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COMMENTARY

Tying the knot: linking cytokinesis to the nuclear cycle

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SUMMARY

For the survival of both the parent and the progeny, it is imperative that the process of their physical division (cytokinesis) be precisely coordinated with progression through the mitotic cell cycle. Recent studies in the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* are beginning to unravel the nature of the links between cytokinesis and the nuclear division cycle. The cyclin-dependent kinases and a novel surveillance mechanism that monitors cytokinesis and/or morphogenesis appear to play important regulatory roles in forging these links. It is becoming increasingly clear that the inactivation of the mitosis-promoting cyclin-dependent kinase, which marks the completion of the nuclear division cycle, is essential for actomyosin ring constriction and division septum assembly in both yeasts. Additionally, the spindle pole bodies are emerging as important transient locale for proteins that might play a key role in coupling the completion of mitosis to the onset of cytokinesis.

Key words: Yeast, Mitosis, Cytokinesis, Actomyosin ring, Cell cycle

INTRODUCTORY REMARKS

A newly born cell does not become a truly independent entity until it is separated from its parent by the process known as cytokinesis. Cytokinesis was the first cell cycle event to be described over one hundred years ago by embryologists studying eggs of amphibia and marine invertebrates. Subsequently, events that occur in the nucleus, namely DNA synthesis (S phase) and mitosis (M phase; collectively referred to as the nuclear cycle, in which the gap phases G_1 and G_2 precede S and M phases, respectively) were described. The logical organization of the cell cycle and its rate-limiting steps were inferred from elegant genetic studies carried out in the yeasts *S. cerevisiae* and *S. pombe* (Hartwell et al., 1974; Nurse, 1975; Nurse et al., 1976) and from cell fusion studies carried out in mammalian cells (Rao and Johnston, 1970). These studies, combined with biochemical analyses of early embryonic cell cycles in frogs and marine invertebrates (Masui and Markert, 1971; Murray and Kirschner, 1989), led to the idea that the orderly progression through the cell cycle depends upon timely activation and inactivation of cyclin-dependent kinases (CDKs). In somatic cells the timing of activation and inactivation of CDKs can be regulated by checkpoint controls (Murray, 1992). For example, checkpoint controls operate to prevent activation of the M-phase-promoting factor (MPF – composed of a kinase subunit encoded by homologs of the fission yeast *cdc2* gene and a B-type cyclin subunit) until chromosomal DNA is completely replicated. Likewise, activation of MPF is prevented when chromosomal DNA is damaged. Finally, MPF inactivation and exit from mitosis are inhibited by the spindle-assembly checkpoint, which perhaps monitors attachment of kinetochores to microtubules. Even though great progress has been made towards understanding of the controls that regulate the nuclear cycle, the mechanics of cytokinesis and its relationship with the nuclear cycle are relatively poorly understood.

The existence of regulatory connections between the nuclear cycle and cytokinesis has been revealed by observations in the yeasts *S. cerevisiae* and *S. pombe*. In the fission yeast a block at the G_2/M boundary or in early mitosis due to loss of microtubules prevents cytokinesis, which suggests that passage through mitosis is a prerequisite (Nurse et al., 1976; Snell and Nurse, 1994; Hiraoka et al., 1984; Murone and Simanis, 1996). Furthermore, accelerated entry into mitosis – for example, when cells are deprived of a nitrogen source or in cells in which the dosage-dependent inhibitor of mitosis Wee1p is defective – causes cell division at a reduced size; this indicates that mitosis and cytokinesis are tightly coupled (Nurse, 1975). In *S. cerevisiae*, assembly of a bud depends on the execution of START (a point in the cell cycle at G_1/S controlled by Cdc28p-Cln, following which cells are irreversibly committed to the mitotic cycle, and alternative developmental fates such as mating and sporulation are no longer available), which again suggests that there is an intimate link between progression

through the mitotic cell cycle and morphogenesis/cytokinesis (Pringle and Hartwell, 1981). Here, we discuss the nature of coupling between the nuclear cycle and cytokinesis, which recent studies in the two yeasts have uncovered.

LESSONS FROM *SCHIZOSACCHAROMYCES POMBE*

S. pombe cells are cylindrical and grow by tip elongation. Cell division is accomplished by medial fission through the use of an actomyosin-based contractile ring (Fig. 1). In pre-mitotic cells, F-actin is detected at the growing ends of the cell in the form of patch-like structures, whereas microtubules are seen as an array that runs from one end of the cell to the other (Marks and Hyams, 1985; Hagan and Hyams, 1988). Upon entry into mitosis and in parallel with assembly of the mitotic spindle (Marks and Hyams, 1985; Kanbe et al., 1989; Snell and Nurse, 1994; Bahler et al., 1998), F-actin patches are lost from the cell ends, and the actomyosin ring, composed of F-actin and type II myosin, assembles at the medial plane of the cell (Kanbe et al., 1989; Gould and Simanis, 1997; Kitayama et al., 1997; May et al., 1997; Motegi et al., 1997; Bezanilla et al., 1997; Naqvi et al., 1999). In addition to F-actin and type II myosin, the ring contains numerous proteins, such as the formin Cdc12p (Chang et al., 1997), the IQGAP-related protein Rng2p (Eng et al., 1998), PSTPIP-related proteins Cdc15p and Imp2p (Fankhauser et al., 1995; Demeter and Sazer, 1998), and the tropomyosin homolog Cdc8p (Balasubramanian et al., 1992; Arai et al., 1998). Following actomyosin ring assembly, F-actin patches appear adjacent to the actomyosin ring, and this event is thought to be essential for delivery of the division septum (Marks and Hyams, 1985; McCollum et al., 1996). Upon disassembly of the mitotic spindle at the end of anaphase, the actomyosin ring constricts (Kitayama et al., 1997; Demeter and Sazer, 1998), and the division septum (called the primary septum) is deposited in a centripetal manner, behind the constricting ring (Marks and Hyams, 1985). The division septum contains sugar polymers such as 1,3-β-glucan, 1,3-α-glucan and α-galactomannan (Ishiguro, 1998). Subsequently, secondary septa are assembled on either side of the primary septum. Finally, cleavage of the primary septum liberates two distinct daughter cells (Gould and Simanis, 1997).

