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COMMENTARY

Polarization of cell growth in yeast

II. The role of the cortical actin cytoskeleton

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SUMMARY

The actin cytoskeleton provides the structural basis for cell polarity in *Saccharomyces cerevisiae* as well as most other eukaryotes. In Part I of this two-part commentary, presented in the previous issue of *Journal of Cell Science*, we discussed the basis by which yeast establishes and maintains different states of polarity through Rho GTPases and cyclin-dependent protein kinase signaling. Here we discuss how, in response to those signals, the actin cytoskeleton guides growth of the yeast cell. A polarized array of actin cables at the cell cortex is the primary structural determinant of polarity. Motors such as class V myosins use this array to transport secretory vesicles, mRNA and organelles towards growth sites, where they are anchored by a cap of cytoskeletal and regulatory proteins. Cortical actin patches enhance and maintain this polarity, probably through endocytic recycling, which allows reuse of materials and prevents continued growth at old sites. The dynamic arrangement of targeting and recycling provides flexibility for the precise control of morphogenesis.

Key words: Yeast, Actin, Polarity, Myosin, Secretion, Endocytosis

INTRODUCTION

Studies in a variety of eukaryotic systems have demonstrated that the actin-rich cell cortex responds to spatial cues and provides the machinery that polarizes the cell. For example, animal epithelial cells polarize in response to cell-substratum and cell-cell contacts by assembling a polarized microfilament cortex (Drubin and Nelson, 1996), whereas in early embryonic cells asymmetries essential for normal development are established by cues provided by molecules anchored at the cell cortex (Rose and Kemphues, 1998). Studies in the budding yeast reinforce and extend this view.

Regulation of the overall polarity state in *S. cerevisiae* is under the coordinated control of Rho GTPases and cyclin-dependent protein kinases (CDKs; Pruyne and Bretscher, 2000). Feedback signals between these pathways allow yeast to switch between alternative stable patterns of growth. Thus shmoo formation during mating occurs through linkage of sustained apical growth to a cell cycle arrest, whereas ellipsoid bud growth requires a cell-cycle-dependent transition from an apical phase to an isotropic phase.

Rho-GTPase- and CDK-dependent signals establish these polarity states by regulating the clustering of Rho-GTPase-containing complexes: a high degree of clustering directs apical growth; and a more random distribution in the bud directs isotropic growth. A major deficiency in our understanding of this process is an explanation for how the clustering of these signaling molecules affects assembly, disassembly and organization of cytoskeletal structures. However, much is known about the components of the actin cytoskeleton, and recent studies have begun to clarify their distinct functions and interrelationships in directing cell growth.

THE YEAST ACTIN CYTOSKELETON POLARIZES GROWTH

The yeast actin cytoskeleton is organized into at least four biochemically and morphologically distinct structures: cortical patches, actin cables, a cytokinetic ring and the cap (Adams and Pringle, 1984; Chant and Pringle, 1995; Lew and Reed, 1995; Fig. 1; Table 1). Cortical patches are discrete cytoskeletal bodies, actin cables are long bundles of actin filaments, and the cap consists of a polarized accumulation of cytoskeletal proteins and regulatory proteins. The formation, function and regulation of the cytokinetic ring has been reviewed recently (Field et al., 1999) and we do not discuss it here.

The yeast actin cytoskeleton polarizes growth for budding during the cell cycle and for shmoo formation during mating. Cortical patches, actin cables and the cap all reside at the cell cortex in a polarized distribution that correlates with directed

Table 1. Actin cytoskeletal components and polarity determinants

Protein name	Homologies and motifs	Endocytosis	High osmol.	Budding	Cyto-kinesis-	References
Cortical patch components						
Abp1p	Cofilin related, SH3 domains	+	sens (partial)	+	+	Drubin et al., 1988, 1990; Lila and Drubin, 1997; Yang et al.,1997
Act1p	Actin	-	sens	b or bem	-	Adams and Pringle, 1984; Kübler and Riezman, 1993; Chant and Pringle, 1995; Yang et al., 1997
Aip1p	Actin-interacting protein 1	?	sens	?	+	Iida and Yahara, 1999; Rodal et al., 1999
Arp2p, Arp3p, Arc15p, Arc18p, Arc19p Arc35p, Arc4p	Arp2p-Arp3p Complex	-	sens	b	sep	Moreau et al., 1996; Winter et al., 1999a
Cap1p, Cap2p	**Actin capping proteins αβ**	?	sens	+	+	Amatruda and Cooper, 1992; Amatruda et al., 1992; Karpova et al., 1993
Cmd1p	**Calmodulin**	-	?	bem	?	Davis and Thorner, 1989; Brockerhoff and Davis, 1992; Kübler et al., 1994; Oyha and Botstein; 1994; Geli et al., 1998
Cof1p	**Cofilin**	-	?	?	?	Moon et al., 1993; Iida et al., 1993; Lappalainen and Drubin, 1997
Crn1p	Coronin	+	+	?	+	Heil-Chapdelaine et al., 1998; Goode et al., 1999
End3p	Eps15 homology	-	?	b	sep or -	Raths et al., 1993; Bénédetti et al., 1994
Ent1p, Ent2p Ent3p, Ent4p	Espins	-	?	?	?	Wendland et al., 1999
Hog1p	**p38 (MAPK)**	?	sens	a and b	-	Brewster et al., 1993; Brewster and Gustin, 1994; Kültz et al., 1997; Ferrigno et al., 1998
Las17p/Bee1p	WASP	-	sens	b	sep or -	Li, 1997; Naqvi et al., 1998; Madania et al., 1999
Myo3p, Myo5p	Myosin I, SH3 domain	-	sens	b	?	Geli and Riezman, 1996; Goodson et al., 1996
Pan1p/Dim2p	Eps15 homology, binding repeats	-	?	a and b	-	Tang and Cai, 1996; Wendland et al., 1996
Prk1p, Ark1p	GAK	?	?	?	sep (when over-expressed)	Cope et al., 1999; Zeng and Cai, 1999
Rvs161p/End6p, Rvs167p	Amphiphysins, SH3 domain	-	sens	b	?	Crouzet et al., 1991; Bauer et al., 1993; Munn et al., 1995; Sivadon et al., 1995; Brizzio et al., 1998; Balguerie et al., 1999
Sac6p	**Fimbrin**	-	sens	b	?	Drubin et al., 1988; Karpova et al., 1993; Kübler and Riezman,1993; Adams et al., 1995;Yang et al., 1997
Sla1p	BindinG repeats, 3 SH3 domains	+	?	b	?	Holtzman et al., 1993; Yang et al., 1997; Ayscough et al., 1999
Sla2p/End4p	Talin-related, Hip1	-	?	b	?	Holtzman et al., 1993; Raths et al., 1993; Wesp et al., 1997; Yang et al., 1997, 1999b
Srv2p	CAP	- (srv2-14)	?	b	?	Vojtek et al., 1991; Freeman et al., 1996; Wesp et al., 1997; Yang et al., 1997
Twf1p	A6 proteins, cofilin-like repeats	+	+	b	+	Goode et al., 1998
Vrp1p/End5p	**WIP**, proline rich	-	sens	b	?	Munn et al., 1995; Vaduva et al., 1997, 1999
Yap1801p, Yap1802p	AP180	+	+	+	+	Wendland and Emr, 1998
Actin cable components						
Act1p	Actin	-	sens	b or bem	-	As above
Sac6p	**Fimbrin**	-	sens	b	?	As above
Tpm1p, Tpm2p	**Tropomyosin**	+	+	+	+	Liu and Bretscher, 1989; Drees et al., 1995; Pruyne et al., 1998
(*tpm1 tpm2*)		+	+	bem	-	
Myo2p	Myosin V	+	+	+ or bem	+	Johnston et al., 1991; Kübler et al., 1994; Lillie and Brown, 1994; Schott et al., 1999
Cap components						
Axl2p		?	?	a	+	Roemer et al., 1996
Bem1p	Two SH3 domains	?	?	bem	?	Bender and Pringle, 1991; Chenevert et al., 1992; Ayscough et al., 1997
Bni1p	Formin	?	+	b	?	Jansen et al., 1996; Zahner et al., 1996; Evangelista et al., 1997; Imamura et al., 1997
(*bni1 bnr1*)		?	sens	bem	?	
Bud2p	RasGAP	?	?	a and b	?	Chant and Herskowitz, 1991; Park et al., 1993, 1999
Bud6p/Aip3p		?	sens	b	-	Zahner et al., 1996; Amberg et al., 1997; Evangelista et al., 1997
Cdc24p.,	RhoGEF PH domain	?	?	a, b, bem	?	Hartwell et al., 1974; Sloat et al., 1981; Nern and Arkowitz, 1999; Toenjes et al., 1999
Cdc42p	**Cdc42 RhoGTPase**	?	supp	bem	-	Adams et al., 1990; Johnson and Pringle, 1990; Munemitsu et al., 1990; Shinjo et al., 1990; Ziman et al., 1993; Richmanet et al., 1999
Cmd1p	**Calmodulin**	-	?	bem	?	As above
Cla4p, Ste20p, Skm1p	PAK, CRIB domain PH domain (Cla4p, Skm1p)	?	?	bem	-	Benton et al., 1993; Cvrcková et al., 1995; Peter et al., 1996; Leberer et al., 1997; Martin et al., 1997; Eby et al., 1998; Holly and Blumer, 1999
ks1p, Gsc2p	1,3-β-glucan synthases	?	supp	?	?	Mazur et al., 1995; Cid et al., 1995; Qadota et al., 1996

