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Septin Scaffolds and Cleavage Planes in Saccharomyces

Minireview

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For one cell to divide into two, it must solve the problem of positioning its cleavage plane to bisect the spindle axis such that one genome is segregated to each progeny cell. Many eukaryotic cells rely on a mechanism in which the spindle apparatus dictates the position of the cleavage plane, thereby ensuring this arrangement. Saccharomyces cells solve the problem of cleavage plane placement by a different mechanism: both the spindle and the cleavage plane respond independently to the budding axis of the cell such that, by the time cytokinesis occurs, the spindle and cleavage plane are positioned in the appropriate arrangement. A ring of proteins called septins defines the cleavage plane, beginning in G1, and persists, encircling the mother–bud junction for the duration of the cell cycle, where it acts as a scaffold for the proteins that ultimately execute the mechanics of cytokinesis. During mitosis, the spindle aligns through the opening of the septin ring and elongates; cytokinesis then occurs in the plane of the septin ring. Despite differences between how budding yeast cells and many symmetrically dividing cells position their cleavage planes, septin homologs are known in other eukaryotes and are required for division. Thus, eukaryotic cells use similar molecules arranged in different regulatory hierarchies to solve the problem of orienting the cleavage plane to bisect the spindle.

This minireview is of two parts. The first reviews the role of the septin ring in defining the cleavage plane and discusses the existence of similar structures in other organisms. The second discusses how yeast cells use cortical positional cues (including the septins) to regulate patterns of cell division and cleavage.

The Budding Lifestyle: Commitment to Cleavage Plane Early in the Cell Cycle

Budding is a distinctly asymmetric mode of cell division, as contrasted with the more symmetric divisions of many bacteria, other yeasts, and most metazoan cells. Once a yeast cell commits to division by activating the G1 cyclin–Cdc28p complex, it polarizes its cytoskeleton in response to the polarity establishment machinery, including Bud1p and Cdc42p GTPases (for more discussion see Chant and Stowers, 1995). Actin, microtubules, and septins polarize virtually simultaneously, well before a bud is visible (Figure 1A; Kim et al., 1991; Snyder et al., 1991; Lew and Reed, 1993). Evidence suggests that actin and septins polarize independently in reponse to the polarity establishment machinery and that microtubule polarization may depend upon the polarization of actin (Kim et al., 1991; Palmer et al., 1992). Polarization of each cytoskeletal element serves a purpose. Polarization of actin directs secretion and cell surface expansion to produce bud growth. Cytoplasmic microtubules polarized from a spindle pole body (centrosome analog) toward the bud orient the nucleus and spindle for mitosis (Figure 1A; Byers, 1981; Kilmartin and Adams, 1984).

Figure 1 illustrates the key point that, once a yeast cell has initiated a bud early in the cell cycle, it has committed to its cleavage plane. The molecular manifestation of commitment is the formation of a ring of septin proteins (Cdc3p, Cdc10p, Cdc11p, and Cdc12p) beneath the cell membrane at the future division site (Kim et al., 1991; Figure 1; Figure 2a). The bud grows from within the septin ring, which remains as a collar encircling the mother–bud neck. As the bud appears, DNA replication begins and continues during bud growth (Byers, 1981). Once the genome is replicated and the bud has grown to nearly the size of its mother, the spindle aligns parallel to the mother–bud axis and elongates, depositing one nucleus each to mother and daughter (Figures 1C and 1D; Byers, 1981; Kilmartin and Adams, 1984). Hence, in Saccharomyces, polarization toward the bud site in G1 defines the spatial axis for all that will follow: actin microfilaments target growth, microtubules position the spindle, and septins mark the cleavage plane.

The Septin Ring, a Scaffold Defining the Cleavage Plane

Four Saccharomyces septin proteins are known: Cdc3p, Cdc10p, Cdc11p, and Cdc12p (individual proteins are named for their genes). In cells singly defective for any of

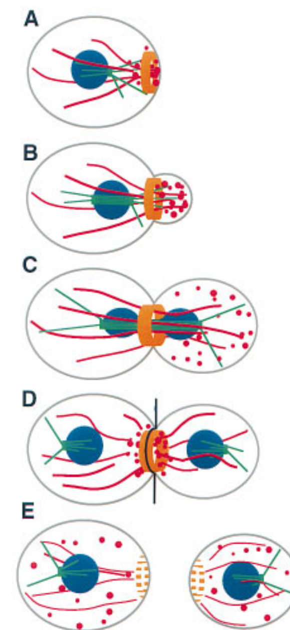

Figure 1. Asymmetric Cell Division by Budding

The septin ring (orange) marks the future cleavage plane early in the cell cycle. Other important elements of cell division are illustrated, including the nucleus (blue), the actin cytoskeleton (red), and the microtubule cytoskeleton (green). (A) Late G1. (B) S phase. (C) M Phase (no nuclear envelope breakdown or condensation of chromosomes is easily observed in yeast) (D) Postmitosis. (E) Early G1/S of the next cell cycle.

