

Septin pairs, a complex choreography

Helge Ewers

Institute of Biochemistry, Eidgenössische Technische Hochschule Zürich, 8093 Zürich, Switzerland

Septins form a filamentous collar at the mother–bud neck in budding yeast. In cytokinesis, this collar splits into two rings and the septin complexes undergo a dramatic reorientation. Using fluorescence polarization microscopy, DeMay et al. (2011. *J. Cell Biol.* doi:10.1083/jcb.201012143) now demonstrate that septin complexes assemble as paired filaments *in vivo* and reveal new insights into septin organization during cytokinesis.

The septins are a family of GTPases that form heteromeric complexes that can assemble into paired filaments *in vitro* (Fig. 1 a). They were discovered as cell division cycle mutants in yeast (Hartwell, 1971), and a role in cell division is conserved in humans (Kinoshita et al., 1997). In mammals, septins are involved in a variety of functions at the interface of the actin and microtubule cytoskeleton and membranes, and are implicated in several diseases.

Most of what we know about the biochemistry of septins has been discovered in budding yeast (Frazier et al., 1998; Vrabioiu et al., 2004). In this organism, the subunit composition of the septin hetero-oligomers determines whether they can polymerize to form filaments. Importantly, if septin complexes cannot assemble into filaments, yeast cells die (McMurray et al., 2011).

The septin collar at the yeast mother–bud neck is the best understood septin structure. Here, septins are present in a highly ordered, membrane-apposed collar that encircles the mother–bud neck (Byers and Goetsch, 1976; Haarer and Pringle, 1987). This structure, which is absent in septin mutants, serves as a diffusion barrier that prevents the exchange of membrane proteins between the mother and daughter cells (Barral et al., 2000; Takizawa et al., 2000) as well as between their ERs (Luedeke et al., 2005) and nuclear envelopes (Shcheprova et al., 2008). The septin collar also serves as a scaffold for enzymes and signaling molecules (DeMarini et al., 1997). During cytokinesis, the septin collar splits into two separate rings. How this process is regulated and how septin complexes are organized at the collar and ring structures is not clear.

In this issue, DeMay et al. investigate the dynamics of septin organization during this process using a sophisticated microscopy assay. Their method relies on the fact that a fluorophore can only absorb and emit light that is polarized parallel to its dipole moment. The relative emission intensity after

excitation at various angles thus reports the orientation of the molecule in space. The authors used septins fused to a GFP that was not free to rotate. The dipole moment was hence fixed relative to the septin complex axis.

This technique had been used earlier to demonstrate that septin complexes are arranged in ordered arrays at the mother–bud neck, and that the septin filaments in the arrays undergo a 90° switch in orientation during ring splitting (Fig. 1 a; Vrabioiu and Mitchison, 2006). Vrabioiu and Mitchison (2006) and others (Weirich et al., 2008) explained this by proposing that the filaments rotate. The present work utilizes a more complex setup allowing for a more detailed analysis of filament orientation and dynamics and reveals several important aspects of septin complex assembly.

First, using immunoelectron microscopy in the filamentous fungus *Ashbya gossypii*, the authors provide strong evidence that septins constitute paired filament bundles with a periodicity of 35 nm, the length of septin complexes. Consistent with this observation, the polarization of the emitted light for several septin–GFP constructs is always in a fixed angle either 0° or 90° in respect to the filaments imaged within a cell, which argues for a twofold rotational symmetry of the total four GFPs within a paired septin complex.

The authors then demonstrate in budding yeast that the bud–neck septin complexes are polarized perpendicular to the filament axis like the elongated filaments in *A. gossypii*. A striking time-lapse imaging experiment of cytokinesis reveals that the polarization of septin fluorescence is completely lost as the septin collar splits. As the two rings emerge, septin fluorescence becomes polarized again, only now perpendicular to the mother–bud axis.