Table 1. Continued

Protein name	Homologies and motifs	Endocytosis	High osmol.	Budding	Cyto-kinesis	References
Gic1p, Gic2p	CRIB domain	?	?	a, bem	?	Brown et al., 1997; Chen et al., 1997
Kar9p		?	?	+	?	Miller and Rose, 1998; Miller et al., 1999
Mlc1p	Myosin light chain	?	?	?	-	Stevens and Davis, 1998
Myo2p, Myo4p	Myosin V	+	+	+ or bem	+	Myo2p refs. above; Haarer et al., 1994; Jansen et al., 1996
Pea2p		?	?	b	?	Valtz and Herskowitz, 1996
Pfy1p	**Profilin**	+	sens	a and b	?	Haarer et al., 1990; Munn et al., 1995; Imamura et al., 1997; Ostrander et al., 1999
Rho1p	**RhoA**	?	supp	bem	?	McCaffrey et al., 1991; Yamochi et al., 1994; Drgonová et al., 1999
Rho3p	Rho GTPase	?	supp	?	?	Matsui and Toh-e, 1992a,b; Robinson et al., 1999a
Rom2p	RhoGEF PH domain	?	supp	?	sep or -	Ozaki et al., 1996; Manning et al., 1997
Sec1p	Sec1/UNC18/Rop	?	?	+	+	Garcia et al., 1994; Haarer et al., 1996; Finger and Novick, 1997; Carr et al., 1999
Sec3p Sec5p, Sec6p Sec8p, Sec10p, Sec15p, Exo70p, Exo84p	Exocyst	+	?	b	sep	TerBush and Novick, 1995; Ting et al., 1995; Hsu et al., 1996; Haarer et al., 1996; TerBush et al., 1996; Finger and Novick, 1997; Hicke et al., 1997; Finger et al., 1998; Guo et al., 1999b
Sec4p	Rab8	+	?	b	sep	Chavrier et al., 1990; Brennwald and Novick, 1993; Finger and Novick, 1997; Hicke et al., 1997
She3p	???	?	?	?	+	Jansen et al., 1996
Slt2p/Mpk1p	**MAPK**	+	supp	+	?	Lee et al., 1993; Mazzoni et al., 1993; Brown, 1997; Wang and Bretscher, 1997
Smy1p	Kinesin-related	?	+	?	+	Lillie and Brown, 1994
Spa2p, Sph1p	Spa2p-box	?	?	b	?	Snyder, 1989; Gehrung and Snyder, 1990; Zahner et al., 1996; Arkowitz and Lowe, 1997; Roemer et al., 1998
Yck2p	Casein kinase 1	-	?	a and b	-	Robinson et al., 1993, 1999b; Panek et al., 1997; Hicke et al., 1998
Wsc1p	Transmembrane	?	supp	?	?	Verna et al., 1997; Delley and Hall, 1999
Neck ring components						
Act1p	Actin	-	sens	b or bem	-	As above
Bni4p		?	?	?	sep	DeMarini et al., 1997
Bnr1p	Formin	+	+	a	+	Imamura et al., 1997; Kamei et al., 1998
(*bni1 bnr1*)		?	sens	bem	?	Imamura et al., 1997
Bud3p		?	?	a	+	Chant and Herskowitz, 1991; Chant et al., 1995; Roemeret al., 1996b
Bud4p		?	?	a	?	Chant and Herskowitz, 1991; Sanders and Herskowitz, 1996
Cdc3p, Cdc10p, Cdc11p, Cdc12p, Shs1p/Sep7p	Septins	?	?	a	-	Hartwell, 1971; Haarer and Pringle, 1987; Ford and Pringle, 1991; Kim et al., 1991;Flescher et al., 1993; Chant et al.,1995; Mino et al., 1998
Chs2p, Chs3p, Chs4p	Chitin synthase subunits	?	supp	?	sep	Shaw et al., 1991; Bulawa, 1992; Chuang and Schekman, 1996
Hsl1p, Gin4p, Kcc1p	Nim1-related kinases	?	+	+	+	Longtine et al., 1998; Barral et al., 1999
Hof1p/Cyk2p	PSTPIP	?	?	?	-	Kamei et al., 1998; Lippincott and Li, 1998a
Iqg1p/Cyk1p	IQ-GAP	?	supp	?	-	Epp and Chant, 1997; Lippincott and Li, 1998b
Pfy1p	**Profilin**	+	sens	?	?	As above
Myo1p	Myosin II	?	supp	a (mild)	-	Rodriguez and Patterson, 1990; Bi et al., 1998; Lippincott and Li, 1998b
Tpm1p, Tpm2p	**Tropomyosin**	+	+	+	+	As above
(*tpm1 tpm2*)		+	+	bem	-	As above

Protein name is the standard SGD designation. Common synonyms for the protein are listed after (e.g. Sla2p/End4p). Related isoforms are separated by commas (e.g. Cla4p, Ste20p, Skm1p). Underlined names indicate proteins that have not been localized to the relevant cytoskeletal domain but that interact directly with components of that structural domain and are likely to function there as well. Abbreviations: src homology 3 (SH3), actin-related protein (Arp), mitogen-activated protein kinase (MAPK), Wiskott-Aldrich Syndrome protein (WASP), cyclin-G-associated kinase (GAK), Huntingtin-interacting protein 1 (Hip1), adenylyl-cyclase-associated protein (CAP), WASP-interacting protein (WIP), clathrin assembly protein 180 (AP180), GTPase activating protein (GAP), guanine-nucleotide exchange factor (GEF), pleckstrin homology (PH), Cdc42p/Rac-interacting binding (CRIB),

Homologies and motifs indicate, where possible, sequences present in animal systems as well. **Bold** listing indicate that the yeast protein can be functionally replaced by an animal homolog.

Endocytosis designates the effect on endocytosis of the loss of function of a particular protein or set of proteins. '-' indicates a defect in fluid-phase endocytosis, receptor-mediated endocytosis or both. '+' indicates endocytosis is unaffected. '?' indicates the affect on endocytosis has not been reported.

