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REVIEW ARTICLE

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Septins: a ring to part mother and daughter

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Abstract The septins are well conserved GTPases found in animals and fungi. In yeast, they are required for the formation of 10-nm filaments, with which they co-localize at the bud neck. Therefore, septins have been proposed to be components of the neck filaments and to have polymerization properties. In support of this hypothesis, septin complexes purified from yeast and flies form filaments in vitro. However, recent studies have questioned the relevance of septin filament formation for septin function. Particularly, septin polymerization may not be required for their function in cytokinesis. New septin functions have also been recently uncovered: in budding yeast, the septin ring is required for the maintenance of cell polarity. It forms a cortical barrier that prevents lateral diffusion of membrane-associated proteins through the bud neck. Here, we review the most recent functional and biochemical data, to discuss whether there is a link between septin polymerization properties and septin function.

Introduction

Septins were first isolated in budding yeast for their role in cytokinesis. In the past 10 years, it has become evident that septins are conserved well beyond fungi (Neufeld and Rubin 1994; Cooper and Kiehart 1996) and are an important component of the cytokinetic machinery of numerous, if not all, animal cells (Neufeld and Rubin 1994; Longtine et al. 1996; Kinoshita et al. 1997; Field and Kellogg 1999). Recent results also underline the importance of septins in cellular processes other than cytokinesis. For example, septins have been reported to associate with focal adhesion complexes in fibroblasts (Kinoshita et al. 1997) and to control the organization of the cell cortex in yeast (Barral et al. 2000; Takizawa et al. 2000). Septins have also been shown to be abundant in neurons (Neufeld and Rubin 1994; Fares et al. 1995; Kinoshita et al. 1997), a cell type that does not divide actively.

While we have made progress in the analysis of septin function at the cellular level, the biochemical and structural characterization of these proteins is still in its infancy. This situation, though, is now changing rapidly, as several groups put efforts in biochemical and ultrastructural characterization of various septin molecules and complexes.

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The identification of septins

Hartwell and colleagues first identified septin genes in 1971 in their screen for temperature-sensitive budding yeast mutants affecting cell division (*cdc* mutants; Hartwell et al. 1970, 1974; Hartwell 1971). Conditional mutations in any of the four septin genes *CDC3*, *CDC10*, *CDC11* and *CDC12* prevented cytokinesis (Hartwell 1971), yet the cells continued budding, DNA synthesis and nuclear division. This resulted in the accumulation of large multinucleated cells with multiple elongated buds at the restrictive temperature.

In 1976, using thin electron microscopy, Byers and Goetsch (1976) found a highly ordered set of 10-nm filaments closely associated with the plasma membrane at the mother-bud neck of *Saccharomyces cerevisiae*. These filaments are parallel to each other and to the plane of the neck, as seen by electron microscopy. They appear shortly after bud emergence and disappear prior to cytokinesis. Temperature-sensitive mutations in several of the septin genes prevent the formation of the "neck filaments" at the restrictive temperature. Moreover, for at least some alleles of *cdc10* and *cdc12*, filaments formed at the permissive temperature quickly disappeared after a shift to the restrictive temperature.

Immunofluorescence techniques showed that the four septin proteins co-localized into a ring at the mother-bud neck (see scheme in Fig. 2A; Haarer and Pringle 1987; Kim et al. 1991; Longtine et al. 1996; Field and Kellogg 1999). Localization of all four septins was disrupted in both *cdc12* and *cdc3* mutants brought to the restrictive temperature, indicating that septins are interdependent for localization.

These results suggested that Cdc3p, Cdc10p, Cdc11p and Cdc12p are the major components of the neck filaments. Biochemical studies strongly support this view (Field et al. 1996; Frazier et al. 1998; Oegema et al. 1998). The four septin proteins co-purify on affinity columns; and the purified complexes are composed of the four septin polypeptides plus a fifth septin molecule identified later, Sep7 (Carroll et al. 1998). This complex is able to self-associate in vitro to form more highly ordered, filamentous structures. The filaments have a diameter range of 7–9 nm and their length varies over 32–100 nm (Frazier et al. 1998). Analysis of micrographs of the purified filaments raised the possibility that septin filaments in fact run parallel to the mother/bud axis. In this model, the 10-nm structures observed in yeast cells may be formed by lateral association of the septin filaments (Frazier et al. 1998; Longtine et al. 1998; Gladfelter et al. 2001).

