REVIEW

The control of septum formation in fission yeast

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If cell division is to produce two daughter cells that are viable, faithful copies of the parent cell, the landmark events of the cell division cycle, S phase, mitosis, and cytokinesis, must be executed in the correct order and with high fidelity. Though considerable advances have been made toward understanding the mechanisms that control the onset of S phase and mitosis, regulation of the events that occur at the end of the cell cycle, such as the reorganization of the cytoskeleton and the initiation of cell separation or cytokinesis, are less well understood. The fission yeast *Schizosaccharomyces pombe* provides a simple eukaryotic model for the study of cytokinesis. *S. pombe* cells are rod shaped, grow mainly by elongation at their ends, and divide by binary fission after forming a centrally placed division septum. Study of *S. pombe* mutants has begun to shed light on how septum formation and cytokinesis are regulated both spatially and temporally, to achieve proper co-ordination with mitosis. Some of the genes defined by these mutants have been functionally conserved through eukaryotic evolution, suggesting that aspects of this control will be common to all eukaryotic cells.

Septum formation and cytokinesis in the fission yeast *S. pombe*

The main morphological events of septum formation and cytokinesis in *S. pombe* are shown in Figure 1. As in higher eukaryotes, the fission yeast cytoskeleton undergoes a characteristic series of rearrangements during mitosis and cytokinesis (for review, see Robinow and Hyams 1989). The distribution of F-actin in *S. pombe* cells is linked intimately with sites of growth and septum formation. During interphase, F-actin is observed mainly as cortical patches at the growing ends of the cell, though actin cables can also be seen (Marks and Hyams 1985; Balasubramanian et al. 1996). Structural studies of cortical actin have demonstrated that it is composed of spiral actin filaments that are associated with an invagination of the plasma membrane (Mulholland et al. 1994). During the early stages of mitosis, before any visible

chromosome separation, a structure called the medial ring forms at the center of the cell, overlying the nucleus. The position of this ring anticipates the site of septum formation. This ring is composed of F-actin and many other components (see below). F-actin patches are subsequently polarized to the medial ring, and the cell is thus primed for septation. At the end of anaphase, when the spindle begins to break down, biosynthesis of the septum is initiated, and the primary septum grows inward from the cell cortex (Johnson et al. 1973). As its biosynthesis proceeds, it is surrounded by F-actin patches (Marks and Hyams 1985), and a ring of F-actin is seen at its leading edge (Jochová et al. 1991). In electron micrographs, F-actin filaments are seen between the edges of the developing septum (Kanbe et al. 1989). The completed septum has a three-layered structure, composed of the inner, primary septum, flanked by the two secondary septa. The primary septum can be visualized by Calcuofluor staining. Fission begins by degradation of the outer cell wall opposite the site of the completed septum and is completed by centripetal degradation of the primary septum to bring about cell separation (Johnson et al. 1973). At this time, the F-actin patches are relocated to the old (pre-existing) end of the cell, from where growth will resume.

S. pombe mutants defective in septum formation or cytokinesis

One of the considerable advantages of a simple model system, such as yeast, is that genes that encode proteins important for the regulation or execution of complex processes can be identified through the study of mutants. A small number of heat-sensitive, cell-division-defective mutants were identified in early screens (e.g., Nurse et al. 1976). Recently, the mutant set has been expanded considerably by the use of a method involving selection for cells that increase ploidy (e.g., Chang et al. 1996). Originally designed to enrich for mutants that rereplicated their DNA, this elegant screen is based on the use of cells that cannot mate but are able to undergo meiosis if they are able to diploidize by other means (Broek et al. 1991). This can occur by rereplication of DNA, by failure to separate the daughter chromosomes before septation, or by failure to synthesize, or correctly position, the di-

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vision septum. The two latter classes increase in ploidy by having two nuclei in the same cytoplasm. Thus, the screen enriches for mutants defective in septum formation. The following sections describe some *S. pombe* cell cycle mutants that show terminal phenotypes indicating that they are unable to execute events important for one or more of the stages in septum formation and cell cleavage described in Figure 1. They are listed in Table 1, and a schematic representation of some of the phenotypes produced by loss-of-function mutations in these genes is shown in Figure 2.

Nomenclature

The original report of fission yeast cell cycle mutants described two categories of septation mutants, called "early" and "late" (Nurse et al. 1976). Early mutants were those that failed to make a septum at all, whereas late mutants arrested after forming a disorganized septum. This terminology assumed (logically enough) that early mutants were defective in an early event of septum formation, whereas late mutants were defective in an event after the initiation of septum biosynthesis. Subsequent analysis of the mutants and cloning of the genes they defined have demonstrated that this is not the case (see below). Therefore, this terminology is not used. Furthermore, throughout this review, "medial ring," rather than "actin ring," is used to describe the structure formed at the cell cortex early in mitosis, because it contains many proteins in addition to actin.

The medial ring

Mutants defective in assembly of the medial ring

Analysis of mutants has shown that many components are involved in the assembly of the medial ring. These include actin (*act1:* Ishiguro and Kobayashi 1996; McCollum et al. 1996), type II myosin (*myo2:* Kitayama et al. 1997), profilin (*cdc3:* Balasubramanian et al. 1994), tropomyosin (*cdc8:* Balasubramanian et al. 1992), a myosin light chain-like protein (*cdc4:* McCollum et al. 1995), a formin (*cdc12:* Chang et al. 1997), an SH3 domain-containing protein (*cdc15:* Fankhauser et al. 1995; Chang et al. 1996), and the product of the *rng2* gene (Chang et al. 1996), which has not been cloned to date. All of these genes are necessary for medial ring assembly. However, in addition to the lack of medial ring formation, mutant alleles of several of these genes retain residual activity at the restrictive temperature and display other aberrant phenotypes (see below). These additional abnormalities most likely reflect the protein's involvement in other actin-requiring processes.

