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Cytokinesis in Budding Yeast: the Relationship between Actomyosin Ring Function and Septum Formation

Erfei Bi

Department of Cell & Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6058, USA

ABSTRACT. **Cytokinesis in budding yeast is accomplished by the concerted action of actomyosin ring function and septum formation. The actomyosin ring is not essential for cell viability, but it is required for efficient cell division. Deletion of the actomyosin ring results in abnormal septum formation, and a delay in cytokinesis and cell separation. In contrast, septum formation is essential for cell viability. Block of septum formation prevents the contraction, but not the formation of the actomyosin ring. Here we review and provide additional evidence that defines the functional and molecular relationship between actomyosin ring function and septum formation.**

Key words: cytokinesis/actomyosin ring/septum formation/actin/myosin/septin/chitin

Cytokinesis, one of the classic problems in cell biology, has fascinated biologists for over a century. Early studies of cytokinesis in marine invertebrate eggs and cultured animal cells uncovered fundamental principles governing cytokinesis in animal cells. One major conclusion is that the cleavege furrow forms at late anaphase of the cell cycle and bisects the mitotic spindle, implicating cytokinesis as both a temporally and spatially regulated process (Field *et al*., 1999; Fishkind and Wang, 1995; Hales *et al*., 1999; Rappaport, 1996; Sanger and Sanger, 2000). The ingression of the furrow is driven by a cortical actin-and myosin-based (actomyosin) contractile ring (Fujiwara and Pollard, 1976; Mabuchi and Okuno, 1997; Schroeder, 1972; Schroeder, 1973). At the end of contraction, the two forming daughter cells remain connected by a narrow intercellular bridge with a central midbody that has a diameter of 1–2 μm (Mullins and Biesele, 1973; Mullins and Biesele, 1977). The completion of cytokinesis requires the actomyosin ring contraction, sealing of the intercellular bridge, and eventual cell separation (Sanger *et al*., 1985).

At the molecular level, studies on cytokinesis in animal cells have concentrated on the function of the actomyosin contractile ring. Key questions include: what proteins, besides F-actin and type II myosin, are required for the formation and contraction of the actomyosin ring? How is the assembly of the actomyosin ring regulated temporally and spatially? How is the function of the actomyosin ring coordinated with other cellular processes such as membrane deposition? Answers to most these questions rely on the identification of additional proteins that are specifically involved in cytokinesis. Thus, it is desirable to study cytokinesis in an organism with facile genetics.

Genetic analysis of cytokinesis was pioneered in *Dictyostelium discoidium* (De Lozanna and Spudich, 1987), which divides by two distinct mechanisms: an actomyosin ring that is required for cells growing in suspension and an actomyosin ring-independent machanism that is required for cells growing on a substratum (Neujahr *et al*., 1997; Zang *et al*., 1997). The study of cytokinesis in *Dictyostelium* has provided valuable information on the assembly and function of type II myosin. Genetic studies in other model organisms such as *Drosophila melanogaster* and the fission yeast *Schizosaccharomyoces pombe*, where an actomyosin ring plays an essential role in cytokinesis, have led to the identification of a large number of proteins involved in cytokinesis, including F-actin, type II myosin, myosin light chains, formins, IQGAP-related proteins, and protein kinases (Bahler *et al*., 1998; Balasubramanian *et al*., 1992; Balasubramanian *et al*., 1994; Balasubramanian *et al*., 1998; Cerutti and Simanis, 1999; Chang *et al*., 1996; Chang *et al*., 1997; Eng *et al*., 1998; Fankhauser *et al*., 1995; Furge *et al*., 1998; Gatti *et al*., 2000; Kitayama *et al*., 1997; May *et al*., 1997; McCollum *et al*., 1995; Naqvi *et al*., 1999; Nurse *et al*., 1976; Ohkura *et al*., 1995; Schmidt *et al*., Sohrmann *et al*., 1996; Sohrmann *et al*., 1998; Sparks *et al*., 1999). A

Department of Cell & Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6058, USA.
Tel: +215–573–6676, Fax: +215–898–9871
E-mail: ebi@mail.med.upenn.edu

comparative study of cytokinesis in multiple organisms has indicated that the major molecular elements involved in cytokinesis are conserved throughout evolution and that these common mechanisms are coordinated with processes or regulated by pathways that bear species-specific features.