High osmol. indicates the effects of high osmolarity medium on cells lacking function of a particular protein or set of proteins. 'sens' indicates inability or reduced ability to grow in high osmotic medium. 'supp' indicates high osmolarity enhances growth, in general by preventing cell lysis. '+' indicates no change in the osmotic sensitivity. '?' indicates the effect of osmolarity has not been reported.

Budding indicates the effects loss of function of a protein or set of proteins has on budding pattern. 'a' indicates axial budding in haploids no longer occurs. 'b' indicates bipolar budding in diploids is defective. 'bem' indicates bud emergence is blocked. '+' indicates wild-type budding pattern. '?' indicates the budding pattern has not been reported.

Cytokinesis indicates whether loss of function for a protein or set of protein affects cell division or separation. '-' indicates that cytokinesis, or separation of the cytoplasm, is defective. 'sep' indicates that cytokinesis is normal, but that separation of the cell wall of divided cells is defective. '- or sep' indicates that cells cannot divide, but that whether this is a failure of cytokinesis or separation has not been determined. '+' indicates that cytokinesis and separation are normal. '?' indicates that this function has not been reported.

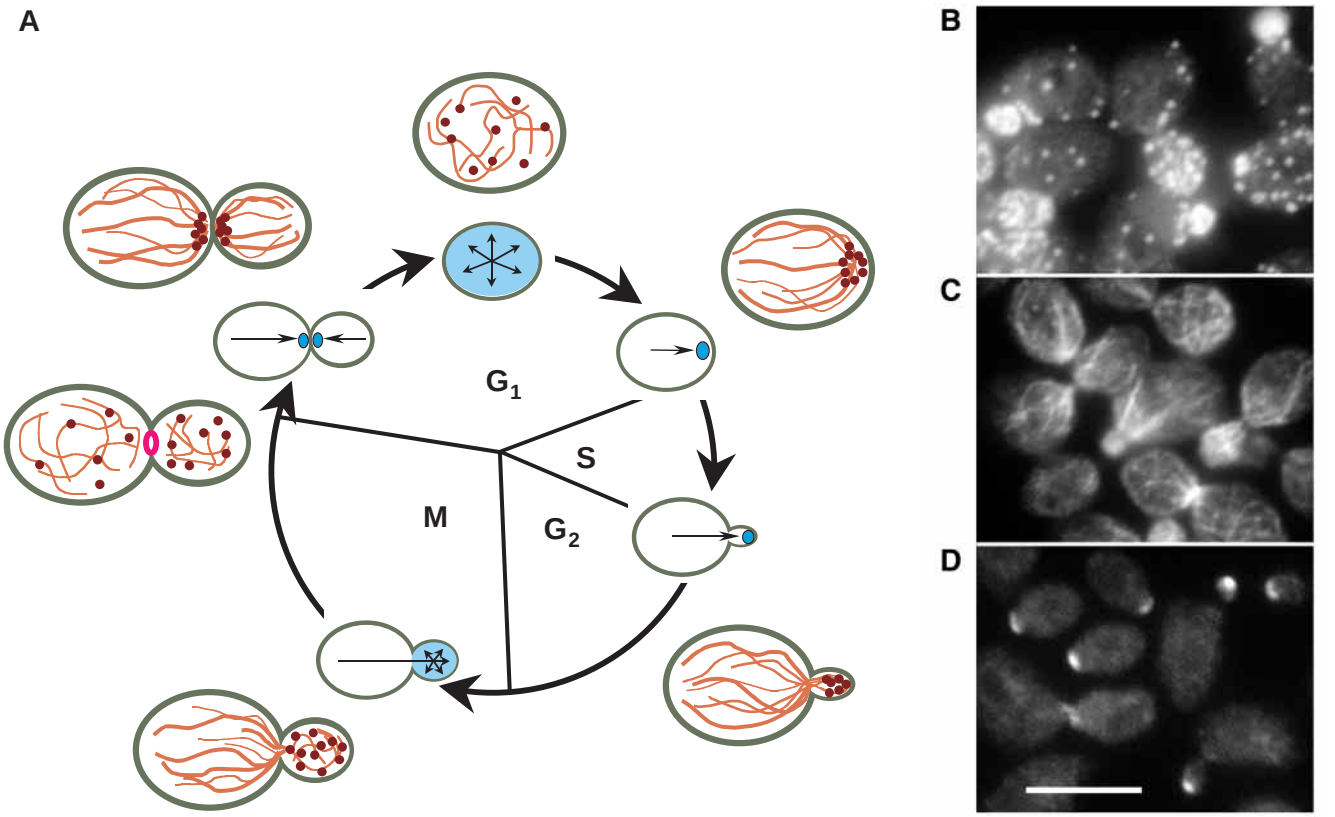

Fig. 1. (A) Cell polarity in budding yeast is established by a polarized actin cytoskeleton throughout the cell cycle. A cap (blue) of regulatory and cytoskeletal proteins establishes the polarity of actin cables (pink) and cortical patches (brown). Tight localization of the cap orients actin cables. Actin cables then guide secretory vesicles to the cap, where they accumulate (also blue) and fuse, thus polarizing growth (arrows). During isotropic growth, the proteins of the cap are more diffusely distributed, cortical patches are isotropically distributed, and actin cables form a meshwork. A fourth cytoskeletal structure, a cytokinetic ring, mediates cell division (bright pink). (B) Cortical patches are clustered about growth sites. Actin patches were visualized in *tpm1-2 tpm2Δ* cells that had been shifted to 34.5°C for one minute and prepared for immunofluorescence using anti-actin antibodies. Under these conditions, actin cables are specifically and completely disassembled, but cortical patches remain unchanged in appearance. (C) Actin cables form arrays oriented toward growth sites. Cells were prepared for immunofluorescence using anti-Tpm1p antibodies. (D) The cap is visualized in cells treated for immunofluorescence by antibodies against the cap-associated myosin Myo2p. Bar, 5 μm.

growth (Adams and Pringle, 1984; Lew and Reed, 1995; Amberg, 1998; Fig. 1). During apical growth, the tight cap at the growing tip overlaps a cluster of cortical patches, and actin cables from throughout the mother cell converge at this area. During isotropic bud growth, cap components and patches redistribute over the bud surface and cables extend from the mother cell into a network in the bud. Finally, after cytokinesis, all three components reorient to the mother-bud junction.

Yeast actin guides growth by directing the delivery of internal membranes and other factors (Bretscher et al., 1994; Finger and Novick, 1998). For example, post-Golgi secretory vesicles are transported by the actin cytoskeleton into the bud from Golgi elements dispersed throughout the mother. Depolymerization of F actin by the monomer-sequestering drug latrunculin A or depolarization of the cytoskeleton by mutation (e.g. *cdc24-4*) blocks polarized growth: mother cells abandon growing buds and grow isotropically into large round cells (Sloat et al., 1981; Ayscough et al., 1997). Conversely, mutations that hyperpolarize the actin cytoskeleton (e.g. *cla4Δ*) generate highly elongated buds (Cvrcková et al., 1995).

Divergent from animals and many fungi, budding yeast do not use microtubules to polarize growth, but only to control nuclear and chromosomal movements (for review, see Botstein et al., 1997). In animals, microtubules mediate long-range transport of membranous organelles to the cell periphery, whereas actin mediates short-range transport and anchorage (Langford, 1995; Brown, 1999). The tiny size of budding yeast cells may have made short-range actin-dependent transport sufficient.