The high-resolution time series shows that the parallel orientation remains longest in the center of the complex, whereas perpendicular orientation emerges first at the outmost area of septin fluorescence. This speaks against a model where filaments rotate (Fig. 1 b), as proposed previously (Vrabioiu and Mitchison, 2006). It would rather be consistent with an equilibrium shift between two organizational forms that are always present (Fig. 1 c) or a model where septin filaments disassemble and reassemble later, as it has been shown in the fungus *Ustilago maydis* (Fig. 1 d; Böhmer et al., 2009).

© 2011 Ewers This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see <http://www.rupress.org/terms>). After six months it is available under a Creative Commons license [Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>].

Correspondence to Helge Ewers: helge.ewers@bc.biol.ethz.ch

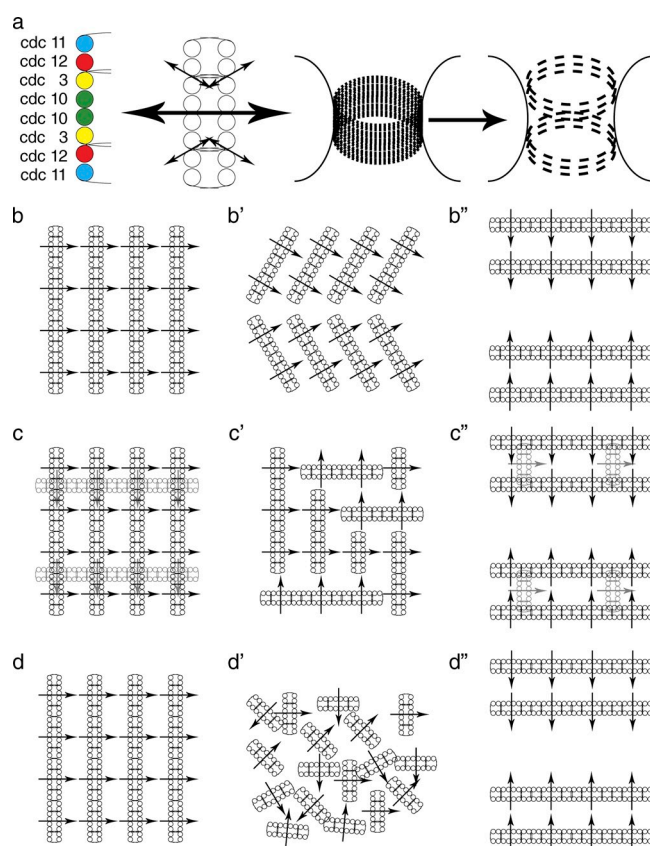


Figure 1. Models of septin complex reorganization. (a) Yeast septins form an octameric complex. The four transition dipole moments of a paired complex average to an overall anisotropy value (arrows). A collar of septin filaments parallel to the mother-bud axis is split into two rings with filaments perpendicular to the mother-bud axis. (b) Septin filaments on opposing sides of the division plane rotate against one another, canceling the overall polarization until, when the process is completed, a perpendicular polarization reemerges. (c) A majority of filaments are oriented parallel to the mother-bud axis while there are also perpendicular filaments. The reorientation represents a shift in equilibrium between the two arrangements. (d) The septin filaments completely disassemble and later reassemble in perpendicular orientation while remaining confined to the bud neck.

The authors find in photobleaching experiments that exchanging molecules maintain the polarization angle of the filament. It will thus be possible to determine filament orientation by single-molecule experiments. Such experiments could distinguish between the above-mentioned models if the collar was photobleached before the ring splitting. During this period, septins are reported to exchange quickly (Dobbelaere et al., 2003), and the polarization of newly incorporated septin molecules should reveal the reorientation more clearly. If filaments rotate, newly incorporated molecules should show a gradual progression of angles (Fig. 1 b). If a balance between parallel and perpendicular filaments shifts to the other form, all incorporated septins would be polarized in one of the two orientations (Fig. 1 c). However, if parallel collar filaments disassemble before perpendicular ring filaments reassemble, there must be a pool of isotropic septin molecules (Fig. 1 d). In general, single-molecule based super-resolution microscopy should be an ideal tool to investigate further the unexpected complexity of septin complex reorganization (Fig. 2).

