Septin complexes assemble end-over-end in cells

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Abstract

The septins are an essential family of filament-forming GTP-binding proteins with conserved functions in cell division. Yeast septins form octameric, nonpolar, rod-shaped complexes of about 32 nm length and assemble further into higher-order structures that perform a variety of functions in the cell cycle. While *in vitro* the assembly of complexes into filaments is quite well understood, how the complexes assemble into the higher-order structures in cells remains unclear.

Here, we used single molecule localization microscopy to visualize both termini of septin rods at nanometer resolution *in vitro* and in cells. Single septin complexes appeared as pairs of localizations around 30 - 35 nm apart and revealed the exact spatial orientation of the complex in space. Under *in vitro* conditions favorable to septin polymerization, we detected septin assemblies as very thin, elongated stretches of equidistant localizations both when Cdc11, the terminal subunit of the rod, and when Cdc10, the central subunit of the rod, was labeled and detected. These filaments were mostly straight and occasionally appeared bundled. In a filamentous fungus, we resolved similar localization pairs and thin filaments of equidistant localizations. Our work demonstrates that septin complexes assemble end-over-end into filaments in cells and that if paired, filaments are aligned in register.