Profilin is an ubiquitous actin monomer-binding protein. It was first purified in a 1:1 complex with actin and can sequester actin monomers under some conditions. However, profilin is now considered to be a critical mediator of actin filament polymerization (for review, see Theriot and Mitchison 1993; Sohn and Goldschmidt-Clermont 1994). The inability of Cdc3p mutants to form a medial ring (Balasubramanian et al. 1994) is consistent with a primary role for profilin in actin filament assembly. Profilin also binds tightly to polyproline and to proteins containing stretches of proline residues. Among the polyproline-containing proteins found to bind profilin in vitro is Cdc12p (Chang et al. 1997).

During interphase, Cdc3p localizes to the growing tips of the cells, where actin patches are concentrated, whereas Cdc8p is seen in discrete patches at the cell cortex. At mitosis, Cdc8p is found as a component of the medial ring, joining the ring after F-actin (Balasubramanian et al. 1992), whereas Cdc3p forms a broad ring at the
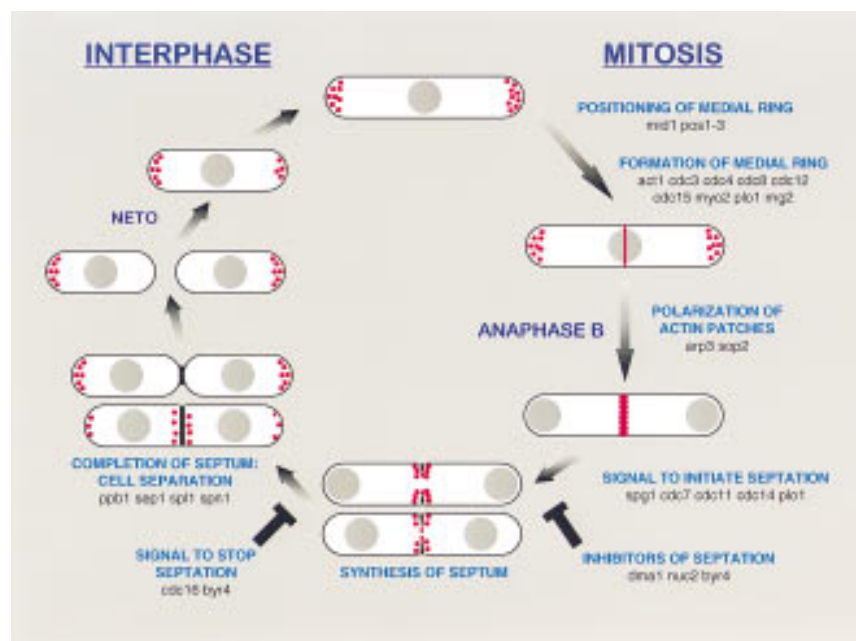

Figure 1. Schematic representation of the main events of septum formation and cytokinesis in *S. pombe.* The nucleus is shown as a gray circle, whereas the small red dots represent F-actin patches. NETO is New End Take-Off (Mitchison and Nurse 1985): Cells switch from one-end to two-end growth. The genes implicated in each of the stages are indicated. Grouping of genes does not necessarily imply that their products interact physically.

cell equator (Balasubramanian et al. 1994). Consistent with these localization patterns, mutants in *cdc3* and *cdc8* exhibit delocalized actin patches at all stages of the cell cycle, indicating that they are involved in the gen-

Table 1. *Genes implicated in the control of septum formation in* Schizosaccharomyces pombe

Gene	Gene product	Loss of function phenotype	Overproduction phenotype	Comments
Medial ring assembly				
act1	actin	Δ: lethal	lethal	
cdc3	profilin	Δ: lethal, no medial ring, no septum ts: gives a disorganized septum, defective in localization of actin patches	altered cell shape, lethal, but rescued by co-overproduction of actin no discrete actin staining	localizes to broad medial band during mitosis, and actin patches double-lethal required for localization of Cdc12p and Mid1p, binds Cdc12p
cdc4	EF hand protein (novel myosin light chain)	Δ: lethal, no actin ring, no septum ts: actin cables, disorganized septum. Cdc12p found as a dot	lethal, blocks septation	localizes to medial ring required for Mid1p ring formation
cdc8	tropomyosin	Δ: lethal, no medial ring, no septum ts: gives a disorganized septum, defective in localization of actin patches	?	localizes to medial ring, required for localization of Cdc12p and Mid1p to the medial ring
cdc12	Diaphanous-like protein Formin-homology domains	Δ: lethal, no medial ring, no septum ts: gives a disorganized septum, defective in localization of actin patches ts: no medial ring	lethal, dot of Cdc12p seen between nucleus and cortex	localizes to medial ring, required for Mid1p ring formation, binds profilin (Cdc3p)
cdc15	cdc15box/SH3 domain	Δ: lethal ts: no septum, assembly of medial ring impaired	lethal, blocks septation rearrangement of actin patches is aberrant	localizes to medial ring, required for localization of Cdc12p to medial ring, not required for Mid1p ring formation
myo2	myosin II	Δ: lethal, malformed medial ring and septum, defect in cell separation	lethal, blocks medial ring formation	component of medial ring; not required for normal interphase F-actin structure
plo1	kinase	Δ: defective in actin ring formation and initiation of septum formation	strong OP induces septation in G_1 and G_2 cells	required for formation of a bipolar spindle
rng2	not cloned	ts: defective in a late stage of medial ring assembly	?	
Positioning of medial ring				
mid1	nuclear, PH domain-containing protein	Δ: nonlethal at low temperatures, incorrect placement of septum	?	aberrant Cdc12p structures formed, located in the medial ring, dependent upon Cdc3p, Cdc4p, Cdc8p, and Cdc12p, also referred to as *dmf1*
pos1 *pos2* *pos3*	not cloned	ts: incorrect placement of septum	?	

