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**Cytokinesis: an anillin-RhoGEF module sets the stage for septin double ring
assembly**

Simonetta Piatti

CRBM, University of Montpellier, CNRS

1919 Route de Mende

34293 Montpellier (France)

Contact: simonetta.piatti@crbm.cnrs.fr

SUMMARY

In many eukaryotes septin filaments form an hourglass-like structure at the division site that re-arranges into a double ring at cytokinesis. A new study elucidates how an anillin-RhoGEF complex guides the assembly of the septin double ring in budding yeast.

Septins are major constituents of the cytokinetic apparatus in animal cells and fungi. Through their ability to polymerise into filaments and higher order assemblies at the plasma membrane, they contribute to cytokinesis by acting as protein scaffolds and/or membrane barriers (reviewed in [1]). Although their relevance for cytokinesis is variable in different organisms, septins often form an hourglass-like structure at the division site that is subsequently rearranged into a double ring at the end of mitosis. This process, also referred to as septin ring splitting, is crucial for constriction of the actomyosin ring (AMR) during cytokinesis in budding yeast [2]. Additionally, the septin double ring is thought to compartmentalise the cortex around the cleavage site, in order to concentrate cytokinetic factors at the division site in yeast and mammalian cells [3,4]. Despite its importance, how the striking reorganization of septin structures occurs during formation of the double ring is unknown. A new study by Chen et al. published in this issue of *Current Biology* provides exciting new insights into this fascinating process [5].

In budding yeast, the septin hourglass marks the cleavage site throughout most of the cell cycle at the bud neck, the constriction between mother cell and bud. Thanks to fluorescence anisotropy measurements, electron tomography and platinum replica electron microscopy, we now know that the mature septin hourglass consists of straight pairs of septin filaments running along the mother-bud axis that co-exist with orthogonal circumferential single filaments coiling along the inner side of the plasma membrane [6–9] (Fig. 1). The paired axial filaments have consistent length (300-400 nm), lie distantly from the membrane with respect to the circumferential filaments and intersect them at regular spacing. On top of this gauze-like structure, facing the cytoplasm, thick filaments, presumably made by myosin II, sit circumferentially [8]

(Fig. 1). Interestingly, the circumferential septin and myosin II filaments occupy different regions of the axial filaments, being located at the outermost and innermost sides of the mature hourglass, respectively. This suggests that septin filaments can acquire asymmetric features through differential binding of partners or uneven post-translational modifications along their length, despite being inherently apolar.

The conversion from septin hourglass to double ring involves the depolymerization of the axial paired filaments through an unknown mechanism, followed by their reassembly at a 90° angle to wrap around the bud neck cortex along with the pre-existing circumferential filaments on the two sides of the bud neck [6,9,10] (Fig. 1).

Disassembly of the axial septin filaments might also trigger myosin II re-organisation into the contractile AMR and its ability to contact the plasma membrane.

What drives this amazing rearrangement?

Anillins are multifunctional proteins that localize at the cytokinetic furrow in mitosis, where they interact with different cytoskeletal components and cytokinetic factors, including septins (reviewed in [11]). Anillin promotes septin assembly along actin bundles *in vitro* [12] and organises septins into a ring in fission yeast [13,14].

Furthermore, the budding yeast anillin Bud4 is required to stabilise the double septin ring at cytokinesis [10,15]. Thus, anillin is an excellent candidate to prompt the rearrangement of the septin hourglass into a double ring at cytokinesis.

Inspired by these premises, Chen et al. investigated the role of the Bud4 anillin and its partner Bud3 in this process [5]. Bud3 and Bud4 are known to associate in a complex [15] and to be involved in the establishment of the axial budding pattern in haploid cells, by depositing a spatial landmark during cytokinesis that determines the

polarity axis in the following cell cycle and leads to formation of a new bud adjacent to the division site [16]. In mitosis the Bud3-Bud4 complex localises as two apparent rings overlapping the outer regions of the septin hourglass and remains associated to the septin double ring after cytokinesis [5,15,17,18] (Fig. 1). Bud3 is a RhoGEF (guanine nucleotide exchange factor) that promotes cell polarisation through direct activation of the GTPase Cdc42 [19]. Interestingly, while many RhoGEFs also possess a PH (pleckstrin-homology) domain for protein- or lipid-binding, Bud3 has none, but its partner Bud4 does. Thus, the Bud3-Bud4 complex may constitute a functional module that assists septin remodelling and cell polarity.

