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Formin' the cytokinetic ring

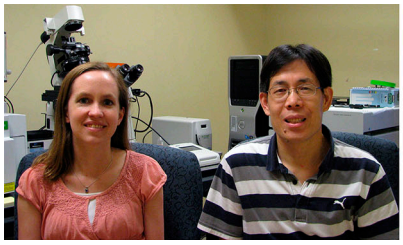
Study reveals how two formin proteins cooperate to assemble the contractile ring in fission yeast.

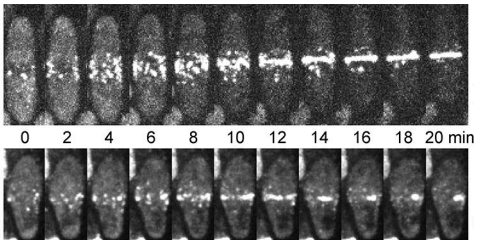
All animal and fungal cells assemble a contractile actomyosin ring to divide themselves in two at the end of mitosis. The rings are constructed from actin filaments formed de novo at the division site and from filaments that are assembled elsewhere in the cell and transported to the cleavage zone. Both types of filament are generated by actin-nucleating formin proteins, but whether one source is more important for ring assembly than the other is unknown. Coffman et al. describe how two formins cooperate to assemble the contractile ring in fission yeast, mainly by generating actin filaments at the site of cytokinesis (1).

The fission yeast formin Cdc12 is essential for contractile ring assembly and cytokinesis (2). It localizes to cytokinetic nodes, protein clusters that are scattered around the cell equator at the early stages of ring assembly. Actin filaments nucleated by Cdc12 at one node are thought to be captured by myosin molecules in neighboring nodes. The nodes are then pulled together to form a mature contractile ring (3). However, Cdc12 also nucleates actin filaments throughout the cell that are incorporated into the ring alongside the filaments generated at the nodes (4). "We wanted to know how Cdc12 is regulated and whether other formins are involved as well," says Jian-Qiu Wu, from The Ohio State University in Columbus.

Wu and colleagues, led by graduate student Valerie Coffman, had noticed that, although cytokinetic nodes couldn't assemble into a contractile ring in yeast lacking Cdc12, they still coalesced into larger clumps (1). This suggested that another fission yeast formin might generate actin filaments to connect neighboring nodes together. The formin For3 localizes to yeast division sites, though its loss has no effect on contractile ring assembly (5, 6). "But when we combined a temperature-sensitive Cdc12 mutant with a deletion of For3, we couldn't see any concentration of actin at the division site, and the nodes failed to clump," Coffman explains. The loss of For3 was also lethal when combined with other mutations that inhibit contractile ring assembly, such as mutations in the myosin light chain and IQGAP.

Focal Point





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Valerie Coffman (left) and Jian-Qiu Wu (right) describe how two actin-nucleating formin proteins cooperate to assemble the contractile actomyosin ring in dividing fission yeast cells. The formin Cdc12 is essential for cytokinesis, but N-terminally truncated versions of Cdc12 can still support ring assembly as long as the formin For3 is present to generate actin filaments that help recruit truncated Cdc12 to the division site. Under these conditions (top row), precursor nodes containing myosin II (white) still coalesce into a functional ring. In For3's absence (bottom row), however, truncated Cdc12 rarely localizes to the division site, and, despite Cdc12's ability to nucleate actin filaments elsewhere in the cell, ring assembly fails. This suggests that contractile ring formation largely relies on the de novo assembly of actin filaments at the division site.

To investigate the relationship between For3 and Cdc12, Coffman et al. generated truncated versions of Cdc12 that no longer bind to the F-BAR protein Cdc15, a cytokinetic node protein that helps recruit Cdc12 to the division site. The Cdc12 truncations still localized to the division site and still supported contractile ring assembly. In fact, they appeared to be more active than wild-type Cdc12, generating more actin filaments throughout the cell. But Coffman et al. found that, when they were unable to bind Cdc15, the Cdc12 truncations mostly relied on actin filaments nucleated by For3 for their recruitment to the division site. Deleting For3 disrupted the Cdc12 truncations' localization and severely compromised contractile ring assembly and cell viability.

> "Transport of actin filaments alone isn't sufficient for ring assembly."

Combining For3 deletion with Cdc12 truncations was so lethal that Coffman et al. could only observe double mutant cells as they are dying, using a novel technique called tetrad fluorescence microscopy. This approach revealed that actin filaments were still assembled and transported to the division site at the same rate as in wild-type cells. But, with For3 and Cdc12 both missing from the cell equator, no filaments could be formed de novo at the division site, and contractile ring assembly was inhibited. "This suggests that transport of actin filaments alone isn't sufficient for ring assembly," says Wu. "De novo nucleation at the division site is a more efficient way to form the contractile ring."

"In the future, it will be important to investigate whether de novo nucleation also predominates in other cell types," Wu continues. In addition, Coffman et al. plan to follow up on their observation that truncated versions of Cdc12 are more active than the wild-type protein. "Our results suggest that Cdc12 could be regulated by auto-inhibition," Wu says.

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