In the budding yeast *Saccharomyces cerevisiae*, cytokinesis is carried out by the concerted action of actomyosin ring contraction and septum formation. As in *Dicyostelium*, the actomyosin ring is not essential for cell viability, providing an excellent opportunity to study how gene deletions affect the assembly and/or contraction of the actomyosin ring. As in fission yeast, the function of the actomyosin ring must coordinate with the centripetal growth of the septum during cytokinesis, providing an excellent opportunity to study how the actomyosin ring is coordinated with other cellular processes. In this article, we review the current status of cytokinesis in budding yeast, focusing on the relationship between actomyosin ring function and septum formation.

The assembly and function of the actomyosin ring

A) Properties of the actomyosin ring

It was originally thought that budding yeast might not need an actomyosin ring, because of the narrowness of the bud neck (1 μm), which is very close to the diameter of a midbody (1–2 μm) in animal cells (Sanders and Field, 1994). However, recent studies showed that there is an actomyosin contractile ring in budding yeast (Bi *et al*., 1998; Lippincott and Li, 1998b). The overall behavior of this ring structure is similar to that in animal cells. The assembly of the actomyosin ring is completed by anaphase in both systems, which is followed by ring contraction that drives the cleavege of cytoplasm. This similarity, together with the genetic tractability, makes the budding yeast an attractive model for studying the general behavior of the actomyosin ring. However, there are three important differences between the actomyosin ring in budding yeast and its counterpart in animal cells. The first is in the timing of its assembly. In budding yeast, the assembly of the actomyosin ring starts at the beginning of the cell cycle (late G1) by marking the presumptive bud site with the only type II myosin, Myo1p. Once the bud emerges, Myo1p remains at the bud neck with a constant diameter until late anaphase when F-actin is recruited to the bud neck to form a functional actomyosin ring (Bi *et al*., 1998; Lippincott and Li, 1998b). The function of Myo1p before late anaphase is unknown. In contrast, type II myosin and F-actin in animal cells arrive at the cleavege site at approximately the same time around anaphase (Rappaport, 1996; Satterwhite and Pollard, 1992). Second, the contraction of the actomyosin ring in budding yeast must be coordinated temporally and spatially with septum formation, whereas, in animal cells, a septum does not exist. Third, unlike in fission yeast and animal cells, the actomyosin ring is not essential for cell viability and cytokinesis in budding yeast (Bi *et al*., 1998; Rodriguez and Paterson, 1990; Watts *et al*., 1987).

B) Proteins involved in the formation of the actomyosin ring

Formation of the actomyosin ring in budding yeast requires the functions of the septins, type II myosin, F-actin, IQGAP, and the formins, all of which are evolutionarily conserved from yeast to mammals. The major question is how these molecules are organized together to build an efficient division apparatus.

Septins: Septins are a family of structurally related proteins that are capable of forming filaments *in vitro* in a guannine-nucleotide-dependent manner (Field *et al*., 1996; Frazier *et al*., 1998). Septins were first discovered in budding yeast, now known to be widely present, ranging from yeast to humans (Byers and Goetsch, 1976a; Byers and Goetsch, 1976b; Longtine *et al*., 1996). Most known septins localize to the cleavege site and play a role in cytokinesis (Kinoshita *et al*., 1997; Neufeld and Rubin, 1994). However, the detailed mechanisms underlying their cytokinesis functions are not clear.

