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COMMENTARY

Polarization of cell growth in yeast

I. Establishment and maintenance of polarity states

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SUMMARY

The ability to polarize is a fundamental property of cells. The yeast *Saccharomyces cerevisiae* has proven to be a fertile ground for dissecting the molecular mechanisms that regulate cell polarity during growth. Here we discuss the signaling pathways that regulate polarity. In the second installment of this two-part commentary, which appears in the next issue of *Journal of Cell Science*, we discuss how the actin cytoskeleton responds to these signals and guides the polarity of essentially all events in the yeast cell cycle.

During the cell cycle, yeast cells assume alternative states of polarized growth, which range from tightly focused apical growth to non-focused isotropic growth. RhoGTPases, and in particular Cdc42p, are essential to guiding this polarity. The distribution of Cdc42p at the cell cortex establishes cell polarity. Cyclin-dependent protein kinase, Ras, and heterotrimeric G proteins all modulate yeast cell polarity in part by altering the distribution of Cdc42p. In turn, Cdc42p generates feedback signals to these molecules in order to establish stable polarity states and coordinate cytoskeletal organization with the cell cycle. Given that many of these signaling pathways are present in both fungi and animals, they are probably ancient and conserved mechanisms for regulating polarity.

Key words: Yeast, Actin, Polarity, Rho, Cdc42, Cell cycle

INTRODUCTION

Essentially all cells can polarize in response to external (e.g. matrix, cell-cell contacts or chemical gradients) and/or internal cues. Eukaryotic cells generally interpret these cues by assembling a polarized actin cytoskeleton at the cortex, which coordinates with microtubules to guide internal membranes; this ultimately polarizes events internally and at the cell surface (Drubin and Nelson, 1996). Because of its simple genetics, budding yeast provides an excellent model system to study these processes. The critical issues in yeast are how polarity cues are established and interpreted to polarize the actin cytoskeleton, and how the cytoskeleton in turn polarizes growth. Non-essential polarity cues that determine sites of cell growth are covered in a recent review (Chant, 1999). We present here the basic logic yeast uses to control polarity in response to those cues and to the cell cycle. The structure of the actin cytoskeleton, and how it directs cell growth, is the subject of part II of this article, which appears in the next issue of *Journal of Cell Science* (Pruyne and Bretscher, 2000).

S. cerevisiae polarizes growth to direct budding during cell replication, and to direct shmoo formation during mating (Fig. 1). Cell growth in yeast has the following requirements: (1) weakening of the cell wall by digestive enzymes to allow cell expansion, (2) insertion of new plasma membrane at the cell surface, and (3) synthesis of new cell wall by biosynthetic enzymes. For growth to be polarized, the secretory pathway must deliver these enzymes and membranes to discrete growth sites at the cell surface. The first studies to localize actin during the cell cycle showed a close correlation between the polarized distribution of the actin cytoskeleton and sites of cell expansion (Adams and Pringle, 1984). Subsequent work has confirmed that the actin cytoskeleton alone targets the secretory vesicles that support growth (for review, see Bretscher et al., 1994; Finger and Novick, 1998).

The yeast actin cytoskeleton also polarizes intracellular structures during growth. Actin orients the mitotic spindle during early bud growth through microtubule-actin interactions (Theesfeld et al., 1999). The inheritance of mitochondria and the vacuole into the bud also depends upon actin: mitochondrial inheritance occurs through an as yet unclear mechanism (Simon et al., 1995, 1997), whereas inheritance of the vacuole is driven by an unconventional myosin V, Myo2p (Hill et al., 1996; Catlett and Weisman, 1998). Another myosin V, Myo4p, delivers mRNA into the bud along actin filaments, which polarizes the synthesis of Ash1p, a transcriptional repressor involved in mating-type switching and filamentous differentiation (Jansen et al., 1996; Bobola et al., 1996; Chandarlapaty and Errede, 1998; Münchow et al., 1999; Beach et al., 1999). Because virtually all aspects of polarized growth

in yeast derive from the polarity of the actin cytoskeleton, elaborate controls must carefully monitor and regulate cytoskeletal structure during all phases of the yeast life cycle.

OVERVIEW OF ACTIN AND GROWTH POLARITY IN YEAST

In yeast, filamentous actin is organized primarily into cortical patches and actin cables. Cortical patches are discrete F-actin-rich bodies, whereas actin cables are long F-actin bundles (Adams and Pringle, 1984; Amberg, 1998). Both structures lie at the cell cortex and are polarized in a cell-cycle-dependent manner.

At commitment to a new cell cycle in G_1 ('START'), yeast select a bud site. Cortical patches ring this site, and actin cables converge there (Fig. 1). As a bud emerges, cortical patches initially cluster at its tip, cables extend from the mother cell into the bud and the bud grows apically (from the tip). Later in vegetatively growing yeast, patches and cables within the bud redistribute randomly while cables in the mother cell still extend to the bud neck; thus growth is still confined to the bud, but the bud expands isotropically into an ellipsoid shape. An alternative filamentous morphology that is induced in some *S. cerevisiae* strains by a variety of conditions, prolongs apical growth to generate highly elongated cells (Fig. 1; Kron and Gow, 1995; Lo et al., 1997; Madhani and Fink, 1998). At the end of either filamentous or vegetative bud growth, the cortical patches and actin cables redistribute randomly in the mother and bud while a cytokinetic F-actin ring assembles at the bud neck, contracts and disassembles (Field et al., 1999). Following cytokinesis, patches and cables in the mother and daughter repolarize to the former bud neck to direct synthesis of cell walls between the two new cells.

Throughout bud formation, virtually all growth is directed into the bud. A growing population therefore contains uniformly sized mother cells bearing variously sized buds. The hallmark of growth polarization defects is abnormal morphology characterized either by highly elongated buds (a consequence of excessive apical growth) or by spherical buds (a consequence of excessive isotropic growth). When growth is completely undirected, bud enlargement ceases altogether, and mother cells grow into huge, round unbudded cells.

Yeast actin also polarizes growth during mating (Fig. 1). Haploid yeast cells secrete pheromones to elicit a mating response in cells of the opposite mating type. Stimulated cells become arrested in G_1, express proteins necessary for cell fusion and orient growth toward mating partners by polarizing their actin cytoskeleton up the pheromone concentration gradient (Kron and Gow, 1995). The resultant mating projections (shmoos) from two mating partners eventually fuse, which results in conjugation to form a diploid zygote.