Alignment of all septin sequences so far identified shows that they are composed of three major domains (Fig. 1). Septins are at least 26% identical to each other over their entire length (Cooper and Kiehart 1996; Field and Kellogg 1999). Similarity is greatest in the central domain, with more than 35% identity. This domain possesses a motif characteristic of ATPase and GTPase domains (Saraste et al. 1990; Flescher et al. 1993; Cooper and Kiehart 1996; Field and Kellogg 1999). At their C-terminus, septins are predicted to contain a coiled-coil domain. In this respect, the Cdc10 septin from *S. cerevisiae* is exceptional in that it appears to lack the coiled-coil region. The N-terminal domain is highly variable. In addition, most septins contain a well conserved polybasic region just upstream of the GTP-binding domain (Trimble 1999; Zhang et al. 1999). In the case of the mammalian septin, H5, this sequence was shown to bind phosphatidylinositol 4,5-bisphosphate (Zhang et al. 1999). More clues about septin organization may come from a better understanding of septin function. Until now, septins have been involved mainly in three distinct processes: cytokinesis, cell polarity and spore formation (Fares et al. 1996). We will focus now on the role of septins in cytokinesis and cell polarity.

Septins in cytokinesis

The role of septins in cytokinesis is not yet completely clear. However, several aspects of how they function in this process have been characterized in yeast. In this organism, cytokinesis is the result of two redundant processes: the contraction of the actomyosin ring and the formation of the septum by vesicle fusion with the plasma membrane (Epp and Chant 1997; Bi et al. 1998; Lippincott and Li 1998a, b; Vallen et al. 2000). In most yeast strains, disruption of either one of these processes leads to a delay in cytokinesis, but not to a complete failure in cell separation (Vallen et al. 2000). In contrast, disruption of septin function fully impairs cytokinesis. As all processes of cytokinesis depend on their function, septins are predicted to act at the most upstream level of the yeast cytokinetic pathway. What is the exact function of septins? In fungi and animal cells, cytokinesis relies on the selection of a cleavage plane at the cell cortex. In yeast, the plane of cleavage is necessarily located at the bud neck (Fig. 2A). Thus, it is established at bud emergence long before cytokinesis and the cell has to remember the location of the bud neck during most of the cell cycle. Interestingly, the septin ring forms at the presumptive bud neck prior to bud emergence (Fig. 2A; Kim et al. 1991). Thus, one simple possibility is that septins form a rigid scaffold (DeMarini et al. 1997) that marks and maintains the position of the bud neck, allowing the subsequent recruitment and maintenance of the cleavage apparatus at that location. This model predicts that the dynamics of septins must be much slower than that of other cytoskeletal structures. Septin function would thus be to maintain the memory of the bud neck's position while the bud grows, organelles move and microfilaments and microtubules turnover rapidly.

Maintaining a stable position in an ever-changing environment supposes a strong anchorage. It would be difficult to conceive how septins accomplish this task if they were not attached to each other and/or to some other stable cellular or extra-cellular structures. It is likely that the ability of septins to assemble into filaments plays an important role in their ability to remain at the bud neck. However, other processes may also participate in the stability of the septin ring.

Septins in cell polarity

Recently, two groups reported new aspects of septin function by demonstrating a role in cell polarity (Barral et al. 2000; Takizawa et al. 2000). They showed that the septin ring at the bud neck acts as a diffusion barrier, which is necessary to restrict the distribution of membrane and membrane-associated factors to specific regions of the cell. In order to explore in more detail the role of septin in these processes, we will first describe the different forms of cellular asymmetry and summarize our knowledge on yeast cell polarity.

Cell polarity can take two main forms

Asymmetric localization of cortical proteins plays a key role in numerous cellular processes, such as cell