ASSEMBLY AND POLARIZATION OF ACTIN CABLES AND CABLE-DEPENDENT MYOSINS

It has recently been established that actin cables are essential for viability in yeast. This contrasts with previous reports of viable cytoskeletal mutants apparently lacking actin cables, such as mutants that lack the genes that encode the actin-bundling protein fimbrin (Sac6p; Adams et al., 1991), tropomyosin (Tpm1p; Liu and Bretscher, 1989) or actin-capping protein β (Cap2p; Amatruda et al., 1990). However, more-recent studies using improved imaging techniques demonstrate that the *tpm1Δ* and *cap2Δ* mutants, at least, have truncated or very fine cables, which suggests that other viable

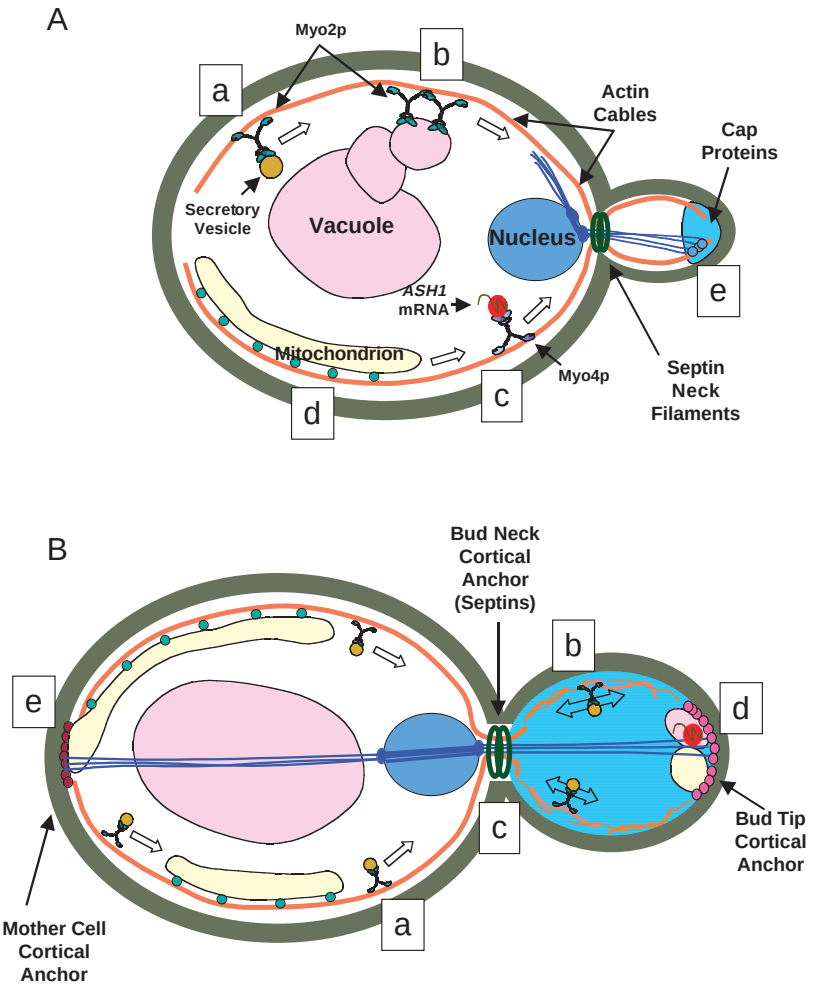

Fig. 2. A variety of cellular components are polarized through interactions with actin cables and the cell cortex. (A) During early bud growth, Myo2p transports (a) secretory vesicles and (b) vacuolar membranes from where they arise in the mother cell to the cap at the bud tip along actin cables. Myo4p delivers (c) mRNA encoding the transcriptional repressor Ash1p along cables as well, whereas (d) mitochondria migrate along actin cables by an unknown mechanism, a proportion of them entering the bud. (e) Cytoplasmic microtubules emanating from one pole of a short spindle enter the bud and impinge on the cap. Their anchorage at this point is dependent upon actin cables as well as polarisome proteins in the cap and the microtubule-binding protein Kar9p. (B) During later bud growth, the nature of transport and polarity changes. Within the mother (a) Myo2p continues to deliver secretory vesicles into the bud along actin cables, but within the bud (b) actin cables form a meshwork that randomizes Myo2p motions, which permits isotropic bud growth. Various anchors maintain polarities that were established by actin cables at earlier phases of the cell cycle. (c) Proteins secreted at the start of bud emergence remain anchored to the bud neck by a scaffold of septin neck filaments that was established at that time. (d) A cortical anchor that was established at the bud tip during earlier apical bud growth immobilizes *ASH1* mRNA, mitochondrial membranes and cytoplasmic microtubules at the bud tip. (e) Cortical anchors in the mother retain mitochondria within the mother and anchor cytoplasmic microtubules emanating from the other pole of the mitotic spindle.

'cable-less' mutants are likely to as well (Karpova et al., 1998; Pruyne et al., 1998). In contrast, loss of filamentous actin (Act1p) or the function of both tropomyosin isoforms (Tpm1p and Tpm2p) completely abolishes actin cables, resulting in a lethal arrest as large, unbudded cells (Ayscough et al., 1997; Pruyne et al., 1998).

Actin cables are highly dynamic structures containing actin (Act1p), fimbrin (Sac6p), and tropomyosin (Tpm1p, Tpm2p; Table 1). The actin present in actin cables is capable of rapid turnover, as demonstrated by the disassembly of cables within 15 seconds by the F-actin-depolymerizing drug latrunculin A (Karpova et al., 1998). Similarly, study of a rapidly reversible temperature-sensitive tropomyosin mutation (*tpm1-2 tpm2Δ*) shows that actin cables can disassemble and reassemble into polarized arrays in less than one minute in vivo (Pruyne et al., 1998).

Actin cables appear to be subjected to a balance between protection and stabilization by tropomyosin and disassembly by the cofilin (Cof1p)-Aip1p complex. Although not apparent under normal conditions, the association of cofilin with cables is revealed under conditions in which filament severing but not binding is inhibited. Thus, cofilin decorates actin cables in *tpm1Δ* cells bearing a depolymerization-resistant *act1*^{V159N} actin allele (Belmont and Drubin, 1998) and in severing-deficient *cof1-19* or *aip1Δ* mutants (Rodal et al., 1999).

Profilin (Pfy1p) is also required for normal cable assembly; it promotes actin polymerization by catalyzing ADP/ATP exchange on G actin (Haarer et al., 1990). A unique protein necessary for mitochondrial inheritance, Mdm20p, is also implicated in cable assembly, although the details of its action are unclear (Hermann et al., 1997). F actin nucleation by the Arp2p-Arp3p complex is not required for actin cable formation (Winter et al., 1999a).

The polarization of actin cable arrays is intimately linked to the polarity of cap proteins, a group of proteins so named for their localization during bud emergence and apical growth into a cap-like structure (Lew and Reed, 1995; Fig. 1). Although not as heavily enriched in F actin as cortical patches or cables, the cap plays an essential role in the regulation of overall cytoskeletal polarity and includes proteins involved in the Rho-GTPase- and CDK-signaling pathways (e.g. Cdc42p, Cdc24p, Bem1p, Ste20p, Cla4p and polarisome proteins Bni1p, Spa2p, Bud6p/Aip3p; Table 1). Regulation by these pathways is the subject of Part I of this review (Pruyne and Bretscher, 2000).

These cap proteins appear to function as nucleation and anchor sites for actin cables. Thus, cables orient towards the cap proteins during all phases of growth (Fig. 1), but form disorganized networks in the absence of essential cap proteins. An example of such a putative actin-cable-nucleating anchor is the cap-associated formin Bni1p, which binds to profilin (Imamura et al., 1997): overexpression of delocalized profilin-

binding fragments of Bni1p generates excess disorganized actin cables (Evangelista et al., 1997). However, because *bni1Δ* cells have only modest cytoskeletal-polarity defects, additional cap proteins that remain to be identified must contribute to cable organization.

