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Shs1 rounds out the septin repertoire

Study reveals how changes in subunit composition and phosphorylation alter the organization of septin filaments.

Septins are a family of GTPases that assemble into filaments and direct a variety of cellular processes, including cytokinesis (1). Garcia et al. describe how the architecture of these filaments is modulated by phosphorylation and by the incorporation of different septin subunits (2).

Budding yeast express five septins during mitosis, all of which accumulate in a collar around the bud neck that recruits proteins required for cell division. In vitro, four of these mitotic septins—Cdc3, Cdc10, Cdc11, and Cdc12—form a rod-shaped octamer with a Cdc11 subunit on each end. Interactions between the terminal Cdc11 subunits help polymerize these septin octamers into long filaments (3), and yeast lacking any of these four septins have severe difficulty assembling a collar at the bud neck and completing cytokinesis (4). The fifth mitotic septin, Shs1, isn't an essential protein, however, and its function in cytokinesis remains enigmatic. "Shs1 is one of the most phosphorylated septins," says Eva Nogales, from the University of California, Berkeley. "So we thought it might have a regulatory role that could change the properties of septin filaments."

Nogales and colleagues, led by graduate student Galo Garcia, found that Shs1 could substitute for Cdc11 in vitro and cap the ends of octameric septin rods containing Cdc3, Cdc10, and Cdc12 (2). Rather than polymerizing end-to-end to form long filaments, however, Shs1-containing octamers bundled together into large ring-shaped structures. "So Shs1 is a regulator of septin assembly and organization," Nogales says.

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In budding yeast lacking Shs1, the remaining septins still accumulated between mother and daughter cells, but they formed disorganized, randomly oriented filaments instead of continuous rings encircling the bud neck. The mutant yeast still complete cytokinesis, but, says Nogales, "they do it badly. The process becomes very slow."

Because all five septins normally colocalize at the bud neck, Galo Garcia wanted to see what would happen if he mixed different proportions of Shs1- and Cdc11-containing octamers in vitro. "I found that, as I titrated in more complexes containing Cdc11, the diameter [of the septin filament rings] increased," Garcia recalls.

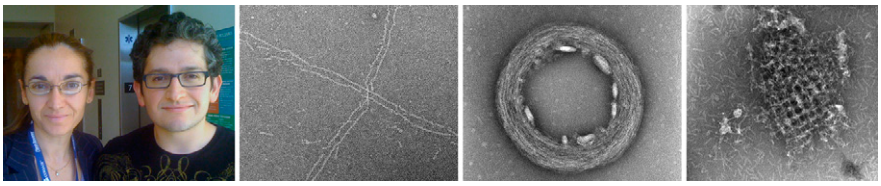
This immediately put Garcia in mind of the hourglass-shaped septin collar that surrounds the yeast bud neck. "If you think of the hourglass structure as a series of stacked septin rings," Garcia explains, "the rings in the center of the bud neck would have a lower diameter than those at the edges. So there might be a higher concentration of Shs1 towards the middle of the collar."

This idea remains to be tested, but Garcia et al. did investigate the potential effects of phosphorylation on Shs1's ability to organize septin filaments. The researchers made several mutations in Shs1 that mimicked previously mapped phosphorylation events and assessed how these mutants organized septin filaments in vitro. Multiple phosphomimetic mutations in Shs1's C terminus—a region required for the assembly of Shs1-containing octamers into hoops—hindered, or even abolished, the formation of septin rings. On the other hand, a different phosphomimetic mutation—affecting a serine residue predicted to lie at the interface between interacting Shs1 molecules—spurred Shs1-containing complexes to form a mixture of rings and a gauze-like meshwork.

"The negative charge at this position may change the geometry with which the octamers interact," Nogales says. "These meshes are very beautiful, and we also see them when we look inside the cell."

The terminal subunits of septin octamers therefore determine how the octamers assemble into higher-order structures, and septin phosphorylation can further modify these interactions. "In mammals, there are many more septins [than in yeast]." Nogales notes. "We know that septins are polymerized in many different ways in different tissues, so we think that changes in the terminal subunit will also affect how mammalian septins self-assemble."

Focal Point



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(Left to right) Eva Nogales, Galo Garcia, and colleagues (not pictured) reveal that alterations in subunit composition and phosphorylation can modulate the assembly and organization of septin filaments, cytoskeletal elements that control a range of cellular processes, including cytokinesis. Septin complexes containing Cdc11 assemble into long, straight rods (left), but substitution of Cdc11 with Shs1 drives the formation of ring-shaped structures (middle). Phosphorylation of different residues in Shs1 can either prevent ring formation or promote the assembly of septins into a gauze-like meshwork (right).

1. Barral, Y., and M. Kinoshita. 2008. *Curr. Opin. Cell Biol.* 20:12–18.
2. Garcia, G., et al. 2011. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201107123.
3. Bertin, A., et al. 2008. *Proc. Natl. Acad. Sci. USA.* 105:8274–8279.
4. Byers, B., and L. Goetsch. 1976. *J. Cell Biol.* 69:717–721.