The execution of cytokinetic events in coordination with the nuclear cycle raises some important questions. What regulates the timing of actomyosin ring assembly and constriction, and how are they coupled to mitosis? Do mechanisms that prevent cytokinesis in pre-mitotic cells exist? As in the case of the nuclear cycle, are there checkpoints that monitor completion of cytokinesis? In the following sections, we discuss these issues in some detail.

TIMING OF ACTOMYOSIN RING ASSEMBLY

It has been well documented that cells arrested at the G_2/M boundary by inactivation of *cdc2* or *cdc25* genes continue to elongate with an undivided nucleus, but fail to assemble the actomyosin ring (Snell and Nurse, 1994). This suggests that entry into mitosis, rather than attainment of a critical size, is essential for assembly of the actomyosin ring. It is possible that phosphorylation of ring-nucleating proteins by the mitotic CDK (Cdc2p-Cdc13p complex) leads to actomyosin ring assembly. Two potential effectors are the actomyosin ring components Plo1p and the SH3-domain-containing phosphoprotein Cdc15p, since ectopic overproduction of Plo1p and Cdc15p leads to actomyosin ring assembly in G_2-arrested cells (Okhura et al., 1995; Fankhauser et al., 1995). Deletion of Plo1p results in actomyosin-ring-assembly defects, implying an essential role for Plo1p in actomyosin ring assembly (Okhura et al., 1995). However, *cdc15* mutants are still capable of forming actomyosin rings; thus the status of Cdc15p as a ring-nucleating protein is unclear (Balasubramanian et al., 1998). Plo1p has also been shown to be a component of the mitotic spindle pole bodies (SPB) and the mitotic spindle, which has led to the suggestion that Plo1p couples actomyosin ring assembly with mitotic spindle assembly or SPB duplication, events that occur upon transition into M phase (Bahler et al., 1998; Mulvihill et al., 1999). It is not known whether Cdc15p and Plo1p are substrates of Cdc2p-Cdc13p. It will be important to resolve this issue and to determine whether other Cdc2p-Cdc13p substrates are involved in actomyosin ring assembly.

A clear role for Plo1p in proper spatial coupling of cell and nuclear division has been shown (Bahler et al., 1998). Mutants in *plo1* and *mid1* have defects in positioning of the actomyosin ring (Chang et al., 1996; Sohrmann et al., 1996; Bahler et al., 1998). In interphase, Mid1p resides in the nucleus and then, upon entry into mitosis, it is phosphorylated and exits the nucleus in a Plo1p-dependent manner. After exiting the nucleus, Mid1p localizes to the cortex overlying the nucleus and recruits proteins involved in actomyosin ring assembly, thereby marking the site of cell division (Sohrmann et al., 1996; Bahler et al., 1998). Other proteins such as the Pom1-kinase (Bahler and Pringle, 1998) and the products of the *pos* genes (Edamatsu and Toyoshima, 1996) have been identified. However, the mechanism of their action in actomyosin ring positioning is not known.

Many components of the actomyosin ring have also been detected in spot-like structures, which in some cases, such as that of Rng2p (Eng et al., 1998), have been demonstrated to be the SPBs. In other cases, it is not known whether the spot-like accumulation of ring components in premitotic cells represents SPBs (Kitayama et al., 1997; Chang et al., 1997; Chang, 1999). Future studies should resolve the regulatory roles, if any, of the SPB in actomyosin ring assembly.

COORDINATING CYTOKINESIS WITH COMPLETION OF MITOSIS

Late in mitosis, a number of events happen in quick succession that have to be precisely coordinated. At the end of anaphase, following chromosome segregation and spindle disassembly, the actomyosin ring constricts and the division septum is formed. How is initiation of constriction of the actomyosin ring and assembly of the division septum coupled to progression through mitosis, such that the constricting ring and the septum do not inappropriately 'cut' the nuclei? Recent evidence suggests that inactivation of Cdc2p-Cdc13p is essential for actomyosin ring constriction. *nda3* mutants, in which the gene that encodes β-tubulin is defective, are unable to form mitotic

spindles, arrest early in mitosis and exhibit high Cdc2p-Cdc13p activity and an actomyosin ring (Hiraoka et al., 1984; Chang et al., 1996; He et al., 1997). *nda3* mutant cells remain arrested at metaphase owing to the activation of the spindle assembly checkpoint, which regulates the activity of the **a**naphase-**p**romoting **c**omplex (APC). The APC is a 20S particle that functions as an E3 enzyme in the ubiquitin-mediated degradation of several mitotically unstable proteins (Zachariae and Nasmyth, 1999). The APC is required either directly or indirectly for the proteolysis of proteins such as cohesins and B-type cyclins. Mitotic arrest is not observed in *nda3* cells, which lack Mad2p, a protein thought to inhibit the APC upon activation of the spindle assembly checkpoint (He et al., 1997; Kim et al., 1998). Interestingly, *nda3 mad2* double mutants also undergo actomyosin ring constriction and assemble a division septum, which suggests that activation/function of APC is important for actomyosin ring constriction and septum deposition (He et al., 1997).

That the B-type cyclin Cdc13p might be the target for APC whose destruction leads to actomyosin ring constriction and septum assembly was inferred from studies of overexpression of Mad2p in *cdc2* and *cdc13* mutants (He et al., 1997; Kim et al., 1998). Overproduction of Mad2p in wild-type cells leads to arrest at metaphase, owing to the activation of the spindle assembly checkpoint. Interestingly, division septa are assembled if Mad2p is overproduced in temperature-sensitive *cdc2* or *cdc13* mutants at the permissive temperature (to impose a metaphase block) followed by inactivation of *cdc2* or *cdc13* by shift to the restrictive temperature. These experiments suggest that degradation of Cdc13p by APC and the eventual loss of Cdc2p-Cdc13p activity are important for actomyosin ring constriction and division septum assembly. Interestingly, overproduction of the APC component Nuc2p results in a defect in cytokinesis (Kumada et al., 1995). Even though the mechanism leading to defective cytokinesis in these cells is unclear, these observations further emphasize the role of the APC in regulation of cytokinesis.