Table 1. *(Continued)*

Gene	Gene product	Loss of function phenotype	Overproduction phenotype	Comments
Cortical actin function				
arp3	actin-related	Δ: lethal, defective in cortical actin function	lethal	found in high molecular-weight complex with Sop2p, interacts genetically with profilin and actin, found associated with actin patches but not medial ring
sop2	transducin repeat containing protein	Δ: lethal, defective for elongation and septation, defective in cortical actin function	?	interacts genetically with profilin; forms cables and punctate structures, found in a high molecular weight complex with Arp3p
Required for initiating synthesis of the division septum				
cdc7	protein kinase	Δ: lethal, medial ring forms, but no septum is made	multiple septa without cleavage	not required for Mid1p ring formation
cdc11	not cloned	ts: multinucleate, no septum but medial ring is formed	?	ts mutant suppressed by increased expression of Cdc7p; not required for Mid1p ring formation
cdc14	novel protein	Δ: lethal, multinucleate cells, no septum, medial ring formed	G2 delay	
spg1	ras-superfamily GTPase	Δ: lethal, medial ring forms, but no septum is made	multiple septa without cleavage from G_1, S-phase or G_2-arrested cells.	
Required to turn off synthesis of the division septum				
byr4	novel protein	Δ: lethal, multiple septa without cell cleavage, nuclear abnormalities	blocks septation	protein post-translationally modified, modification absent in *cdc15* and *cdc16* mutants
cdc16	novel protein	Δ: lethal, loss of function deregulates septation	?	homology to *S. cerevisiae* Bub2p and GAP proteins, required for proper mitotic arrest
Potential regulators of septation				
dma1	RING finger protein	Δ: nonlethal, but increased rate of chromosome loss	blocks septation	
nuc2	APC component	Δ: lethal, metaphase arrest ts: cuts at semipermissive temperature	blocks septation	synthetically lethal with *cut9* ability to exit cycle in G_1 in response to starvation is compromised

eral organization of actin (Marks et al. 1987; Balasubramanian et al. 1992, 1994; Chang et al. 1996). Despite these abnormalities in actin patch distribution, *cdc3* and *cdc8* null mutants grow in a polarized fashion and die because they fail to assemble a medial ring and undergo cytokinesis, suggesting that this is the step most sensitive to their absence.

The interphase actin distribution remains normal at the restrictive temperature in *cdc4, cdc12,* and *cdc15* heat-sensitive mutants and in the *myo2* null mutant. However, during mitosis, *cdc12* and *cdc15* mutants show delocalized actin patches, and conditional *cdc4* mutants form cables and asters emanating from one point on the cell cortex, though the *cdc4* null mutant

Table 1. *(Continued)*

Gene	Gene product	Loss of function phenotype	Overproduction phenotype	Comments
Required for cell separation				
ppb1	type 2b, calcineurin-like phosphatase	Δ: cytokinesis defect	?	
sep1	forkhead transcription factor	branched, multicompartmented cells.	?	
sep2	uncloned	cell separation defect, multicompartmented cells	?	also forms double, or complex septa
spl1	?	branched multicompartmented cells	?	
spn1	septin	Δ: not lethal, cleavage defect	?	found at medial ring late in mitosis, and at new ends of cells after separation

does not do this (Marks et al. 1987; Fankhauser et al. 1995; McCollum et al. 1995; Chang et al. 1996, 1997). The proteins Cdc4p, Cdc12p, Cdc15p, and Myo2p show no discrete localization during the majority of interphase and are seen in the medial ring at the beginning of mitosis. These data are consistent with the notion that these proteins play a more specific role in the assembly and/or function of the medial ring, rather than in other actin-requiring processes. In addition to these components, at least one of the proteins required for positioning the medial ring (Mid1p; see below) is also found in the medial ring.

It is clear that at the onset of septation, the medial ring constricts (Marks and Hyams 1985; Jochová et al. 1991; Fankhauser et al. 1995; McCollum et al. 1995; Chang et al. 1997; Kitayama et al. 1997). By analogy with higher eukaryotes, the medial ring has often been referred to as a contractile ring. Recently, a type II myosin heavy chain (Myo2p) has been localized to the medial ring during mitosis. The *myo2* null mutant forms an aberrant medial ring, often going on to form incomplete or uncleaved septa (Kitayama et al. 1997). Furthermore, the Myo2p ring has been observed to constrict in live cells (Kitayama et al. 1977). This result is perhaps the most persuasive evidence to date that an active contractile force is involved in *S. pombe* cell division. Targeted mutagenesis of domains of Myo2p predicted to be essential for Myo2p activities should allow a test of this hypothesis in the near future. Interestingly, Myo2p differs from conventional type II myosin heavy chains in two ways. First, it contains only one IQ domain for binding to a light chain. Cdc4p, which is known to bind a large protein with ATPase activity, is related to myosin light chains (McCollum et al. 1995). However, it appears not to bind a second light chain. Given its intracellular distribution and sequence homology, Cdc4p may turn out to be the single light chain binding partner for Myo2p. Second, Myo2p contains proline residues, which are predicted to break α-helices, in the carboxy-terminal coiled-coil re-

gion. Thus, the structure of the Myo2p rod domains might be bent in several places.

Positioning the medial ring

Wild-type fission yeast almost always divides in the middle. An interesting problem is how the cell defines where the middle is when placing the medial ring at the onset of mitosis, so that the septum that forms subsequently will divide the cell equally. In a β-tubulin mutant, which has activated Cdc2p kinase but cannot form a spindle and is arrested in early mitosis, the medial ring is present and correctly placed (Chang et al. 1996). This observation indicates that formation and placement of the medial ring do not depend on either the integrity of the mitotic spindle. It is likely that the position of the premitotic nucleus defines the position of the medial ring (Chang et al. 1996; for an extended discussion of this issue, see Chang and Nurse 1996).