A second group of proteins occupies the cap as a consequence of actin cable polarity. These include two myosin V heavy chains (Myo2p and Myo4p) and their associated light chains (Cmd1p and Mlc1p; Table 1). These dimeric myosins polarize by translocating along cables, driven by N-terminal motor domains. Accordingly, Myo2p can be seen associated with actin cables in addition to its localization at the cap (Schott et al., 1999). After the loss of cables in a conditional tropomyosin mutant (*tpm1-2 tpm2Δ*), Myo2p delocalizes from the cap rapidly, whereas within one minute of cable reassembly Myo2p relocalizes (Pruyne et al., 1998). Similarly, a temperature-sensitive mutation in the Myo2p motor domain (*myo2-66*) causes Myo2-66p to delocalize from the cap within five minutes of a temperature shift, despite the presence of a polarized actin cytoskeleton and no myosin degradation (Schott et al., 1999). Myo4p-dependent transport is also blocked in cable-defective *act1-133*, *tpm1Δ* and *pfy1-111* mutants (Long et al., 1997). Given that class V myosins are processive, barbed-end-directed motors (Wolenski et al., 1995; Mehta et al., 1999), these results suggest that the actin filaments in cables are of uniform polarity, with their barbed ends directed towards the cap.

Myo2p also associates with cap proteins independently of motor activity. Thus, in 20% of cells depleted of F actin by latrunculin A, a small amount of Myo2p localizes to the cap (Ayscough et al., 1997). Furthermore, ectopically expressed Myo2p coiled-coil and C-terminal tail domains localize to the cap (see below for the likely mechanism of recruitment) and can remain there even after full-length Myo2p becomes delocalized (Reck-Peterson et al., 1999). A direct interaction between the cap protein Rho3p and the coiled-coil of Myo2p might be responsible (Robinson et al., 1999a). However, these interactions are unlikely to be the normal mechanism by which Myo2p polarizes, because motor-dependent Myo2p polarization is extremely robust compared with actin-independent polarization (Pruyne et al., 1998; Schott et al., 1999). This suggests that these interactions instead are involved in myosin regulation or other functions.

POLARIZED SECRETION

Polarized growth at the yeast cell surface depends upon delivery of secretory vesicles along actin cables by Myo2p (Fig. 2A). This rapid transport of vesicles from Golgi and endosomal elements in the mother into the bud leads to their accumulation at the cap (Govindan et al., 1995; Chuang and Schekman, 1996; Santos and Snyder, 1997). After the loss of cables in a *tpm1-2 tpm2Δ* mutant or the loss of *myo2-66* motor activity, secretory vesicles rapidly vanish from the cap: previously delivered vesicles presumably fuse with the plasma membrane, whereas new vesicles are no longer delivered. This depolarizes growth and cell wall synthesis, and causes both mutants to enlarge isotropically (Johnston et al., 1991; Govindan et al., 1995; Pruyne et al., 1998; Schott et al., 1999; Fig. 3). Myo2p-driven transport along actin cables is rapid: in the case of cable reassembly in a *tpm1-2 tpm2Δ* mutant, vesicles re-accumulate at the cap within one minute in a Myo2p-dependent manner. Notably, this polarization of vesicles occurs independently of the distribution of cortical patches within the cell (Pruyne et al., 1998), which indicates that patches do not play a direct role in targeting vesicles to the cell surface.

The Myo2p C-terminal tail mediates the association with vesicles. Thus, conditional tail mutants (e.g. *myo2-16*) depolarize vesicles rapidly under restrictive conditions, although the myosin still translocates along cables to the cap (Schott et al., 1999). Furthermore, fusion proteins containing the C-terminal Myo2p tail polarize to the cap by piggy-backing on secretory vesicles delivered by full length Myo2p, and a high level of expression of the Myo2p tail actually depolarizes growth, presumably by saturating the vesicular Myo2p-binding sites (Schott et al., 1999).

Three proteins likely to be involved in assembling Myo2p-vesicle complexes are Smy1p, Sec2p and Sec4p. Defects in these all are synthetically lethal, which suggests that their functions are interrelated (Lillie and Brown, 1992; Govindan et al., 1995; Lillie and Brown, 1998; Schott et al., 1999). Smy1p is a divergent kinesin heavy chain homolog that binds to the Myo2p tail and is polarized to the cap by the myosin (Lillie and Brown, 1992, 1994; Brown, 1999). Smy1p is not essential for polarized growth in yeast, but its overproduction enhances the polarization of Myo2p and suppresses partial defects in myosin mutants (Lillie and Brown, 1992, 1994; Schott et al., 1999). Despite its homology to kinesin, Smy1p functions independently of microtubules or its kinesin motor activity (Lillie and Brown, 1998). Rather, it might play a conserved role in promoting the assembly of transport complexes that in fungal ancestors utilized microtubules and microfilaments. The fact that a mouse myosin V interacts directly with a functional kinesin through residues that are partially conserved in Smy1p supports such a hypothesis (Lillie and Brown, 1994; Brown, 1999; Huang et al., 1999).

Sec4p is a Rab GTPase that is essential for fusion of secretory vesicles with the plasma membrane. Sec4p, being bound to vesicles, also polarizes to the cap in a Myo2p-dependent manner (Walch-Solimena et al., 1997; Schott et al., 1999). Sec2p, the Sec4p nucleotide-exchange factor, is required for delivery of vesicles along actin cables; this suggests that Sec2p or GTP-Sec4p on vesicles promotes Myo2p-Smy1p binding (Walch-Solimena et al., 1997).

A second polarizing factor at the cap reinforces directed vesicular transport: the exocyst. Exocytosis in yeast requires plasma membrane t-SNAREs, a t-SNARE-interacting protein Sec1p, and a complex termed the exocyst (reviewed by Finger and Novick, 1998). Although t-SNAREs are distributed isotropically over the cell surface (Brennwald et al., 1994), polarization of Sec1p and regulated assembly of the exocyst at the cap appears to confine exocytosis to this region. The exocyst protein Sec3p localizes to the cap (by an unknown mechanism) to establish a landmark for assembly (Finger et al., 1998), whereas the localization of other components (e.g. Sec8p, Sec10p, Sec15p) depends on with the arrival of vesicle-bound GTP-Sec4p (Guo et al., 1999a). Similarly, the arrival of Sec1p depends upon Sec4p (Carr et al., 1999). The exact mechanism of exocyst-Sec1p-SNARE function is unclear, but GTP-Sec4p-dependence ensures that only vesicles competent

for Myo2p delivery fuse with the Sec3p-defined docking site; this reinforces polarity.

OTHER POLARIZATION EVENTS: TRANSPORT ALONG ACTIN CABLES FOLLOWED BY CORTICAL ANCHORAGE

Actin cables and the cap proteins guide the majority, if not all, polarizing events in yeast, including organelle inheritance, mRNA sequestration and orientation of the mitotic spindle. A general theme is cable-dependent transport (Fig. 2A) followed by anchorage at the cell cortex (Fig. 2B).

Through a mechanism similar to that which it uses to guide secretory vesicles, Myo2p polarizes vacuolar membranes into the bud (Hill et al., 1996): it again associates with membranes through its C-terminal tail, but probably uses a region distinct from the secretory-vesicle-association site (Catlett and Weisman, 1998). It will be interesting to determine whether other secretory membranes that enter the bud early (i.e. ER and Golgi elements) also depend upon Myo2p.

Myo4p transports mRNA encoding the transcriptional repressor Ash1p into the bud (Bobola et al., 1996; Long et al., 1997; Takizawa et al., 1997). This allows mother and daughter cells to adopt different developmental fates during mating-type switching and, possibly, pseudohyphal differentiation (Jansen et al., 1996; Bobola et al., 1996; Sil and Herskowitz, 1996; Chandarlapaty and Errede, 1998). Myo4p-mRNA association requires structural elements in the mRNA 3′ UTR and two other proteins (She2p and She3p; Gonzalez et al., 1999; Münchow et al., 1999). Whether other mRNAs become polarized by this mechanism is not known.