In budding yeast, there are a total of seven septins, five of which are expressed during the vegetative cycle and two of which are expressed exclusively during sporulation (De Virgilio *et al*., 1996; Fares *et al*., Longtine *et al*., 1996). All vegetative septins localize to the bud neck throughout the cell cycle in an hourglass structure. Mutational inactivation of any of the septin genes results in the loss of the entire septin structure and a complete block in cytokinesis. Thus, septins play an esssential role in cytokinesis. At the molecular level, septins have two known functions in cytokinesis. First, septins are required for the recruitment and maintenance of Myo1p at the bud neck; thus, defining their role in the assembly of the actomyosin ring (Bi *et al*., 1998; Lippincott and Li, 1998b). Second, septins are required for the anchoring of chitin synthases (CS), including CSII and SCIII, to the bud neck, defining their role in septum formation (DeMarini *et al*., 1997; Shaw *et al*., 1991) (Yu and Pringle, personal communication). Proteins involved in actomyosin ring function and septum formation target to the septins independently, which may explain why the septins are essential for cytokinesis, whereas the actomyosin ring is not.

In addition to thier role in cytokinesis, septins are also required for the anchoring of proteins involved in bud-site selection and morphogenetic checkpoint to the bud neck (Barral *et al*., 1999; Chant *et al*., 1995; Longtine *et al*., 2000; McMillan *et al*., 1999; Shulewitz *et al*., 1999), suggesting that septins may function as a scaffold for targeting proteins to the bud neck in general (Longtine *et al*., 1996). In addition, recent data suggest that septins may function as

a diffusion barrier at the bud neck that prevents some integral-membrane proteins and membrane-associated proteins from moving freely between the mother and the daughter cells (Barral *et al*., 2000; Takizawa *et al*., 2000).

Type II myosin and F-actin: The only type II myosin in budding yeast, Myo1p, marks the division site shortly before bud emergence. Once the bud is formed, Myo1p is maintained at the bud neck until F-actin is recruited to form a contractile actomyosin ring in late anaphase. The contraction, but not the formation, of Myo1p ring requires the presence of F-actin. The disassembly of the Myo1p ring during the contraction phase is F-actin-independent and is instructed by a cell cycle signal (Bi *et al*., 1998). The localization and maintenance of Myo1p at the bod neck depend on the septins, whereas the formation of the actin ring depends on Myo1p. Thus, the order of protein assembly at the bud neck is as follows: septins→Myo1p→F-actin. Deletion of *MYO1* is not lethal, but causes a delay in cytokinesis and cell separation (Bi *et al*., 1998; Rodriguez and Paterson, 1990; Watts *et al*., 1987).

In order to establish budding yeast as a model system for studying cytokinesis, it is necessary to understand the basic properties of the actomyosin ring. In the past two years, our effort has focused on understanding the targeting and regulation of Myo1p in cytokinesis. The dramatic, yet viable, phenotype of *myo1* null cells has provided a unique opportunity to perform structure-function analysis on Myo1p. Through deletion analysis, we have defined Myo1p's targeting domain, a minimal sequence of 115 amino acids located in a proline-rich region of the tail (Dravis and Bi, unpublished data). This domain is both necessary and sufficient for Myo1p's localization to the bud neck. However, the targeting domain does not interact with the septins directly, suggesting that there may be a linker protein(s) between them. A search for these linker proteins is currently underway. Once they are identified, their role in targeting Myo1p to the bud neck will be assessed.

All known type II myosins are regulated by a pair of light chains, an essential light chain and a regulatory light chain. Using the Myo1p head as the bait, we have identified one interactiong protein Ypr188cp (here designated Mlc2p) that shares sequence homology to calmodulin and other light chain-related molecules. Mlc2p and Myo1p have an identical localization pattern throughout the cell cycle. Mlc2p localization to the bud neck depends on Myolp, but Myolp localization does not depend on Mlc2p. Thus, Myo1p brings Mlc2p to the bud neck. Mlc2p binds to the second IQ motif of Myo1p, just upstream of the head-tail junction, suggesting that Mlc2p is the regulatory light chain for Myo1p. However, delection of *MLC2* or its binding site does not seem to affect the assembly or contraction of Myo1p (Bi, unpublished data). Currently, we are using genetic approaches to investigate the function of *MLC2*.