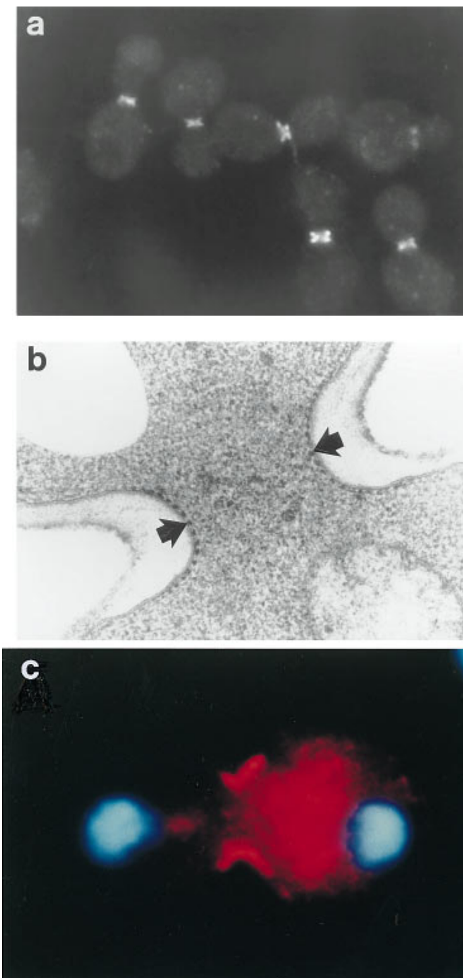

Figure 2. Septin and Neck Filament Structures

(a) The septin ring of yeast. Rings are visible in this field of several yeast cells. Rings are viewed edge on and therefore appear as lines (Cdc3p is shown). Figure courtesy of B. Haarer.

(b) The neck filaments as observed by electron microscopy. An extreme closeup of the neck region of a mother–bud junction is shown. Figure courtesy of B. Byers (appears in Byers, 1981). Neck filaments are immediately beneath the plasma membrane, as indicated by arrows.

(c) The septin ring of Drosophila cells. A tissue culture cell is shown in late mitosis. Similar staining is seen in cells of embryos Figure courtesy of T. P. Neufeld and G. M. Rubin (appears in Neufeld and Rubin, 1994).

these proteins, cytokinesis is defective (Hartwell, 1971). Even though all four septins are required individually for cytokinesis, the four proteins are closely related by sequence along their entire lengths (Flescher et al., 1993; Neufeld and Rubin, 1994). Each sequence predicts a nucleotide-binding loop, and three of the proteins have patches of predicted coiled coil. What structure do these proteins form? Much evidence suggests that the septin proteins are components of the regular neck filaments, observed by electron microscopy to encircle the mother–bud neck immediately against the cytoplasmic face of the plasma membrane (Figure 2b; Byers and Goetsch, 1976). The individual neck filaments are 10 nm in cross section and colocalize with the positions of the septins as observed by immunofluorescence (Kim et al., 1991). Although it is considered likely that the neck filaments are composed of the septin proteins, direct demonstration of filament formation by purified or recombinant septins has not been reported.

Mechanistically, the septin ring functions as a scaffold for the recruitment of cytokinesis machinery to the mother–bud neck. Following Cdc28p inactivation in late M phase, there is large-scale reorganization of actin and myosin (type II) to the mother–bud neck to build the cleavage plane (Figure 1D; Lew and Reed, 1993). Although the septin ring directs these cytokinetic activities, it does not contract during cytokinesis. Instead, the septin ring is sliced in two by cleavage, and remnants of the ring persist on each progeny cell (Figure 1E; Kim et al., 1991).

To date, many critical questions remain unanswered concerning the septin ring. Are purified or recombinant septins sufficient to form the regular neck filaments? If the septins bind nucleotide trisphosphates, does hydrolysis regulate the dynamics of their assembly and disassembly, as observed for actin and tubulin? Do the neck filaments encircle the neck as closed rings, a long helical structure, or short overlapping segments? How would such a structure be assembled?