Several studies have identified genes (*mid1, pos1, pos2,* and *pos3*) that are required for correct placement of the division septum (Chang et al. 1996; Edamatsu and Toyoshima 1996; Sohrmann et al. 1996). The best characterized of these is *mid1* (also called *dmf1*). Cells with mutations in this gene make a septum, but its position is aberrant. This can range from slightly eccentric placement, which produces daughters of unequal size, to far more extreme phenotypes, such as formation of the septum along the long axis of the cell. F-actin is always found to colocalize with the misplaced septum, suggesting that the defect lies not in forming the medial ring but in the normal mechanism for locating it at the center of the cell (Chang et al. 1996; Sohrmann et al. 1996). The *mid1* gene is not essential, but the null allele is heat sensitive. The gene encodes a novel protein containing a putative nuclear localization signal and a carboxy-terminal PH domain. In wild-type cells, Mid1p is nuclear during interphase and relocates to form a medial ring at the cell cortex coincident with the onset of mitosis. This
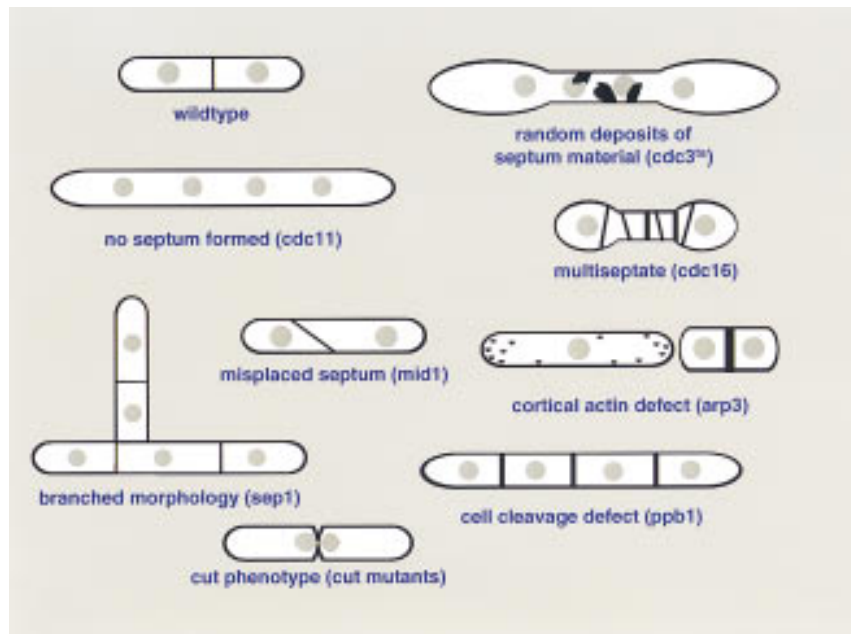
Gould and Simanis

Figure 2. Schematic representation of the phenotypes produced by loss-of-function mutations in genes implicated in control of septum formation. The phenotypes shown are those that would be observed in a typical experiment, two generations after a shift in the restrictive temperature. Septum material is shown in black; the nucleus in gray. A representative mutant is given for each class, and a wild-type cell is shown for comparison.

relocalization occurs before polarization of actin patches to the medial ring and is associated with increased phosphorylation of Mid1p. Formation of the Mid1p ring does not require an intact spindle. When the septum is completed and the cells separate, Mid1p staining is once again nuclear (Sohrmann et al. 1996). One possible role of Mid1p is to direct growth of the medial ring and prevent it from drifting around at the cortex. The three *pos* mutants (Edamatsu and Toyoshima 1996), like *mid1,* are defective in placing the septum at the restrictive temperature. The identity of the products they encode and their interactions with *mid1* will be of considerable interest.

Dependencies in medial ring assembly

One important question that remains to be resolved is the role of individual medial ring components in its assembly and function. For example, are some components required only to seed the ring, whereas others act as a scaffold for its assembly and propagation around the cell cortex? To try to address this question, the effects of inactivating different components of the medial ring upon the distribution of the other components have been investigated, as reagents recognizing the various proteins have become available. To date, the localization of Cdc12p, Cdc15p, and Mid1p have been analyzed extensively.

Cdc12p does not form a ring in *cdc3, cdc8,* or *cdc15* mutants, suggesting that the products of these genes are all required for formation or stabilization of the Cdc12p ring. In addition, in a *cdc4* background, a single dot of Cdc12p is seen at the cell cortex, surrounded by an aster of F-actin, suggesting that Cdc4p is required for exten-

sion of the ring around the cell cortex. In a *mid1* mutant, strands of Cdc12p are seen that emanate from one point and extend in random directions (Chang et al. 1997). Mutations in *cdc12* show strong negative genetic interactions with a *cdc3* mutation, and Cdc12p interacts with GST–Cdc3p fusion protein directly in vitro. Cdc12p is a large multidomain protein, and it has been suggested that it may act as a nucleating scaffold for medial ring assembly (Chang et al. 1997).

Formation of the Mid1p ring is dependent on Cdc3p, Cdc4p, Cdc8p, and Cdc12p, although not Cdc15p, and in synchronous cultures, the appearance of a Mid1p ring precedes appearance of Cdc15p, and polarization of actin patches to the ring. The misplaced medial rings found in *mid1* mutants are associated with Cdc15p (Sohrmann et al. 1996).