Although the data described above suggest a role for APC-mediated destruction of Cdc13p and loss of Cdc2p-Cdc13p kinase activity in actomyosin ring constriction and division septum assembly, mutants defining components of the anaphase-promoting complex display a 'cut' phenotype generated by septation in the absence of chromosome segregation (Yanagida, 1998). One possible explanation for this is that, in these mutants, Cdc2p kinase gets inactivated by other means, such as inhibitory tyrosine phosphorylation (Gould and Nurse, 1989) or accumulation of a protein such as the Cdc2p-Cdc13p inhibitor Rum1p (Stern and Nurse, 1996), which thereby allows septum formation. Alternatively, the temperature-sensitive APC mutants might be hypomorphs and might retain low levels of APC function sufficient for destruction of Cdc13p but not for the destruction of other substrates, such as Cut2p (Yanagida, 1998), which would lead to septation in the absence of chromosome segregation.

LINKING MITOTIC EXIT TO ACTOMYOSIN RING CONSTRICTION AND SEPTUM ASSEMBLY

How then do cells transduce the signal that cyclins have been destroyed to the division machinery such that actomyosin ring constriction and division septum synthesis are activated? The answer to this question might lie in the signaling cascade encoded by the *sid* genes (***s***eptum ***i***nitiation ***d***efective). The *sid* genes were identified in genetic screens aimed at identifying genes that regulate cell division (Nurse et al., 1976; Schmidt et al., 1997; Balasubramanian et al., 1998). Mutations in the *sid* genes block cytokinesis, but the cells undergo multiple cycles of S and M phases and become multinucleate and highly elongated before they lyse. Interestingly, F-actin rearrangements, such as the assembly of actomyosin rings at mitosis, accumulation of F-actin patches at late anaphase, and accumulation of F-actin patches at cell ends in interphase are unaffected in these mutants. Mutations in seven genes (*cdc7*, *cdc11*, *cdc14*, *sid1*, *sid2*, *spg1* and *sid4*) produce a *sid* phenotype. Extensive genetic analysis by Marks et al. (1992) and more recently by Balasubramanian et al. (1998) has suggested that the products of the *sid* genes interact to effect septum assembly. Molecular analysis of these genes reveals that the Sid pathway represents an elaborate signal transduction network, which includes three protein kinases (Cdc7p, Sid1p, and Sid2p; Fankhauser and Simanis, 1994; Sohrmann et al., 1998; Guertin et al., 2000; Sparks et al., 1999), and a GTPase (Spg1p; Schmidt et al., 1997; Balasubramanian et al., 1998).

The nucleotide state of the GTPase Spg1p appears to be of key importance in activation of ring constriction and septum delivery (Schmidt et al., 1997). Cells expressing constitutively activated forms of Spg1p exhibit uncontrolled septation, whereas, in cells expressing a dominant negative form of Spg1p, septum assembly is prevented. The effects of GTP-bound Spg1p are mediated through its effector protein Cdc7p and require the function of Sid1p, Sid2p, Sid4p and Cdc14p (Schmidt et al., 1997; Sparks et al., 1999; Balasubramanian et al., 1998; Guertin et al., 2000). Cdc16p and Byr4p, which form a two-component GTPase-activating protein complex for Spg1p negatively regulate the Sid pathway (Furge et al., 1998). The fact that mutations in *cdc16* and *byr4* lead to uncontrolled septation (Minet et al., 1979; Fankhauser et al., 1993; Song et al., 1996) whereas mutations in the *sid* genes lead to a lack of septation (Nurse et al., 1976; Marks et al., 1992; Balasubramanian et al., 1998) is consistent with this. Plo1p might inhibit Cdc16p-Byr4p function, since overproduction of Plo1p seems to result in conversion of Spg1p to the GTP-bound state, thereby resulting in deregulated septation (Minet et al., 1979; Fankhauser et al., 1993; Okhura et al., 1995; Mulvihill et al., 1999). Alternatively, Plo1p might regulate the as-yet-unidentified guanine-nucleotide-exchange factor for Spg1p.

Cytological analysis suggests that the proteins of the Sid pathway regulate actomyosin ring constriction and septum assembly in response to signals originating from the mitotic SPBs (Sohrmann et al., 1998; Cerutti and Simanis, 1999; Sparks et al., 1999). Localization of all Sid-pathway components depends on the function of Sid4p, a novel protein, which is itself associated with the SPB (Chang and Gould, 2000). The Spg1p GTPase is detected at the SPB in both interphase and mitotic cells (Sohrmann et al., 1998). However, in interphase, Spg1p is GDP bound and, in late mitotic cells, one SPB carries GTP-bound Spg1p and the other carries GDP-bound Spg1p (Sohrmann et al., 1998). The effector for Spg1p, the Cdc7p-kinase (Fankhauser and Simanis, 1994), is also detected asymmetrically during mitosis at the pole that carries GTP-bound Spg1p (Sohrmann et al., 1998). As would be

expected, the GAP complex consisting of Cdc16p and Byr4p is detected at the SPB containing GDP-bound Spg1p (Cerutti and Simanis, 1999). It was recently found that the protein kinase Sid1p associates with Cdc14p and localizes late in anaphase to the SPB that contains Cdc7p-Spg1p-GTP. Furthermore, the localization of Sid1p to the SPB is intimately linked with Cdc2p inactivation. For example, Sid1p is not detected at the SPB in mitotic cells arrested at or prior to metaphase by overproduction of Mad2p or inactivation of *nda3* (Guertin et al., 2000). Sid1p is, however, detected in *nda3 mad2* double mutants (thereby allowing bypass of the spindle assembly checkpoint) as well as following heat-inactivation of Cdc2p in cells pre-arrested at metaphase (Guertin et al., 2000). It is clear from the present data that Sid1p localization to the SPB takes place only at anaphase following cyclin proteolysis and Cdc2p-Cdc13p kinase destruction. Future studies should test if Sid1p localization to the SPB acts to couple destruction of Cdc2p-Cdc13p to the onset of actomyosin ring constriction and septum assembly.

It is unclear why Cdc7p/Spg1p-GTP and Sid1p-Cdc14p are localized asymmetrically in a symmetrically dividing fission yeast cell. Asymmetric localization of Cdc7p and Sid1p does not seem to be necessary for actomyosin ring constriction and septum formation, since actomyosin ring constriction and septum formation are unaffected in *cdc16*-116 mutants, in which Cdc7p and Sid1p are detected at both the SPBs. It is possible that the asymmetry is important for turning off septum synthesis or for cell separation.