Cdc42p SIGNALING ESTABLISHES ACTIN POLARITY

The essential Rho GTPase Cdc42p is central to polarizing the actin cytoskeleton in yeast. A key polarizing event is the recruitment of Cdc42p to growth sites on the plasma membrane, where the GTPase activates effectors that signal to the actin cytoskeleton (Ziman et al., 1993; Fig. 2). In the

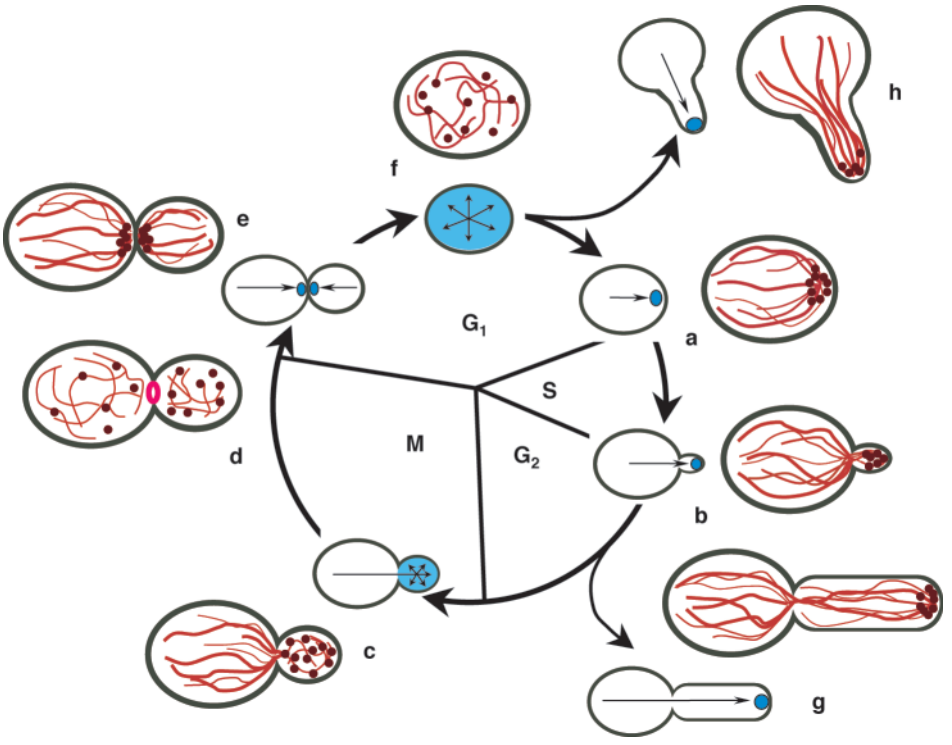

Fig. 1. Cell polarity in budding yeast is established by the localized plasma membrane recruitment of the Rho GTPase Cdc42p (blue) and proteins related to its function. These proteins orient the actin cytoskeleton, which consists of actin cables (pink) and cortical patches (brown). In turn, the actin cytoskeleton guides secretory vesicles to the cell surface, where they accumulate (also blue) and fuse, thus polarizing growth (arrows). (a) The cell cycle begins in G_1 with establishment of a nascent bud site. (b) Clustering of Cdc42p directs early bud growth toward the tip. (c) Redistribution of Cdc42p over the bud surface during G_2-M redirects bud growth isotropically, and results in an ellipsoidal shaped bud. (d) With the completion of bud growth, cables and patches disorganize, and a cytokinetic ring forms, then contracts and disassembles after mitosis. (e) Cdc42p reorients actin and growth between the two new cells to generate new cell walls. The mother cell resumes budding immediately. (f) The new daughter undergoes a period of undirected growth. (g) Under certain growth conditions, some strains of *S. cerevisiae* differentiate into a filamentous state that forgoes the transition in G_2-M from tip-directed to isotropic growth. The resulting cells are highly elongated. (h) Mating pheromones arrest haploid yeast in G_1 and polarize Cdc42p toward potential mating partners to generate a mating projection (shmoo).