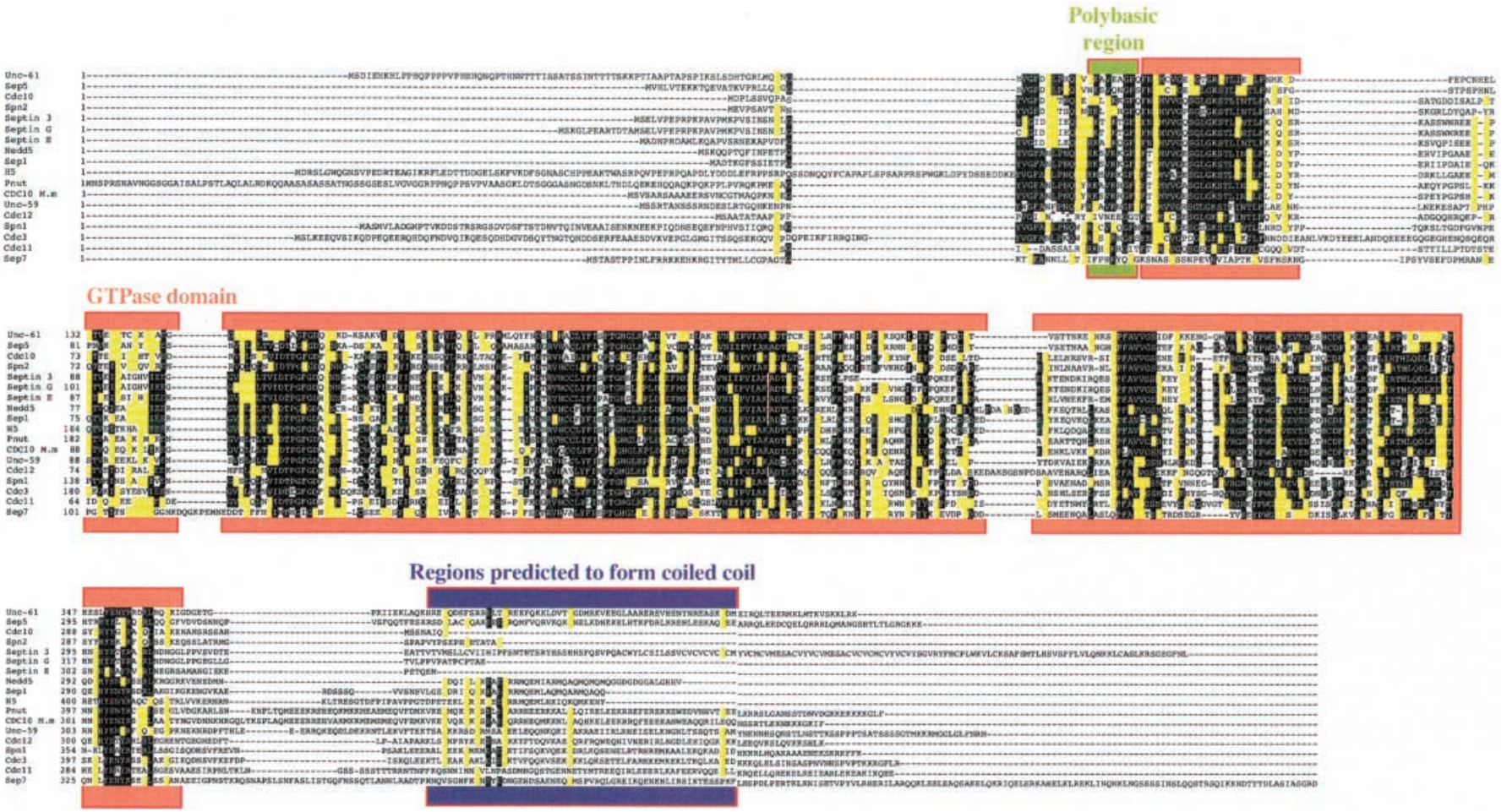

Fig. 1. Alignment of septin sequences. The following septin sequences have been aligned: *Caenorhabditis elegans* (*Unc-59*, *Unc-61*), *Saccharomyces cerevisiae* (*Cdc3*, *Cdc10*, *Cdc11*, *Cdc12*, *Sep7*), *Drosophila melanogaster* (*Sep1*, *Sep5*, *Pnut*), *Schizosaccharomyces pombe* (*Spn1*, *Spn2*), *Mus musculus* (*Septin 3*, *CDC10 M.m*), *Rattus norgevicus* (*Septin E*, *Septin G*) and *Homo sapiens* (*Nedd5*, *H5*). The *green box* marks the polybasic sequence involved in phospholipid-binding, the *orange box* corresponds to the GTPase domain and the *blue box* corresponds to the coiled-coil domains

morphogenesis and cell fate determination. Under "cortical", we define the plasma membrane and the intracellular structures closely associated with it. Cortical polarity can take two forms. Asymmetry can be organized around one point on the cell surface. It is then reflected by the formation of a gradient of protein distribution around the center, or pole, of polarization (Fig. 3A, part a). Some factors become more abundant around the pole, while others may have the reverse distribution. We refer to this case as apical polarity. Alternatively, the cortex can also be organized into domains separated by sharp boundaries (Fig. 3A, part b). We refer to this situation as cortical compartmentalization. The maintenance of a gradient, in the case of apical polarity, presumably requires the maintenance of a punctual organizer. In contrast, the maintenance of compartments may necessitate that specific structures define compartment boundaries and prevent compartments from mixing.