An attractive possibility to have emerged from recent studies is that a key function of Cdc7p, Cdc11p, Cdc14p, Sid1p, Spg1p and Sid4p is to promote the localization and/or function of Sid2p (Sparks et al., 1999). Sid2p is related to the budding yeast Dbf2p and Dbf20p kinases (Balasubramanian et al., 1998) and is detected at the SPB in both interphase and mitotic cells (Sparks et al., 1999). Interestingly, Sid2p is also detected at the division site during actomyosin ring constriction and division septum assembly. Efficient localization of Sid2p to the division site requires the microtubule cytoskeleton, which suggests that Sid2p is transported to the division site along the post-anaphase array of microtubules that lead from the SPBs to the division site. The localization of Sid2p to the division site and the activation of Sid2p kinase activity require the function of the Sid pathway. This suggests that the Sid2p kinase functions at the end of the Sid pathway. Combined genetic and cytological analysis suggests that the order of the Sid pathway as follows: Spg1p => Cdc7p => Sid1p-Cdc14p => Sid2p (Fig. 2; Guertin et al., 2000).

Genetic studies have also identified Zfs1p, a zinc-finger-containing protein, as an inhibitor of the Sid pathway, since a null mutation in *zfs1* is capable of reversing the cell division defect of a number of *sid* mutants and since overproduction of Zfs1p rescues the *cdc16*-116 mutant (Beltraminelli et al., 1999). These findings suggest that downregulation of Zfs1p is a mechanism by which the Sid pathway activates septation. Future studies should address the precise mechanism of interaction of the Sid pathway with Zfs1p.

Localization of Sid2p to the division site (Sparks et al., 1999) might activate and/or stabilize the constricting actomyosin ring and allow septum formation (Liu et al., 1999; Sparks et al., 1999). Potential targets of Sid2p at the actomyosin ring are unknown. However, genetic interactions between *sid2*-250 and *cps1*-191 (a mutation in a 1,3-β-glucan synthase essential for septum assembly) have been detected. Cps1p might therefore be a target of Sid2p (Liu et al., 1999). Genetic interactions between *cdc7* and the type II myosin heavy chain encoding genes, *myo2* and *myp2*, have also been identified (Mulvihill et al., 1999). Thus, the Sid pathway (and possibly Sid2p) might also regulate septum assembly by modulation of type II myosin function.

Dma1p, a novel protein that suppresses a *cdc16*-116 mutant upon overproduction, might also play an important role in linking completion of mitosis to initiation of septation (Murone and Simanis, 1996). Mutants lacking *dma1* do not display any overt phenotype. However, *dma1 nda3* mutants are unable to maintain Cdc2p kinase activity and exit from mitosis and form septa. Interestingly, overproduction of Dma1p leads to a *sid* phenotype and allows progression through multiple mitotic cycles without septation. Cells overproducing Dma1p also undergo normal actin rearrangements associated with the cell cycle; they assemble actomyosin rings at mitosis and possess actin patches at the cell tips during interphase (Murone and Simanis, 1996). One interpretation of these data is that Dma1p is an effector of the spindle assembly checkpoint that blocks actomyosin ring constriction and septum assembly. Biochemical studies of Dma1p, its intracellular localization and the analysis of localization of the Sid proteins in cells overproducing Dma1p should provide clues to the mechanism by which Dma1p links completion of mitosis with actomyosin

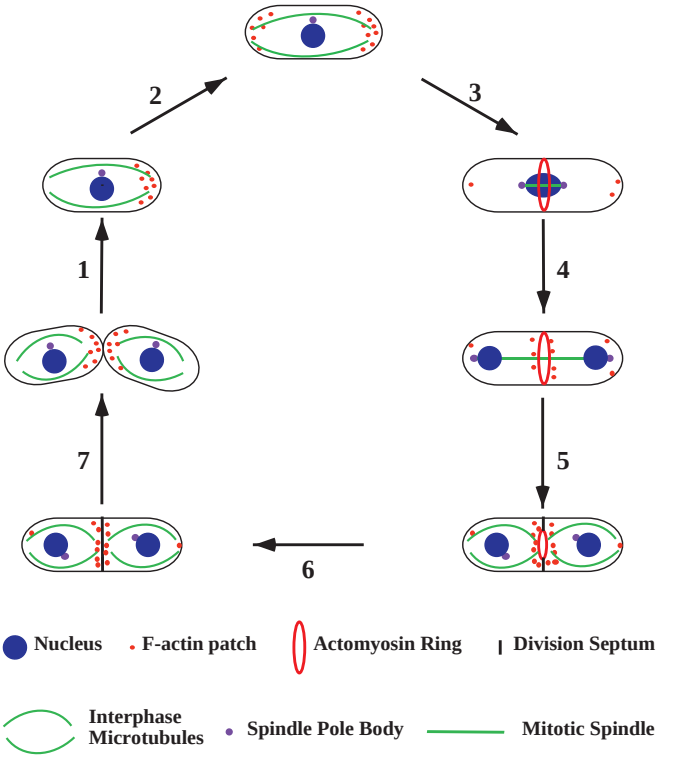

Fig. 1. Cytoskeletal rearrangements in the *Schizosaccharomyces pombe* cell cycle. Shown are the changes in distribution of F-actin, microtubules, and the spindle pole body. The series 1-7 represents the life cycle of an *S. pombe* cell from birth (1) to division and cell separation (7).

ring constriction and division septum assembly and to the Sid protein it presumably inactivates.

MECHANISMS TO PREVENT CYTOKINESIS IN PRE-MITOTIC CELLS

In wild-type *S. pombe* cells, there are mechanisms that prevent re-replication of chromosomal DNA when cells are in the G_2 phase of the cell cycle. Given that Cdc13p is the only B-type cyclin essential for viability, an elegant mechanism proposed by Nurse and colleagues is that the levels of Cdc2p-Cdc13p are critical for prevention of chromosomal DNA rereplication (Stern and Nurse, 1996). They propose that low levels of Cdc2p-Cdc13p activity promote DNA synthesis, and higher levels of Cdc2p-Cdc13p activity allow entry into M phase, whereas intermediate levels of Cdc2p-Cdc13p activity prevent rereplication of chromosomal DNA in G_2 cells. Similarly, recent work suggests that a combination of the activities of Cdc16p-Byr4p and Cdc2p kinase prevents inappropriate septation prior to entry into M phase (Cerutti and Simanis, 1999).