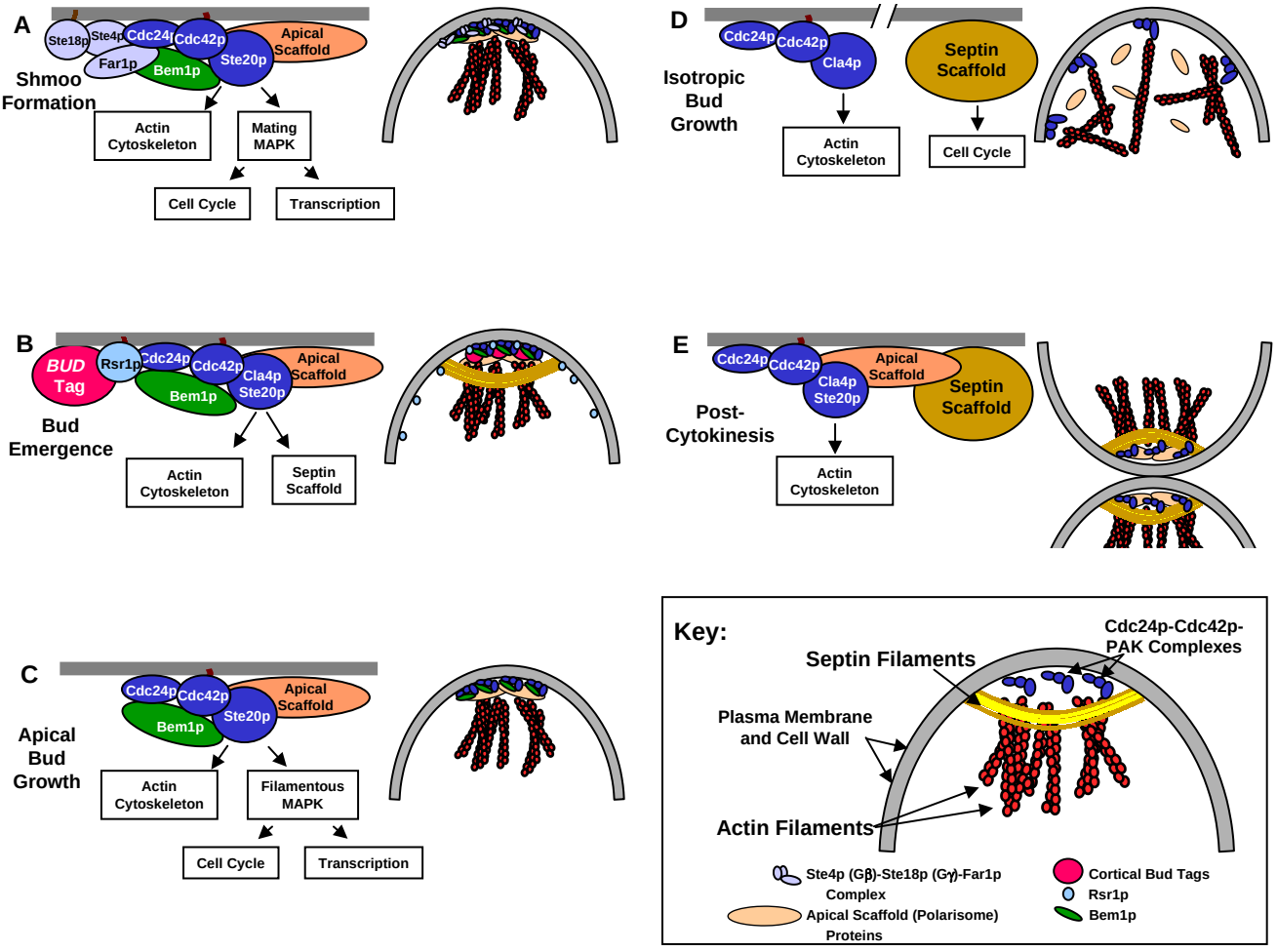

Fig. 2. Clustering of Cdc24p-Cdc42p-effector complexes (dark blue) at the cell surface requires factors that form putative scaffolds. The Cdc42p-activated effectors, such as Ste20p, Cla4p, Gic1p and Gic2p, orient the actin cytoskeleton (red) from these signaling clusters. (A) Shmoo formation. Cdc24p-Cdc42p-Ste20p assemble at the cell surface into a pheromone-induced complex with Bem1p (green), Far1p (purple) and free Gβγ (Ste4p, Ste18p; purple). This complex polarizes the actin cytoskeleton to guide shmoo growth. Tight clustering of this complex for proper shmoo morphogenesis also requires polarisome proteins (Spa2p, Sph1p, Bud6p, Bni1p and Pea2p) as a putative apical scaffold (tan). Finally, the mating complex recruits a MAPK cascade to promote signaling of Cdc42p through Ste20p to trigger MAPK-dependent transcriptional changes and cell cycle arrest. (B) Bud emergence. A tight patch of Cdc24p-Cdc42p on the plasma membrane establishes the nascent bud site. Bem1p strongly facilitates bud emergence, possibly as a scaffold to assist clustering of Cdc24p-Cdc42p. Cortical cues (pink) established by *BUD* gene products and the Rsr1p (Bud1p) GTPase (light blue) normally guide bud emergence, but are non-essential. Cdc42p probably functions through several effectors during bud emergence, including Gic1p, Gic2p, Ste20p and Cla4p, to both polarize the actin cytoskeleton and to direct assembly of a ring of septin proteins (yellow). (C) Apical bud growth. Early apical bud growth and filamentous bud elongation require polarisome proteins, possibly as a scaffold for Cdc42p-containing complexes and MAPK cascade proteins. Ste20p is the primary Cdc42p-effector during sustained apical growth, signaling to the actin cytoskeleton and a MAPK cascade. (D) Isotropic bud growth. During isotropic bud growth, accessory scaffolds are apparently not required. Inactivation of these scaffolds depends at least indirectly upon Cla4p, and Cla4p is the primary Cdc42p effector that signals to the actin cytoskeleton during isotropic growth. (E) Post-cytokinesis. After contraction of the cytokinetic ring, Cdc24p, Cdc42p and polarisome proteins repolarize to the former mother-bud neck site in order to redirect the actin cytoskeleton to the mother-bud junction. This guides the formation of a new cell wall between the mother and daughter, and in the absence of a contractile ring, this directed wall synthesis provides a secondary mechanism of cytokinesis. The septin scaffolds at the former mother-bud neck site are required to reorient the actin cytoskeleton, perhaps acting in part through direct recruitment of polarisome proteins (e.g. Spa2p).

absence of Cdc42p, cortical patches and actin cables still form but are completely disorganized; this depolarizes growth to yield large, round unbudded cells (Adams et al., 1990). The association between Cdc42p and the plasma membrane is essential: *cdc42*^{C188S}, which lacks the geranylgeranyl membrane anchor, and *cdc43* geranylgeranyl transferase mutants also cannot polarize growth (Adams et al., 1990; Ziman et al., 1991).

Like other Rho GTPases, Cdc42p signals to effectors only in an active GTP-bound state. GTP binding requires the guanine-nucleotide-exchange factor (GEF) Cdc24p, and *cdc24* mutations (e.g. *cdc24-1*, *cdc24-4*) also depolarize actin and growth (Hartwell et al., 1974; Sloat et al., 1981; Zheng et al., 1994). Normal Cdc42p function also requires inactivation by GTP hydrolysis. This might permit the redistribution of Cdc42p through the cell cycle, given that constitutive activation

by mutation (*CDC42*^{G12V}) or loss of the relevant GTPase-activating proteins (GAPs), Bem3p and Rga1p, locks Cdc42p into a polarized distribution and hyperpolarizes growth (Ziman et al., 1991; Stevenson et al., 1995). Rdi1p, a Rho guanine-nucleotide-dissociation inhibitor, binds to GDP-Cdc42p in the cytosol, possibly facilitating such redistribution (Koch et al., 1997).

The p21-activated kinases (PAKs) are Cdc42p effectors that signal to the actin cytoskeleton (Davis et al., 1998; Eby et al., 1998; Fig. 2). Binding of GTP-Cdc42p to the PAK N-terminal inhibitory domain activates these kinases (Vojtek and Cooper, 1995), localizes at least one PAK (Ste20p) to growth sites and conveys cell-cycle-dependent regulation on another (Cla4p; Peter et al., 1996; Benton et al., 1997; Leberer et al., 1997). Two PAKs, Ste20p and Cla4p, are essential to Cdc42p-actin signaling at all stages of growth, and simultaneous loss of Ste20p and Cla4p blocks initial bud emergence, bud growth and cytokinesis (Cvrcková et al., 1995; Eby et al., 1998; Holly and Blumer, 1999; Richman et al., 1999).

