are synthesized at either side of the PS (Lesage and Bussey 2006). The SS, which are structurally similar to the lateral cell wall (Lesage and Bussey 2006), likely involve Rho1-controlled glucan synthesis as in the budding process (Yoshida *et al.* 2009). The PS and a portion of the SS are then degraded by an endochitinase and glucanases from the daughter side, resulting in cell separation (Lesage and Bussey 2006). The expression of these hydrolytic enzymes is controlled, in part, by the conserved nuclear-Dbf2-related (NDR)/large tumor suppressor (LATS) signaling network (Hergovich and Hemmings 2009) or the (regulation of Ace2 activity and cellular morphogenesis (RAM) (Nelson *et al.* 2003) pathway that is normally activated only in the daughter cells of *S. cerevisiae* (to be discussed in the planned YeastBook chapter by Eric L. Weiss).

The AMR generates a contractile force that powers the ingression of the PM and is also thought to guide membrane deposition and the formation of the PS (Vallen *et al.* 2000; Fang *et al.* 2010). The functions of the AMR and the PS appear to be interdependent (Bi 2001; Schmidt *et al.* 2002; VerPlank and Li 2005), as disruption of the AMR causes severely misoriented PS formation (Fang *et al.* 2010), whereas disruption of PS formation results in abnormal AMR contraction (Bi 2001; Schmidt *et al.* 2002; VerPlank and Li 2005; Nishihama *et al.* 2009). It is noteworthy that *S. cerevisiae* cells lacking the AMR are viable and able to divide in most genetic backgrounds, though less efficiently than wild-type cells (Figure 6C) (Watts *et al.* 1987; Rodriguez and Paterson 1990; Bi *et al.* 1998; Lippincott and Li 1998a). Thus, in *S. cerevisiae*, the AMR-dependent and -independent mechanisms act together to ensure that cytokinesis occurs with optimum efficiency and fidelity, but the AMR-independent route can suffice. Here, we discuss the mechanisms underlying the structures and functions of the AMR and the PS and their spatiotemporal relationship. All cytokinesis proteins relevant to our discussions are described in Table 2.

Actomyosin ring assembly and disassembly

The AMR is thought to generate a contractile force through the sliding of myosin-II motors on actin filaments, as with sarcomere behavior during muscle contraction (Schroeder 1968; Schroeder 1972; Balasubramanian *et al.* 2004; Eggert *et al.* 2006; Pollard 2008). Yet despite its prevalence, this model has not been demonstrated in any experimental system. Specifically, it is not clear whether myosin-II forms bipolar filaments at the cleavage furrow and how myosin-II filaments are arranged with respect to actin filaments in the AMR. It is also unclear how other cytokinesis proteins such as IQGAP facilitate AMR assembly. During AMR contraction, the volume of the ring decreases, suggesting that contraction is coupled with disassembly (Schroeder 1972), which contrasts with Huxley's "sliding-filament model" for muscle in which the number of contractile units remains unchanged during contraction (Huxley and Hanson 1954; Huxley 1969). How the AMR is disassembled during and at the end of cytokinesis remains poorly understood in any system.

AMR assembly: In *S. cerevisiae*, six families of proteins (septins, Myo1, Mlc1, Iqg1, Bni1, and actin) are required for the AMR assembly. Septins are the first family of proteins to arrive at the division site and they are required for the localization of all other known cytokinesis proteins at the division site. Consequently, septins are required for both the AMR-dependent and -independent cytokinesis pathways, and cytokinesis is not even attempted in their absence (Hartwell 1971a). The septin hourglass structure (described earlier) is maintained at the bud neck until telophase when it splits into two cortical rings sandwiching the AMR (Cid *et al.* 2001; Lippincott *et al.* 2001; Dobbelaere and Barral 2004). Septins are thought to play two distinct roles in cytokinesis. Prior to cytokinesis, the septin hourglass functions as a scaffold required for the localization of AMR components to the division site and thus for AMR assembly (Bi *et al.* 1998; Lippincott and Li 1998a). During cytokinesis, the split septin rings may provide a diffusion barrier to retain diffusible cytokinesis factors at the division site (Dobbelaere and Barral 2004), although this diffusion barrier is dispensable for cytokinesis (Wloka *et al.* 2011).

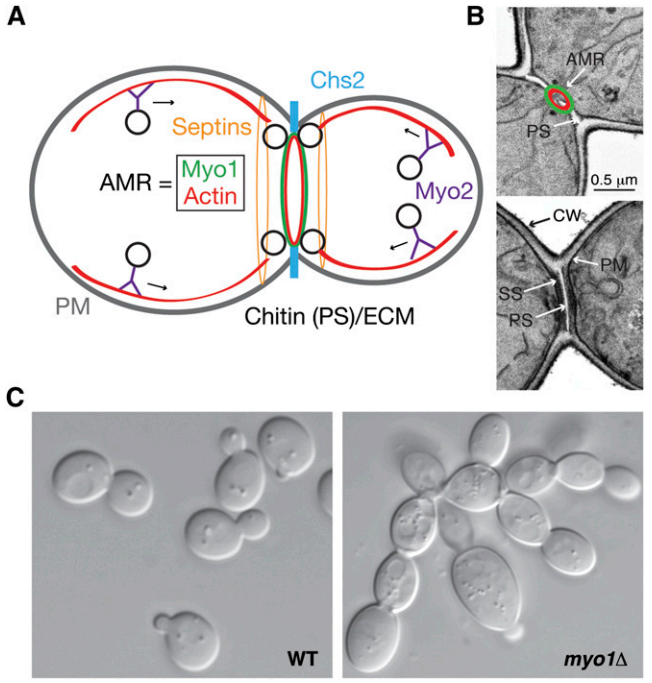

Figure 6 Events of cytokinesis. (A) Coupling of AMR contraction to membrane trafficking and primary septum (PS) formation during cytokinesis. Myo1, myosin-II; Myo2, myosin-V; Chs2, a transmembrane chitin synthase responsible for PS formation. Black circles, post-Golgi vesicles; red lines, actin cables. (B) EM visualization of the primary and secondary septa. SS, secondary septa; CW, cell wall. An AMR is drawn to illustrate its spatial relationship with the PS during cytokinesis. (C) Cytokinesis defects of *myo1Δ* cells. Unlike wild-type (WT) cells, which are either unbudded or single-budded, *myo1Δ* cells form extensive chains or clusters, indicative of cytokinesis and cell-separation defects. Micrographs in B and for WT cells in C were published previously (Fang *et al.* 2010).

Myo1 is the heavy chain of the sole myosin-II in *S. cerevisiae*. Like all conventional myosin-IIs, Myo1 forms a dimer with two globular heads each harboring an ATPase and an actin-binding site(s), and a long coiled-coil tail (Fang *et al.* 2010). Each Myo1 binds to one essential light chain (ELC), Mlc1, and one regulatory light chain (RLC), Mlc2, via distinct IQ motifs (Luo *et al.* 2004). Deletion of *MYO1* is not lethal but abolishes actin ring assembly and causes defects in cytokinesis and cell separation, including misoriented PS formation (Bi *et al.* 1998; Lippincott and Li 1998a; Fang *et al.* 2010). Strikingly, Myo1 lacking the entire head domain, including the light chain-binding sites, is able to assemble an actin ring (Fang *et al.* 2010). This "headless" AMR constricts with a rate of only 20–30% less than the wild-type AMR does and is largely sufficient for directing PS formation and cytokinesis (Lord *et al.* 2005; Fang *et al.* 2010), implying that AMR assembly is driven by the Myo1 tail.