As described earlier, mutations in *cdc16*, a component of the two-component GAP for Spg1p, lead to uncontrolled septation. Two types of unregulated septation event are seen in these cells (Minet et al., 1979). In an asynchronous population of *cdc16*-116 cells shifted to the restrictive temperature, the majority of cells (85%, referred to as type I cells) proceed through mitosis and become blocked, and the two nuclei are separated by 2-3 septa. Following shift to the restrictive conditions, a small fraction (15%, referred to as type II cells) assemble a division septum prior to entry into mitosis. These cells are uninucleate and possess a septum on one side of the nucleus. Cerutti and Simanis (1999) have recently analyzed the basis for the formation of these two phenotypes upon inactivation of Cdc16p. These authors have found that the frequency of production of the type II phenotype can be enhanced to up to 50% by shifting *cdc16*-116 cells to the restrictive temperature during early S phase; this suggests that Cdc16p has a central role in prevention of septation in this phase of the cell cycle. Shifting of cells after S phase produces predominantly a type I population of cells (septation after mitosis). Following S phase, therefore, additional mechanisms probably prevent septation until entry into mitosis. Interestingly, *cdc16*-116 *cdc2*-33 cells or *cdc16*-116 *cdc25*-22 cells shifted to the restrictive temperature generate an increased number of uninucleate septated cells (up to 60%), even when shifted after S phase. These observations led Cerutti and Simanis to propose that during S phase, when Cdc2p kinase activity is low, the activity of Cdc16p is important to prevent inappropriate septation. In G_2 cells, intermediate levels (which prevent re-replication of chromosomal DNA) of Cdc2p kinase activity in combination with Cdc16p function prevent inappropriate septation. Thus, Cdc2p function, in addition to being important for ring assembly and division septum assembly, is also important for prevention of inappropriate septation in G_2 cells.

WHEN CYTOKINESIS FAILS…

Entry into M phase is blocked if chromosomal DNA is damaged or not fully replicated (Murray, 1992). It is less clear whether mechanisms that delay progression through the nuclear cycle if cytokinesis fails exist. Such mechanisms would prevent accumulation of more than one nucleus in the same cytoplasm, a situation that might lead to a deleterious increase in ploidy caused by fusion of interphase nuclei. Since mutants in which cytokinesis is defective accumulate more than two nuclei, it was presumed that, although cytokinesis may depend on nuclear events, the nuclear cycle does not depend on cytokinesis. However, this view has recently been questioned as a result of the cell cycle analysis of mutations in the gene that encodes a 1,3-β-glucan synthase, *cps1* (Ishiguro et al., 1997; Liu et al., 1999, 2000; LeGoff et al., 1999).

The *cps1*-191 (Liu et al., 1999, 2000) and the *cps1*-N12 (Le Goff et al., 1999) mutants differ from the other cytokinesis mutants in that they progress through the nuclear cycle more slowly. After a four-hour shift to the restrictive temperature, the *cps1*-191 mutant blocks predominantly with two interphase G_2 nuclei and an actomyosin ring, which persists from the failed cytokinesis (due to lack of division septum assembly). Interestingly, the mitotic defect, but not the septum assembly defect, is suppressed in *cps1*-191 *wee1* and *cps1*-191 *cdc2*-1w double mutants (*cdc2*-1w is an allele that encodes an activated form of Cdc2p that mimics loss of Wee1p function). Thus, Wee1p, in addition to regulating Cdc2p in response to nutrients and other factors affecting entry into mitosis, also appears to inhibit Cdc2p function if an interphase cell finds itself with some molecular marker left behind as a result of failed cytokinesis. This mechanism of regulating Cdc2p function is reminiscent of the morphogenetic checkpoint in *S. cerevisiae*, which delays mitosis by inhibition of Cdc28p via Swe1p (the budding yeast homolog of Wee1p) when bud assembly is affected (Lew and Reed, 1995; Barral et al., 1999).

What is the cellular marker that signals failure to complete cytokinesis? Although the answer to this question has not been fully resolved, F-actin-based structures that persist in *cps1*-191 mutants at the arrest point probably activate the cytokinesis checkpoint. Support for this conclusion comes from the ability of *cps1*-191 cells to enter a second round of mitosis (and accumulate four nuclei) if treated at the arrest point with an actin-polymerization inhibitor (Liu et al., 2000). Since *cps1*-191 mutants arrest with a prominent actomyosin ring, unlike all other cytokinesis mutants, it is likely that the presence of an actomyosin ring in the interphase cells activates the checkpoint and prevents further mitotic cycles. However, it is also possible that either F-actin patches or other unidentified F-actin-dependent structures that persist in *cps1*-191 cells activate the checkpoint.

Why do the cytokinesis mutants isolated to date accumulate multiple nuclei if a cytokinesis checkpoint monitors completion of cytokinesis? The two major classes of cytokinesis mutant isolated to date are the actomyosin ring assembly mutants (*rng* mutants) and the septum-initiation-defective mutants (*sid* mutants). Whereas the *rng* mutants fail to assemble normal actomyosin rings at mitosis, the *sid* mutants assemble normal actomyosin rings that disassemble at the end of mitosis. The *rng* mutants accumulate nuclei more slowly than do the *sid* mutants (Nurse et al., 1976; Liu et al., 2000). Thus, one possibility is that the G_2 delay is partially imposed in a *rng* mutant because an improper septum is synthesized rather slowly upon an improperly organized actomyosin ring. However, the *sid* mutants that completely

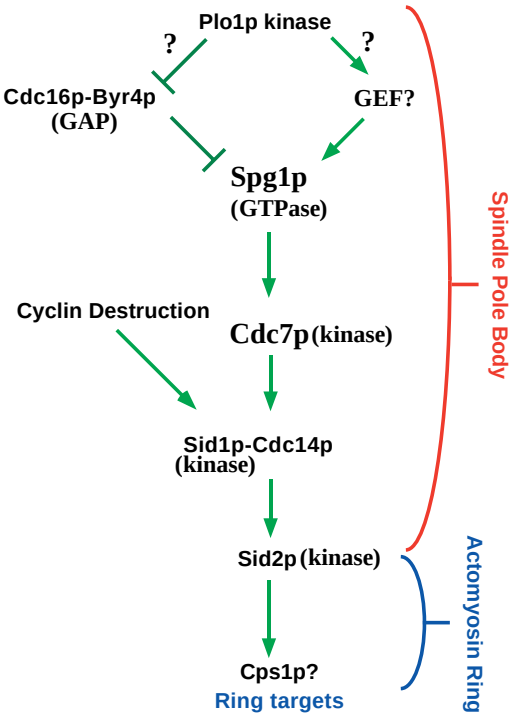

Fig. 2. The Sid pathway regulates the timing of actomyosin ring constriction and septum assembly in response to signals originating from the spindle pole body following mitotic exit. The Sid pathway might activate Cps1p, a 1,3-β-glucan synthase to effect septum assembly.