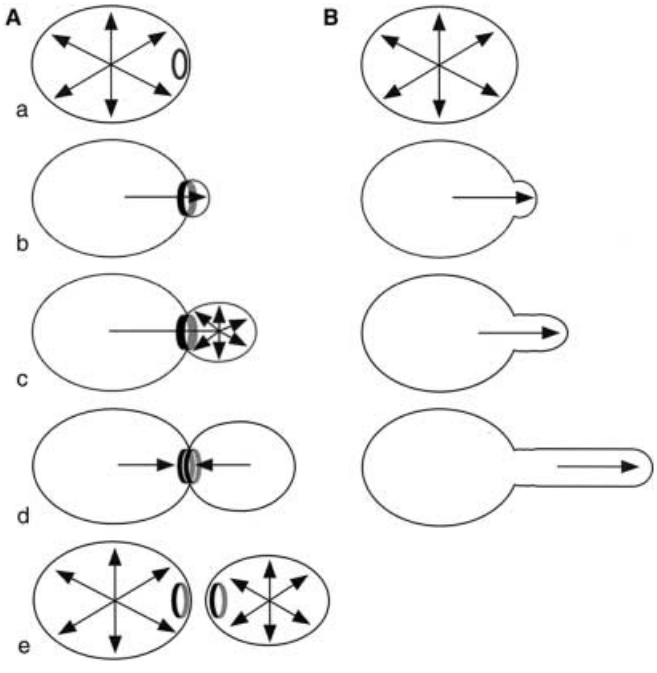

Fig. 2A, B. Bud formation in yeast and the dynamics of the septin ring. **A** Formation of the bud requires the polarization of cell growth. *a* The septin ring forms prior to bud emergence. At this stage cell growth is isotropic in the unbudded cell. *b* At bud emergence, cellular growth becomes polarized towards the incipient bud site, which turns into the bud tip as the bud emerges. The septin ring forms a collar at the bud neck. *c* Shortly after bud emergence, bud growth switches from apical to isotropic mode. At this point, the mother cell has completely stopped growing. The septin ring shows little change. *d* After nuclear division, the septin ring splits into two rings. Cell growth is redirected towards the neck for the formation of the septum. *e* After cell separation, cellular growth becomes isotropic in both mother and daughter cells. Each cell has inherited a septin ring, which remains during most of G1, until a new ring forms at the new incipient bud site. **B** In cells that lack the septin ring, a cell-cycle checkpoint prevents the transition from apical to isotropic growth. As a consequence, the cell forms an elongated bud

Yeast cells polarize during division and mating (Huffaker et al. 1987; Lew and Reed 1995; Madden and Snyder 1998). Forms of apical polarity can be observed during bud emergence (Fig. 2A, part b, Fig. 3B, part a) and the formation of a mating projection. In contrast, during most of bud growth, cortical polarity relies on the compartmentalization of the cell periphery into mother and bud cortex (Fig. 2A, part c, Fig. 3B, part b; Barral et al. 2000; Gladfelter et al. 2001). Septins are involved in the maintenance of cell polarity during both mating and bud growth (Giot and Konopka 1997; Barral et al. 2000).

Cell polarity during the budding cycle

Formation of the yeast bud is the object of intense study and is starting to become well understood. Since it has been the topic of many reviews (Finger and Novick 1998; Madden and Snyder 1998; Pruyne and Bretscher 2000a, b), we will not describe this process in detail here. In summary, formation of the yeast bud depends on the polarization of cell growth towards a selected area. Bud growth happens in two successive phases (Figs. 2A, 3B).

First, cell growth is restricted to the incipient bud site, which becomes the bud tip as the bud emerges. During

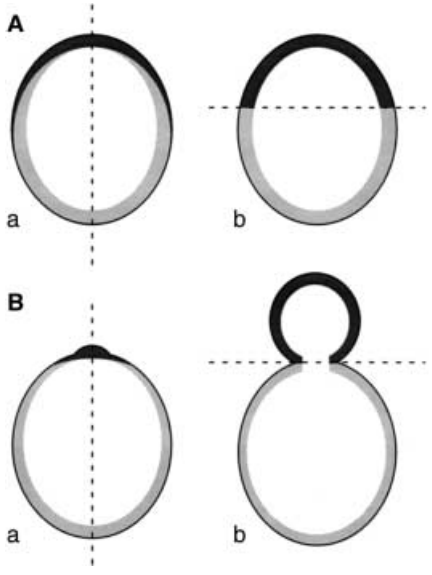

Fig. 3A, B. Apical polarity and the budding process. **A** Apical polarity can take two forms. *a* The distribution of cortical components is organized in a gradient around one pole. The concentration of the polarized factors peaks at the pole, while other factors may have the reverse distribution. This is referred to as apical polarity. *b* Cortical components may be distributed asymmetrically into two distinct compartments separated by a sharp boundary. In this case, components of distinct compartments never mix. This is referred to as cortical compartmentalization. **B** The budding process presents the two forms of polarity successively. During bud emergence, polarity is organized around the incipient bud site (*a*). After the switch to isotropic bud growth, the yeast cortex is clearly compartmentalized into mother and bud cortex (*b*)

this phase, cell polarity is organized apically. Second, bud-growth switches to an isotropic mode. Growth occurs over the entire surface of the bud, which becomes rounded, and is excluded from the mother cell. Thus, the bud and mother cortexes become two distinct compartments separated by the bud neck. Septin mutants do not switch to isotropic growth (Fig. 2B): they keep on growing apically and form elongated buds. This is due to the existence of a cell cycle checkpoint preventing the switch to isotropic growth in the absence of the septin ring (Barral et al. 1999). We recently showed that, in cells losing the septin ring after the checkpoint, cell growth is no longer restricted to the bud. Thus, the septin ring plays some role in the compartmentalization of the cortex during isotropic growth.