Table 2 Proteins involved in cytokinesis

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Proteins involved in actomyosin ring (AMR) assembly			
Rho1	Rho GTPase, required for actin ring assembly		
Act1	Actin, required for actin ring assembly		
Pfy1	Profilin, required for actin assembly		
Bni1	Formin, plays a major in actin ring assembly		
Bnr1	Formin, may contribute to actin ring assembly		
Myo1	Myosin-II	Required for AMR assembly and constriction Null cells are viable in most genetic backgrounds (except W303) but display severe defects in cytokinesis (Figure 6C) Guides membrane deposition and primary septum formation	Watts *et al.* 1987; Bi Rodriguez and Paterson 1990; *et al.* 1998; Lippincott and Li 1998a
Mlc1	Essential light chain (ELC) for Myo1	Serves as a light chain for Iqg1 and Myo2 Essential for cell viability and actin ring assembly Required for Iqg1 localization at the bud neck throughout the cell cycle Required for Myo1 localization at the division site during cytokinesis	Stevens and Davis 1998; Boyne *et al.* 2000; Shannon and Li 2000; Luo *et al.* 2004; Fang *et al.* 2010
Mlc2	Regulatory light chain (RLC) for Myo1	Bud-neck localization depends on Myo1 May regulate Myo1 disassembly during cytokinesis Null cells are viable	Luo *et al.* 2004; Tully *et al.* 2009
Septins	Act as a scaffold for AMR assembly before the onset of cytokinesis and may act as a diffusion barrier and/or scaffold during cytokinesis		Bi *et al.* 1998; Lippincott and Li 1998a; Dobbelaere and Barral 2004; Wloka *et al.* 2011
Bni5	Required for Myo1 targeting to the division site from bud emergence to the onset of cytokinesis		Fang *et al.* 2010
Iqg1	IQGAP	Essential for cell viability and actin ring assembly Required for Myo1 localization at the division site during cytokinesis Involved in septum formation	Epp and Chant 1997; Lippincott and Li 1998a; Shannon and Li 1999; Boyne *et al.* 2000; Korinek *et al.* 2000; Shannon and Li 2000; Luo *et al.* 2004; Corbett *et al.* 2006; Fang *et al.* 2010
Proteins involved in targeted membrane deposition and primary septum (PS) formation			
Bni1	Formin, required for actin cable assembly during cytokinesis		
Myo2	Myosin-V, required for vesicle transport during cytokinesis		
Exocyst	Required for tethering post-Golgi vesicles to the PM during cytokinesis		
Chs2	Chitin synthase II, required for PS formation during cytokinesis		
Chs3	Catalytic subunit of chitin synthase III, required for "remedial-septum" formation during cytokinesis		
Proteins involved in the coordination between the AMR and PS formation			
Septins	See above		
Iqg1	See above		
Inn1		Required for PS formation and cleavage-furrow ingression May interact with Iqg1 Binds to the SH3 domains of Hof1 and Cyk3 via distinct PXXP motifs	Sanchez-Diaz *et al.* 2008; Jendretzki *et al.* 2009; Nishihama *et al.* 2009; Meitinger *et al.* 2010;

(continued)

Table 2, *continued*

Name of protein or protein complex[a]	Protein activity	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Hof1	F-BAR protein	Involved in PS formation Binds to Inn1 via its SH3 domain May regulate AMR contraction Undergoes cell-cycle–dependent phosphorylation and degradation	Lippincott and Li 1998b; Kamei *et al.* 1998; Vallen *et al.* 2000; Blondel *et al.* 2005; Sanchez-Diaz *et al.* 2008; Nishihama *et al.* 2009; Meitinger *et al.* 2010; Meitinger *et al.* 2011
Cyk3		Involved in PS formation Binds to Inn1 via its SH3 domain	Korinek *et al.* 2000; Jendretzki *et al.* 2009; Nishihama *et al.* 2009; Meitinger *et al.* 2010

[a] Information on individual proteins is partly based on *Saccharomyces* Genome Database (SGD: http://www.yeastgenome.org/).
[b] Only functions related to polarized cell growth and cytokinesis are indicated here.

Myo1 localizes to the incipient bud site and the bud neck in a septin-dependent manner prior to the splitting of the septin hourglass (Bi *et al.* 1998; Lippincott and Li 1998a), then remains at the division site between the split septin rings during cytokinesis (Dobbelaere and Barral 2004). In contrast, actin ring assembly is initiated around the onset of anaphase and is progressively matured thereafter (Epp and Chant 1997; Bi *et al.* 1998; Lippincott and Li 1998a). Thus, a functional AMR is assembled in late anaphase or telophase in budding yeast, similar to animal cells. Myo1 localizes to the division site via two distinct targeting signals in its tail that act sequentially during the cell cycle and via two molecular pathways (Fang *et al.* 2010). From late G1 to telophase, Myo1 localization depends on the septins and Bni5 (septins → Bni5 → Myo1) (Fang *et al.* 2010). Deletion of *BNI5* causes only mild defects in cytokinesis (Lee *et al.* 2002; Fang *et al.* 2010). From anaphase to the end of cytokinesis, Myo1 localization depends on Mlc1 and Iqg1 (Mlc1 → Iqg1→ Myo1), although a direct interaction between Iqg1 and Myo1 has not been established (Fang *et al.* 2010). Besides being the ELC for Myo1 (Boyne *et al.* 2000; Luo *et al.* 2004), Mlc1 is also a light chain for the myosin-V, Myo2, and Iqg1 (Stevens and Davis 1998; Boyne *et al.* 2000; Shannon and Li 2000) and is required for the localization of Iqg1 to the bud neck (Boyne *et al.* 2000; Shannon and Li 2000). Both Mlc1 and Iqg1 are essential for actin ring assembly, cytokinesis, and cell survival (Epp and Chant 1997; Stevens and Davis 1998; Boyne *et al.* 2000; Shannon and Li 2000). The Bni5- and Iqg1-mediated Myo1-targeting pathways functionally overlap from the onset of anaphase to the onset of telophase (Fang *et al.* 2010). The Bni5 pathway might be more species-specific and involved in Myo1-mediated retrograde flow of actin cables before cytokinesis (Huckaba *et al.* 2006; Fang *et al.* 2010). In contrast, the Iqg1 pathway is conserved in fission yeast (Almonacid *et al.* 2011; Laporte *et al.* 2011; Padmanabhan *et al.* 2011). This pathway is responsible for AMR assembly and largely accounts for the role of Myo1 in cytokinesis (Fang *et al.* 2010).

The formins are collectively required for actin ring assembly (Kamei *et al.* 1998; Vallen *et al.* 2000; Tolliday *et al.* 2002). Bni1 is the only formin present at the division site during cytokinesis (Buttery *et al.* 2007), consistent with its more prominent role in this process (Vallen *et al.* 2000). Rho1 is required for actin ring assembly, which is thought to occur, at least in part, by activating Bni1 (Tolliday *et al.* 2002; Yoshida *et al.* 2006, 2009).

A model and key questions on AMR assembly: The core components that directly participate in the AMR assembly are Myo1, Iqg1, and Bni1. Thus, it is paramount to determine whether and how these proteins interact with each other to promote AMR assembly. One possibility is that formin (mainly Bni1 with some contribution from Bnr1)-nucleated actin filaments are captured by the actin-binding calponin homology (CH) domain of Iqg1 (Epp and Chant 1997; Shannon and Li 1999) and then organized into a ring structure using Myo1 bipolar filaments as a "template." It is currently unknown whether Myo1 forms bipolar filaments and whether such a higher-order structure is important for cytokinesis.

AMR disassembly: AMR contraction must be coupled with disassembly (Schroeder 1972), yet the underlying mechanism remains obscure. Current evidence suggests that the RLC, the motor activity of myosin-II, and the IQGAP are involved. In *S. cerevisiae*, Mlc2 (RLC) is localized to the division site by binding to Myo1 and is thought to play a role in Myo1 disassembly, as deletion of *MLC2* causes Myo1 to linger at the division site at the end of cytokinesis (Luo *et al.* 2004). Deletion of the head domain of Myo1, including the Mlc2-binding site, causes a more severe defect in AMR disassembly than does the deletion of *MLC2* (Fang *et al.* 2010), suggesting that the motor activity of myosin-II regulates AMR disassembly. Finally, Iqg1 is a target of anaphase promoting complex or cyclosome (APC/C), an E3 ligase that targets substrates for ubiquitin-mediated degradation by the 26S proteasome (Ko *et al.* 2007; Tully *et al.* 2009). Increased levels of Iqg1 in APC/C mutants cause prolonged duration of Iqg1 at the division site and at ectopic sites (Ko *et al.* 2007; Tully *et al.* 2009). At each site, Iqg1 colocalizes with Myo1, Mlc1, and Mlc2, suggesting that the AMR components are disassembled together. Thus, APC/C-mediated degradation of Iqg1 appears to define a major mechanism for AMR disassembly (Tully *et al.* 2009). Importantly, the Mlc2- and Iqg1-mediated disassembly mechanisms act

synergistically, as *mlc2Δ* enhances the disassembly defect of the APC/C mutants (Tully *et al.* 2009).