Mlc1p, the light chain for Myo2p (a type V myosin) and Iqg1p (IQGAP) (Shannon and Li, 2000; Stevens and Davis, 1998), can be co-immunoprecipitated with Myo1p (Boyne *et al*., 2000). We have mapped its binding site to the IQ1 motif of Myo1p, suggesting that Mlc1p is the essential light chain for Myo1p (Bi, unpublished data). The function of Mlc1p-Myo1p interaction is not clear, because deletion of IQ1 motif or IQ1 and IQ2 together, which eliminates the interaction between Mlc1p and Myo1p completely, does not cause any obvious defect in the assembly and contraction of Myo1p at 25°C. We are currently assessing the effect of Mlc1p-Myo1p interaction on cytokinesis under other growth conditions (Bi, unpublished data). Because F-actin is required for the contraction of Myo1p (Bi *et al*., 1998), this data suggests that in the absence of Mlc1p-Myo1p interaction, cells are still able to form a functional actomyosin ring.

In contrast to Myo1p, Mlc1p is essential for cell viability and cytokinesis. Thus, Mlc1p must play additional role(s) in cytokinesis. Indeed, Mlc1p interacts with Myo1p and Iqg1p (discussed below), both of which are required for the formation of the actomyosin ring (Boyne *et al*., 2000; Shannon and Li, 2000). Mlc1p also interacts with Myo2p (Stevens and Davis, 1998), suggesting that Mlc1p may be involved in targeted secretion to the bud neck, which is required for septum formation. The localization of Mlc1p to the bud neck depends on the septins, but not Myo1p (Boyne *et al*., 2000; Shannon and Li, 2000), suggesting that Mlc1p only associates with Myo1p transiently during cytokinesis. Because Mlc1p-Myo1p interaction is not required for the function of the actomyosin ring, at least at 25°C, the essential role of Mlc1p in cytokinesis under this condition must be largely due to its function in septum formation.

IQGAP: Iqg1p (also called Cyk1p) belongs to a family of proteins that is characterized by an N-terminal calponin-homology domain (CHD), middle IQ motifs, and a C-terminal GAP-related domain (GRD). Iqg1p forms a single ring at the bud neck around anaphase and contracts like Myo1p, suggesting that Iqg1p may be a component of the actomyosin ring (Shannon and Li, 1999). Indeed, Iqg1p is required for the actin ring formation (Epp and Chant, 1997; Lippincott and Li, 1998b). However, the recruitment of Iqg1p to the bud neck depends on the septins but not Myo1p, suggesting that Iqg1p and Myo1p target to the septins independently. How does Iqg1p promote actin ring formation? One possibility is that Mlc1p recruits Iqg1p to the bud neck by binding to the IQ motifs of Iqg1p (Shannon and Li, 2000), and then the CHD domain of Iqg1p cross-links F-actin (Bashour *et al*., 1997) into a ring structure using the Myo1p ring as the template. This hypothesis would explain the requirement of both Iqg1p and Myo1p in actin ring formation. Like Mlc1p, Iqg1p is essential for cell viability and cytokinesis; thus, it must play dual roles in cytokinesis, contributing to both the actomyosin ring function and septum formation.

Formins: Formin-Homologous (FH) proteins are known to play a role in cytokinesis in other organisms (Wasserman,

1998). There are two FH proteins in budding yeast: Bni1p and Bnr1p. Bni1p localizes to the presumptive bud site, the tip of a small bud, and the neck of a large-budded cell, whereas Bnr1p localizes to the bud neck throughout the cell cycle (Evangelista *et al*., 1997; Fujiwara *et al*., 1998; Kamei *et al*., 1998; Kikyo *et al*., 1999). Deletion of *BNI1* or *BNR1* alone is not lethal, but deletion of both causes cell lethality, suggesting that Bni1p and Bnr1p share at least one essential function (Kamei *et al*., 1998; Vallen *et al*., 2000). Deletion of *BNI1* causes defects in multiple processes including bud-site selection, polarity establishment, cytokinesis, and spindle orientation (Evangelista *et al*., 1997; Kohno *et al*., 1996; Lee *et al*., 1999; Miller *et al*., 1999; Zahner *et al*., 1996). Most *bni1* null cells fail to complete actomyosin ring contraction (Vallen *et al*., 2000), whereas the role of Bnr1p in cytokinesis is less obvious. Our recent analysis of a *bni1-ts* mutation in a *bnr1*Δ background suggests that Bni1p and Bnr1p together are required for the formation of the actomyosin ring and septum formation (Tu and Bi, unpublished date).