By combining fluorescence microscopy and platinum replica electron microscopy, Chen et al. find, surprisingly, that Bud3 and Bud4, though part of the same complex, have distinct and complementary roles in stabilising the septin double ring [5]. In the absence of Bud3 circumferential septin filaments are completely missing in the “transitional” hourglass (i.e. just before septin splitting), while lack of Bud4 destabilises both axial and circumferential filaments preferentially at the mother side of the bud neck. Remarkably, a *bud3Δ bud4Δ* double mutant shows precocious and virtually complete destabilisation of the transitional hourglass, thus preventing the assembly of the septin double ring. Conversely, loss of Bud3 and Bud4 has no effect on AMR formation and constriction, consistent with recent data [2].

Altogether the data indicate that the Bud3-Bud4 complex contributes to the architecture of the mature septin hourglass and, additionally, provides a positional information to instruct the assembly of the septin double ring at cytokinesis. In agreement with this conclusion and consistent with previous data [15,17,18], Bud3

and Bud4 are found specifically at the outer zone of the transitional hourglass by immunogold-labelling [5].

What dictates this peculiar localisation pattern is unclear at the moment and is an important question to be addressed in the future. The curvature of circumferential septin filaments, specific post-translational modifications or their intersection with axial filament could all contribute to elicit the distinctive distribution of Bud3 and Bud4.

The exact molecular mechanism underlying the role of Bud3 and Bud4 in guiding the assembly of the septin double ring also remains to be established. This process does not rely on regulation of the Cdc42 GTPase, since it does not involve the GEF domain of Bud3 [5]. Given the ability of Bud3 and Bud4 to interact physically with septins and to induce formation of ectopic septin rings and spirals when overexpressed [15,20], it is tempting to speculate that these proteins directly induce specific architectures of septin filaments and/or modify their physico-chemical properties at the edges of the hourglass. Interestingly, the septin filaments in the double ring appear to be connected by bulging bridges that are lost specifically in *bud4Δ* cells, raising the possibility that Bud4 acts as a septin crosslinker [5].

Consistent with this hypothesis, the authors propose that the Bud3-Bud4 complex might stabilise the junctions between axial and circumferential filaments in the transitional hourglass, with Bud3 and Bud4 lying more contiguous to the circumferential and to the axial filaments, respectively.

Alternative and non-mutually exclusive scenarios can be envisioned. For instance, the Bud3-Bud4 complex could aid at linking septins to the plasma membrane by

virtue of phospholipid-binding domains (e.g. the PH domain of Bud4). If this were the case, a local change in membrane composition, triggered by a specific signal at cytokinesis, could drive the destabilisation of the axial septin filaments that are not physically anchored to the plasma membrane by Bud3 or Bud4. This hypothesis could also explain why the C-terminus of Bud4, which bears the adjacent anillin-homology (AH) and PH domains but not the septin-interacting region, is required for formation of septin double ring [5]. Whether and how Bud3 and Bud4 interact with the plasma membrane at the bud neck, thereby influencing septin assemblies, is an important question for future studies.

A related burning question is what triggers the disassembly of the axial filaments at cytokinesis and formation of the septin double ring, in yeast as well as in other organisms. The notion that a Hippo-like pathway in budding yeast called Mitotic Exit Network, and in particular its effector phosphatase Cdc14, are involved in this process suggests that protein dephosphorylation might be crucial to bring about septin ring splitting at cytokinesis [2]. The identification of the critical Cdc14 substrates will shed further light into this remarkable process.

FIGURE LEGEND

The hourglass-to-double ring transition in budding yeast.

At the end of mitosis, the septin transitional hourglass (in blue) is made by paired axial filaments oriented along the mother-bud axis and circumferential filaments oriented perpendicularly and lying close to the plasma membrane. At this stage the Bud3-Bud4 (in yellow) marks the edges of the septin hourglass (note that the Bud3-Bud4 is depicted as a ring for simplicity but its structure is unknown). On top of the septin hourglass, facing the cytoplasm, lie thick filaments (in purple) presumably made by myosin II. At cytokinesis, the axial filaments are disassembled and re-assembled into additional circumferential filaments guided by Bud3 and Bud4, thus forming a double ring at each side of the bud neck. Contextually, myosin II joins the AMR, which gains access to the membrane and can constrict to drive cytokinesis.

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