Mitochondria also migrate along actin cables (Simon et al., 1997), using integral membrane proteins (Mmm1p and Mdm10p) to dock with an ATP-sensitive, non-myosin, actin-based motor of unknown identity (Simon et al., 1995; Boldogh et al., 1998). The mitotic spindle is also oriented in a cable-dependent manner during early bud emergence (Theesfield et al., 1999).

Once delivered, many factors are anchored to the cell cortex at discrete locations (Fig. 2B). Anchorage of secreted membrane proteins (e.g. chitin synthase III Chs3p), mitochondria, *ASH1* mRNA and cytoplasmic microtubules has been demonstrated (DeMarini et al., 1997; Simon et al., 1997; Long et al., 1997; Takizawa et al., 1997; Theesfield et al., 1999). Cortical anchors also establish tags that guide future budding events (reviewed by Chant, 1999).

Depending on their locations in the cell, different anchors require different structural proteins. Many anchors located at bud tips, including those for *ASH1* mRNA, the spindle and bipolar budding tags, depend upon polarisome proteins, particularly the formin Bni1p (Lee et al., 1999; Miller et al., 1999; Theesfield et al., 1999; Beach et al., 1999; Chant, 1999). This suggests that apical growth is important for the establishment of these anchors. Anchors at the bud neck, as in the case of chitin synthase III and the axial budding tags Bud3p and Bud4p, require a scaffold of septin filaments and, sometimes, the neck-associated formin Bnr1p (DeMarini et al., 1997; Kamei et al., 1998; Chant, 1999). Anchors also retain structures in the mother cell during growth, as has been shown for mitochondria (Yang et al., 1999a).

The molecular identity of most of the cortical anchors remains unknown. The best studied is that for chitin synthase III, which uses two proteins, Chs4p and Bni4p, to link with the septin-neck-filament scaffold (DeMarini et al., 1997). Kar9p is a strong candidate for the link between cytoplasmic microtubules and the early actin-dependent cortical anchor (Miller and Rose, 1998; Miller et al., 1999). Finally, the phenotypes caused by mutations in two membrane proteins (Bud8p and Bud9p) make them possible tags for bipolar budding cues (Zhaner et al., 1996).

CORTICAL PATCH FUNCTION: ENDOCYTOSIS AND CELL WALL MORPHOGENESIS

Cortical patches are another type of major actin cytoskeletal structure in yeast. They exhibit great biochemical complexity (Table 1; Fig. 4), and are associated with invaginations of the plasma membrane (Mulholland et al., 1994). Cortical patches alternatively assume either highly mobile or stationary states (Doyle and Botstein, 1996; Waddle et al., 1996). Generally clustered near regions of exocytosis in growing cells (Adams and Pringle, 1984; Fig. 1), cortical patches are essential to normal growth; loss of single or combinations of components is lethal (reviewed by Botstein et al., 1997).

The mobility of cortical patches seems at odds with their association with plasma membrane invaginations. Although myosins are not required for patch motility (Waddle et al., 1996), the actin-nucleating Arp2p-Arp3p complex is (Winter et al., 1997), which suggests that F actin is important. However, motility is probably not through a *Listeria*-like mechanism, given that stabilization of actin filaments in the $act1^{V159N}$ mutant or in *cof1-22* cells does not alter patch dynamics (Lappalainen and Drubin, 1997; Belmont and Drubin, 1998).

Rather, the dependence upon actin appears to be complex. In latrunculin-A-treated cells, patches do not appear to slow down gradually as they are depleted of F actin (Belmont and Drubin, 1998). However, after prolonged treatment with latrunculin A, cortical patches completely lacking F actin but retaining proteins such as Rvs167p are immobile (Balguerie et al., 1999). This suggests that F actin is required for the initial recruitment of a still-mysterious motility factor that, once associated with patches, remains until after patch disassembly is complete.

The vital function of cortical patch proteins is unknown, but there is a remarkable correlation between mutations in patch components and defects in the internalization step of endocytosis (Table 1). Two recent reviews (Geli and Riezman, 1998; Wendland et al., 1998) provide insight into some of the roles that the patch proteins might play in the internalization process.

The best-studied internalization event is the uptake of the pheromone-receptor, which occurs as part of its down-regulation after pheromone-stimulation (Schandel and Jenness, 1994). Interestingly, pheromone-receptor uptake occurs at membrane invaginations distinct from cortical patch membrane invaginations, even though patch proteins are still required (Mulholland et al., 1999). A possible explanation is that low concentrations of patch proteins mediate endocytosis at receptor-internalization sites, whereas their more visible localization to cortical patches is a consequence of a higher

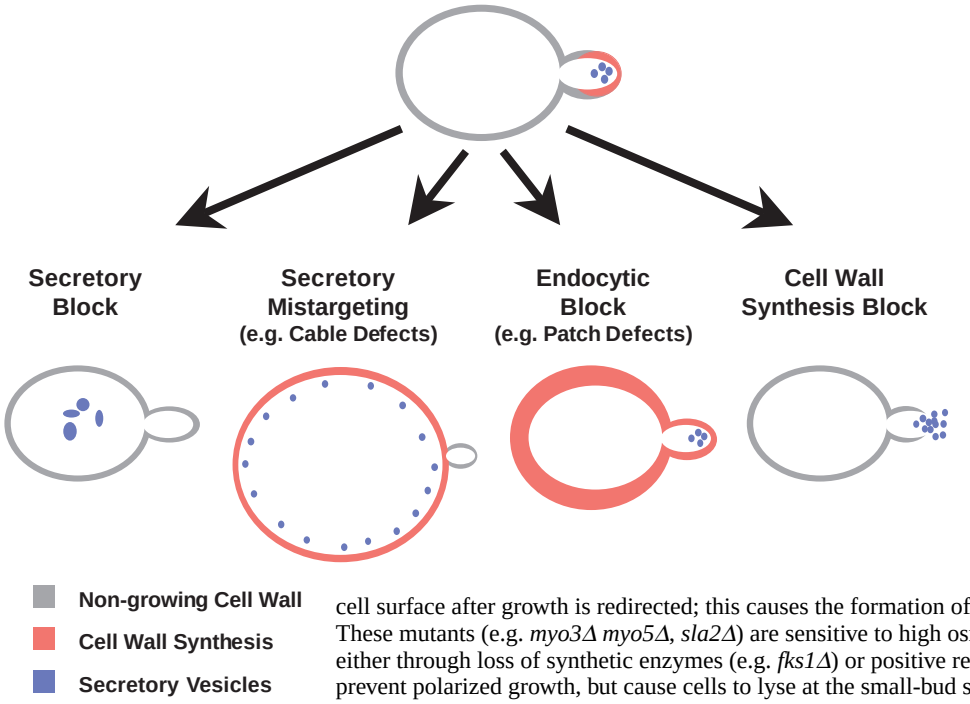

Fig. 3. Polarized growth defects in yeast fall into four classes. Secretory Blocks prevent all export, blocking cell expansion and cell wall synthesis. The sec mutants that comprise this class are involved in packaging of cargo into transport intermediates, and membrane fusion and fission events. Secretory Mistargeting leads to isotropic growth and isotropic cell wall synthesis. Such mutants include defects in Cdc42p signaling (e.g. *cdc24-4*, *cdc42-1*) and in actin cable structure and function (e.g. *tpm1-2 tpm2Δ*, *myo2-16*). Defects in cortical patch components generally exhibit Endocytic Blocks. Polarized growth occurs, but factors involved in wall synthesis are not cleared from the cell surface after growth is redirected; this causes the formation of multiple cell wall layers in the mother. These mutants (e.g. *myo3Δ myo5Δ*, *sla2Δ*) are sensitive to high osmolarity. Cell Wall Synthesis Blocks, either through loss of synthetic enzymes (e.g. *fks1Δ*) or positive regulators (e.g. *rho1-104*, *pkc1Δ*), do not prevent polarized growth, but cause cells to lyse at the small-bud stage and are sensitive to low osmolarity.

concentration of patch proteins at these sites. Given that cortical patches also associate with membrane invaginations (Mulholland et al., 1994), they are likely to be sites that mediate internalization events other than pheromone-receptor uptake.