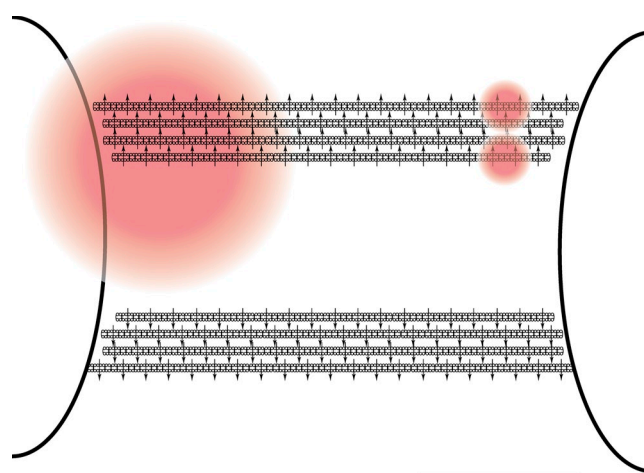


Figure 2. The scale of the septin complex at the bud neck. Graphic representation of the septin rings at the mother-bud neck drawn approximately to scale. The entire structure is only a few hundred nanometers wide, making it difficult to investigate by light microscopy. Shown as red intensity centroids are typical point-spread functions of a confocal microscope (left) and the localization precision possible with novel super-resolution methods such as stimulated emission depletion (STED) or photo-activated localization (PALM) microscopy (right). Bar, 200 nm.

Lastly, the authors apply their system to mammalian septins and find that overexpressed septin2 is polarized in elongated structures, thereby providing the first evidence that septins are arranged in strict patterns in mammalian cells. All elongated septin structures described in mammals so far are dependent on the structural integrity of either actin filaments (Kinoshita et al., 2002) or microtubules (Nagata et al., 2003), and electron microscopy of highly ordered septin structures is lacking. Although the finding that septins in filaments that are the result of overexpression are polarized does not mean that septins will be organized in a similar fashion when they interact with microtubules, actin, or membranes in cells, this finding provides an important step forward in understanding the organization of mammalian septin structures.

Overall, the work by DeMay et al. (2011) revealed important details in septin complex and filament assembly. Future studies using novel super-resolution, electron, or polarization microscopy methods will help to elucidate how the structural organization of septin complexes mediates cellular function.

Submitted: 16 May 2011

Accepted: 23 May 2011

References

- Barral, Y., V. Mermall, M.S. Mooseker, and M. Snyder. 2000. Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. *Mol. Cell.* 5:841–851. doi:10.1016/S1097-2765(00)80324-X
- Böhmer, C., C. Ripp, and M. Bölker. 2009. The germinal centre kinase Don3 triggers the dynamic rearrangement of higher-order septin structures during cytokinesis in *Ustilago maydis*. *Mol. Microbiol.* 74:1484–1496. doi:10.1111/j.1365-2958.2009.06948.x
- Byers, B., and L. Goetsch. 1976. A highly ordered ring of membrane-associated filaments in budding yeast. *J. Cell Biol.* 69:717–721. doi:10.1083/jcb.69.3.717
- DeMarini, D.J., A.E. Adams, H. Fares, C. De Virgilio, G. Valle, J.S. Chuang, and J.R. Pringle. 1997. A septin-based hierarchy of proteins required for