Targeted membrane deposition and primary septum formation

Polarized exocytosis and membrane addition at the division site: During cytokinesis, the "growth machine" used for budding, including Cdc42, actin cables, and post-Golgi vesicles, is redirected from the bud cortex to the bud neck (Figure 7A). The underlying mechanism for this spatiotemporal switch is unknown, but it presumably involves cell-cycle–regulated disassembly and reassembly. Both cytokinesis and budding require Myo2 (myosin-V)–powered delivery of post-Golgi vesicles along polarized actin cables (Pruyne *et al.* 1998; VerPlank and Li 2005), but notable differences exist. First, Cdc42 is essential for polarized organization of actin cables during budding (Adams *et al.* 1990; Johnson and Pringle 1990). In contrast, a clear and specific role of Cdc42 in cytokinesis has yet to be established, even though Cdc42 and its regulators are localized at the bud neck during cytokinesis (Ziman *et al.* 1993; Toenjes *et al.* 1999; Nern and Arkowitz 2000; Richman *et al.* 2002; Caviston *et al.* 2003). Conditional lethal mutations in *CDC42* arrest cells at bud emergence but not cytokinesis (Adams *et al.* 1990; Johnson and Pringle 1990; Iwase *et al.* 2006), suggesting that Cdc42 may play a fine-tuning or redundant role in cytokinesis. Alternatively, the Cdc42 activity threshold required for cytokinesis may be lower than that for budding. Second, during budding, vesicles fuse with the PM upon arrival at the bud cortex. In contrast, during cytokinesis, the Myo2-loaded vesicles likely switch tracks from polarized actin cables to the actin filaments in the AMR, leading to a more uniform distribution of vesicles along the division site (Fang *et al.* 2010). In the absence of the AMR (*e.g.*, *myo1Δ* cells), secretory vesicles are delivered to the bud neck and directly fuse with the PM between the split septin rings, by a process similar to that during budding. As the bud neck is ~1.0 μm in diameter (Bi *et al.* 1998; Lippincott and Li 1998a), a mere ~50 post-Golgi vesicles would suffice to fill both sides of the neck, and perhaps this small requirement explains why force production by the AMR, required in systems with a larger diameter furrow, is not required in yeast (Fang *et al.* 2010).

Regulation of Chs2 and primary septum formation: Chs2 expression peaks near the end of mitosis (Chuang and Schekman 1996) and is held at the endoplasmic reticulum (ER) by Cdk1 phosphorylation (Figure 7B) (Teh *et al.* 2009). Upon mitotic exit, Chs2 is induced by the MEN to exit the ER and delivered to the bud neck through the secretory pathway (Chuang and Schekman 1996; VerPlank and Li 2005; Zhang *et al.* 2006). Chs2 may require further activation at the bud neck to catalyze PS formation, as, in mutants such as *inn1Δ* cells, Chs2 is localized to the bud neck with correct timing but fails to form any PS (Nishihama *et al.* 2009). Proteolysis of Chs2 by trypsin stimulates its activity *in vitro*, suggesting a possible zymogen-like behavior *in vivo* (Sburlati and Cabib 1986; Uchida *et al.* 1996; Martinez-Rucobo *et al.* 2009). Indeed, a soluble protease was found to stimulate Chs2 activity but its identity remains undetermined (Martinez-Rucobo *et al.* 2009). Together, these observations indicate that Chs2 activity is spatiotemporally controlled at multiple levels to ensure its optimal function during cytokinesis.

Although Chs2 is essential for PS formation during cytokinesis (Sburlati and Cabib 1986; Shaw *et al.* 1991), deletion of *CHS2* is not lethal in most genetic backgrounds but causes severe defects in cytokinesis. However, deletion of *CHS2* and *CHS3* together causes cell lethality with cytokinesis arrest. It is thought that in the absence of Chs2, Chs3 contributes to cell survival by promoting the assembly of a frequently misshaped "remedial" septum (Cabib and Schmidt 2003). Importantly, Chs3 localization at the bud neck during cytokinesis depends on Rho1 (Yoshida *et al.* 2009). Thus, Rho1 plays critical roles in both AMR-dependent and -independent cytokinesis pathways (Yoshida *et al.* 2009). In *chs2Δ* cells, the AMR is assembled but often undergoes asymmetric contraction, leading to the idea of mutual dependency between the PS and the AMR (Bi 2001; Schmidt *et al.* 2002; VerPlank and Li 2005).

Coordination of the actomyosin ring and primary septum formation

AMR contraction and PS formation must be coordinated in time and space to ensure robust cytokinesis. Cells lacking the formin Bni1 are defective in actin ring assembly and often display asymmetric, little, or no AMR contraction (Vallen *et al.* 2000). In these mutant cells, septum formation is usually asymmetric or misaligned, suggesting that AMR contraction may guide PS formation. This hypothesis has been corroborated by a number of studies (Bi 2001; Schmidt *et al.* 2002; VerPlank and Li 2005; Fang *et al.* 2010). Here, we discuss the potential role of four proteins (Iqg1, Inn1, Cyk3, and Hof1) in the coupling of AMR contraction and PS formation during cytokinesis (Figure 8).

Iqg1 must play a role in AMR-independent cytokinesis, presumably promoting septum formation (Epp and Chant 1997; Lippincott and Li 1998a; Korinek *et al.* 2000; Ko *et al.* 2007), as deletion of *IQG1*, but not *MYO1*, is lethal in all genetic backgrounds. Deletion of *INN1* prevents PS formation but permits AMR assembly (Sanchez-Diaz *et al.* 2008; Jendretzki *et al.* 2009; Nishihama *et al.* 2009; Meitinger *et al.* 2010). Inn1 interacts with and localizes to the bud neck after Iqg1 (Epp and Chant 1997; Lippincott and Li 1998a; Sanchez-Diaz *et al.* 2008). Thus, Inn1 likely acts downstream of Iqg1 in PS formation (Figure 8) (Nishihama *et al.* 2009; Meitinger *et al.* 2010). Cyk3 localizes to the bud neck shortly after Inn1, constricts with the AMR, and lingers at the division site for a few minutes before it disappears (Korinek *et al.* 2000; Jendretzki *et al.* 2009). Deletion of *CYK3* does not cause any obvious defects in AMR assembly but causes a partial defect in PS formation, and