Class I myosins (Myo3p and Myo5p) are the only cytoskeletal substrates of PAKs identified so far in yeast (Wu et al., 1997). These molecular motors, which localize to cortical patches, are necessary for proper cytoskeletal organization (Goodson et al., 1996; Geli and Riezman, 1996). Although PAK-mediated phosphorylation is required for myosin I activity, and an activated mutant of myosin I (*MYO3*^{S357D}) can rescue *myo3Δ myo5Δ* polarity defects, an activated mutant of myosin I cannot rescue the lethal loss of PAK function (Wu et al., 1997). Therefore, unidentified signaling pathways from PAKs to the cytoskeleton must exist.

Two related proteins, Gic1p and Gic2p, also bind to GTP-Cdc42p and are required for normal cytoskeletal polarization during bud emergence and shmoo formation (Brown et al., 1997; Chen et al., 1997). Gic2p, at least, is present only in G_1, and both Gic1p and Gic2p colocalize with Cdc42p at growth sites before bud emergence. In the absence of Gic1p and Gic2p, the actin cytoskeleton is partially depolarized. These features suggest Gic1p and Gic2p somehow facilitate Cdc42p in its role during bud emergence.

ESTABLISHMENT AND MAINTENANCE OF Cdc42p POLARITY: CUES AND SCAFFOLDS

Cytoskeletal polarity is guided in yeast by the distribution of Cdc42p and its GEF Cdc24p on the plasma membrane (Figs 1 and 2). The distribution of Cdc24p-Cdc42p complexes ranges from a tight polarization during bud emergence through a cap-like distribution during apical growth to a diffuse distribution during isotropic growth (Ziman et al., 1993; Peter et al., 1996; Leberer et al., 1997; Holly and Blumer, 1999; Nern and Arkowitz, 1999). These changes probably reflect variable assembly of Cdc42p-containing complexes into scaffolds through different growth phases. Several putative scaffold factors, discussed below, include Bem1p, the septin neck filaments and a complex of proteins termed the polarisome.

The initial polarization of Cdc24p and Cdc42p during shmoo- and bud-site selection depends strongly on Bem1p (Fig. 2A,B). Bem1p colocalizes with Cdc24p and Cdc42p to growth sites, and its transcription in G_1 coincides with these early polarization events (Bender and Pringle, 1991; Chenevert et al., 1992; Ayscough et al., 1997; Ayscough and Drubin, 1998; Cho et al., 1998). Bem1p promotes coupling between polarity determinants and Cdc24p-Cdc42p by directly binding both Cdc24p and shmoo- and bud-site-selection proteins.

During mating, external gradients of pheromone guide polarized shmoo growth (Segall, 1983). Pheromone stimulation activates G-protein-coupled receptors that generate free Gβγ , which in turn recruits a polarity determinant Far1p to the plasma membrane (Butty et al., 1998). Together, Gβγ and Far1p recruit Bem1p, Cdc24p and the PAK Ste20p to assemble a Cdc42p-dependent signaling complex (Butty et al., 1998; Leeuw et al., 1998; Nern and Arkowitz, 1999). External pheromone gradients lead to a higher concentration of Cdc42p associated with the plasma membrane to one side of the cell. Actin-dependent clustering of pheromone receptors further tightens these signaling complexes into a patch directed toward the pheromone source (Ayscough and Drubin, 1998). This patch then orients the actin cytoskeleton and directs shmoo growth towards a mating partner.

In contrast, the initiation of bud emergence is guided by pre-existing cortical cues (Fig. 2B). These cues, established during previous budding events by the *BUD* gene products, allow a Ras-related protein, Rsr1p (Bud1p), to bind to Cdc24p and Bem1p at a discrete region of the plasma membrane during early G_1 (Zheng et al., 1995; Chant, 1999; Park et al., 1999). Binding triggers the recruitment of Cdc42p, which defines the nascent bud site and allows bud emergence to begin.

The mechanism by which Cdc24p-Cdc42p complexes consolidate into a single patch during bud emergence remains unclear. Although the cortical budding cues normally guide the site of bud emergence, they are not essential to forming a nascent bud site; normal buds grow in the absence of budding cues, although they arise at random sites on the cell surface. Bem1p is also not essential, although it does greatly facilitate bud emergence and is required for normal bud morphology. Bem1p might facilitate Cdc24p-Cdc42p clustering by binding to other proteins, such as F-actin and Ste20p, that cross-link Bem1p-Cdc24p-Cdc42p complexes (Leeuw et al., 1995). It remains to be determined whether the Cdc24p-Cdc42p clustering that occurs in *bem1Δ* cells is mediated through a self-association between Cdc24p-Cdc42p complexes that is activated at START, or whether additional proteins are involved.

Cdc24p and Cdc42p continue to remain clustered for apical growth during shmoo formation, early vegetative bud growth, and filamentous bud elongation (Fig. 1). A group of polarity-determining proteins that comprises Bni1p, Sph1p, Spa2p, Pea2p and Bud6p (Aip3p) share features that suggest they function as an apical scaffold for Cdc24p-Cdc42p during these processes (Fig. 2A,C). Spa2p, Pea2p and Bud6p have been detected in a 12S complex termed the polarisome (Sheu et al., 1998), but the existence of features shared by all these proteins lead us to refer to them collectively here as polarisome proteins.

Polarisome proteins are required for apical actin organization. In their absence, vegetative buds grow as spheres rather than ellipsoids, filamentous bud elongation is blocked and shmoo growth depolarizes to generate short, broadened projections (Gehrung and Snyder, 1990; Chenevert et al., 1994; Amberg et al., 1997; Evangelista et al., 1997; Mösch and Fink, 1997). Polarisome mutants also have widened mother-bud

necks, which suggests that initial bud emergence is improperly focused as well, and occurs from a larger area of the cell surface than in wild-type cells (Zahner et al., 1996).

Protein-protein interactions involving polarisome components suggest that the polarisome links RhoGTPase signaling to actin filament assembly. Bni1p is central to these interactions, binding Bud6p and Spa2p, as well as activated RhoGTPases (Cdc42p, Rho1p, Rho3p, and Rho4p; Kohno et al., 1996; Evangelista et al., 1997). Spa2p, Sph1p and Pea2p localize to growth sites and provide a polarized docking site for Bud6p and Bni1p (Snyder, 1989; Valtz and Hersokowitz, 1996; Amberg et al., 1997; Arkowitz and Lowe, 1997; Evangelista et al., 1997; Fujiwara et al, 1998; Roemer et al., 1998; Sheu et al., 1998). Finally, Bni1p binds profilin (Pfy1p), a protein that stimulates actin polymerization (Mockrin and Korn, 1980; Imamura et al., 1997), and Tef1p/Tef2p, an actin-bundling protein (Umikawa et al., 1998), whereas Bud6p binds to actin filaments (Amberg et al., 1997).