How does the septin ring at the bud neck ensure that growth remains properly polarized during isotropic growth? Due to the presence of the cell wall, yeast cell growth depends on the local delivery of new cell wall material and cell wall-remodeling enzymes. Thus, the sites of exocytosis determine the sites of growth. Two processes determine where exocytosis takes place: vesicle delivery and the docking/fusion of vesicles with the plasma membrane (Finger and Novick 1998).

Vesicle delivery is ensured by the actin cytoskeleton (Pruyne et al. 1998; Schott et al. 1999, 2002; Karpova et al. 2000). Actin cables, which run from the bud cortex into the mother cell, serve as tracks for the myosin-dependent transport of vesicles to the bud. Polarization of actin cables towards the bud cortex requires the function of the polarisome, a protein complex containing the formin Bni1 and the formin-associated proteins Spa2, Pea2 and Bud6 (Snyder 1989; Chenevert et al. 1994; Valtz and Herskowitz 1996; Amberg et al. 1997; Evangelista et al. 1997, 2002; Sheu et al. 1998; Sagot et al. 2002). The polarisome localizes to the bud cortex.

The final control of vesicle docking and fusion to the plasma membrane is ensured by the exocyst (Finger and Novick 1998). This multi-protein complex is also associated with the plasma membrane of the bud. During apical growth, both the polarisome and the exocyst localize to the tip of the bud. Upon switching to isotropic bud growth, they relocalize to the entire bud periphery and remain excluded from the mother cell cortex.

Role of septins in the maintenance of cell polarity

Septins act in the maintenance of cell polarity by ensuring the sequestration of the exocyst and the polarisome to the bud during isotropic growth (Barral et al. 2000). In the *cdc12-6* mutant, the septin ring quickly disassembles at the restrictive temperature. In these cells, the polarisome marker, Spa2, and the exocyst markers, Sec3p (Finger and Novick 1997) and Sec5p (TerBush et al. 1996), are no longer excluded from the mother cell. In fact, most factors tested until now (Barral et al. 2000; Takizawa et al. 2000; Barral, unpublished results) require septin function to maintain their proper localization in the bud during isotropic growth. As a consequence, cells that switch to isotropic growth without having a septin ring at the bud neck are unable to restrict exocytosis and growth to the bud. During apical growth, when polarity is organized around the bud tip, absence of the septin ring does not affect the localization of cortical markers and growth. These results suggest that the septin ring may act at the bud neck as a barrier, preventing the lateral diffusion of membrane-associated factors along the plasma membrane into the mother cell.

Prevention of passive movement

Two lines of evidence indicate that the septin ring does in fact prevent passive diffusion of cortical factors through the bud neck. First, if *cdc12-6* cells are treated with cycloheximide to prevent de novo protein synthesis prior to a shift to the restrictive temperature, polarity markers still redistribute between the bud and the mother cell (Barral et al. 2000). The kinetics of redistribution are not affected, compared with the same experiment in untreated cells. Conversely, cycloheximide does not trigger protein redistribution in wild-type cells, where the septin ring remains stable. Thus, the pool of polarity proteins that re-localizes to the mother cell upon disruption of the septin ring is not due to de novo protein synthesis, but comes from the bud. Therefore, in the absence of a septin ring, these proteins diffuse into the mother cell and, in wild-type cells, the septin ring prevents this diffusion from happening (Barral et al. 2000). Together, these results suggest that, once protein asymmetry is established, the septin ring at the mother-bud neck avoids diffusion of membrane-associated proteins back to the mother compartment.

Takizawa and colleagues (2000) reported a second line of evidence for the septin-dependent formation of a diffusion barrier. In this case, the Ist2p (Entian et al. 1999) membrane protein was shown to localize specifically to the plasma membrane of the bud. GFP-Ist2p localizes like Ist2p. Photo-bleaching of a plasma membrane segment in the bud of cells expressing GFP-Ist2p showed that fluorescence recovery was rapid. Recovery was not due to de novo protein synthesis, since synthesis of the reporter GFP-Ist2p protein was turned off prior to photo-bleaching. Thus, Ist2p freely diffuses in the plane of the plasma membrane. Yet it does not cross the bud neck, since it is not found at the plasma membrane of the mother cell. Thus, a diffusion barrier is present at the bud neck. In the *cdc12-6* mutant, GFP-Ist2p remains in the plasma membrane of the bud as long as the cells are grown at the permissive temperature. However, it redistributes rapidly to the surface of both the bud and the mother compartments after disruption of the septin ring. Thus, the presence of the septin ring is required to form the diffusion barrier at the bud neck.