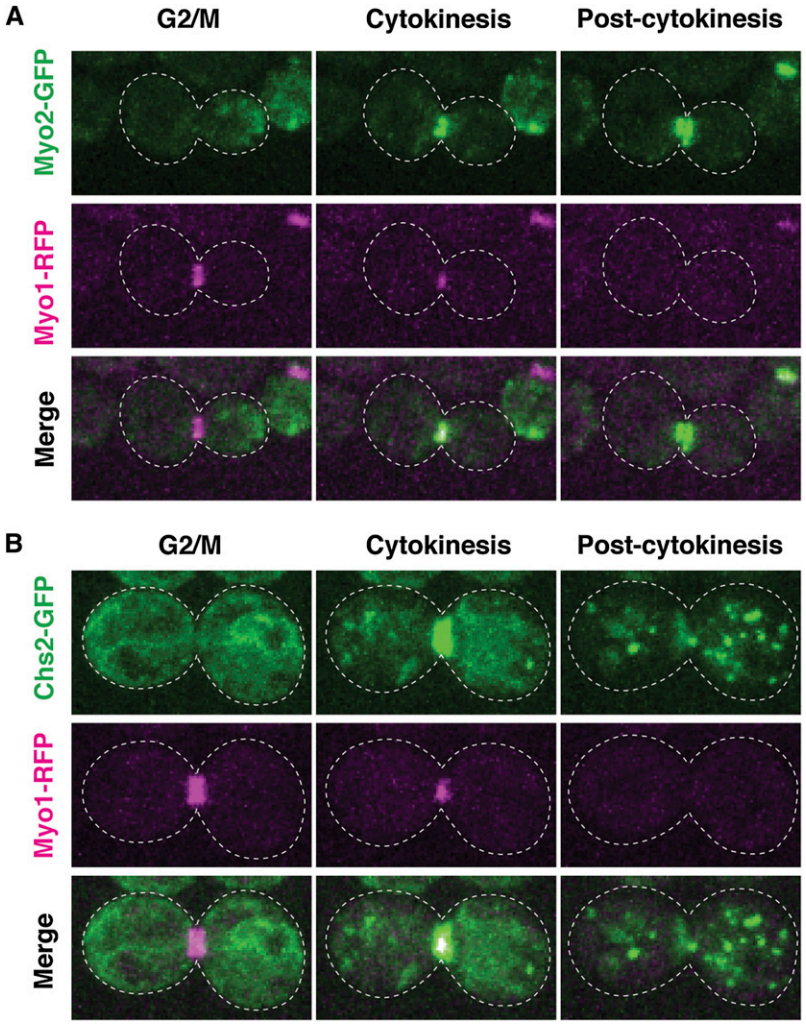

Figure 7 Spatiotemporal coupling of actomyosin ring contraction with membrane trafficking and septum formation during cytokinesis. (A) Spatiotemporal relationship between Myo1 (magenta) and Myo2 (green) before, during, and after cytokinesis. (B) Spatiotemporal relationship between Myo1 (magenta) and Chs2 (green) before, during, and after cytokinesis.

overexpression of Cyk3 leads to ectopic PS formation (Korinek *et al.* 2000; Meitinger *et al.* 2010), suggesting a stimulatory role for Cyk3. Multicopy *CYK3* suppresses the growth and cytokinesis defects of *iqg1Δ* and *inn1Δ* cells by promoting PS formation and without restoring actin ring assembly in *iqg1Δ* cells (Korinek *et al.* 2000; Jendretzki *et al.* 2009; Nishihama *et al.* 2009). Thus, Cyk3 likely acts downstream of Iqg1 and Inn1 in PS formation (Figure 8) (Nishihama *et al.* 2009). Inn1 also binds to the F-BAR protein Hof1 (Nishihama *et al.* 2009). Hof1 colocalizes with the septins from S/G2 to the onset of cytokinesis, when it is phosphorylated first by the Polo kinase Cdc5 and then by the MEN kinase Dbf2 (Lippincott and Li 1998b; Vallen *et al.* 2000; Blondel *et al.* 2005; Meitinger *et al.* 2011). Phosphorylated Hof1 dissociates from the septins to bind and contract with the AMR (Meitinger *et al.* 2011). After cytokinesis, Hof1 lingers at the division site for a few minutes before being degraded (Blondel *et al.* 2005). Deletion of *HOF1* causes no obvious defects in AMR assembly but is synthetically lethal with *myo1Δ*, suggesting that Hof1 plays a role in the AMR-independent cytokinesis pathway (Figure 8) (Vallen *et al.* 2000). Indeed, PS formation is abnormal in *hof1Δ* cells (Meitinger *et al.* 2011).

Key questions on cytokinesis

The core components and events of cytokinesis are conserved from yeast to humans, with inevitable differences in regulation. However, key questions remain: how are the myosin-II and actin filaments organized into "contractile units?" How do other ring components such as IQGAP and the formins facilitate AMR assembly? How is the AMR coupled with membrane trafficking and localized ECM remodeling or PS formation during cytokinesis? How does abscission occur after AMR contraction? What are the functions of the septin double rings during cytokinesis and cell separation? How are the various cellular events in cytokinesis regulated by the cell-cycle machinery? Answers to these questions in multiple model systems will reveal the common and unique mechanisms underlying cytokinesis in different organisms.

Spatial Control of Polarized Cell Growth and Division

Patterns of bud-site selection

S. cerevisiae cells choose a cortical site for polarized growth in a nonrandom pattern depending on their cell type. Haploid **a** and α cells (as well as **a**/**a** and α/α diploids) bud in an

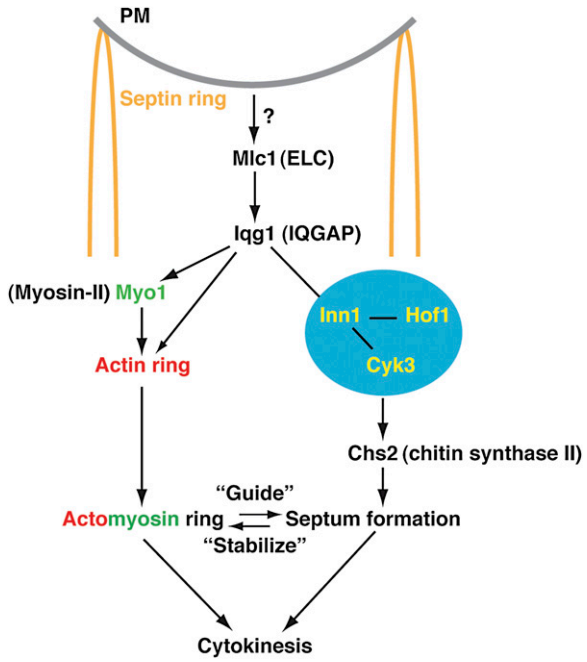

Figure 8 A molecular model for cytokinesis in budding yeast. At the onset of cytokinesis, the septin hourglass is split into two cortical rings (light brown), Mlc1 and Iqg1 maintain Myo1 at the division site, and all three proteins are required for AMR assembly and constriction. Iqg1 is also involved in septum formation, possibly by interacting with Inn1. Inn1 interacts with Hof1 and Cyk3 to somehow "activate" Chs2 for PS formation. AMR "guides" membrane deposition and septum formation whereas the latter "stabilizes" the AMR and its constriction. Efficient cytokinesis requires spatiotemporal coordination of the AMR and septum formation.

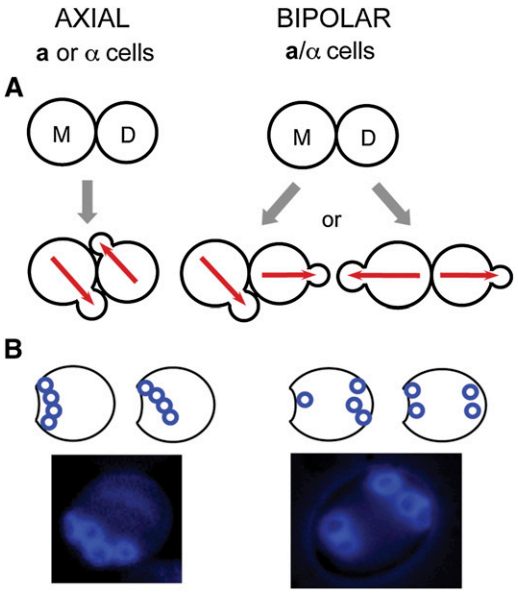

Figure 9 Patterns of bud-site selection in *S. cerevisiae*. (A) Axial and bipolar patterns of cell division. Red arrows denote polarization axes. (B) The patterns of bud scars on the yeast cell surface resulting from the two different modes of budding. On each cell a single birth scar marks where the cell detached from its mother (M). A bud scar shown as a blue ring marks a division site on the mother cell surface. Bud scars can be visualized by staining with the dye Calcofluor (as shown) or by scanning electron microscopy. In the axial pattern, scars form a continuous chain. In the bipolar pattern, scars cluster around the birth pole (proximal pole) and the pole opposite the birth end (distal pole). Modified from (Park and Bi 2007) with permission.

axial pattern in which both mother and daughter cells select a bud site immediately adjacent to their previous division site. In contrast, diploid **a**/α cells bud in a bipolar pattern: mother cells select a bud site adjacent to their daughter or on the opposite end of the cell, whereas daughter cells almost exclusively choose a bud site directed away from their mother (Freifelder 1960; Hicks *et al.* 1977; Chant and Pringle 1995) (Figure 9). The choice of a bud site determines the axis of cell polarization during budding and ultimately the cell division plane, which is perpendicular to the axis of cell polarization. Successive divisions produce distinct patterns of bud scars that mark the sites of cell division on the mother cell surface (Figure 9). In cells undergoing axial budding, the division site is marked by a transient spatial signal, whereas in cells undergoing bipolar budding, both poles of the cell are marked by persistent signals that direct future budding events (Chant and Pringle 1995).