collapse the actomyosin ring might cause the loss of the signaling center that activates the checkpoint. Alternatively, the Sid proteins might be bifunctional, and they might be required for activation of septum deposition as well as for monitoring of the completion of cytokinesis. Previous studies have shown that the Sid protein Cdc14p can block the G_2/M transition in a Wee1p-dependent manner (Fankhauser and Simanis, 1993). This observation lends support to the idea that the Sid proteins are indeed bifunctional and might be required for the cytokinesis checkpoint in addition to activation of actomyosin ring constriction and division septum assembly.

SACCHAROMYCES CEREVISIAE: BUILDING A FENCE BETWEEN THE MOTHER AND DAUGHTER

The budding yeast mothers are a practical and unsentimental lot when it comes to separation from their daughters. Unlike fission yeast, they begin to plan for this eventual separation when the daughters are barely in their infancy. The assembly of the cytokinesis site begins early in the cell cycle (Fig. 3). Sometime in G_1 phase, the mother marks a site (a bud site and eventually also the site for cytokinesis) at its cortex by depositing a number of proteins, such as Spa2p, Myo2p (Myosin heavy chain class V) and Rho1p (GTP-binding protein; reviewed by Madden and Snyder, 1998). The passage through START triggers reorganization of the actin cytoskeleton: actin patches accumulate at the prospective bud site, and actin cables orient towards it. It is thought that

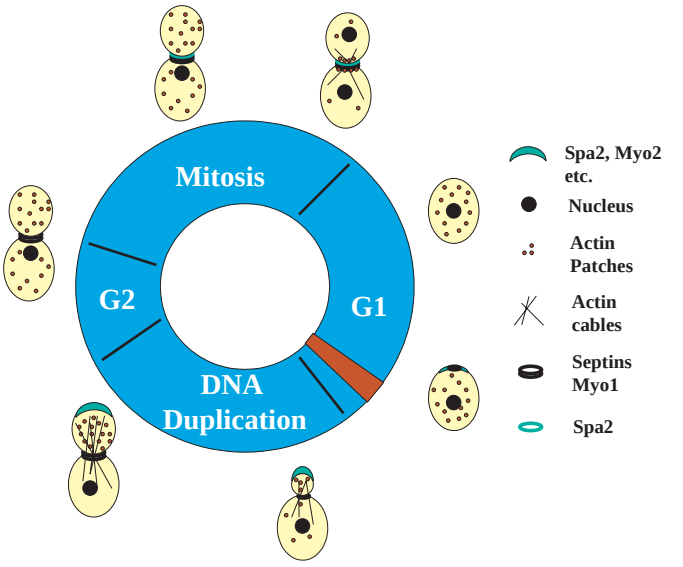

Fig. 3. Rearrangements of F-actin and bud assembly components through the cell cycle in *Saccharomyces cerevisiae*.

polarized transport of secretory vesicles occurs along these cables. A battalion of proteins that includes a Rho-family GTPase Cdc42p, its exchange factor Cdc24p, the rho-GAP homologue Bem3p and some of the polarity-establishment proteins, such as Bem1p and Bni1p, participate in the reorganization of actin cytoskeleton (reviewed by Pruyne and Bretscher, 2000a,b). Eventually a daughter (bud) emerges from the bud site by localized remodeling of the cell wall.

At about the time of bud emergence, a boundary, referred to as the neck region, between the mother and the newly formed bud is marked. This site is where the separation of the mother and its daughter (cytokinesis) occurs at the end of M phase. One of the most conspicuous features of the neck, when observed by thin-section electron microscopy, is the presence of ordered filaments that are about 10 nm in diameter. The structural components of these filaments are septins, which are encoded by *CDC3*, *CDC10*, *CDC11* and *CDC12*. Among these, *CDC3* and *CDC12* are essential for viability, but *CDC10* and *CDC11* are not (reviewed by Longtine et al., 1996; Field and Kellogg, 1999). In vitro studies suggest that the polymerization into a properly assembled higher-ordered structure requires all four septins, since septins isolated from *cdc10Δ* or *cdc11Δ* mutant strains fail to polymerize into discernibly ordered structures. Although localization to the neck appears to be required for septin function, assembly into a higher-ordered structure might not be necessary. Nevertheless, the fact that the mutants lacking septin function exhibit severe defects in both morphogenesis and cytokinesis underscores the importance of septins in the process of cell separation; however, their precise role remains to be clearly understood.

Another structural component of the neck region is the actomyosin ring, which is probably responsible for the contractile force and ingression of the cell wall into the division plane (Fig. 3). Unlike fission yeast, initiation of actomyosin ring assembly seems not to require entry into mitosis, since myosin II, which is encoded by the *MYO1* gene, arrives first at

the neck at the time of bud emergence and assembles into a ring (Lippincott and Li, 1998a; Bi et al., 1998). It can be argued that actomyosin ring assembly in *S. cerevisiae* can still be considered linked to mitosis, because budding yeast cells undergo an extended form of mitosis in which the spindle formation occurs early in the cell cycle (Nurse, 1985). However, more extensive analysis has shown that the timing of SPB separation (and hence the formation of the mitotic spindle) in wild-type budding yeast cells progressing through the division cycle coincides with the completion of S phase (Lim et al., 1996). Therefore, it is reasonable to suggest that the assembly of the actomyosin ring in *S. cerevisiae* begins early in the division cycle and is independent of entry into mitosis.