The existence of such interactions suggests that the polarisome assembles and binds to actin filaments, perhaps under RhoGTPase regulation. Although the polarisome is not essential for assembly of cortical patches or actin cables (deletion mutants still produce these structures), it might construct an actin-based apical anchor for Cdc24p, Cdc42p or other cytoskeleton-organizing factors. This possibility is supported by studies of cells expressing Bni1p truncations lacking the polarizing Spa2p- and Rho-binding sites. These cells exhibit highly abundant but disorganized cortical patches and actin cables (Evangelista et al., 1997), which suggests that ectopic actin-organizing sites are formed. However, an interdependence of Cdc24p-Cdc42p distribution and polarisome function is yet to be demonstrated.

A third scaffold affecting yeast polarity is formed by filaments composed of proteins called septins (Cdc3p, Cdc10p, Cdc11p, Cdc12p, Shs1p; for reviews see Longtine et al., 1996; Field and Kellogg, 1999). Unlike the other two scaffolds, septins do not colocalize with growth sites throughout the cell cycle, but remain immobilized at the locations where they are initially organized. Prior to bud emergence, the septins are organized into a ring surrounding the nascent bud site by Cdc42p-Cla4p signaling (or imperfectly organized by Cdc42p-Ste20p; Cvrcková et al,. 1995; Holly and Blumer, 1999; Richman et al., 1999). Throughout bud growth, the septins remain as a collar of filaments surrounding the mother-bud neck. During mating, the septins form a similar but less tightly localized collar around the base of the growing shmoo. The initial organization of septins by Cdc42p-PAK signaling is independent of any signaling by Cdc42p to the actin cytoskeleton, although continued maintenance of the septin ring may depend upon normal polarized growth and may be lost over time when Cdc42p-PAK signaling or actin polarity are disrupted (Holly and Blumer, 1999).

The septins have several functions that are essential for cytokinesis and cell separation. The septins anchor plasma membrane enzymes that synthesize a chitin ring surrounding the mother-bud neck; loss of this ring allows the neck to widen abnormally (DeMarini et al., 1997). Septins also form a template for a contractile ring of F-actin, myosin II (Myo1p), an IQ-GAP homolog (Iqg1p) and other proteins that facilitate cytokinesis (for review see Field et al., 1999), and in the absence of septin function, this ring does not form. Finally, septins are required for reorientation of cortical patches and actin cables to the mother-bud neck after bud growth in order to complete cell separation, and cells lacking septins do not repolarize their actin to the mother-bud neck (Adams and Pringle, 1984).

The failure to reorient the cytoskeleton to the bud neck may explain why loss of septin function lethally blocks cytokinesis, while the loss of chitin synthase or the contractile ring only partially blocks cytokinesis. The septins may reorient the cytoskeleton in part through recruitment of the polarisome protein Spa2p; the repolarization of Spa2p depends upon septins, and Spa2p directly binds to the septin Shs1p (Arkowitz and Lowe, 1997; Mino et al., 1998). It remains to be seen whether Cdc24p and Cdc42p or other regulators of actin polarity also depend upon the septin scaffold for repolarization at the end of the cell cycle.

REGULATION OF Cdc42p AND PAK POLARITY BY CDK

The change from an apical Cdc24p-Cdc42p distribution to an isotropic distribution ultimately reflects a decrease in the proportion of Cdc24p and Cdc42p present in tight bud-tip-associated scaffolds. Similarly, the repolarization of Cdc24p and Cdc42p from a diffuse distribution during isotropic bud growth back to the bud neck indicates an increase in the amount of Cdc24p-Cdc42p associated with bud neck-associated scaffolds. How is this regulated? Two important factors are the activity of the Cdc28p cyclin-dependent protein kinase (CDK) and the specific PAK associated with Cdc42p.

Changes in the activity of Cdc28p, the primary yeast CDK, drive changes in the localization of Cdc42p and actin polarity (Lew and Reed, 1993; Lew et al., 1997; Fig. 3A). During the G_1-S transition, Cdc28p complexes with the G_1 cyclins Cln1p and Cln2p and polarizes the cytoskeleton for bud emergence: inhibition of Cln1p or Cln2p blocks emergence, whereas overproduction of either G_1 cyclin hyperpolarizes growth. The appearance of the B cyclins Clb1p and Clb2p in G_2-M counters the G_1 cyclins and depolarizes actin during vegetative budding: yeast overexpressing Clb1p or Clb2p accelerate the isotropic switch and are unable to initiate a new bud. Late in the cell cycle, cyclin degradation follows anaphase, inactivating Cdc28p and triggering cytokinesis and the transient repolarization of Cdc42p and the actin cytoskeleton to the bud neck.

After completion of the cell cycle, yeast cells can rest, resume budding immediately, or enter a phase of depolarized growth. During this period after cell division, when the cell is relatively free of cyclins, pheromone signaling can recruit Cdc42p into an apical distribution to guide shmoo formation, and induce a cell cycle arrest (see below) to maintain the cyclin-free state (Oehlen and Cross, 1994; Kron and Gow, 1995).

The Cdc42p-dependent PAKs Ste20p and Cla4p are important for cyclin-dependent polarity changes (Fig. 3B-D). Although either kinase alone promotes bud emergence and cytokinesis, the two PAKs polarize growth differently.

Ste20p mediates prolonged apical growth, and is required for shmoo formation, filamentous cell elongation, and Cln1p- or Cln2p-induced hyperpolarization (Eby et al., 1998; Madhani