Three groups of genes, collectively called "*BUD* genes," are involved in producing these patterns. The first group includes *BUD3*, *BUD4*, *AXL1*, and *AXL2/BUD10*, which are specifically required for the axial pattern (Chant and Herskowitz 1991; Chant *et al.* 1995; Halme *et al.* 1996; Roemer *et al.* 1996; Sanders and Herskowitz 1996). The second group includes *BUD7–BUD9*, *RAX1*, and *RAX2*, which are specifically required for bipolar budding (Zahner *et al.* 1996; Chen *et al.* 2000; Fujita *et al.* 2004; Kang *et al.* 2004a). The third group, which includes *RSR1* (also known as *BUD1*), *BUD2*, and *BUD5* (Bender and Pringle 1989; Chant *et al.* 1991; Chant and Herskowitz 1991; Bender 1993; Park *et al.* 1993), is required for both budding patterns and is thus thought to encode the "general site-selection machinery" (Chant and Pringle 1995). These gene products convey cell-type–specific information to the downstream polarity establishment machinery (Figure 10) (Chant and Pringle 1995; Pringle *et al.* 1995; Park and Bi 2007). All bud-site selection proteins relevant to our discussion are listed in Table 3.

The Rsr1 GTPase module: the center of spatial regulation

The Rsr1 GTPase module: A visual screen for mutants with altered budding patterns led to the identification of *BUD* genes including *BUD1*, *BUD2*, and *BUD5* (Chant *et al.* 1991; Chant and Herskowitz 1991). *BUD1* is identical to *RSR1*, which was originally identified as a multicopy suppressor of a *cdc24* mutation (Bender and Pringle 1989). *BUD2* and *BUD5* were also identified from independent genetic screens (Powers *et al.* 1991; Benton *et al.* 1993; Cvrckova and Nasmyth 1993). *RSR1* encodes a Ras-like GTPase (Bender and Pringle 1989). *BUD2* and *BUD5* encode the GAP and GEF for Rsr1, respectively (Chant *et al.* 1991; Powers *et al.* 1991; Bender 1993; Park *et al.* 1993; Zheng *et al.* 1995; Park and Chant 1996). Rsr1, Bud2, and Bud5 thus constitute a functional GTPase module involved in proper

bud-site selection (Figure 10). Expression of *RSR1*^{G12V} or *RSR1*^{K16N}, which encodes Rsr1 constitutively in the GTP- or in the GDP-bound (or nucleotide-empty) state, respectively, results in selection of a random bud site as does deletion of *RSR1* (Ruggieri *et al.* 1992). Consistent with this observation, deletion of *BUD2* or *BUD5* also randomizes the budding pattern (Chant *et al.* 1991; Bender 1993; Park *et al.* 1993). Thus, cycling of Rsr1 between the GTP- and GDP-bound states is critical for its function in bud-site selection. Indeed, Rsr1 interacts with specific binding partners depending on its GTP- or GDP-bound state (see below).

Localization of Rsr1, Bud2, and Bud5: Rsr1 localizes to the sites of polarized growth as well as internal membranes, particularly the vacuolar membrane. After cell division, Rsr1 remains enriched at the division site (Park *et al.* 2002; Kang *et al.* 2010). The C-terminal C*AAX* (*A* is aliphatic; X is any amino acid) box and the polybasic region (PBR) of Rsr1 are important for its efficient localization to the PM (Park *et al.* 2002; Kang *et al.* 2010).

Bud5 also localizes to the sites of polarized growth in **a** or α cells and to the mother-bud neck during G2/M. In late M, Bud5 appears as a double ring encircling the mother-bud neck, which then splits into two single rings at cytokinesis. Newly born G1 cells thus have the Bud5 ring at the division site (Kang *et al.* 2001; Marston *et al.* 2001). Bud5 localizes in distinct patterns in **a**/α cells, particularly during M and G1. Before bud emergence, Bud5 is often present at both poles of **a**/α cells: as a ring at one pole, which is the previous division site, and in a patch at the opposite pole, which becomes a new bud site. At a later stage of the cell cycle, Bud5 localizes to the neck and one pole of the mother cell (and/or bud tip) and occasionally only at the neck as in **a** or α cells (Kang *et al.* 2001; Marston *et al.* 2001). Overexpression of Bud5 results in mislocalization and random budding, suggesting that its localization is critical for proper bud-site selection (Kang *et al.* 2001).

Unlike Rsr1 and Bud5, Bud2 is not enriched at the division site during M and early G1. Bud2 concentrates at the incipient bud site in late G1 and at the mother-bud neck after bud emergence and then delocalizes during G2/M (Park *et al.* 1999; Marston *et al.* 2001). These localization patterns of Rsr1, Bud2, and Bud5 imply that localized action of the Rsr1 GTPase module promotes proper bud-site selection (Park *et al.* 1993; Michelitch and Chant 1996). Bud5 may be the key player that interacts with a spatial landmark and recruits Rsr1, while Bud2 is likely to be important for targeted release of the bud-site assembly proteins (*e.g.*, Cdc42 and Cdc24) at the chosen site (see below).

Polarization of the Rsr1 GTPase module: Rsr1 associates with itself and with Cdc42 (see below). The homotypic interaction of Rsr1 depends on its GEF Bud5 (Kang *et al.* 2010), suggesting that Rsr1 needs to maintain its ability to pass through the GDP-bound state to function, consistent with previous findings (Ruggieri *et al.* 1992; Park *et al.* 1993, 1997). In fact, Rsr1^{G12V} fails to form a dimer and concentrate

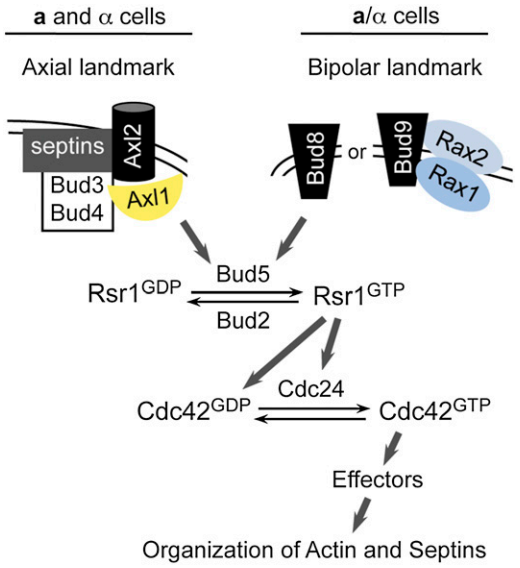

Figure 10 Pathways governing axial and bipolar budding in haploid (**a** or α) and diploid (**a**/α) cells. Although physical interaction has been demonstrated in some cases, many interactions are postulated on the basis of genetic and localization data. All proteins do not necessarily interact at the same time. See text for further details. Modified from (Park and Bi 2007) with permission.

at the division site, whereas Rsr1^{K16N} forms persistent homodimers (Park *et al.* 2002; Kang *et al.* 2010). The transient homotypic interaction of Rsr1, unlike that of Rsr1^{K16N}, suggests that Rsr1 dimerization occurs in a spatially and temporally controlled manner. Mutations in *RSR1* that cause defects in its homotypic interaction and heterotypic interaction with Cdc42 result in random budding and poor membrane association (Park *et al.* 2002; Kang *et al.* 2010). Thus it remains uncertain whether the GTPase interactions and/or membrane association of Rsr1 is critical for its polarization.