From the information above, it is clearly not possible to formulate a simple, internally consistent model describing the assembly of these components into the medial ring. These studies are obviously far from complete and need to be extended to determine the localization of these proteins in null as well as conditional mutants, as the latter may retain some residual activity, perhaps giving misleading results. Further analysis using reagents specific for other markers will no doubt reveal additional dependency relationships in the assembly of the medial ring and will also address the relative timing with which various components appear in the ring. It will be particularly interesting to determine how far assembly can proceed in the absence of F-actin and the effects of F-actin depolymerization on it, by the use of drugs such as Latrunculin-A (Ayscough et al. 1997). It is already apparent, even from these early studies, that the medial ring proteins show considerable interdependency for proper localization. Models that attempt to portray medial ring

assembly as a linear pathway may therefore be too simplistic.

Regulation of medial ring assembly

Little is known about how medial ring assembly is triggered or regulated. Mutants that are arrested in late G_2, such as *cdc2* and *cdc25*, still have an interphase actin cytoskeleton, with actin patches located at the growing tips. Thus, the cytoskeletal rearrangements depend on entry into mitosis. No medial ring (as judged by visualization of F-actin) is formed in cells lacking Plo1p function (Ohkura et al. 1995), suggesting that Plo1p is important for triggering this rearrangement. A key element in the process may be Cdc15p: Increased expression of this protein in G_2-arrested cells can provoke a rearrangement of F-actin to form either a ring or an aster at the middle of the cell, even in the absence of Cdc2p kinase activity (Fankhauser et al. 1995). Medial ring formation correlates with dephosphorylation of Cdc15p, though whether this dephosphorylation is responsible for regulating its activity remains to be determined.

Intriguingly, it has been found that Cdc12p and Myo2p both form a dot between the nucleus and cell cortex in early mitotic cells (Chang et al. 1997; Kitayama et al. 1997), though these dots have only been observed when expression of the proteins is increased. The significance of these observations remains unclear. The Cdc12p dot is present in late interphase cells, leading to the suggestion that this is part of the mechanism by which the site of medial ring formation is marked (Chang et al. 1997). The observation that the F-actin asters and medial rings triggered by increased expression of Cdc15p in G_2-arrested cells are located at the middle of the cell (Fankhauser et al. 1995) is consistent with this view, but demonstration of a Cdc12p dot in G_2-arrested cells is lacking.

Polarization of actin patches to the medial ring: the role of arp3 and sop2

As mentioned above, an obligatory step in formation of the septum is the polarization of actin patches adjacent to the medial ring. The function of these actin patches is presumably to localize properly vesicles and enzymes necessary for plasma membrane and cell wall biosynthesis during septation. The patches might also be important for the localization of other components necessary for medial ring function. In addition to actin, two components of the patches that are required for normal cytokinesis have been identified in *S. pombe*, Arp3p and Sop2p (Balasubramanian et al. 1996; McCollum et al. 1996). Arp3p and Sop2p localize to actin patches throughout the cell cycle but do not localize to the medial ring. Sop2p is also detected in cables, which presumably also contain actin filaments (Balasubramanian et al. 1996). Both Arp3p and Sop2p functions are essential for cell viability. Loss of either protein results in growth arrest throughout the cell cycle, an accumulation of cells with septa that are abnormally thick, disorganization of actin patches, and cell lysis. Interestingly, an early effect

of the loss of Arp3p function is a pronounced delay in actin patch relocation to the medial ring, which results in septum material being deposited at the ends of the cell rather than the medial region (McCollum et al. 1996). Mutants in Sop2p are defective in polarized cell growth after release from starvation (Balasubramanian et al. 1996). These results suggest that Arp3p and Sop2p functions are required for the normal mobility and localization of cortical actin patches. Arp3p and Sop2p have been structurally and functionally conserved throughout evolution and are part of a multiprotein complex (Machesky et al. 1994, 1997; Balasubramanian et al. 1996; Mullins et al. 1997; Welch et al. 1997), although in *Saccharomyces cerevisiae* the purified complex lacks a *sop2* homolog (Winter et al. 1997). The Arp3p-containing complex binds directly to profilin (Machesky et al. 1994), and mutations in *arp3* and *sop2* rescue *cdc3* mutants (Balasubramanian et al. 1996; McCollum et al. 1996). It is yet unclear what consequence these interactions have for normal cortical actin function.

Regulation of the onset of septum formation

At the restrictive temperature, mutants defective in the genes *cdc7, cdc11, cdc14,* and *spg1* do not form a division septum, but growth, DNA replication, and mitosis continue in the absence of cytokinesis (Nurse et al. 1976; Mitchison and Nurse 1985; Fankhauser and Simanis 1993, 1994; Schmidt et al. 1997), resulting in the formation of multinucleate cells. The *cdc7, cdc14,* and *spg1* genes (but not *cdc11*) have been cloned and sequenced. All three genes are essential. In each case the null mutant shows the same phenotype as the heat-sensitive mutant. These mutants can all form a medial ring during mitosis; so it is likely that the gene products act either downstream or independently from the genes that are required for formation of the medial ring. In null mutants where this has been examined (*spg1* and *cdc7*), the medial ring does not appear to contract significantly. Mutations in these genes show strong genetic interactions, suggesting that the gene products function in a single biochemical pathway (Marks et al. 1992). Because the *plo1*-encoded protein kinase is required not only for medial ring formation but also for initiating septum formation (Ohkura et al. 1995), activation of these genes may also require Plo1p function. However, to date, there is no genetic or biochemical evidence linking Plo1p with this pathway. The identity of the substrates of Plo1p will be of considerable interest.

The *cdc7* gene encodes a protein kinase. Overexpression of the gene deregulates septum formation and causes a cell cycle arrest with a phenotype similar to that of a *cdc16^{ts}* mutant (see below). This overexpression phenotype requires functional Cdc11p, Cdc14p, and Cdc15p. In addition, slightly increased expression of Cdc7p, to levels that do not perturb significantly the normal regulation of septum formation, is sufficient to rescue mutations in *cdc11.* These observations suggest that Cdc7p and Cdc11p interact in the same pathway (Fankhauser and Simanis 1994).