The proper assembly of the myosin II ring, which persists through out the cell cycle, is dependent on septins. Later in telophase, F-actin is recruited to this ring. Video microscopy studies show a clearly discernible change in the size of the myosin II ring during cytokinesis (Lippincott and Li, 1998a; Bi et al., 1998). This has been interpreted as the contractile motion of the ring, which lends support to the notion that the actomyosin ring is the source of contractile force during cell separation in budding yeast. The recruitment of F-actin appears to require an IQGAP-like protein encoded by an essential gene, *IQG1*/*CYK1*, in *S. cerevisiae* (Epp and Chant, 1997; Lippincott and Li, 1998a; Shannon and Li, 1999). *CYK1* was identified in the *S. cerevisae* genome database as an open reading frame that was predicted to encode a protein that shows substantial structural similarity to IQGAPs. That Cyk1p plays an important role in F-actin recruitment to the myosin II ring is supported by the observation that cells depleted of Cyk1p fail to accumulate filamentous actin at the cytokinetic ring and therefore fail to undergo cytokinesis (Lippincott and Li, 1998a). Cyk1p itself shows diffuse staining until the onset of anaphase, after which it localizes to the mother-daughter neck. The binding of IQGAP to calmodulin, small GTPases and F-actin (Machesky, 1998) suggests that IQGAPs are involved in linking signal pathways to actin reorganization.

Once the actomyosin ring is assembled, the maintenanace of integrity during the contractile motion at the end of mitosis seems to require Cyk2p protein. *CYK2* was identified as an open reading frame in the *S. cerevisiae* genome database because of its similarity to the *S. pombe* gene *cdc15*$^{+}$, which encodes a protein involved in the function of the actomyosin ring (Lippincott and Li, 1998b; Fankhauser et al., 1995). Cyk2p localizes as a pair of rings at the neck and localizes with the septin rings throughout most of the cell cycle. During cytokinesis the Cyk2p rings merge with the actomyosin ring and undergo a size change – as myosin II does. Interestingly, budding yeast cells lacking Cyk2p do not show any gross defect in actomyosin ring assembly. However, in the absence of Cyk2p, the ring rapidly disappears during what is thought to be cytokinetic contraction, which suggests that Cyk2p is required for the integrity of the actomyosin ring during neck constriction.

The mother-daughter neck also houses a number of other proteins primarily involved in cell polarity and morphogenesis. Some of these components exhibit complex spatial behavior. For example, both Bud3p and Bud4p proteins, first identified in a screen for mutants in which bud-site selection is defective, localize as a double ring at the mother-daughter neck during mitosis (Chant et at., 1995; Sanders and Herskowitz, 1996). The staining is not seen at the G_1-S transition or during S phase but only apparent once the mitotic kinase (Cdc28p-Clb) is activated. Spa2p, a nonessential protein that affects bud-site selection in diploids, translocates to the incipient bud site, but moves to the bud tip upon bud emergence (Snyder, 1989; Gehrung and Snyder, 1990). Later in late-S or G_2 phase, it assembles into a double ring structure at the neck region. Axl2p, an integral membrane glycoprotein, behaves similarly in its spatial distribution but translocates to the neck earlier than Spa2p (Roemer et al., 1996). It is not clear whether these proteins play a significant role, if any, in the process of cytokinesis, although the *bud3Δ* mutant does show a modest defect in cytokinesis (Roemer et al., 1996).

TETHERING CELL SEPARATION TO THE COMPLETION OF MITOSIS

In the original 'functional map' of the *S. cerevisiae* cell cycle (Pringle and Hartwell, 1981), nuclear division was depicted as a pathway independent of both bud emergence and cytokinesis. Indeed, *cdc24* mutant cells, although they fail to form buds at the restrictive temperature, nevertheless undergo nuclear division. Similarly, cells in which cytokinesis is defective continue to go through multiple rounds of nuclear division, which suggests that completion of cell separation is not a prerequisite for entry into a new cycle or for chromosome segregation.

Conversely, however, the completion of nuclear division cycle appears to be a prerequisite for both cell separation and the emergence of a new bud, since neither of these processes is initiated until cells have exited mitosis. During a normal cell cycle, the separation of a daughter from its mother usually follows the appropriate partitioning of the chromosomes; a failure to do so can result in the death of both. How do cells ensure that this temporal order is maintained? One of the hallmarks of completion of the nuclear division cycle is the inactivation of the Cdc28-Clb mitotic kinase through rapid destruction of the mitotic cyclins, Clbs (Nasmyth, 1993), catalyzed by the APC (reviewed by Morgan, 1999; Zachariae and Nasmyth, 1999). The proteolysis of Clbs requires a number of other proteins, including Tem1p (a GTP-binding protein related to the *S. pombe* Spg1p), Cdc15p (a MAP-kinase-kinase-like protein related to the *S. pombe* Cdc7p kinase) and Cdc14p (a dual-specificity phosphatase).

Whereas Cdc14p phosphatase is responsible for activating Hct1p, itself an activator of the APC, the main function of Tem1p and Cdc15p is to ensure the release of Cdc14p from a multifunctional complex called RENT (for regulator of nucleolar silencing and telophase), which sequesters it to the nucleolus until late anaphase (Shou et al., 1999; Visintin et al., 1999; Zachariae, 1999). Cells in which Tem1p, Cdc15p or Cdc14p is defective not only fail to inactivate the kinase but also are unable to undergo cytokinesis. Similarly, expression of proteolysis-resistant Clb1p or Clb2p causes cells to arrest in late telophase and also prevents them from triggering cytokinesis (Ghiara et al., 1991; Surana et al., 1993). The mitotic kinase might thus be a negative regulator of cytokinesis in that, without its inactivation, cell separation is not initiated.

Although, in *S. cerevisiae*, the link between the mitotic

kinase and cytokinesis has not been explored extensively, investigations in other experimental systems have suggested that myosin-filament assembly and activity are negatively regulated in metaphase by phosphorylation by p34^{cdc2} kinase (Satterwhite et al., 1992; Yamakita et al., 1994). However, there are some budding yeast mutants in which cytokinesis appears to be independent of the completion of nuclear division at the restrictive temperature. For example, cells lacking Esp1p function exhibit defective chromosome separation but eventually undergo cytokinesis to produce progeny, many of which are anucleate (McGrew et al., 1992; Surana et al., 1993). However, in this mutant, cell separation is still dependent on the inactivation of the mitotic kinase, since the *esp1 cdc15* double mutant arrests with high kinase activity and an undivided nucleus (Surana et al., 1993). Thus far there is no report of any mutant that can undergo cell separation despite high mitotic kinase activity.