It remains to be determined whether the septin act by recruiting specialized molecules to the neck, which in turn ensure a fence function, or whether the septins themselves form the barrier. Mutations that would specifically affect the fence function are predicted to lead to the formation of small buds and large mothers. Until now, despite extensive studies, no septin-associated proteins have been isolated whose absence leads to such a phenotype. We would like therefore to argue that the septins themselves are the best candidates to assume the fence function (see next section).

The role of septin structures as a barrier may also be important during mating. In response to pheromones, mating partners use polarized growth to extend a projection towards each other and fuse. In the absence of fully functional septins, cells are unable to form an extended projection (Giot and Konopka 1997). This defect leads to a reduction in the efficiency with which partners enter in contact and fuse. However, the role of septins during mating has not yet been extensively studied and therefore roles other than the formation of a diffusion barrier cannot be excluded at this point.

How do septins work?

Functional studies suggest that, at least in yeast, the role of septins is tightly associated with their assembly into a stable ring. This ring has two main functions: it separates the bud cortex from the mother cortex and it recruits specialized structures to the bud neck. The first function helps to maintain cell polarity and organize actin distribution. The second function serves to organize the cell wall at the bud neck and position the cleavage machinery. In addition, the septin ring may also help the cell to monitor its morphology (Barral et al. 1999; Longtine et al. 2000). In all cases, the primary function of the septin ring is to be at the bud neck and to remain there.

C. Field and colleagues initiated the biochemical characterization of septin complexes. These studies revealed that both yeast and *Drosophila* septins purify as complexes (Oegema et al. 1998). In the case of *S. cerevisiae* septins, this complex contains Cdc3, Cdc10, Cdc11 and Cdc12, with a stochiometric ratio of roughly 2:2:1:2. The purified complexes from yeast and *Drosophila* are both able to form filamentous structures in vitro. Polymerization is affected by salt concentration. The length of the filaments varies from 32 nm to 100 nm in 1 M KCl and to more than 1500 nm in 75 mM KCl. At a lower salt concentration, filaments have been found to associate into bundles by side interactions (Frazier et al. 1998). These studies did not determine whether nucleotide binding or hydrolysis plays a role in the polymerization/depolymerization processes.

Studies with the mammalian septin H5 gave very exciting clues about one of the possible functions of nucleotide binding and hydrolysis (Zhang et al. 1999). Like most other septins, septin H5 is closely associated with membranes in vivo. This observation invited the authors to investigate whether H5 directly interacts with lipids and whether such an interaction plays any role in the association of septins with membranes. Their analysis revealed that a short polybasic sequence located directly upstream of the GTPase domain is necessary and sufficient for PIP_2 binding. Binding of phosphatidyl inositol biphosphate by this sequence was required for septin interaction with the plasma membrane. Remarkably, the affinity of the septin for PIP_2 was affected by the nature of the nucleotide present in the nucleotide-binding pocket. GTP inhibited PIP_2 binding, while GDP favored the interaction. Importantly, the polybasic sequence found in the septin H5 is conserved among septins, suggesting that phospholipid binding is a general septin property (Zhang et al. 1999). These findings unravel at least one of the manners in which septins interact with the plasma membrane and indicate that one of the functions of the GTPase domain is to regulate this interaction. In addition, this work allows several predictions.

First, since the GDP-bound state corresponds to the lipid-binding conformation, septin localization indicates which nucleotide is in its binding pocket. Since filaments are generally associated with the plasma membrane, they are probably composed of GDP-loaded septin molecules. This situation would be very similar to that observed for actin and tubulin.

Second, septin filaments are likely to concentrate specific lipids in the plasma membrane domain with which they are in contact. One consequence should be that septin filaments are tightly attached to the membrane. In addition, septin filaments would locally affect the composition and the fluidity of the plasma membrane.

In this respect, it is interesting to notice that C. Beh and J. Rine (personal communication) found that some ergosterol derivatives are enriched in the plasma membrane of the mother cell, compared with that of the bud. This observation suggests that lipid diffusion in the plasma membrane is inefficient at the bud neck. It will be interesting to determine whether the septin ring also affects lipid diffusion at the bud neck. If this is the case, the lipid-binding properties reported for septins are predicted to be important for this function.