C) Function of the actomyosin ring

Deletion of *MYO1* eliminates the actomyosin ring, yet the *myo1* null cells form colonies with cells in short chains, indicating that the actomyosin ring is not essential but is required for efficient cytokinesis (Bi *et al*., 1998; Rodriguez and Paterson, 1990; Watts *et al*., 1987). Electron microscopic studies showed that *myo1* null cells formed abnormally thick, irregularly shaped septa (Rodriguez and Paterson, 1990), suggesting that the actomyosin ring plays a role in septum maturation and/or cell separation. In addition, in cells such as *bni1* nulls, where the actomyosin ring plays can form but fail to complete contraction, the septum is usually misaligned with respect to the bud neck, suggesting that contraction of the actomyosin ring may normally guide septum formation so that the latter process can occur efficiently (Vallen *et al*., 2000).

Role of septum formation in cytokinesis

In the absence of the actomyosin ring (*myo1* null cells), budding yeast is still able to accomplish cytokinesis and cell separation, albeit less efficiently, suggesting that septum formation alone is sufficient for cytokinesis. The formation of a septum requires cell wall synthesis across the bud neck and, presumably, requires membrane addition as well.

Chitin synthases II (CSII), whose catalytic subunit is encoded by *CHS2*, is mainly responsible for the primary septum formation. In some strain backgrounds, chitin synthase III (CSIII), whose catalytic subunit is encoded by *CHS3*, also contributes to the primary septum formation. CSII is exclusively used for septum formation, whereas CSIII is responsible for ~90% of the total cellular chitin (Bulawa, 1993; Orlean, 1996) and is responsible for the chitin at the base of the bud (Shaw *et al*., 1991). Both Chs2p and Chs3p localize to the bud neck around telophase of the cell cycle, in a septin-depandent manner (Chuang and Schekman, 1996; DeMarini *et al*., 1997) (Yu and Pringle, personal communication). Depletion of CSII and CSIII together results in cell lethality, with cells arrested in chains, indicative of a defect in cytokinesis and/or cell separation (Shaw *et al*., 1991). In our stain background, deletion of *CHS2* alone causes a similar phenotype (Yu and Pringle, personal communication). When Chs2p is depleted, the Myo1p ring (Fig. 1) and actin ring (data not shown) can form, but the actomyosin ring cannot contract. Thus, a block in septum formation prevents the contraction but not the formation of the actomyosin ring. There are two possible explanations for this result. First, the contractile force generated by the actomyosin ring may not be sufficient to overcome the physical restraint imposed by the rigid cell wall, which is usually cross-linked to the plasma membrane. Second, contraction of the actomyosin ring and septum formation may be coupled by a regulatory pathway such that a defect in septum formation can prevent the contraction of the actomyosin ring. Further experimentation is required to distinguish these possibilities.

Coordination of actomyosin ring function and septum formation

In budding yeast, the contraction of the actomyosin ring is followed closely by the centripetal growth of the septum (Bi *et al*., 1998; Lippincott and Li, 1998b). As discussed earlier, the ring contraction may provide the directionality for efficient septum formation (Vallen *et al*., 2000), whereas septum formation is required for the ring contraction. Thus, the ring and the septum must coordinate temporally and spatially to carry out cytokinesis. The coordination mechan-

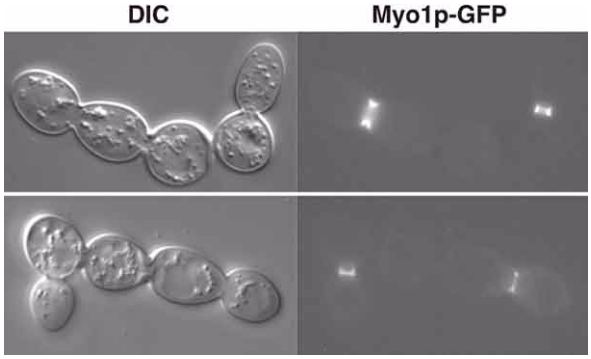

Fig. 1. A block in septum formation does not affect the formation of the actomyosin ring but prevents its contraction. A diploid strain homozygous for *pGAL1-CHS2* and *MYO1-GFP* was grown exponentially under repressive condition at 25°C (Chs2p expression was repressed by additon of 2% glucose to the rich YPD medium). Cell morphology and Myo1p-GFP ring were documented by DIC and fluorescence microscopy, respectively. Under this condition, the old Myo1p-GFP rings were disassembled from the division sites before the completion of septum formation.

