

Polybasic PPII Helix-Based NC Interface and Divergent G-domain Features Support an Alternative Septin Filament Architecture in the Single Septin of *Chlamydomonas reinhardtii*

Diego A. Leonardo¹, Bryan Marquez¹, Eloy Condori¹, Andressa P. A. Pinto¹, Richard C. Garratt¹, Ana Paula U. Araujo¹

¹Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos – São Paulo, Brazil

dleonardo@usp.br

Septins are a family of GTP-binding proteins involved in essential cellular processes such as cytoskeletal organization, membrane compartmentalization, and cell division¹. In most eukaryotes, septins polymerize into heteromeric complexes and filaments through conserved G and NC interfaces. However, *Chlamydomonas reinhardtii*, a model green alga, encodes only a single septin (CrSEPT), offering a unique opportunity to investigate how filament formation and function are achieved in the absence of heteromeric partners². This simplicity, combined with its phylogenetic position, makes *Chlamydomonas* a valuable system for studying the structural and functional evolution of septins. Previous structural studies of the CrSEPT G-domain revealed an unusually high GTPase activity and a lack of filament formation in crystal, despite filament-like structures observed in solution². These findings suggest that CrSEPT may follow a noncanonical assembly. This study aims to investigate the structural and functional mechanisms that enable CrSEPT to form filaments in the absence of canonical septin partners. Specifically, we sought to explore: (1) how the inclusion of the N-terminal polybasic region (PB1) and hook-loop-like motif influences the oligomeric behavior and filament formation; (2) how mutations at the conserved arginine finger affect nucleotide binding, hydrolysis, and oligomerization; and (3) what structural features, including potential NC interface adaptations, distinguish CrSEPT from septins in higher eukaryotes. We expressed and purified recombinant CrSEPT construct comprising the G-domain and an extended N-terminal regions (CrSEPT₇₁₋₄₀₁) and two Arginine Finger mutants (R239→H and R239→A). Oligomeric states in solution were analyzed under different nucleotide conditions using SEC-MALS. Crystal structures of wild-type and mutant CrSEPT₇₁₋₄₀₁ in complex with GDP or GppNHp were solved by X-ray diffraction and analyzed for interface formation, nucleotide binding, and structural features. CrSEPT₇₁₋₄₀₁ was purified in the apo form and exhibited altered oligomeric states upon incubation with GDP or GTP, particularly in the R239H and R239A mutants, suggesting differences in nucleotide interaction and intersubunit interface formation. The wild-type protein, crystallized with GppNHp (without Mg²⁺), showed a monomer in the ASU with a disordered switch II, a distorted G-interface, and no NC contacts. Unusually, the γ -phosphate occupied the canonical Mg²⁺ site, correlating with an inactive conformation and disordered arginine finger. In contrast, the R239H mutant crystallized with GDP and Mg²⁺ formed filaments in the crystal lattice, showing both G- and NC-interfaces. The NC interface features a polyproline type II (PPII) helix replacing the canonical α -helix, likely enabled by extended β -strands (β 1– β 3) and a unique polyacidic motif in *Chlamydomonas*. The polybasic residues of PB1 in the PPII interact with this motif, establishing a distinct intersubunit contact network. The arginine finger in the wild-type is disordered in absence of Mg²⁺, but becomes ordered in previous structures with GTP analogs, while the His mutant does not engage the nucleotide due to its shorter side chain. A low-resolution structure of the R239H mutant with GppNHp and Mg²⁺ revealed the same crystal packing as the wild-type, reinforcing the idea that nucleotide identity and coordination state critically influence filament formation. Our findings support a model in which filament assembly in CrSEPT is tightly modulated by GTPase activity, the presence of Mg²⁺, and the conformation of the N-terminal domain. The discovery of a PPII helix at the NC interface, in conjunction with species-specific sequence features (a new polyacidic motif), reveals an alternative filament architecture in this single septin system. These insights not only uncover unique structural strategies for self-assembly in CrSEPT but also provide a framework to explore the evolutionary divergence of septins in organisms lacking canonical multimeric complexes.

[1] Cavini, I. A., Leonardo, D. A., Rosa, H. V.D., Castro, D. K. S. V., D’Muniz Pereira, H., Valadares, N. F., Araujo, A. P. U. & Garratt, R. C. (2021). *Front Cell Dev Biol* 9.

[2] Pinto, A. P. A., Pereira, H. M., Zeraik, A. E., Ciol, H., Ferreira, F. M., Brandão-Neto, J., DeMarco, R., Navarro, M. V. A. S., Risi, C., Galkin, V. E., Garratt, R. C. & Araujo, A. P. U. (2017). *Journal of Biological Chemistry* 292, 10899–10911.

This research was funded by grant 2021/08158-9 and 2020/02897-1 from FAPESP.