Selection of a bud site in the axial pattern

Mutations in *BUD3*, *BUD4*, *AXL1*, or *AXL2/BUD10* (*AXL2* hereafter) disrupt axial budding of **a** or α cells, resulting in bipolar budding, while these mutations do not affect normal bipolar budding of **a**/α cells (Chant and Herskowitz 1991; Fujita *et al.* 1994; Adames *et al.* 1995; Chant *et al.* 1995; Halme *et al.* 1996; Roemer *et al.* 1996; Sanders and Herskowitz 1996). Septins also play an important role in axial budding, as some septin mutants are defective in axial budding (Flescher *et al.* 1993; Chant *et al.* 1995). Proteins encoded by these genes are thus thought to function as (or regulate) a transient cortical marker for the axial budding pattern. Genetic and localization data support the view that the cycle of assembly and disassembly of a protein complex at the mother-bud neck (and the division site) provides a spatial memory from one cell cycle to the next.

Molecular nature of the axial-budding–specific proteins: *AXL1* is the only known *BUD* gene that is expressed in **a** and α cells but not in **a**/α cells, and ectopic expression of *AXL1* in **a**/α cells increases axial budding (Fujita *et al.* 1994). Axl1

Table 3 Proteins involved in bud-site selection

Name of protein or protein complex[a]	Protein activity (or domain/motif)	Function[b] (mutant phenotypes and/or key protein interactions)	Key references
Rsr1/Bud1	Ras-like GTPase	Required for axial and bipolar budding Interacts with Cdc24 and Cdc42	Bender and Pringle 1989; Chant *et al.* 1991; Park *et al.* 1997; Kozminski *et al.* 2003; Kang *et al.* 2010
Bud2	GAP for Rsr1	Required for axial and bipolar budding	Park *et al.* 1993; Bender 1993; Park *et al.* 1999
Bud5	GEF for Rsr1	Required for axial and bipolar budding Interacts with Axl2, Bud8, and Bud9	Chant *et al.* 1991; Powers *et al.* 1991; Kang *et al.* 2001; Kang *et al.* 2004b; Kang *et al.* 2012
Bud3	Axial landmark	Required for axial budding	Chant *et al.* 1995; Lord *et al.* 2000; Kang *et al.* 2012
Bud4	Axial landmark	Required for axial budding	Sanders and Herskowitz 1996; Kang *et al.* 2012
Axl1	Axial landmark (Metalloendopeptidase activity)	Required for axial budding and a-factor processing	Fujita *et al.* 1994; Adames *et al.* 1995; Lord *et al.* 2002; Kang *et al.* 2012
Axl2	Axial landmark	Required for axial budding	Roemer *et al.* 1996; Halme *et al.* 1996; Sanders *et al.* 1999; Lord *et al.* 2000; Gao *et al.* 2007; Kang *et al.* 2012
Bud8, Bud9	Bipolar landmarks	Required for bipolar budding Interact with Bud5	Zahner *et al.* 1996; Harkins *et al.* 2001; Schenkman *et al.* 2002; Krappmann *et al.* 2007
Rax1	Bipolar landmark	Required for bipolar budding Required for localization of Bud8, Bud9, and Rax2	Kang *et al.* 2004a; Fujita *et al.* 2004
Rax2	Bipolar landmark	Required for bipolar budding	Chen *et al.* 2000; Kang *et al.* 2004a
Bud7	Member of the ChAPs (Chs5p-Arf1p-binding proteins) family of proteins	Required for bipolar budding	Zahner *et al.* 1996

[a] Information on individual proteins is partly based on *Saccharomyces* Genome Database (SGD: http://www.yeastgenome.org/).
[b] Only functions related to polarized cell growth and cytokinesis are indicated here.

shares homology with the insulin-degrading enzyme family of endoproteases and is also required for processing of the mating pheromone **a**-factor precursor (Adames *et al.* 1995). However, mutations in the presumptive active site of Axl1 do not perturb bud-site selection despite disrupting **a**-factor precursor processing, suggesting that its protease activity is not necessary for bud-site selection (Adames *et al.* 1995). Bud4 and Bud3 contain a putative GTP-binding motif (Sanders and Herskowitz 1996) and a Rho GEF homology domain (also called DH domain), respectively. Bud4 is indeed a GTP-binding protein and plays a critical role in the assembly of the axial landmark (Kang *et al*. 2012). Axl2 is a type-I transmembrane glycoprotein (Roemer *et al.* 1996) but has no similarity to ligand-binding or catalytic domains of known transmembrane receptors. It is possible that Axl2 functions like non-catalytic receptors such as the integrins, for which clustering drives downstream signaling (Halme *et al.* 1996), but it is not known whether Axl2 undergoes clustering or oligomerization. Although Axl2 contains four cadherin-like motifs in its extracellular domain (Dickens *et al.* 2002), the role of these motifs is obscure.

Localization of Bud3, Bud4, Axl1, and Axl2: Bud3 and Bud4 localize as a double ring encircling the mother-bud neck during and after the G2 phase and as a single ring at the division site after cytokinesis (Chant *et al.* 1995; Sanders and Herskowitz 1996). Localization of Bud3 and Bud4 depends on septin integrity (Chant *et al.* 1995; Sanders and Herskowitz 1996). Bud4 is inefficiently localized in *cdc12* mutants, and extra copies of *BUD4* suppress the temperature-sensitive growth of a *cdc12* mutant (Sanders and Herskowitz 1996). Thus, Bud4 may assemble at the mother-bud neck through direct interaction with septins and recruit Bud3 (Kang *et al*. 2012), although molecular details are not known.

Axl1 and Axl2 also localize as a double ring encircling the mother-bud neck prior to cytokinesis, and this double ring splits into two single rings after cytokinesis (Halme *et al.* 1996; Roemer *et al.* 1996). While the localized Axl1 signal is absent in late G1 and weak in S phase (Lord *et al.* 2000), the Axl2 signal is most intense in cells with emerging buds and appears at the periphery of small buds (Halme *et al.* 1996; Roemer *et al.* 1996). Axl1 and Bud4 localize normally in the absence of a component of the Rsr1 GTPase module, consistent with the idea that the axial landmark functions upstream of the Rsr1 GTPase module. Localization of Axl1 and Axl2 to the mother-bud neck depends on Bud3 and Bud4 to different extents (Halme *et al.* 1996; Lord *et al.* 2002; Gao *et al.* 2007; Kang *et al*. 2012). In contrast, both

Bud3 and Bud4 seem to localize to the mother-bud neck normally in the absence of Axl2 (Halme *et al.* 1996; Roemer *et al.* 1996). These observations support the view that Bud3 and Bud4 recruit Axl1 and Axl2 to the division site (Gao *et al.* 2007; Kang *et al*. 2012).

AXL2 is expressed in a cell-cycle–dependent manner, peaking in late G1, and is delivered to the cell surface via the secretory pathway (Halme *et al.* 1996; Roemer *et al.* 1996; Sanders *et al.* 1999; Powers and Barlowe 2002). Axl2 fails to localize specifically to the bud side of the mother-bud neck in *pmt4* mutants, which are defective in O-linked glycosylation of some secretory and cell surface proteins, and daughter cells of the *pmt4* mutants exhibit a specific defect in the axial pattern (Sanders *et al.* 1999). These findings support the idea that localization of Axl2 to the mother-bud neck is important for proper bud-site selection in the subsequent cell division cycle while its localization in late G1 is likely to be important for organization of the septin filaments (Gao *et al.* 2007).

Choosing a nonoverlapping bud site: Despite enrichment of the landmark proteins at the division site, a new bud appears next to, but not overlapping with, the previous division site in axially budding cells (Chant and Pringle 1995). This phenomenon depends on the activity of the Cdc42 GAP Rga1. Rga1 establishes an exclusion zone at the division site that blocks subsequent polarization within that site (Tong *et al.* 2007). Strikingly, in the absence of localized Rga1, a new bud forms within the old division site. The level of Cdc42-GTP is also elevated at the division site in *rga1Δ* cells, unlike in wild-type cells in which Cdc42-GTP localized adjacent to but outside the old division site. Deletion of *RGA1* also causes a similar phenotype in diploid cells, although to a lesser extent.