Septins of Other Eukaryotes

Recent reports and database entries indicate that septins are conserved beyond budding yeast. Septin-related sequences have been reported from fission yeast, fruit flies, and mice (Nottenburg et al., 1990; Neufeld and Rubin, 1994; Fares et al., 1996). In fruit flies, work has centered around a septin homologs encoded by the *peanut* and *sep1* genes (Neufeld and Rubin, 1994; Fares et al., 1996). The sequences of Peanut and SEP1 are highly similar to yeast septins, including the predicted nucleotide-binding pocket. In close correspondence with what has been observed in yeast, a ring of Drosophila septins encircles the cleavage furrow prior to and throughout cytokinesis (Figure 2c). During cytokinesis, the septin ring shrinks as the membrane pinches inward, and a remnant of the ring persists associated with the midbody. Drosophila cells depleted of Peanut septin are defective for cytokinesis (Neufeld and Rubin, 1994). Thus, septin proteins appear conserved in sequence and function. How the septins are recruited to the cleavage furrow is not known, but they may be positioned in response to spindle orientation.

Do Plants Have Septins?

Since much of plant morphogenesis is dependent upon regulation of cleavage plane, it will be particularly interesting to learn whether septins function in plants. In plants, a conspicuous belt of microtubules, the preprophase band, marks the cleavage plane prior to mitosis (Traas et al., 1995). In mutants where the preprophase microtubules are absent, cleavage planes are misaligned, resulting in embryos which are misshapen and stumpy (*ton1* and *ton2* mutants; Traas et al., 1995). Whether the entire mutant phenotype reported is strictly due to loss of the preprophase band microtubules is not known. Beyond this report, much remains unknown. How is the preprophase band of microtubules directed to the future cleavage site? What is the role of these

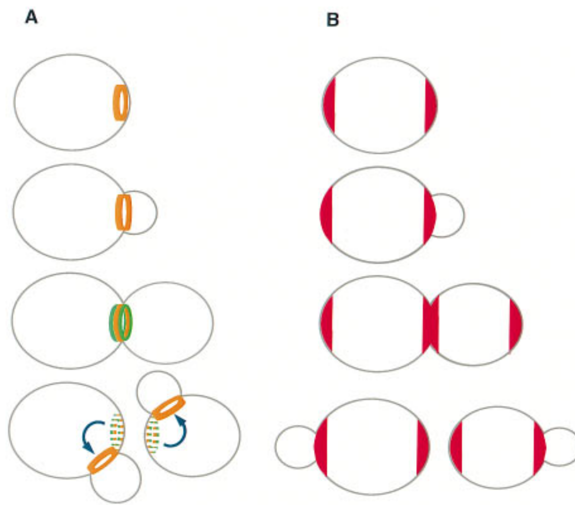

Figure 3. The Axial and Bipolar Patterns of Cell Division

(A) The axial pattern. This pattern is produced by a cycle in which Bud3p (green) is brought to the neck by the existing septin ring (orange) and then directs the next round of axial budding, including a new septin ring, to occur next to the previous division site. The blue arrow represents the actions of Bud1p and Cdc42p GTPase modules and associated machinery. Panel 1 shows G1; septins polarize in response to polarity establishment machinery. Panel 2 shows S phase; septin ring remains encircling the mother–bud neck as the bud grows. Panel 3 shows G2/M; Bud3p accumulates in the mother–bud neck region in association with the septin ring. Cytokinesis splits this double ring structure. Panel 4 shows G1 of subsequent cell cycle; Bud3p, in association with the old septin rings, directs axial formation of buds and new septin rings. Bud3p and the old septin ring are lost.

(B) The bipolar pattern. Cells are constrained to bud at the poles of their ellipsoidal shapes. Note that mother and daughter can bud at either pole (only one of four possible division patterns is shown). Postulated bipolar marks, of unknown composition, are indicated in red.

microtubules per se in directing cleavage? What other proteins are constituents of the preprophase band? In light of these questions, a not-too-far-out hypothesis is that plant septins, yet to be reported, underly the preprophase band of microtubules. This hypothesis has appeal because, in a temporal sense, marking of a cytokinesis site early in the cell cycle by the preprophase band is a similar phenomenon to the septin ring in yeast. If septins are discovered in plants, learning their relationship to the preprophase band or the *ton* genes shall be a high priority.

Cortical Cues Orient Division Axes and Cleavage Planes

Layered upon the basic mechanics of cell division, yeast cells are able to divide in two different spatial patterns depending upon cell type. Under most conditions, haploid **a** and α cells divide in a pattern called axial, whereas diploid **a**/α cells divide in a pattern called bipolar (Freifelder, 1960; Hicks et al., 1977; Figure 3). Because the cleavage plane is defined by bud position, the regulation of cleavage plane placement originates with selection of a bud site. In both the axial and bipolar patterns of budding, it is believed that the cell responds to cortical cues marking positions on the cell surface.