The polarized clustering of cortical patches suggests that they play a role in polarized growth. The phenotypes of various mutants indicate that endocytic cycling by patch proteins is important for regulation of cell wall assembly and for recycling of secreted proteins and lipids. Proper wall assembly requires tight regulation of synthetic enzymes such as chitin and glucan synthases. Polarization of these integral membrane proteins requires not only actin-cable-dependent polarized secretion and anchorage by cortical determinants, but also eventual re-internalization. In the absence of this last step, wall synthesis would be expected to continue inappropriately after scaffolds are disassembled and growth is redirected. Accordingly, in many patch mutants (e.g. *act1-1*, *myo3Δ myo5Δ*, *sla1Δ*, *sla2Δ*), mother cells generate multiply layered cell walls and accumulate chitin diffusely over their surface (Goodson et al., 1996; Ziman et al., 1996; Mulholland et al., 1997; Ayscough et al., 1999; Fig. 3). Patch mutants are also unable to grow in high osmolarity medium (Table 1), which may reflect their need to develop a higher turgor pressure within the yeast cell to breach the multiple cell wall layers for bud emergence.

Mammalian homologs of patch proteins mediate endocytic recycling of lipids, v-SNAREs and other components at synaptic membranes, and sustain the high levels of secretion there (Geli and Riezman; Table 1). In yeast, such recycling also appears to be important, particularly at elevated temperatures when growth rates increase. Mutants in which endocytosis is blocked in general show temperature-sensitive growth (e.g. *end3Δ*, *sla2Δ*, *myo3Δ myo5Δ*, *las17Δ*, *vrp1Δ*, *rvs161Δ*, *rvs167Δ*, *she4Δ*; Bénédetti et al., 1994; Munn et al., 1995; Zoladek et al., 1995; Geli and Riezman, 1996; Goodson et al., 1996; Wendland et al., 1996; Li, 1997). Furthermore, several patch mutants (*act1-1*, *myo3Δ myo5Δ*, *sla2Δ*) develop a partial block in the secretion of invertase (Novick and Botstein, 1985; Goodson et al., 1996; Mulholland et al., 1997).

This secretory block appears to occur after the Golgi but before formation of the final Sec4p-containing exocytic vesicles. The branch of the late secretory pathway responsible for the export of invertase is thought to pass through an endosomal recycling compartment on its way from the Golgi to the plasma membrane (Harsay and Bretscher, 1995; Harsay and Schekman, 1998; Holthuis et al., 1998a,b; Henkel et al., 1999). The partial block in invertase export in patch mutants is consistent with the depletion of this compartment as a consequence of an endocytic block.

Patch mutants show other growth defects. Many grow large rounded cells and are unable to establish bipolar budding patterns or to generate elongated filamentous cells (Mösch and Fink, 1997; Yang et al., 1997; Table 1). These polarity defects are not due to the inability to target secretory vesicles: vesicles polarize to growth sites even when cortical patches are isotropically distributed (Pruyne et al., 1998). Many patch mutants are also sensitive to nutrient starvation and are unable to sporulate or to grow on non-fermentable medium, which indicates that they possess defects in stress responses and in mitochondrial function (Crouzet et al., 1991; Gerst et al., 1991; Bauer et al., 1993; Munn et al., 1995; Sivadon et al., 1995; Zoladek et al., 1995; Lila and Drubin, 1997; Vaduva et al., 1997). The basis of these particular defects remains to be determined.

CORTICAL PATCH ASSEMBLY AND REGULATION

Cortical patch assembly appears to be hierarchical: association of assembly factors with the plasma membrane is followed by