Selection of a bud site in the bipolar pattern

A genetic screen for mutants with specific defects in bipolar budding identified *BUD8* and *BUD9* (Zahner *et al.* 1996). *RAX1* and *RAX2*, which were identified as extragenic suppressors of an *axl1* mutant, are also involved in bipolar budding (Chen *et al.* 2000; Fujita *et al.* 2004; Kang *et al.* 2004a). Null mutations in any of these genes disrupt bipolar budding but not axial budding. Strikingly, *bud8* mutants bud almost exclusively at the proximal pole (the birth pole), whereas *bud9* mutants predominantly bud at the distal pole (the pole opposite the birth end) (Zahner *et al.* 1996). These unipolar patterns differ from the axial pattern, since bud sites do not appear in a sequential chain but cluster in the vicinity of either pole in no particular order. These findings suggest that *BUD8* and *BUD9* encode key components that mark the poles distal and proximal to the birth pole of a daughter cell, respectively (Zahner *et al.* 1996).

Molecular nature of the bipolar-budding–specific proteins: Both Bud8 and Bud9 are transmembrane proteins with a similar cytoplasmic domain and a predicted N-terminal extracellular domain, which appears to be heavily glycosylated. It has been postulated that the cytoplasmic domains of Bud8 and Bud9 may provide a signal that is recognized by a common downstream target such as a component of the Rsr1 GTPase module (Harkins *et al.* 2001). However, a Bud5-responsive region has been mapped within the extracellular domains of Bud8 and Bud9 (Krappmann *et al.* 2007). It is thus not clear whether Bud5 interacts directly with Bud8 or Bud9, or with a transmembrane protein, which indirectly bridges the interaction (see below).

Unlike Axl2, which undergoes a rapid turnover, Rax1 and Rax2 are very stable (Chen *et al.* 2000; Kang *et al.* 2004a), which fits the persistent nature of the bipolar landmark (Chant and Pringle 1995). Rax1 and Rax2 also appear to be integral membrane proteins. Rax2 has a type-I orientation, with a long extracellular N terminus (Kang *et al.* 2004a). Current evidence suggests that Rax1 and Rax2 interact closely with each other and with Bud8 and Bud9 in helping to mark both poles for bipolar budding.

Rax1 is necessary for efficient delivery of Bud8 and Bud9 to the proper sites for bipolar budding (Fujita *et al.* 2004; Kang *et al.* 2004a). While Rax1 and Rax2 are almost totally unable to localize to the bud tip or distal pole in the absence of Bud8, they localize normally to the proximal poles of daughter cells in the absence of Bud9 (Kang *et al.* 2004a). Nonetheless, Rax1 and Rax2 do not suffice to provide a spatial signal, as a *bud9* null mutant almost never buds at the proximal pole (Zahner *et al.* 1996; Harkins *et al.* 2001). Thus, all of these proteins are likely to function together to provide spatial signals at both poles of **a**/α cells.

Localization of Bud8, Bud9, Rax1, and Rax2: Consistent with their predicted roles, Bud8 localizes to the distal pole of a newly born cell and Bud9 localizes to the bud side of the mother-bud neck (which becomes the proximal pole of the daughter cell) just before cytokinesis (Harkins *et al.* 2001). In some strain backgrounds, Bud9 is observed more frequently at the distal pole of daughter cells (Taheri *et al.* 2000; Krappmann *et al.* 2007). Interestingly, the levels of *BUD8* mRNA and *BUD9* mRNA peak in late G2/M and G1, respectively, and this timing appears to dictate the localization of these proteins (Schenkman *et al.* 2002). Thus, translation and/or delivery of the proteins to the cell surface might be regulated in a cell-cycle–dependent manner, although the underlying mechanism remains unknown. Bud9 localization depends on actin and septins (Harkins *et al.* 2001; Schenkman *et al.* 2002), and Bud8 localization requires the formin Bni1, indicating a dependence on bud tip-directed actin cables (Harkins *et al.* 2001; Ni and Snyder 2001; Tcheperegine *et al.* 2005).

Rax1 and Rax2 localize to the bud tips of cells with small- or medium-sized buds and to the mother-bud necks of cells with fully formed septa, and thus these proteins remain at distal pole and the division site in both mother and daughter cells. Their presence at the division site is persistent such that multiple Rax1 and Rax2 rings are found in cells that

have budded multiple times (Chen *et al.* 2000; Fujita *et al.* 2004; Kang *et al.* 2004a). Although this property fits expectations for a persistent bipolar landmark, several questions remain. What provides the spatial signal for bipolar budding at the previous division sites on mother cells? Since Rax1 and Rax2 localize persistently to these sites unlike Bud8 or Bud9, which is rarely present at the division sites on mother cells, Rax1 and Rax2 could provide the spatial signal. But then, why are Rax1 and Rax2 unable to do so at the proximal pole of daughter cells without Bud9? Discovering the precise functions of each protein will help clarify how mother and daughter cells respond to bipolar spatial cues (see below).

Other players in bipolar budding: Some mild actin mutations such as *act1-116* and *act1-117* minimally affect cell growth but perturb the bipolar budding pattern (Yang *et al.* 1997). Interestingly, in **a**/α cells carrying specific *act1* mutations, daughter cells correctly position their first bud at the distal pole of the cell, whereas the mother cells bud randomly, indicating that different rules govern bud-site selection of mother and daughter cells in **a**/α diploids (Yang *et al.* 1997). A similar phenomenon is also observed in other bipolar mutants including *spa2* and *bud6/aip3* (Zahner *et al.* 1996). Bipolar budding is also disrupted by mutations in many other genes, which may affect bipolar budding indirectly (Snyder 1989; Sivadon *et al.* 1995; Valtz and Herskowitz 1996; Zahner *et al.* 1996; Finger and Novick 1997; Vaduva *et al.* 1997; Yang *et al.* 1997; Sheu *et al.* 1998; Tennyson *et al.* 1998; Bi *et al.* 2000; Ni and Snyder 2001; Tcheperegine *et al.* 2005).

Mechanisms of the cell-type–specific budding patterns

Coupling of spatial landmarks to the Rsr1 GTPase module: How is the spatial signal transmitted to the Rsr1 GTPase module? Recruitment of Bud5 to the landmarks appears to be critical for the establishment of the correct budding pattern (Kang *et al.* 2001; Marston *et al.* 2001). This idea is further supported by isolation of *bud5* alleles that specifically disrupt bipolar budding and mislocalize only in **a**/α cells (Zahner *et al.* 1996; Kang *et al.* 2001, 2004b). Bud5 associates with Axl2, Bud8, and Bud9 (Kang *et al.* 2001, 2004b; Krappmann *et al.* 2007), potentially linking the landmark to the Rsr1 GTPase module (Figure 10). Bud5 indeed associates with the axial landmark only in **a** and α cells but not in **a**/α cells (Kang *et al.* 2012). However, it is not known whether these interactions are direct and whether distinct domains of Bud5 recognize each landmark, as no axial-specific alleles of *BUD5* have yet been identified.

Bud2 localizes to the presumptive bud site initially even in the absence of Rsr1 or Bud5 (although it is not stably maintained) (Park *et al.* 1999; Marston *et al.* 2001). Bud5 localizes to the division site in the absence of Bud2. Thus, both Bud2 and Bud5 are likely to interact with spatial landmarks independently. Isolation of *bud2* alleles with a specific defect in bipolar budding also supports this idea (Zahner *et al.* 1996). However, as with *BUD5*, no axial-specific alleles of *BUD2* are currently known. It is also unknown which landmark protein(s) interacts with Bud2.

An important issue is whether and how Bud2 and Bud5 are regulated. It is not known whether spatial landmarks (*e.g.*, Axl2 or Bud8) only recruit Bud5 to the presumptive bud site or also activate its GEF activity. A number of localization studies (see above) suggest that interaction of a spatial landmark with Bud5 should occur during the late M or early G1 phases, potentially activating the Rsr1 GTPase cycle, coincident with Rsr1 dimerization. Genetic and high throughput data suggest a possible regulation of Bud2 by a G1 CDK (Benton *et al.* 1993; Cvrckova and Nasmyth 1993; Drees *et al.* 2001; Holt *et al.* 2009), but this remains to be tested.