In the axial pattern, cells are constrained to form daughter buds next to the previous division site. Genetic analysis suggested that *BUD3*, *BUD4*, and associated factors might provide the cortical marks for axial budding, as mutations in these genes specifically affect the axial pattern. Immunolocalization has revealed that, during later stages of the cell cycle, Bud3p encircles the mother–bud neck region as a double ring—at essentially the positions where cells will form axial buds in the next cell cycle (Figure 3A, panel 3; Chant et al., 1995). This double ring structure persists until cytokinesis, at which time it is sliced into two single rings, one on each progeny cell (Figure 3A, panels 3 and 4). The single rings persist until approximately the time the next axis of polarization forms in anticipation of axial bud formation. The Bud3p rings then dissipate (Figure 3A, panel 4). It appears that Bud3p is initially directed to the neck region by the preexisting septin ring. Conversely, because Bud3p directs the formation of axial buds, it constrains the position of the next septin ring. Thus, the axial pattern of budding is potentially produced by a closed cycle of the septin ring acting as a template for the accummulation of Bud3p (and associated proteins) in the neck, with these factors then directing the positions of the next septin rings (Figure 3A). As such, Bud3p behaves as spatial memory, inherited from one cell cycle to the next.

At the molecular level, it is quite possible that Bud3p and associated factors bind the septin ring directly. The pathway by which Bud3p and associated factors direct the new axially positioned septin ring and budding axis is undoubtedly more complex: it involves the machinery responsible for polarizing towards the bud site early in the cell cycle, namely the GTPase cascade of Bud1p and Cdc42p (indicated by the blue arrow in Figure 3A; Chant and Stowers, 1995). Exactly how these GTPases produce polarized cytoskeletal structures is not understood.

In the bipolar pattern, the cortical cues that mark the polar regions of the ellipsoidal diploid cell remain unknown, but these cues are distinct from Bud3p and Bud4p. Physiological experiments suggest that stable structures, which can be inherited for many generations, mark the poles of the ellipsoidal cells (Figure 3B; for an extended discussion, see Chant and Pringle, 1995). A large number of genes that are required specifically for the bipolar pattern are known (Zahner et al., 1996). However, which of these genes, if any, encode the bipolar cortical cues remains to be discovered.

Cell Type Regulation of Budding Pattern

How is it that haploid **a** and α cells bud in an axial pattern, whereas diploid **a**/α cells bud in a bipolar pattern? This difference is entirely analagous to the difference in cleavage patterns exhibited by AB and P1 blastomeres in Caenorhabditis elegans (White and Strome, 1996 [this issue of *Cell*]). In yeast, the basis for cell type specializations is well understood. **a**/α cells differ from haploid **a** and α cells because **a**/α cells produce the **a**1–α2 corepressor by virtue of expressing both **a** and α mating-type information (*MAT**a*** and *MAT*α; wild-type haploid cells only express one or the other *MAT* locus; Herskowitz, 1989). Therefore, a reasonable possibility was that the difference between the axial and bipolar patterns would be produced by the differential expression of a

gene that is a target of **a**1–α2 and, therefore, expressed in haploids but repressed in diploids. Recently, such a gene has been described: *AXL1* (Fujita et al., 1994). Axl1p is required for axial budding: when *axl1* is mutant, haploids bud in a bipolar pattern, much as is observed for *bud3* or *bud4* mutants. However, unlike *BUD3* and *BUD4*, which are expressed in all cell types, *AXL1* expression is repressed in **a**/α cells by **a**1–α2. Moreover, ectopic expression of *AXL1* in diploids converts the bipolar pattern to axial, indicating that *AXL1* is the critical factor whose expression is regulated between haploids and diploids (Fujita et al., 1994). Mechanistically, how *AXL1* acts has not been determined, but *AXL1* encodes a protease that may modify the actions of Bud3p, Bud4p, or associated factors (Fujita et al., 1994). However, the large size of the Axl1p suggests that it may have additional activities. By analogy to *AXL1*, which is a switch controlling use of the axial division remnant in yeast, some C. elegans factor, perhaps PAR-3, must be differentially distributed between the P and AB lineages such that the midbody division remnant is recognized in the P lineage but not the AB lineage.

Closing Remarks

Although the highly asymmetric cell divisions of yeast are rather specialized, the use of cortical cues to orient spindles and cleavages is a common theme (White and Strome, 1996). What differs among systems is the regulatory hierarchy that specifies cleavage plane orientation and position. In C. elegans cells and probably most metazoan cells, the hierarchy is cortical cues to spindle to cleavage plane—a flow of information in series. In yeast the regulatory hierarchy is cortical cues (e.g., old septin ring and Bud3p) to spindle and, independently, the same cortical cues to cleavage plane (i.e., septin ring)—a more parallel hierarchy. Although these hierarchies are overtly different, a conversion of one to the other could be produced by a few simple changes in molecular links between elements common to all eukaryotic systems, such as septins, actin, and microtubules.

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