How does the mitotic kinase prevent onset of cytokinesis? One possibility is that the Cdc28p-Clb kinase inhibits localization to the neck of components that play important roles in neck constriction. For instance, *cdc15* cells, which fail to inactivate the kinase at restrictive temperature, are unable to localize actin properly, and to a large extent Cyk1p, to the neck region (Lippincott and Li, 1998a). Moreover, the fact that *cdc15*^{ts} mutants also fail to septate implies that appropriate assembly of the actomyosin ring is necessary for cell wall ingression in the division plane. Alternatively, some of the proteins involved in the inactivation of mitotic kinase might also be important for organizing cytokinetic structures. Recently, Song et al. (2000) showed that overexpression of the Cdc5p polo-like kinase, which is required for proteolysis of the mitotic cyclins, causes buds to elongate and induces additional septin rings within these buds in a kinase-activity- and polo-box-dependent manner (Song et al., 2000). Cdc5p shows localization to both the neck filaments and to the extra septin rings within the elongated buds. The dual roles of Cdc5p might further help link the process of cytokinesis to the inactivation of the kinase. Clearly, some of the structural components, such as septin and myosin II, can translocate to the neck in the presence of high mitotic kinase activity. However, the kinase might inhibit the final steps in the structural assembly and contractility to prevent premature cell separation. Any firm conclusions regarding this issue will have to await detailed investigations.

Cytokinesis seems to have another regulatory connection with nuclear division. In budding yeast, a mutation in the motor protein dynein often causes misalignment of the mitotic spindle and delays cytokinesis until the spindle is properly positioned. This delay apparently requires a yeast homologue (Yeb1p) of the human protein EB1, a protein that binds to the tumor-suppressor protein APC (adenomatous polyposis coli; Muhua et al., 1998). A mutation in Yeb1p abolishes the delay in cytokinesis in response to the misorientation of the mitotic spindle. However, the mechanism that underlies this regulation is not known.

Although nuclear division was originally proposed to be independent of both bud emergence and cytokinesis, it has become clear that it is affected at by the state of the budding cycle (Lew and Reed, 1995). Mutants such as *cdc42* and *cdc24*, which fail to bud at the non-permissive temperature, exhibit a Swe1p/Cdc28p-dependent delay in nuclear division (Lew and Reed, 1993). This regulatory framework is known as the morphogenetic checkpoint control. Does failure to complete cytokinesis likewise influence the progression to nuclear division? Recent evidence in *S. pombe* favors the existence of a cytokinesis-failure-induced nuclear division delay (Le Goff et al., 1999; Liu et al., 1999, 2000). In budding yeast, the analysis of *cdc15-lyt1* and *cdc15*-1 mutants has been interpreted to indicate the existence of a septin-dependent checkpoint that links initiation of a new cycle (not nuclear division per se) to the completion of cytokinesis. *cdc15-lyt1* cells fail to septate but produce apical projections after some delay at the restrictive temperature (Jimenez et al., 1998). The formation of apical projections, which is accompanied by an increase in DNA content, was found to be dependent on the entry into the next cycle and was taken as the cells' attempts to bud. A mutation in the gene encoding the Cdc10p septin abolished the delay in projection formation, which suggests that the delay in projection formation is due to a failure to complete cytokinesis. However, further evidence will be required to establish the regulatory links between cytokinesis and the initiation of a new cycle.

Hence, the regulatory frameworks for coupling cytokinesis to the nuclear cycle appear to be similar in the fission and budding yeasts. However, despite these general similarities, there are some notable differences in the finer details. The most conspicuous example of such differences is the function of proteins involved in the exit pathway in budding yeast. Both Tem1p and Cdc15p are required for the release of Cdc14 phosphatase from the nucleolar RENT complex, thus allowing rapid destruction of mitotic cyclins. The function of the recently identified *S. pombe* protein (D. McCollum, unpublished observations) related to *S. cerevisiae* Cdc14p is unknown. However, *S. pombe* Spg1p and Cdc7p (related to *S. cerevisiae* Tem1p and Cdc15p, respectively) are implicated in septum formation and are not required for mitotic exit. Thus, it is possible that the Sid pathway of *S. pombe* and the mitotic-exit network of *S. cerevisiae* represent a common regulatory module that the two yeasts employ for two different physiological ends.

CONCLUDING REMARKS

In recent years, studies on morphogenesis and polarization, nuclear division and the anatomy of the cytokinetic site in budding and fission yeasts have yielded a wealth of information on these important aspects of the cell division cycle. Although these studies were initiated as distinct streams of investigations, an unmistakable regulatory overlap is beginning to emerge. It is becoming clear that an actomyosin-based ring is important for cell division in both *S. cerevisiae* and *S. pombe*. Interestingly, in *S. cerevisiae*, this ring structure is assembled in stages: assembly begins at START, with assembly of type II myosin (Bi et al., 1998) and ending after anaphase with the incorporation of F-actin and Iqg1p (Lippincott and Li, 1998a; Epp and Chant, 1997). In contrast, in *S. pombe*, the actomyosin ring with all its identified constituents is detected upon entry into M phase and prior to anaphase. Furthermore, the mechanisms by which the actomyosin ring is positioned are clearly different. However, regardless of these differences, inactivation of the mitotic kinase (by destruction of B-type

cyclins) appears to be a key step in regulation of actomyosin ring constriction and/or septum assembly in both of these yeasts. Studies on the two yeasts have also identified the SPB as a site from which cytokinesis is regulated, which thus provides a means to couple nuclear events with cytokinesis. Finally, mechanisms to delay entry into mitosis in response to failed cytokinesis or failed morphogenetic events exist in both yeasts. Negative regulators of mitosis (such as Wee1p or Swe1p) and cytoskeletal elements (such as F-actin and septins) appear to be important for delay of subsequent nuclear cycle events in response to failed cytokinesis or morphogenesis.

Given the fundamental nature of cytokinesis and nuclear division, we expect that the mechanisms coupling these activities in the two yeasts are similar. It is conceivable that such a regulatory framework is also used by other organisms. With researchers' continuing efforts, uncovering of the detailed molecular circuitry that underlies the space-time coordination of these events may not be too far in the future.

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