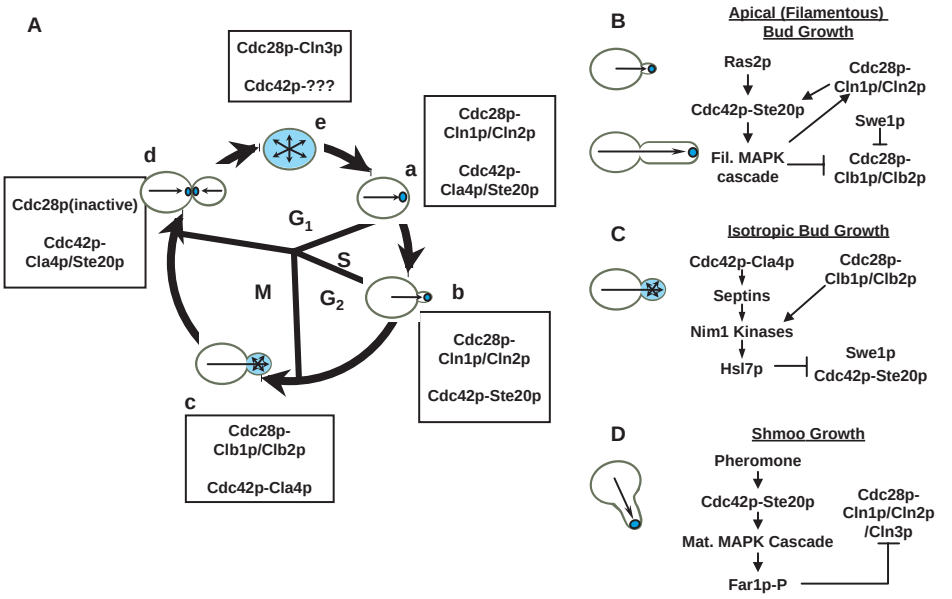

Fig. 3. Cdc28p-cyclin and PAK activities regulate the apical clustering of Cdc42p. (A) The cyclins and the PAKs show changes through the cell cycle that parallel the changes in Cdc42p polarity (blue). Apical Cdc42p clustering and cell growth during G_1-S occurs as Cln1p and Cln2p are synthesized and Ste20p is phosphorylated and polarized to growth sites. Isotropic Cdc42p distribution and bud growth are associated with the appearance Cdc28p-Clb1p/Clb2p complexes and the increased activation of Cla4p during G_2-M. Cyclin degradation after mitosis triggers reconvergence of Cdc42p to the bud neck. (B) Apical (filamentous) bud growth. Phosphorylation of Ste20p by Cdc28p-Cln1p/Cln2p during G_1-S initiates apical bud growth. Signaling by Ras2p sustains this apical growth through G_2-M by causing Ste20p to activate a filamentous MAPK cascade (MAPKKK Ste11p, MAPKK Ste7p, MAPK Kss1p). This cascade prolongs Cln1p expression through G_2-M and inhibits Cdc28p-Clb1p/Clb2p complexes. The Swe1p kinase also inhibits Cdc28p-Clb1p/Clb2p during filamentous growth. (C) Isotropic bud growth. In the absence of filament-inducing signals, bud growth becomes isotropic during G_2-M in response to Cdc28p-Clb1p/Clb2p activity. Cdc28p-Clb1p/Clb2p activity is sustained through a positive feedback loop: Cdc28p-Clb1p/Clb2p complexes activate Nim1-related kinases (Hsl1p, Gin4p, Kcc4p), which activate Hsl7p, which in turn inhibits Swe1p and Ste20p and prevents inhibition of Cdc28p-Clb activity. Cla4p is required for this feedback, possibly indirectly through its role at bud emergence in organizing the septins, which are required for Nim1 kinase function. (D) Shmoo growth. Pheromone stimulation in early G_1 causes Cdc42p-Ste20p to activate a mating MAPK cascade (MAPKKK Ste11p, MAPKK Ste7p, MAPK Fus3p), which in turn activates the Cdc28p-Cln-inhibitor Far1p. This locks the cell into a cyclin-free state, and prevents both Cdc28p-Cln1p/Cln2p from inhibiting the mating MAPK cascade and Cdc28p-Clb1p/Clb2p from depolarizing growth.

and Fink, 1998; Pan and Heitman, 1999). Cdc28p-Cln1p and Cdc28p-Cln2p appear to regulate the nature of Cdc42p-Ste20p function, possibly by direct phosphorylation of Ste20p (Oda et al., 1999). Such phosphorylation at the G_1-S transition may allow the PAK to direct early apical bud growth, whereas continuous Cdc28p-Cln1p activity is required to prolong apical growth during filamentous cell elongation (Oehlen and Cross, 1998; Wu et al., 1998). Conversely, the inhibition of Cdc28p-Cln1p and Cdc28p-Cln2p by pheromone stimulation allows Ste20p to direct shmoo growth rather than bud emergence.

Cla4p facilitates the Clb1p/Clb2p-driven apical-isotropic switch. Cells lacking Cla4p or bearing a *cdc42*^{V44A} allele, whose product binds poorly to Cla4p, generate highly elongated buds (Cvrcková et al., 1995; Richman et al., 1999). The fact that Cla4p kinase activity in wild-type cells peaks during G_2-M (Benton et al., 1997), and that the apical-isotropic switch correlates with Clb-dependent phosphorylation of Cla4p (Tjandra et al., 1998), is consistent with the notion that Cdc42p-Cla4p is active throughout isotropic bud growth.

In addition to responding to cyclin signals, the PAKs play a feedback role in maintaining specific Cdc28p-cyclin states by signaling through mitogen-activated protein kinase (MAPK) cascades and a family of kinases related to the *Schizzosaccharomyces pombe* Nim1 (Fig. 3B-D). Yeast contain several MAPK cascades, each of which generates a distinct response to stimuli such as mating pheromones, filament-inducing starvation, or osmotic shock (Herskowitz, 1995). Ste20p activates two of these (a mating MAPK cascade and a filamentous growth MAPK cascade) as part of a feedback loop to sustain apical growth states.

Pheromone-stimulation allows Gβγ and Far1p to recruit the proteins of the mating MAPK cascade to the plasma membrane, along with Ste20p, Bem1p, Cdc24p and Cdc42p. This permits Cdc42p-Ste20p to activate the cascade directly by phosphorylation of the MAPK kinase kinase Ste11p (Wu et al., 1995; Pryciak and Huntress, 1998). One function of the Fus3p mating MAPK is to phosphorylate Far1p. This converts the latter into a Cdc28p-Cln complex inhibitor and arrests the cell cycle (Oehlen and Cross, 1994; Kron and Gow, 1995). This cell cycle arrest provides a feedback that sustains Cdc42p-Ste20p signaling for shmoo formation.

Filamentous growth is triggered by a variety of signals, all of which activate the RasGTPase Ras2p (Kron and Gow, 1995; Lo et al., 1997; Madhani and Fink, 1998). By an unknown mechanism, Ras2p causes Cdc42p-Ste20p to activate an alternative MAPK cascade (Mösch et al., 1996; Cook et al., 1997), possibly in conjunction with recruitment of MAPK cascade proteins to growth sites by the polarisome proteins Spa2p and Sph1p (Madhani and Fink, 1998; Roemer et al., 1998; Sheu et al., 1998). The filamentation MAPK Kss1p has several effects on the cell cycle. One is to induce the transcription of the Cln1p cyclin, which might maintain active Cdc42p-Ste20p (Madhani et al., 1999). Kss1p also cooperates through an unknown mechanism with the inhibitory kinase Swe1p to inactivate Cdc28p-Clb1p and Cdc28p-Clb2p complexes (Ahn et al., 1999; Edgington et al., 1999). Both the Cln1p expression and Clb1p/Clb2p inhibition lead to a prolonged G_2 phase and a delay in the apical-isotropic switch.