- localized deposition of chitin in the *Saccharomyces cerevisiae* cell wall. *J. Cell Biol.* 139:75–93. doi:10.1083/jcb.139.1.75
- DeMay, B.S., X. Bai, L. Howard, P. Occhipinti, R.A. Meseroll, E.T. Spiliotis, R. Oldenbourg, and A.S. Gladfelter. 2011. Septin filaments exhibit a dynamic, paired organization that is conserved from yeast to mammals. *J. Cell Biol.* 193:1065–1081.
- Dobbelaere, J., M.S. Gentry, R.L. Hallberg, and Y. Barral. 2003. Phosphorylation-dependent regulation of septin dynamics during the cell cycle. *Dev. Cell.* 4:345–357. doi:10.1016/S1534-5807(03)00061-3
- Frazier, J.A., M.L. Wong, M.S. Longtine, J.R. Pringle, M. Mann, T.J. Mitchison, and C. Field. 1998. Polymerization of purified yeast septins: evidence that organized filament arrays may not be required for septin function. *J. Cell Biol.* 143:737–749. doi:10.1083/jcb.143.3.737
- Haarer, B.K., and J.R. Pringle. 1987. Immunofluorescence localization of the *Saccharomyces cerevisiae* CDC12 gene product to the vicinity of the 10-nm filaments in the mother-bud neck. *Mol. Cell. Biol.* 7:3678–3687.
- Hartwell, L.H. 1971. Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. *Exp. Cell Res.* 69:265–276. doi:10.1016/0014-4827(71)90223-0
- Kinoshita, M., S. Kumar, A. Mizoguchi, C. Ide, A. Kinoshita, T. Haraguchi, Y. Hiraoka, and M. Noda. 1997. Nedd5, a mammalian septin, is a novel cytoskeletal component interacting with actin-based structures. *Genes Dev.* 11:1535–1547. doi:10.1101/gad.11.12.1535
- Kinoshita, M., C.M. Field, M.L. Coughlin, A.F. Straight, and T.J. Mitchison. 2002. Self- and actin-templated assembly of Mammalian septins. *Dev. Cell.* 3:791–802. doi:10.1016/S1534-5807(02)00366-0
- Luedeke, C., S.B. Frei, I. Sbalzarini, H. Schwarz, A. Spang, and Y. Barral. 2005. Septin-dependent compartmentalization of the endoplasmic reticulum during yeast polarized growth. *J. Cell Biol.* 169:897–908. doi:10.1083/jcb.200412143
- McMurray, M.A., A. Bertin, G. Garcia III, L. Lam, E. Nogales, and J. Thorner. 2011. Septin filament formation is essential in budding yeast. *Dev. Cell.* 20:540–549. doi:10.1016/j.devcel.2011.02.004
- Nagata, K., A. Kawajiri, S. Matsui, M. Takagishi, T. Shiromizu, N. Saitoh, I. Izawa, T. Kiyono, T.J. Itoh, H. Hotani, and M. Inagaki. 2003. Filament formation of MSF-A, a mammalian septin, in human mammary epithelial cells depends on interactions with microtubules. *J. Biol. Chem.* 278:18538–18543. doi:10.1074/jbc.M205246200
- Shcheprova, Z., S. Baldi, S.B. Frei, G. Gonnet, and Y. Barral. 2008. A mechanism for asymmetric segregation of age during yeast budding. *Nature.* 454:728–734.
- Takizawa, P.A., J.L. DeRisi, J.E. Wilhelm, and R.D. Vale. 2000. Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. *Science.* 290:341–344. doi:10.1126/science.290.5490.341
- Vrabioiu, A.M., and T.J. Mitchison. 2006. Structural insights into yeast septin organization from polarized fluorescence microscopy. *Nature.* 443:466–469. doi:10.1038/nature05109
- Vrabioiu, A.M., S.A. Gerber, S.P. Gygi, C.M. Field, and T.J. Mitchison. 2004. The majority of the *Saccharomyces cerevisiae* septin complexes do not exchange guanine nucleotides. *J. Biol. Chem.* 279:3111–3118. doi:10.1074/jbc.M310941200
- Weirich, C.S., J.P. Erzberger, and Y. Barral. 2008. The septin family of GTPases: architecture and dynamics. *Nat. Rev. Mol. Cell Biol.* 9:478–489. doi:10.1038/nrm2407