The *spg1* gene was cloned as a multicopy suppressor of a dominant-negative mutant of the Cdc7p kinase and as a suppressor of *cdc11* mutations (Schmidt et al. 1997). It is an essential gene, which encodes a GTPase of the ras superfamily. Increased expression of Spg1p induces septum formation in G_2-, S phase-, or pre-Start G_1-arrested cells. In addition to breaking the normal dependency on initiation of mitosis, *spg1* overexpression (like *cdc7* overexpression) deregulates septum formation, resulting in formation of multiple septa that are not cleaved. This effect requires the activities of Cdc7p, Cdc15p, and Cdc14p, but not Cdc2p. Spg1p and Cdc7p can be coimmunoprecipitated from cell extracts; whether this interaction is required for localization of Cdc7p, for its kinase activity, or both will be of interest.

The predicted sequence of Cdc14p, a 28-kD protein, gives no clues to its biochemical function. Overproduction of the protein delays the onset of mitosis and cytoskeletal rearrangement (Fankhauser and Simanis 1993).

Synthesis of the division septum

The main components of the *S. pombe* cell wall are branched $(1 \rightarrow 3)$ β-glucan, linear $(1 \rightarrow 3)$ α-glucan, and α-galactomannan (Horisberger and Rouvet-Vauthey 1984): $(1 \rightarrow 3)$ β-Glucan is also a component of the division septum. Recent studies indicate that Rho-family GTPases are involved in controlling the activity of β-glucan synthase (Arellano et al. 1996) and that activated mutants make a very thick wall and very little septum material. It is not known at present how the synthetic enzymes are directed to the site of septum formation.

Turning off septum formation

To date, two mutants have been described that cannot turn off septum formation once it has been initiated. In a *cdc16* mutant, cells complete mitosis normally and form a septum between the two nuclei. However, instead of proceeding to cell cleavage, the cell synthesizes multiple, additional septa. These are not formed simultaneously but instead are laid down one at a time, producing anucleate cell compartments (Minet et al. 1979). A similar phenotype is produced by loss of *byr4* function, although in this case, mitotic abnormalities are also observed (Song et al. 1996). Therefore, Byr4p and Cdc16p are implicated in limiting the cell to a single septum per cell cycle. Synthetic lethal interactions and suppression have been observed between *cdc16* and genes required for the onset of septum formation, such as *cdc7, cdc11,* and *cdc14,* suggesting that Cdc16p is a component of the same pathway (Marks et al. 1992); however, this remains to be established biochemically. Because loss of Cdc16p activity also compromises the function of the checkpoint that monitors spindle integrity and/or function during mitosis, it has been suggested that Cdc16p may play a dual role in the cell cycle (Fankhauser et al. 1993). Cdc16p is most closely related to *S. cerevisiae* Bub2p and shares homology with GTPase-activating proteins (GAPs) (Schmidt et al. 1997). It is tempting to speculate that it will be the GAP for Spg1p and that the Spg1p GTPase switch is the key, rate-limiting step in the initiation of septum formation at the end of mitosis (Schmidt et al. 1997). However, this remains to be demonstrated. The sequence of Byr4p gives no clue as to its function, although the protein has a short region of homology with Cdc7p, the significance of which is presently unknown (Song et al. 1996). Byr4p is subject to post-translational modification that alters its migration on SDS–polyacrylamide gels. This modification is reduced in *cdc15* and *cdc16* mutants and is very extensive in a cell arrested in early mitosis (Song et al. 1996). Whether this modification plays any role in coupling the onset of septation with mitosis remains to be determined.

Cell separation

A number of mutants defective in cell separation have been identified, although to date, little is known of the biochemical pathways involved. Mutations in *sep1* interfere with cell separation and result in a branched, mycelial morphology. The *spl1-1* mutation results in a terminal phenotype showing characteristics of both *sep1* and heat-sensitive medial ring formation mutants (Sipiczki et al. 1993). Under restrictive conditions, *spl1-1* cells are mononucleate, are elongated, have an abnormal shape, and contain one or more uncleaved or partially cleaved septa, together with irregular depositions of cell wall material. *sep1* has been cloned; it encodes a fork head-type putative transcription factor (Ribar et al. 1997). This raises the possibility that expression of certain genes late in the cell cycle is required for efficient cell separation. Consistent with this hypothesis, addition of cycloheximide late in mitosis prevents cell cleavage but not synthesis of the septum (Fantes 1982; Sohrmann et al. 1996).

A screen for mutants showing increased resistance to cell wall-digesting enzymes has yielded a new series of *sep* mutants. The one analyzed in detail, *sep2,* shows a linear rather than branched morphology. In addition, cells often form complex or double septa (Grallert et al. 1997).

Septins are also implicated in cell separation in fission yeast. *S. pombe* has at least six septin genes (Longtine et al. 1996). Spn1p appears to function in cell division; in particular it seems to be required for a late stage of septum formation or perhaps cell wall dissolution, because a null mutant is viable, but has a delay in cell separation or in completing the septum. The phenotype resembles that of *sep1.* Septins localize to the site of the medial ring very late in mitosis, after association of actin and related proteins (cited in Longtine et al. 1996).

It is likely that phosphatases are also important in regulating the septation machinery and activating cell cleavage. Treatment of cells with okadaic acid interferes with septation and cytokinesis, producing binucleate cells with or without a septum (Kinoshita et al. 1993). Cytokinesis is also inhibited by the drug cyclosporin A or mutation of its main target, the calcineurin-like type

2B phosphatase encoded by *ppb1* (Yoshida et al. 1994); the affected cells can form septa, but cell separation is impeded. Cytokinesis and morphology defects are also produced by mutation of the protein phosphatase 2A regulatory subunit gene, *pab1* (Kinoshita et al. 1996). Genetic interactions between *ppb1* and other phosphatase genes have also been observed. Double mutants of *ppb1* with either *dis2* or *ppa2* show a cytokinesis defect, which is even more extreme in the *ppb1 sts1* double mutant. The protein kinase C pathway has also been shown to affect cell division. Overexpression of *pkc2* is lethal, producing branched, multiseptate cells (Toda et al. 1993), whereas lack of Pkc2p affects cell shape and polarity and renders protoplasts unable to resynthesize cell wall or reorganize F-actin for polar growth (Kobori et al. 1994).