isms may involve proteins that are physical components of both pathways or regulatory proteins that may link the pathways together.

The septins are required for targeting the actomyosin ring and the septal components such as the chitin synthases to the bud neck. Thus, septins are involved in the coordination in a physical sense. Likewise, Mlc1p, Iqg1p, and the formins are involved in the coordination physically, because these proteins are required for the assembly of the actin ring as well as for septum formation, although their precise roles in the latter process remain to be determined.

Two other ptoteins, Hof1p and Cyk3p, appear to play a role in septum formation and, perhaps, in the coupling of actomyosin ring function to septum formation. Hof1p, a member of the cdc15p/PSTPIP family that is characterized by an N-terminal coiled-coil domain, followed by a PEST sequence and a C-terminal SH3 domain (Balasubramanian *et al*., 1998; Fankhauser *et al*., 1995; Spencer *et al*., 1997), plays a role in septum formation (Kamei *et al*., 1998; Korinek *et al*., 2000; Lippincott and Li, 1998a; Lippincott and Li, 2000; Vallen *et al*., 2000). Deletion of *HOF1* results in temperature-sensitive growth, with cells arested in chains at the nonpermissive temperature. However, *hof1* deletion does not affect the formation and contraction of the actomyosin ring at the permissive temperature (Vallen *et al*., 2000). At the nonpermissive temperature, the actomyosin ring can form but fails to contract in *hof1* nulls, which is similar to what happens in Chs2p-depleted cells. Hof1p targets to the septins in a Myo1p-independent manner. In addition, *myo1* and *hof1* deletions are synthetically lethal. All these data support the conclusion that Hof1p functions in septum formation independent of the actomyosin ring (Vallen *et al*., 2000). The localization of Hof1p to bud neck changes in the cell cycle. Haf1p localizes to the mother-side of the bud neck first, then distributes equally at both sides of the neck. Hof1p contracts like Myo1p during cytokinesis and this contractile behavior is Myo1p-dependent (Lippincott and Li, 1998a). During septum formation, Hof1p splits into two rings, one at each side of the bud neck (Lippincott and Li, 1998a; Vallen *et al*., 2000). This localization pattern is distinct from that of Myo1p, which disappears from the neck at the end of the actomyosin ring contraction (Fig. 2). This localization data suggests that Hof1p may couple the actomyosin ring contraction to septum formation (Vallen *et al*., 2000). Because *hof1* nulls are only blocked at septum formation at the nonpermissive temperature, this data suggests that, at the permissive temperature, there must be another Hof1p-like activity that acts in parallel to Hof1p.

In fact, genetic and cytological evidence suggest that Cyk3p functions in parallel to Hof1p in septum formation and in coupling actomyosin ring function to septum formation. Cyk3p has an SH3 domain at its N-terminus and has a homologue in *S. pombe*, which appears to be involved in cytokinesis as well (M. Balasubramanian, personal communication). *CYK3* was isolated as a multicopy bypass suppressor of *iqg1* deletion (Korinek *et al*., 2000). Multicopy *CYK3* restored the viability of *iqg1* deletion without restoring the actin ring, suggesting that Cyk3p stimulates cytokinesis independent of the actomyosin ring. *HOF1* behaved similarly in the same genetic assay, suggesting that Cyk3p and Hof1p have similar activity in cytokinesis. Like *HOF1*, *cyk3* and *myo1* deletions are synthetically lethal (Korinek *et al*., 2000). Interestingly, *cyk3* and *hof1* deletions are also synthetically letheal, suggesting that Cyk3p and Hof1p act in parallel in septum formation. Cyk3p localizes to the bud neck shortly after actin ring formation (Korinek *et al*., 2000). Like Hof1p, Cyk3p contracts to a dot during cytokinesis, then splits into double rings after septum formation (Fig.2), suggesting that Cyk3p may be involved in the coupling of actomyosin ring function to septum formation.