Regulation by cell type: Budding patterns depend on cell types (Hicks *et al.* 1977; Chant and Pringle 1995), which are controlled by transcriptional regulators encoded by the mating loci *MAT***a** and *MAT*α (Herskowitz 1988). It was thus initially proposed that the cell-type–specific budding pattern is produced by transcriptional repression of genes critical to axial budding by the corepressor **a**1-α2 present in diploid **a**/α (*MAT***a**/*MAT*α) cells (Chant and Herskowitz 1991). This idea was supported by the isolation of *AXL1*, which is expressed only in **a** or α cells and is necessary for axial budding (Fujita *et al.* 1994). However, determination of cell-type–specific budding pattern is likely to be more complex than initially thought. In the absence of both Bud8 and Bud9, **a**/α cells exhibit a partially randomized budding pattern with an increased tendency to bud at the proximal pole, rather than at random sites. When a potential axial landmark is also absent in **a**/α *bud8 bud9* mutant, a more fully randomized budding pattern is observed (Harkins *et al.* 2001). Thus, **a**/α cells have some ability to use axial cues in the absence of both putative bipolar landmarks. Despite our current knowledge of several proteins that control or constitute spatial landmarks, the mechanism by which the cell-type–specific budding pattern is determined remains largely unknown. One feasible model is that Axl1 may play a key role in assembly of an active axial landmark, and it may block the function of Rax1 and/or Rax2, thus inhibiting bipolar budding in haploid **a** or α cells. In diploid **a**/α cells where *AXL1* is not expressed, Rax1 and Rax2 may be active for establishment of the bipolar landmark.

Directing polarity establishment by coupling of the Rsr1 and Cdc42 GTPase modules

Numerous studies suggest the coupling of bud-site selection with bud-site assembly. Overexpression of *RSR1* suppresses the temperature-sensitive growth of a *cdc24* (*cdc24-4*) mutant (Bender and Pringle 1989), and certain alleles of *CDC24* including *cdc24-4* show a bud-site selection defect (Sloat and Pringle 1978; Sloat *et al.* 1981). Rsr1-GTP binds Cdc24 (Zheng *et al.* 1995; Park *et al.* 1997) but not Cdc24-4 (Shimada *et al.* 2004), and Rsr1-GDP preferentially binds

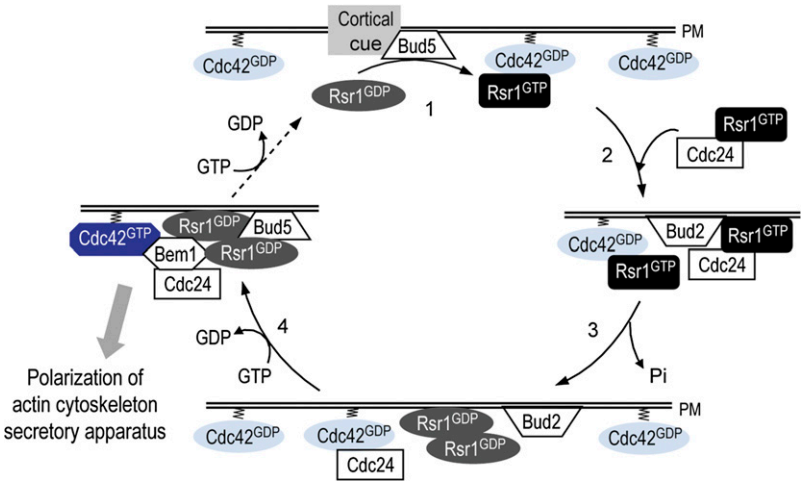

Figure 11 A model for how the Rsr1 GTPase cycle directs polarity establishment to a specific site. (Step 1) Bud5, which is recruited to a specific site by a spatial landmark, catalyzes GDP/GTP exchange on Rsr1. (Step 2) Rsr1-GTP associates with Cdc24 and Cdc42 and guides them to the presumptive bud site. (Step 3) Bud2 stimulates Rsr1 to hydrolyze its bound GTP. (Step 4) Cdc24 no longer interacts with Rsr1 and catalyzes the exchange of GDP for GTP on Cdc42, leading to local Cdc42 activation. Cdc42-GTP then triggers actin assembly and exocyst localization to establish an axis of cell polarization. Bud5 at the presumptive bud site may convert Rsr1 to a GTP-bound state (dashed line), allowing for another GTPase cycle. Homotypic Rsr1–Rsr1 interaction and heterotypic Rsr1–Cdc42 interaction may stabilize these GTPases at a single site, contributing to proper bud-site selection and polarity establishment. Modified from (Park and Bi 2007) with permission.

Bem1 (Park *et al.* 1997). *RSR1* is necessary for localization of Cdc24 to a correct bud site in late G1 (Park *et al.* 2002; Shimada *et al.* 2004). It has been suggested that Rsr1 also activates Cdc24 by triggering its conformational change (Shimada *et al.* 2004). However, the underlying mechanism is less certain as there are some discrepancies among studies regarding a domain of Cdc24, which is necessary for its cortical localization or interaction with Rsr1 (Park *et al.* 1997; Toenjes *et al.* 1999; Shimada *et al.* 2004; Toenjes *et al.* 2004).

RSR1 also exhibits genetic interactions with *CDC42*. *RSR1* was identified as an allele-specific dosage suppressor of a temperature-sensitive *cdc42* allele (*cdc42-118*) that is defective in polarity establishment (Kozminski *et al.* 2003). Unlike suppression of *cdc24*-Ts, overexpression of GTP-locked Rsr1 ($RSR1^{G12V}$) cannot suppress *cdc42-118*, suggesting that cycling of Rsr1 between GDP- and GTP-bound states is required (Kozminski *et al.* 2003). Rsr1 also binds Cdc42 *in vitro* and this association is enhanced by Cdc24 (Kozminski *et al.* 2003). Interestingly, a mutation of *RSR1* (*rsr1-7*), which maintains its ability to suppress *cdc24-4*, is no longer able to suppress *cdc42-118*, suggesting that this mutation disrupts the Rsr1–Cdc42 interaction without affecting the Rsr1–Cdc24 interaction (Kozminski *et al.* 2003; Kang *et al.* 2010). The Rsr1–Cdc42 interaction is thus likely to be direct rather than bridged by Cdc24. Similar GTPase heterodimerization has been reported in various organisms (Sekiguchi *et al.* 2001; Weibel *et al.* 2003; Shan *et al.* 2004). Interestingly, *rsr1Δ* exhibits synthetic lethality with *cdc42-118* at 30° (Kozminski *et al.* 2003), and cells lacking *RSR1*, *GIC1*, and *GIC2* also fail to form a bud (Kawasaki *et al.* 2003). These genetic interactions suggest that Rsr1 functions not only in selection of a growth site but also in polarity establishment. This notion is reinforced by high-resolution microscopy of live cells, which indicates that Rsr1 is required for both selecting and stabilizing the polarity axis in G1 (Ozbudak *et al.* 2005).

How does the Rsr1 GTPase module function in linking the spatial landmark to bud site assembly? A scheme in which the Rsr1 GTPase cycle orchestrates bud-site assembly at a proper bud site has been proposed (Park *et al.* 1997; Kozminski *et al.* 2003) (Figure 11). Recruitment of Bud5 and Bud2 to the presumptive bud site by the spatial landmark may lead to rapid cycling of Rsr1 between GTP- and GDP-bound states at an adjacent site. This Rsr1 cycling coupled with differential affinities of each of these species for binding partners such as Cdc24 and Bem1 may trigger the ordered assembly of a complex at the proper bud site. Several rounds of this cycle may be necessary to assemble critical levels of Cdc42-GTP and its associated proteins at the proper bud site. In the absence of the Rsr1 GTPase module, localization of Cdc24 and Cdc42 to a random bud site may occur through a distinct default pathway yet to be identified or by a "symmetry breaking" mechanism (Figure 3B, and the YeastBook chapter by Howell and Lew, 2012).

While the Rsr1 GTPase module is at the center of spatial regulation of budding by virtue of its role in linking the spatial landmark to the polarity machinery, the current model is mostly based on binary interactions of the proteins involved in bud-site selection and bud-site assembly. A future challenge is to integrate information on individual players into a comprehensive model for the spatial and temporal regulation of bud-site selection and polarity establishment.

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