Cla4p appears to promote the apical-isotropic switch indirectly through the Nim1-related kinases (Gin4p, Hsl1p,

Kcc4p). In the absence of these partially redundant kinases, yeast generate elongated buds similar to *cla4Δ* cells (Altman and Kellogg, 1997; Tjandra et al., 1998; Barral et al., 1999). The Nim1 kinases are activated by Cdc28p-Clb1p/Clb2p (Altman and Kellogg, 1997; Tjandra et al., 1998). Interestingly, the kinases are associated with the septin neck filaments and require the presence of septins to be activated by Cdc28p-Clb complexes (Carroll et al., 1998; Longtine et al., 1998; Barral et al., 1999). As a consequence, septin mutants also generate elongated cells (Hartwell, 1971). Since the septin scaffold is established by Cdc42p-Cla4p at the START of the cell cycle (Cvrcková et al., 1995; Richman et al., 1999), the activation of Nim1 kinases ultimately depends upon Cdc42p-Cla4p (Tjandra et al., 1998).

The Nim1 kinases facilitate the apical-isotropic switch through activation of a novel, conserved protein, Hsl7p (McMillan et al., 1999). Hsl7p, in turn, degrades Swe1p, which prevents the inhibition of the Cdc28p-Clb1p/Clb2p-dependent apical-isotropic switch. This role for Nim1 kinases is consistent with the fact that the cell elongation phenotypes caused by the Cla4p-defective *cdc42*^{V44A} mutation, by septin mutations, or by Nim1 kinase deletions are all corrected by *swe1Δ* (although other phenotypes, such as cytokinesis defects in septin mutants, are not rescued; Barral et al., 1999; Richman et al., 1999). Additionally, Hsl7p might compete with Cdc42p for binding to Ste20p; this would inactivate the Ste20p PAK and free additional Cdc42p for association with Cla4p to organize actin during isotropic bud growth (Fujita et al., 1999).

The dependence of the Nim1 kinases on septin structure provides yeast with a Swe1p-dependent cell cycle checkpoint that monitors the septin scaffold in a manner that is still mysterious (Carroll et al., 1998; Longtine et al., 1998). Disruption of the actin cytoskeleton also activates Swe1p (potentially through indirect disruption of the septins caused by depolarized growth; Holly and Blumer, 1999), which indicates that Swe1p functions as part of a general morphogenetic checkpoint to coordinate polarized growth with the cell cycle (McMillan et al., 1998).

Feedback between PAKs and Cdc28p sustains stable states of either highly polarized or isotropic growth. How do different Cdc42p-PAK and Cdc28p-cyclin states alter the apical/isotropic distribution of Cdc42p? In part, this may be through a higher affinity of Cdc42p-Ste20p for scaffolds than Cdc42p-Cla4p. The fact that Ste20p directly binds the polarity determinants Bem1p and Gβ, whereas Cla4p is not known to bind Bem1p and binds Gβ more weakly than Ste20p (Leeuw et al., 1995, 1998), supports such an idea. Direct regulation of scaffold assembly and disassembly by Cdc28p-cyclin and Cdc42p-PAK complexes are also likely to regulate overall cell polarity as well. Additional kinase targets or PAK-binding partners that would define apical or isotropic complexes need to be identified. Furthermore, the mechanisms that regulate the repolarization of Cdc42p and the actin cytoskeleton to the bud neck also remain largely unexplored.

Rho3p AND Rho4p: TIES TO CABLE-DEPENDENT DELIVERY OF SECRETORY VESICLES

The RhoGTPases Rho3p and Rho4p also contribute an important role in polarizing growth (Matsui and Toh-e, 1992a; Imai et al., 1996). The influence of Rho3p and Rho4p on cytoskeletal polarity is similar to that of Cdc42p: loss of the partially redundant Rho3p and Rho4p depolarizes actin and growth, whereas constitutively activated Rho3p (*RHO3*^{D119A}) hyperpolarizes actin and growth.

Genetic evidence suggests that Cdc42p and Rho3p/Rho4p share a common polarizing function, and that the contribution of Cdc42p depends upon Bem1p and two Bem1p-interacting proteins, Boi1p and Boi2p. Thus, polarity defects resulting from the loss of Rho3p and Rho4p are corrected by overexpression of Cdc42p or Bem1p in the presence of Boi1p or Boi2p, whereas the loss of Boi1p and Boi2p results in polarity defects corrected by overproduction of Rho3p or Rho4p (Matsui and Toh-e, 1992b; Bender et al., 1996; Matsui et al., 1996).

This common Cdc42p/Boi/Rho3p-type activity appears to compete with other Cdc42p functions, such as promoting bud emergence. Therefore, Rho3p or Rho4p overproduction exacerbates *cdc24-4* and *cdc42-1* bud emergence defects, and Boi1p or Boi2p overproduction blocks bud emergence, requiring Cdc42p overproduction to correct the defect (Bender et al., 1996).

Genetic, two hybrid and in vitro interactions suggest that at least one function of Rho3p is in the targeting of secretory vesicles. Two genes involved in vesicular targeting, *SEC4* and *TPM1*, interact genetically with *RHO3*. *SEC4* encodes a secretory-vesicle-bound RabGTPase involved in both the polarized transport of secretory vesicles and fusion with the plasma membrane (Finger and Novick, 1998). *TPM1* encodes tropomyosin, a major structural component of actin cables (Liu and Bretscher, 1989). A *sec4-2* mutation is synthetically lethal when combined with *rho3Δ* defects, whereas either *SEC4* or *TPM1* overexpression suppresses *rho3Δ* polarity defects (Imai et al., 1996; Kagami et al., 1997). GTP-Rho3p directly binds two other proteins involved in vesicle targeting: Myo2p, the myosin that ferries vesicles along actin cables; and Exo70p, a component of the exocyst, a polarized fusion complex (TerBush et al., 1996; Pruyne et al., 1998; Robinson et al., 1999; Schott et al., 1999). Rho3p polarizes to regions of cell growth similarly to Myo2p, Sec4p, and Exo70p (Robinson et al., 1999). These interactions, along with the *rho3Δ rho4Δ* cytoskeletal polarity defects, suggest that Rho3p positively regulates actin-cable-based vesicular transport.