A model of cytokinesis in budding yeast

Our recent work has led to a working model on cytokinesis in budding yeast (Fig. 3A). In normal cells, cytokinesis is accomplished by the concerted action of actomyosin ring contraction and septum formation. Although not essential for cell viability, the actomyosin ring is required for efficient cell division by providing a guiding cue for septum formation. In contrast, septum formation is essential for cell viability and failure to form a septum blocks the con-

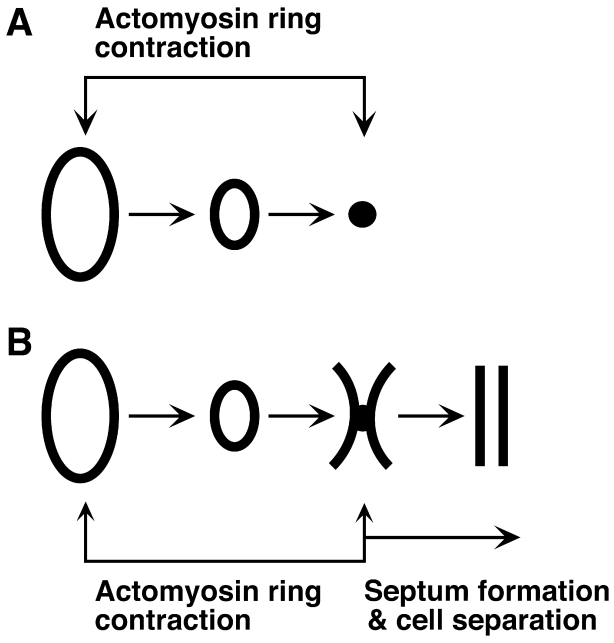

Fig. 2. Proteins that are integral parts of the actomyosin contractile ring such as Myo1p and Mlc2p (A) and proteins that are involved in septum formation or in the coordination between actomyosin ring function and septum formation such as Hof1p and Cyk3p (B) have distinct localization patterns during cytokinesis and cell separation.