Induction of septum formation by increased expression of Spg1p or Cdc7p or inactivation of Cdc16p or Byr4p results in multiple septa being formed without cell cleavage. This could be because the septa produced under these conditions are in some way defective, because the presence of anucleate compartments prevents cleavage (i.e., a septum needs a nucleus on either side to be cleaved), or because the cleavage signal is ignored or not given while the septum-synthesizing machinery is active. In certain cases, such as the long cells produced by *cdc25* arrest and release or by combining *sep2* with mutations that increase cell length, double septa are produced, generating an anucleate compartment that separates the two nucleated ones. In these cases, one and sometimes both of the septa are cleaved (Grallert et al. 1997). It is thus unlikely that there is an obligatory requirement for a nucleus to be situated on each side of a septum for cleavage to occur. Though it seems likely that there will be a signal to initiate septum dissolution, its biochemical nature remains unknown.

Coordinating mitosis and cytokinesis

Analysis of the phenotypes of different cell cycle mutants has led to the conclusion that septum formation is dependent on the initiation, although not on the completion, of mitosis. Thus, no septum formation is observed in cells blocked in late G_2 by a defect in *cdc2* function (Nurse et al. 1976; Minet et al. 1979). As mentioned above, mutants that arrest in the early stages of mitosis because of defects in β-tubulin form a medial ring but do not make a division septum. The cell cycle arrest in this case is imposed by activation of the spindle assembly checkpoint (for review, see Wells 1996). If progression through mitosis is delayed, the cell must also restrain septum formation to avoid cutting the undivided nucleus. Loss of Cdc16p function compromises the checkpoint-induced arrest in mitotically arrested cells (Fankhauser et al. 1993). Dma1p, which was isolated as a multicopy suppressor of *cdc16-116,* may be an effector of the checkpoint signal transduction pathway (Murone and Simanis 1996). *dma1* is nonessential, but if Dma1p function is lacking, septation is not blocked in cells arrested in early mitosis. Increased expression of Dma1p initially blocks septum formation, whereas S phase and mitosis continue, producing elongated, multinucleate cells; medial rings are formed; so it is apparently the onset of septum formation that is inhibited. The targets and regulation of Dma1p will be of interest.

Certain mutants that arrest at late stages in mitosis are unable to restrain cytokinesis and form a division septum, resulting in the cleavage of the undivided nucleus by the septum to produce the so-called *cut* (cell untimely torn) phenotype. To date, >20 *cut* genes have been described (Hirano et al. 1986; Samejima et al. 1993; Saka et al. 1994), and mutations in the *top2* gene also produce this phenotype (Uemura and Yanagida 1984). It is clear that many, although not all, of the proteins encoded by the *cut* genes are required either for anaphase onset or postanaphase events, or that lack of the protein interferes with proper chromosome segregation. The observation that cells arrested early in mitosis do not cut, taken together with the existence of *cut* mutants, suggests that there is a "point of no return" during mitosis when a cell commits to septation and thereafter cannot delay the process very much if later events do not occur properly. However, it is also clear that some delay must be possible, because in the long cells produced by increased expression of *wee1* or by block and release of a *cdc25* mutant, anaphase is extended, and septation is delayed correspondingly until after spindle breakdown (Hagan et al. 1990).

The finding that some *cut* genes (*nuc2, cut9,* and *cut4*) encode components of the 20S APC/cyclosome (Yamashita et al. 1996; Yamada et al. 1997) suggests that this complex is not involved directly in triggering the onset of septum formation. However, an unexplained result is that strong ectopic expression of *nuc2* inhibits septum formation, producing long, multinucleate cells similar to those produced by mutations in *cdc7* or *cdc11* (Kumada et al. 1995). Because mitosis continues normally in these cells, the 20S APC/cyclosome is presumably functioning properly. It is not known at this time which stage of septum formation is blocked by *nuc2* overproduction, because it has not been determined whether these cells form medial rings. It is possible that the excess Nuc2p titrates away some component essential for septum formation. Although the data cited above argue that the involvement of the 20S APC/cyclosome may not be direct, septum formation may be initiated by the same cues that activate the 20S APC/cyclosome to bring about anaphase onset. The role played by proteolysis in controlling the onset of septum formation therefore remains to be elucidated.

The role of Cdc2p

cdc2 mutants arrest in G_2 and do not initiate mitosis or any of the cytoskeletal rearrangements associated with it. Thus, these rearrangements are normally coordinated with entry into mitosis. However, increased expression of some genes (*cdc15:* Fankhauser et al. 1995; *plo1:* Ohkura et al. 1995; *spg1:* Schmidt et al. 1997) can bypass this requirement and induce septation or at least some of

the cytoskeletal rearrangements, even though Cdc2p is inactivated by mutation. Thus, the activation of these septum-promoting proteins may normally be coupled to the activation of Cdc2p or to its inactivation during mitosis. How this is achieved is unclear presently. However, in view of the central role that it appears to play both in establishing a bipolar spindle and in medial ring and septum formation, it is probable that Plo1p kinase will also be of importance.