The observation that septins can form filaments in vitro strongly supports the idea that they are the constituents of the 10-nm filaments seen in vivo. However, what is the function of septin polymerization? Indications that septin filament formation might not be required for septin function came from the analysis of the *cdc10*Δ mutant. C. Fields and colleagues found that cells lacking Cdc10 do not form the bud neck filaments, as judged by electron microscopy. Septin complexes purified from these cells are smaller than those purified from wild-type cells, but still contain Cdc3, Cdc11 and Cdc12. These complexes do not form filaments in vitro (Frazier et al. 1998). Thus, cells lacking Cdc10 may have lost the

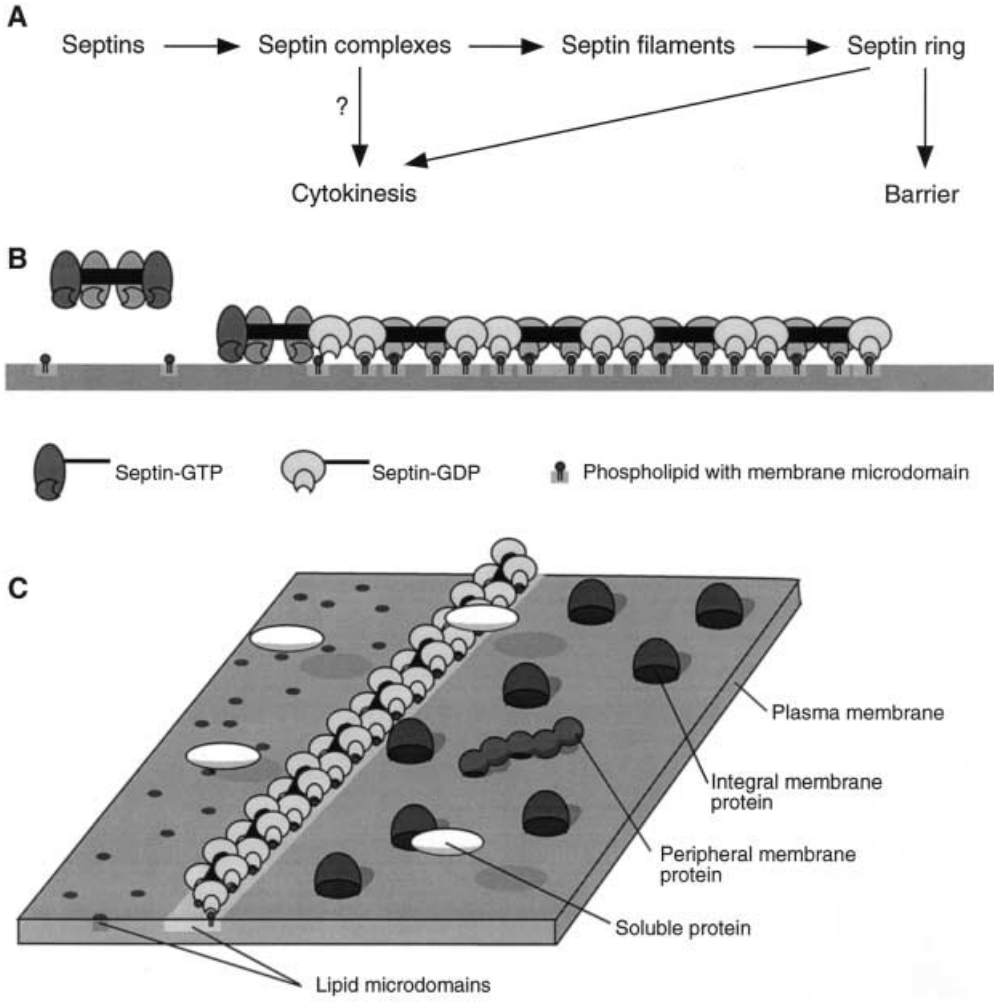

Fig. 4A–C. Model for septin organization and function. **A** Scheme of septin ring assembly. Yeast septin monomers assemble into septin complexes. Septin complexes are subunits of septin filaments. In turn, septin filaments form the septin ring. This structure serves both as a scaffold for the organization of the cytokinetic machinery and as a barrier to limit the diffusion of cortical components through the bud neck. **B** Model for the polymerization of septin filaments at the surface of the plasma membrane. Septins in soluble complexes are in their GTP-bound state. Upon association of septin complexes at the surface of the membrane, GTP hydrolysis opens the phospholipid-binding pocket. The binding of PIP2 leads to the recruitment and immobilization of these molecules underneath the filament. It is proposed here that this step helps to recruit lipid microdomains underneath the filaments, thereby modifying the local fluidity of the plasma membrane. **C** The septin filament forms a barrier against the lateral diffusion of lipids, integral membrane proteins and membrane-associated proteins. This property is used by the cell to develop two membrane domains with distinct compositions. Septin filaments do not restrict the diffusion of soluble molecules

ability to form septin filaments. However, *cdc10*Δ cells are perfectly viable at lower temperatures. At these temperatures, the other septins seemed to localize properly, since the septin Cdc3 localized properly to the bud neck. Also, known bud neck components, such as Bud4, were recruited properly to the bud neck. No major cytokinetic defects were observed at these temperatures. Thus, these results suggest that formation of septin filaments is not required for septin localization and function.