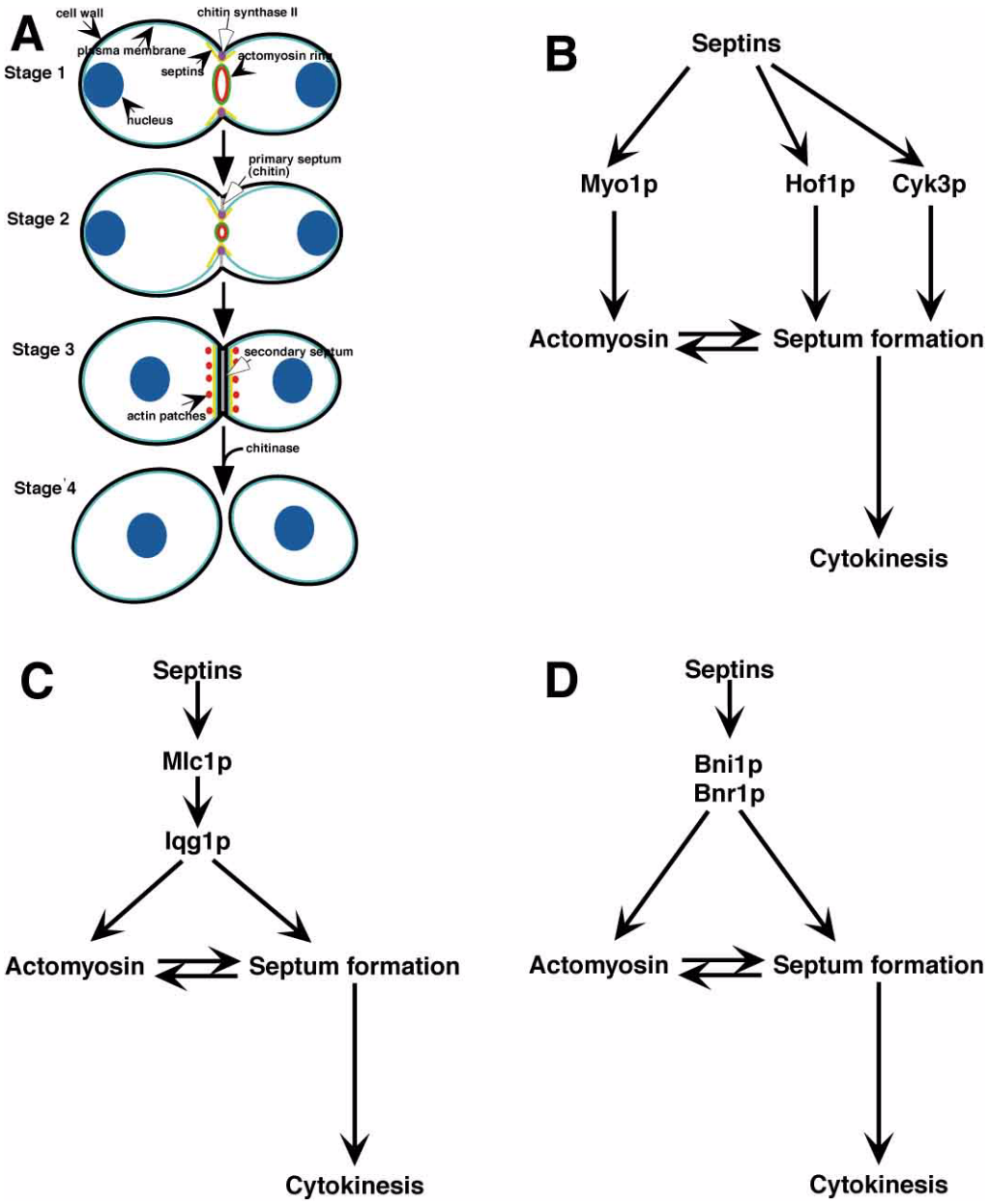

Fig. 3. A model of cytokinesis in budding yeast. (A) A depiction of cytokinesis and cell separation in budding yeast. (B–D) Molecular pathways involved in actomyosin ring function and septum formation.

traction, but not the formation of the actomyosin ring.

In molecular context, our model can be summarized as follows (Fig.3B): septins are essential for cytokinesis, and they are the first known proteins to localize to the cytokinesis site. The components of the actomyosin ring such as Myo1p and the components of the septum formation machinery such as Hof1p and Cyk3p target independently to the septins. Deletion of each pathway alone does not cause cell death. However, deletion of any two pathways together causes cell lethality. For example, *myo1*Δ and *hof1*Δ, *myo1*Δ and *cyk3*Δ, *hof1*Δ and *cyk3*Δ are all lethal combinations. In addition, Myo1p is required for the formation of the actomyosin ring, wherease Hof1p and Cyk3p are not. These defined genetic relationships between different pathways will allow us to design rational screens to identify additional genes that are specifically involved in regulating the function of the actomyosin ring.

Besides Myo1p, Hof1p, and Cyk3p, two other pairs of proteins, Mlc1p and Iqg1p (Fig.3C), Bni1p and Bnr1p (Fig.3D), are also required for the assembly of the actomyo-

sin ring as well as for septum formation. In the near future, the main focus in the field is to understand how all these proteins are assembled into an efficient machinery that drives cytokinesis and cell separation.

Concluding remarks

Despite the differences in molecular details, the overall mechanisms of cytokinesis are conserved between budding yeast and animal cells. This conclusion is mainly affirmed by the fact that cytokinesis in budding yeast and animal cells involves the contraction of an actomyosin ring as well as directed membrane addition at the division site. In addition, all the major molecules involved in cytokinesis are conserved among these eukaryotic systems. Because of its genetic tractability, its well-annotated genomic database, and the improved microscopy on live cells, budding yeast offers an attractive model for studying cytokinesis.

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