Inactivation of Cdc2p kinase may be a critical step in signaling the onset of septum formation. If cells are arrested in mitosis by increased expression of *S. pombe* Mad2p, and Cdc2p or Cdc13p (the B-type cyclin with which it associates) is inactivated, cells form a division septum (He et al. 1997). A similar result was obtained in *S. cerevisiae,* where inactivation of Cdc28p in a mitotically arrested cell is sufficient to trigger cell cleavage (Ghiara et al. 1991).

In higher eukaryotes, studies of myosin II regulatory light chain phosphorylation also indicate a role for Cdc2p inactivation in triggering cytokinesis. Myosin II regulatory light chain activity appears to be both negatively and positively regulated by changes in phosphorylation. During mitosis, it is inhibited through phosphorylation by Cdc2p/Cyclin B (Satterwhite et al. 1992). When Cdc2p/Cyclin B is inactivated in the normal cell cycle, coincident with the metaphase–anaphase transition, these phosphorylations no longer occur. At this time, phosphorylation of another site by myosin light chain kinase is increased markedly. The changes at these phosphorylation sites are thought to contribute to signaling the initiation of cytokinesis (Yamakita et al. 1994). Thus, inactivation of Cdc2p kinase at the end of mitosis may be a universal feature in signaling the onset of cytokinesis.

Checkpoints

Are there checkpoints operating during medial ring assembly, septum formation, and cytokinesis? The existence of a checkpoint that delays mitosis if bud formation is compromized has been demonstrated in *S. cerevisiae* (Lew and Reed 1995; Sia et al. 1996). The observation that cleavage is delayed in *mid1* mutant cells that have drastically misplaced their septum or created an anucleate compartment suggests that some form of sensor mechanism must operate during septation (Chang et al. 1996; Sohrmann et al. 1996). In addition, the septum-forming machinery responds to activation of the mitotic checkpoint, if spindle function is compromized (see above). However, mutants such as *cdc11,* which assemble a medial ring but are unable to trigger the onset of septum formation, rearrange actin to the tips of the cell at the end of mitosis and resume growth without cytokinesis, repeating this at each nuclear division (Mitchison and Nurse 1985; Marks et al. 1987). This may mean that there is no septum formation checkpoint, that it is only activated if septation is actually triggered, or that the proteins required to trigger it are themselves part of the sensor/transduction mechanism. Although it

is presently not clear which of these possibilities (if any) is correct, it may be that in the wild this is not as serious a problem as it would at first appear to be. Because "wild" *S. pombe* are homothallic and therefore switch mating type, a cell skipping septation may end up with two nuclei that express different mating-type cassettes in the same cytoplasm. Under these conditions, if the cell is starved (likely in the wild), it will undergo karyogamy and meiosis, solving the problem of not having divided. The nature of the checkpoint, if one exists, will be of considerable interest.

Microtubules

To date, the localization of F-actin correlates best with the sites of growth and division. However, it is also clear that microtubules cannot be ignored in this context. For example, there is a continuous requirement for intact microtubules to direct Tea1p to the tips of the cells, where it is essential to maintain proper growth polarity (Mata and Nurse 1997). In addition, the postanaphase microtubule array (PAA) that appears at the end of mitosis is seeded from microtubule-organizing centers at the cell equator (Hagan and Hyams 1988). Interestingly, in cells that misplace the division septum, the PAA is also misplaced, suggesting that the site of division determines where the new microtubule array will be seeded from at the end of mitosis (Chang et al. 1996). Furthermore, it has been reported that in addition to a medial F-actin-containing ring at the cell cortex, there is also a ring of microtubules at the cell equator during mitosis (Pichová et al. 1995). Although, to date, attention has focused mostly on the role of actin, it is possible that future models will also have to incorporate an active role for microtubules in formation of the division septum.

Evolutionary conservation of the fission yeast division machinery

Many of the components implicated in assembly of the medial ring or in control of actin polymerization or distribution have been functionally conserved through eukaryotic evolution. In many cases, for example, the septins, Cdc12p/Diaphanous-like proteins, profilin, tropomyosin, type II myosin, and myosin light chain (for review, see Satterwhite and Pollard 1992; Longtine et al. 1996; Frazier and Field 1997), these components are also implicated in cytokinesis. Anillin, an actin-binding protein from *Drosophila melanogaster* (Field and Alberts 1995), has many of the structural motifs of Mid1p and cycles from the nucleus to the cell cortex at the time of division; it is tempting to speculate that it plays a similar role to Mid1p in defining the site where the contractile ring will form. The study of yeast mutants has also identified molecules that are potential regulators of cytokinesis. Some of these proteins, such as Plo1p, Cdc7p, Spg1p, and Cdc16p, have functional counterparts in *S. cerevisiae,* though their role in this organism seems to be concerned mainly with regulating the end of mitosis, perhaps reflecting the different mode of growth and di-

vision of the two organisms (Kitada et al. 1993; Surana et al. 1993; Shirayama et al. 1994a,b, 1996). It will be interesting to determine whether these proteins have homologs in higher eukaryotes.

Future prospects

It is clear that mutant screens are far from saturated, and one would expect many new regulators both of medial ring formation and septum formation to emerge from new mutants hunts. In addition, the increasing amount of information from genome-sequencing projects and purification of actin-regulating complexes from organisms more amenable to biochemistry than yeast cells will allow cloning of genes for proteins that regulate division but for which yeast mutants are not yet available. Thus, the extent to which the control mechanisms identified in yeasts are applicable to higher eukaryotes should soon become evident. A few of the many outstanding questions in the *S. pombe* system that will surely be addressed in the future are: (1) How is the assembly of the medial ring influenced by genes controlling cell polarity? (2) What is the role of the nucleus in determining the site of division? (3) What role do microtubules play in controlling cell division, and how do the actin and microtubule cytoskeletal elements interact? (4) What signals the onset of medial ring contraction/septum biosynthesis? and (5) How does the cell know that the septum has been completed, and what triggers dissolution of the septum?

Acknowledgments

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