Until now, all septin mutations that affect cell viability have in common the disruption of the septin ring at the restrictive temperature. Thus, the formation of the ring is a major requirement for septin function. It is unclear how septin ring formation and septin filament formation could be uncoupled. On the one hand, analysis of septin filament formation in the *cdc10*Δ cells does not completely exclude the formation of higher-order septin structures. Since filaments are always formed in close apposition with the plasma membrane in vivo, it is possible that the interaction with lipids plays an important role in the biochemistry of septin polymerization. In the absence of Cdc10, lipid requirement may become more acute, explaining why filaments are not observed in vitro. The inability to observe 10-nm filaments in vivo may also reflect their fragility more than their absence. On the other hand, the localization of the septins to the bud neck may indeed depend not only on their polymerization. Interactions with other molecules may ensure proper septin localization and be sufficient to maintain non-filamentous septins in their proper localization. For example, it has long been

assumed that a septin receptor may be responsible for the association of septins with the plasma membrane at the bud neck.

The localization of the septins to the bud neck throughout the budding process and their putative role in the establishment of a diffusion barrier in the plane of the membrane would be consistent with septins forming filaments. Recent results using FRAP techniques also point in that direction (Kozubowski et al. 2001; E. Bi, personal communications; J. Dobbelaere and Y. Barral, unpublished results). Consistent with the ring forming a highly stable structure, septins were found to not turnover at the bud neck of medium- and large-budded cells. Fluorescence recovery could only be observed in cells with very small buds or unbudded. Thus, septin dynamics are extremely slow in budded cells. It will be interesting to determine whether the same results are obtained in a *cdc10*Δ strain.

Model for septin function in yeast

Putting together all the results discussed here, we propose the following model for septin organization and function (Fig. 4). Septins assemble into soluble multimeric complexes (Fig. 4A, B). It is possible that GTP-binding is required for further septin polymerization into filaments. Rapidly after incorporation into filaments, GTP is most likely hydrolyzed, allowing the exposition of the lipid-binding site. The presence of phospholipids in the vicinity may favor transition to the GDP-bound form and stabilize it.

Tight interaction of septin filaments with the plasma membrane results from the binding of multiple lipid molecules by the septin molecules. Conversely, these interactions lead to the recruitment and enrichment of the plasma membrane in specific phospholipids. The restriction of lipid movement by the septin polymers in turn locally affects the fluidity of the plasma membrane. For example, the septin filaments may recruit and anchor lipid microdomains at the plasma membrane of the bud neck.

One of the consequences of lipid microdomain recruitment by septin filaments would be the formation of a diffusion barrier for lipids and integral membrane proteins in the plasma membrane (Fig. 4C). Septin filaments are also likely to impair free diffusion of proteins that are tightly associated with the cytoplasmic face of the plasma membrane through the bud neck. In contrast, factors that are soluble or more transiently associated with the plasma membrane may diffuse more freely.

In addition to septin filament formation and lipid association, septin interaction with transmembrane proteins may play an important role in anchoring the septin ring at the bud neck. Particularly, proteins that extend into the cell wall are good candidates to help maintain the localization of the septin ring. These molecules may ensure that the septin ring remains structured and partly functional, even when septin filaments are highly unstable, such as in the *cdc10*Δ cells. More advanced biochemical and biophysical studies will be required to test the different aspects of our model. It also becomes urgent to develop a molecular model of septins, septin complexes and septin filaments.

Conclusion

If bones are understood to comprise the skeleton, the hard part of our body around which the rest organizes, then septin structures must be at the core of the cellular skeleton. More than microtubules and microfilaments, septin structures are highly stable structures around which yeast cells organize. The low dynamics of septin filaments allow their use by the cell as a pivotal structure to organize cell polarity and cell division. The complex interaction of septins with nucleotides, lipids and among themselves tremendously complicates their biochemical analysis. It is therefore not really surprising that the efforts made to understand the control of septin polymerization and de-polymerization have obtained little success so far. However, future work will have to address septin biochemistry and structural organization. It will also be necessary to identify the molecules and parameters that determine the size, morphology and localization of the ring.

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