Rho1p AND Rho2p MAINTAIN CELL INTEGRITY IN THE FACE OF POLARIZED GROWTH

Rho1p and Rho2p are partially redundant GTPases that play a variety of roles. Rho1p is essential (Madaule et al., 1987), but non-essential Rho2p can replace Rho1p if overexpressed (Ozaki et al., 1996). Rho1p polarizes to growth sites in an actin-dependent manner (McCaffrey et al., 1991; Yamochi et al., 1994; Ayscough et al., 1999); there it is activated by redundant GEFs Rom1p and Rom2p (Ozaki et al., 1996; Manning et al., 1997).

Rho1p mediates a variety of functions, and *rho1* alleles show a variety of phenotypes. Many conditional *rho1* mutants grown under restrictive conditions are able to generate small buds, but then lyse at their bud tips. This indicates that these *rho1* mutants have a cell wall synthesis defect; cell expansion

outpaces synthesis of cell wall material at the bud tip. Rho1p stimulates cell wall synthesis directly through two β-1,3-glucan synthases (Fks1p and Fks2p), and indirectly through protein kinase C (Pkc1p), which upregulates wall-enzyme transcription through the Mpk1p MAPK cascade (Cabib et al., 1998; Schmidt and Hall, 1998).

The regulation of the Rom2p GEF is consistent with its role activating Rho1p to maintain cell integrity. During polarized bud growth, Rom2p is activated by a Tor2p-Mss4p lipid kinase cascade, possibly through the direct binding of phosphorylated lipids to its pleckstrin-homology domain (Schmidt et al., 1997; Desrevières et al., 1998; Helliwell et al., 1998a). Cell wall stresses also activate Rom2p, probably through transmembrane glycoprotein stress receptors (Gray et al., 1997; Verna et al., 1997; Bickle et al., 1998; Jacoby et al., 1998; Ketela et al., 1999; Rajavel et al., 1999).

Some *rho1* alleles depolarize the actin cytoskeleton, which indicates that Rho1p modulates actin organization (Helliwell et al., 1998b). Rho1p signaling to actin is through Pkc1p and the Mpk1p MAPK (Schmidt and Hall, 1998). However, the effects of this signaling on polarity are unclear. Defects in Mpk1p or Tor2p, as well as some *rho1* alleles, cause cytoskeletal depolarization (Mazzoni et al., 1993; Helliwell et al., 1998b), which suggests that Rho1p activity promotes polarity. Conversely, loss of Rom2p or Pkc1p hyperpolarizes actin and growth (Paravacini et al., 1992; Ozaki et al., 1996; Manning et al., 1997), whereas Rho1p overexpression or the loss of the Rho1p GAPs Bem2p or Sac7p depolarizes the cell (Dunn and Shortle, 1990; Bender and Pringle, 1991; Espinet et al., 1995). This suggests that Rho1p, and possibly Rho2p, antagonizes actin polarity. Such cytoskeletal depolarization would be expected to cooperate with Rho-stimulated wall synthesis to maintain cell integrity by opposing highly polarized growth. Resolution of these conflicting results will require a better understanding of how Rho1p and Rho2p signal to the actin cytoskeleton.

A final *rho1* mutant class recently isolated arrests as unbudded cells. The defects in these cells do not appear to be in the machinery that polarizes actin, but instead reflect an inability to progress through START (Drgonová et al., 1999). Neither Pkc1p nor glucan synthases are involved in this signaling pathway. The identification of this *rho1* mutant suggests that the Rho1p GTPase, in a similar way to Cdc42p GTPase, modulates CDK-cyclin activity and progression through the cell cycle.

ANIMALS AND FUNGI: HOW SIMILAR ARE THEY?

How conserved are the pathways that regulate polarity in yeast? A proper comparison between yeast and other model systems is beyond the scope of this commentary, but recent reviews emphasize similarities, particularly in regards to Rho GTPase function in polarity (Schmidt and Hall, 1998; Johnson, 1999). Thus, the central role of Cdc42p in organizing the actin cytoskeleton appears to be highly conserved in eukaryotes. For example, in both animals and fungi, Cdc42p clusters at the plasma membrane to induce polarization of actin filaments; in budding yeast, this directs growth of the bud, whereas in animals clustered Cdc42p and Cdc42p-related Rac guide the formation of outgrowths such as filopodia and lamellipodia (Nobes and Hall, 1995). Furthermore, some specific downstream signaling pathways are conserved, such as the regulation of actin polarity and MAPK signaling through PAKs (Bagrodia and Cerione, 1999).

Less is known about the mechanisms that control the polarization of Cdc42p, but evidence suggests some are conserved. Some putative scaffold proteins are present in both fungi and animals (e.g. septins, formins (homologs of Bni1p) and Spa2p-related proteins), and at least some of these colocalize with actin structures during particular cellular activities in these organisms (Roemer et al., 1998; Wasserman, 1998; Field and Kellogg, 1999). Ras and G-protein-coupled receptors have also been implicated in the regulation of Cdc42p function in diverse systems (Schmidt and Hall, 1998; Johnson, 1999). The regulation of Cdc42p polarity by the cell cycle might also be conserved. In particular, the cortical actin cytoskeleton depolarizes during mitosis in a variety of eukaryotes. In yeast, this depolarization is observed as an isotropic redistribution of cortical patches and actin cables over the mother and bud surfaces, whereas in animal cells the depolarization leads to the retraction of large cellular extensions and the formation of actin-rich microvilli isotropically over the cell surface. It remains to be determined to what extent these similarities reflect conserved molecular pathways for controlling the polarity of eukaryotic cells.

CONCLUSIONS

Recent studies establish dual functions for small GTP-binding proteins in the regulation of yeast morphology: (1) organization of cytoskeletal polarity, and (2) generation of feedback signals that coordinate polarity both with the cell cycle and in response to environmental signals. The feedback signals are mediated through PAKs, MAPK cascades, Nim1-related kinases, and other pathways yet to be determined. A major gap in our understanding is how RhoGTPases organize the cytoskeleton. Few cytoskeletal targets have been identified, and little is known about how these regulatory networks actually assemble a polarized actin cytoskeleton. However, much is known about the components of the actin cytoskeleton and its highly dynamic nature, about how actin, and actin cables in particular, are involved in polarizing growth, and about how this process participates in the determination of cell polarity. These topics are covered in part II of this commentary (Pruyne and Bretscher, 2000).

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