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Structural biology of yeast septin complexes

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Septins are a family of proteins closely related to small GTPases discovered by Hartwell in *Saccharomyces cerevisiae*.as cell cycle elements for their ability to polymerize into long non-polar filaments at the mother bud neck. (1) In yeast, four septins are organized in octamers as [Cdc11-Cdc12-Cdc3-Cdc10-Cdc10-Cdc3-Cdc12-Cdc11]n to form filaments, with Cdc11 being able to be substituted by another terminal subunit, Shs1. (2) Structurally, each septin subunit has three domains: a guanine nucleotide-binding domain (G domain); a C-terminal domain that is usually predicted to form coiled-coils with other subunits (C domain); and a variable N-terminal, which may interact with membrane phospholipids, particularly through an α-helix dubbed α0 (N domain). (3) The subunits interact with two other septins by alternating interfaces, NC and G. For this project, DNA sequences of yeast septin subunits were cloned by Gibson assembly and expressed on E. coli Rosetta(DE3). Proteins were co-expressed with their G-domain partners and purified by affinity and size-exclusion chromatography, and by ion-exchange chromatography for GTP content assays. The oligomeric state was confirmed by SEC-MALS, and protein stability by circular dichroism (CD). Crystallization assays were carried out by the sitting drop vapor diffusion method, and the diffraction patterns of harvested crystals were collected on the Sirius Synchrotron (Campinas, Brazil). Experiments were first conducted using the constructs Shs1NG-Cdc12α0G, Cdc11NG-Cdc12α0G, and Cdc3α0G-Cdc10α0GC. The two first pairs were purified as dimers, but Cdc3α0G-Cdc10α0GC was obtained as a tetramer, through the formation of a homodimeric NC interface between subunits of Cdc10α0GC. CD data also confirmed that the tetramer was the most stable of the three complexes. All of these constructions were crystallized, but had poor diffraction spectra, probably due to the flexibility of the α0 helix when not involved in an interface. Concomitantly, a crystal structure of another construction of Cdc3G-Cdc10G was solved, which was organized as a dimer within the crystal. Therefore, in order to obtain data on the Cdc10-Cdc10 NC interface, a new construction of Cdc3G-Cdc10α0GC was expressed and purified, again as a tetramer in solution. This construction was crystallized and generated a structure at 2.66 Å resolution through X-ray diffraction. Unlike the first structure, Cdc3G-Cdc10α0GC was organized as two dimers in the crystal asymmetric unit, allowing the visualization of a physiological NC interface between two subunits of Cdc10. Analysis of this interface has allowed a better understanding of the α0 helix and its function in stabilizing septin complexes. Cdc3 and Cdc10 were also bound to GTP and GDP, respectively, as expected by their catalytic activities and the GTP content assays performed. Overall, this work has allowed the first look into the structure of a central NC interface for a septin complex and, consequently, a better understanding of how yeast septin structure correlates evolutionarily to human ones. This work has been supported by FAPESP and CAPES. The author is funded by grant **2022/00125-7, São Paulo Research Foundation (FAPESP).**

Palavras-chave: Septin. Crystal structure. Protein-protein interface.

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Referências:

1 HARTWELL, L. H. Genetic control of the cell division cycle in yeast. **Journal of Molecular Biology**, v. 59, n. 1, p. 183-194, July 1971. DOI: 10.1016/0022-2836(71)90420-7.

2 BERTIN, A. *et al.* Saccharomyces cerevisiae septins: supramolecular organization of heterooligomers and the mechanism of filament assembly. **Proceedings of the National Academy of Sciences**, v. 105, n. 24, p. 8274-8279, June 2008. DOI: 10.1073/pnas.0803330105.

3 FIELD, C. M. *et al.* Septins: cytoskeletal polymers or signalling gtpases?. **Trends in Cell Biology**, v. 9, n. 10, p. 387-394, Oct. 1999. DOI: 10.1016/s0962-8924(99)01632-3.