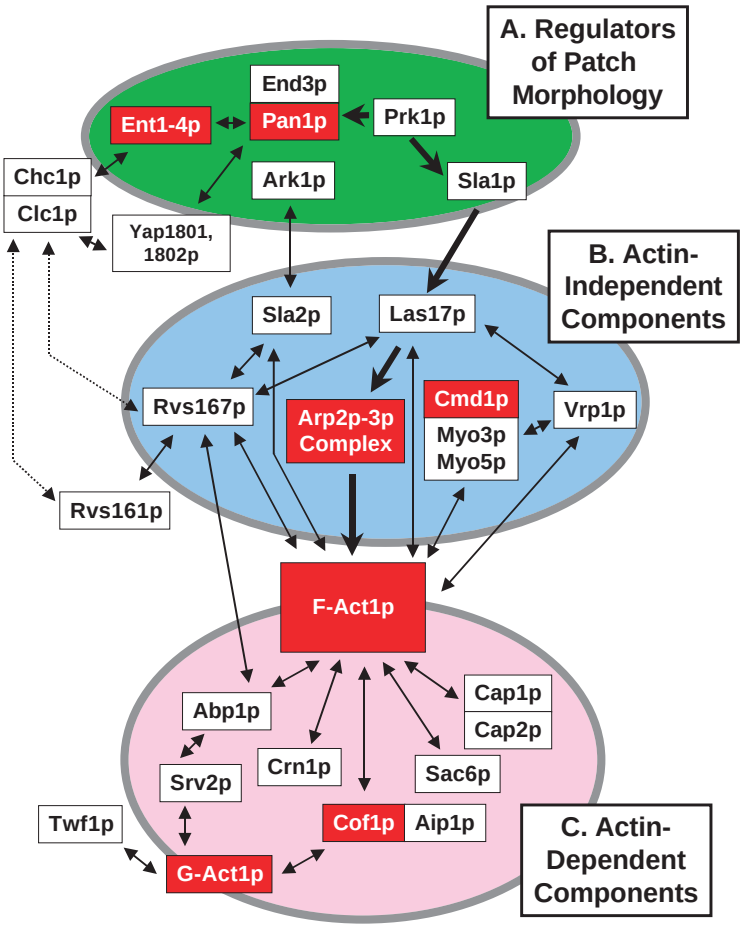

Fig. 4. A bewildering number of proteins localize to cortical patches. Chc1p, Clc1p, Cmd1p, Myo3p, Ent1-4p and End3p are suspected to localize to cortical patches, whereas the remainder of the proteins depicted here have been demonstrated to do so. Some patch components are essential or nearly essential (red), whereas others provide redundant functions (white). Double arrows show the many protein-protein interactions that have been demonstrated in vitro or implied from two-hybrid assays or coimmunoprecipitation. Different patch components share different features, such as mutant phenotypes or localization in latrunculin A; this leads us to propose three functional categories of patch components. (A) Regulators of patch morphology (green oval). (B) Actin-independent components (blue oval). (C) Actin-dependent components (pink oval). (non-assigned components are outside the ovals). Class A proteins are required for normal patch formation: in their absence, actin cytoskeletal proteins aggregate into cortical and cytoplasmic globs. Class A proteins include two kinases, Prk1p and Ark1p, and several proteins bearing Prk1p-consensus sites (Pan1p, Sla1p, Ent1-4p; Wendland et al., 1996, 1999; Cope et al., 1999; Zeng and Cai, 1999). Class B components associate with patches either in the presence or in the absence of F actin, but require the activity of the Class A proteins. Included in these are the components of the Arp2p-Arp3p complex, which nucleate actin filaments and allow incorporation of F actin and the Class C actin-dependent patch proteins. The best-documented example of this hierarchical assembly is shown by the thick arrows. Sla1p is a Class A scaffold protein required for the cortical patch association of Las17p and Arp2p (Class B) as well as F actin, Abp1p and Cof1p (Class C). In its absence, these proteins form aberrant globs at the cell cortex (Holtzman et al., 1993; Ayscough et al., 1999). Once associated with patches, Las17p can stimulate the Arp2p-Arp3p complex to nucleate actin filaments, allowing F actin incorporation and binding of Abp1p and Cof1p. In the absence of patch-associated F actin (e.g. in the *arp2Δ* or (in some genetic backgrounds) *las17Δ* mutants or because of latrunculin A treatment), Sla1p, Arp2p, and Las17p can still localize to cortical patches, whereas Abp1p and Cof1p cannot (Ayscough et al., 1997; Li, 1997; Winter et al., 1999a,b). Investigation of the interdependence of various patch components for localization is far from complete, and undoubtedly many refinements will be made through time to models such as this one. (Drubin et al., 1988; Amatruda and Cooper, 1992; Iida et al., 1993; Moon et al., 1993; Amberg et al., 1995; Freeman et al., 1995, 1996; Ayscough et al., 1997; Li, 1997; Lila and Drubin, 1997; McCann and Craig, 1997; Novarro et al., 1997; Tang et al., 1997; Vaduva et al., 1997; Anderson et al., 1998; Brizzio et al., 1998; Geli et al., 1998; Goode et al., 1998, 1999; Heil-Chapdelaine et al., 1998; Naqvi et al., 1998; Wendland and Emr, 1998; Balguerie et al., 1999; Colwill et al., 1999; Cope et al., 1999; Madania et al., 1999; Rodal et al., 1999; Wendland et al., 1999; Winter et al., 1999a,b; Zeng and Cai, 1999).

recruitment of actin-nucleating factors and then incorporation of actin filaments and actin-dependent proteins (Fig. 4). Loss of one factor prevents the incorporation of dependent proteins into patches, often causing them to aggregate at the cortex or in the cytoplasm. For example, the patch-scaffold protein Sla1p is required for incorporation of some proteins (e.g. the Arp2p-Arp3p complex and the WASP-related Las17p/Bee1p) but not others (e.g. the talin-related protein Sla2p/End4p), and in *sla1Δ* cells Sla2p is still associated with cortical patches, whereas Arp2p and Las17p are associated with aberrant cortical globs (Holtzman et al., 1993; Ayscough et al., 1999). In turn, Las17p stimulates the Arp2p-Arp3p complex to nucleate F actin upon patches, which allows the recruitment of actin-dependent proteins such as fimbrin, cofilin and capping protein (Li, 1997; Ayscough et al., 1997; Winter et al., 1999a,b). Finally, cofilin-Aip1p severing and monomer sequestration by twinfilin (Twf1p) drive patch disassembly (Lappalainen and Drubin, 1997; Goode et al., 1998; Rodal et al., 1999). As in the case of actin cables, F actin turnover in cortical patches is highly dynamic: latrunculin A studies show that patch-associated F actin turns over within 10 seconds (Karpova et al., 1998).

Several clues suggest that cortical patch assembly at the plasma membrane is initiated through interactions with proteins targeted for internalization. In particular, cortical patch proteins essential for patch assembly include yeast homologs of mammalian clathrin-adaptor-associated proteins Eps15 (yeast Pan1p and End3p) and espin (Ent1p, Ent2p, Ent3p, Ent4p; Table 1). Whereas complete loss of these proteins is lethal, partial loss of function causes F actin to associate with the plasma membrane only at a few locations; it assembles into a few cortical globs, rather than many cortical patches (Bénédetti et al., 1994; Tang and Cai, 1996; Wendland et al., 1996, 1999). Two kinases, Ark1p and Prk1p, function in this process, probably in part through phosphorylation of the Eps15 homolog Pan1p. In the absence of Ark1p and Prk1p, many cytoskeletal proteins do not associate with the cortex at all but form cytoplasmic aggregates (Cope et al., 1999; Zeng and Cai, 1999).

This model of patch assembly upon internalization targets is also consistent with the polarized distribution of cortical patches. For example, glucan synthase (Fks1p), the major enzyme for cell wall synthesis, is a plasma membrane protein localized at growth sites that would require internalization after functioning (Qadota et al., 1996). The secretory pathway delivers Fks1p and other such proteins and, when secretion is depolarized, patches would be expected to redistribute isotropically. This is the phenotype seen in conditional *myo2-16* and *tpm1-2 tpm2Δ* mutants (Pruyne et al., 1998; Schott et al., 1999). Similarly, when secretion is repolarized after extended depolarization, patches gradually cluster back near growth sites (Pruyne et al., 1998).

The observation that blocks in endocytosis universally depolarize cortical patches (see refs. in Table 1) is also consistent with this model, and is expected if patch assembly factors cannot be removed from old growth sites. Finally, long-term secretory blocks (e.g. *sec3-101* and *sec4-8*) cause patch depolarization and aggregation of cytoskeletal proteins in the cytoplasm (Lillie and Brown, 1994; Haarer et al., 1996; Mulholland et al., 1997). Again, this would be expected given a failure to transport patch assembly factors to the cell surface.

Regulation of patch assembly and function is also likely to occur through direct interactions between regulatory proteins of the cap and cortical patch proteins. Such interactions might either anchor patches, or activate either patch assembly or patch function. In vivo assays show that cap-associated Rho-GTPase-kinase complexes Cdc42p-Cla4p and Cdc42p-Ste20p stimulate incorporation of F actin into patches (Eby et al., 1998). Two known targets of these kinases are the patch-associated myosin I heavy chains Myo3p and Myo5p (Wu et al., 1997). The patch component verprolin (Vrp1p/End5p) can associate directly with the cap: in the absence of F actin and polarized secretion (caused by latrunculin A treatment), verprolin redistributes to the cap (Vaduva et al., 1997). Other patch proteins (Rvs167p, Las17p) also remain polarized in the presence of latrunculin A (Madania et al., 1999; Balguerie et al., 1999), although it is not clear whether they do so through direct interaction with the cap or through association with patches previously polarized but immobilized by the loss of F actin.

Other signaling pathways impinge upon cortical patch function. For example, the Pho85p-Pcl2p CDK-cyclin complex phosphorylates the patch amphiphysin Rvs167p (Lee et al., 1998), whereas the adenylyl-cyclase-associated protein (CAP) Srv2p, involved in Ras2p-dependent signaling, is also a cortical patch component (Freeman et al., 1996). Interestingly, Pho85p-Pcl2p and Ras2p signaling pathways are required for the responses to nutritional stresses that also depend upon cortical patch proteins (see Table 1 for references). The convergence of Rho GTPase, CDK and Ras signals upon cortical patches suggests that the regulation of cortical patches is rich and complicated.

CONCLUSIONS

The functional organization of the actin cytoskeleton in yeast is beginning to emerge. As has been demonstrated repeatedly throughout the eukaryotes, microfilaments at the cell cortex provide the overall polarity that guides a variety of events, such as vesicular fusion, organelle localization, mRNA anchorage and mitotic spindle orientation. In yeast, a cap of cytoskeletal and regulatory proteins establishes overall polarity, whereas a network of actin cables is the primary structure that polarizes the cell towards the cap, guiding active transport by class V myosins and other cargo-bearing motors. Cortical patches probably maintain polarity by recycling components such as enzymes necessary for cell wall synthesis that, having functioned, must now be removed from the plasma membrane as growth is redirected through the cell cycle. Given the availability of the complete yeast genome sequence and the easy molecular and classic genetics, it should not be long before we reach a coherent understanding of the molecular mechanisms involved in polarization of growth in budding yeast.

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