**3D reconstruction of mammalian septin filaments**

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**Introduction**

Septins are a conserved family of GTP-binding proteins implicated in diverse processes, including cytokinesis, protein scaffolding, and vesicle trafficking. It has been hypothesized that septin exists as a cytoskeletal polymer. Here we present the 3D density map of septin filaments determined using single-particle analysis of images obtained by negative stain electron microscopy.

**Methods**

A mixture of septin isoforms 3, 5, 7a and 7b were isolated from rat brain. The preparations were examined by negative stain electron microscopy and the images digitized and imported into the single particle image processing programs Spider and Eman.

**Results**

SDS-PAGE of the septin preparations showed four main bands with molecular weights 40-50 kDa. Identification of these bands as septins was by western blotting and N-terminal sequencing. Gel filtration chromatography indicated the size of the complex was ~240 kDa, consistent with the presence of two copies each of septins 3,5 and 7. Negative stain electron microscopy showed rod-like filaments with a variable length of 24-32 nm and width 7-8 nm. 3500 particle images were windowed out from digitized micrographs. Reference-free alignment and classification indicated a main group of filaments 27 nm long, together with a smaller group 32 nm long. The image averages showed considerably more detail than the raw data, where the filaments were featureless (Fig 1).

3D reconstruction of 2115 shorter filaments was carried out using a row of spheres or continuous helical density as starting models. After approximately 40 iterations of refinement, both models of converged to very similar reconstructions. The reconstruction revealed the apparent presence of three subunits, each separated by a transverse cleft; these subunits were similar but not identical, possibly indicating multiple septin isoforms within each filament. In some views a smaller cleft appeared to separate the subunits into two smaller regions, perhaps reflecting the presence of septin dimmers (Fig 2). Subsequent iterations (40–60) yielded no changes in the structure, cross-correlation between raw images and the projections of reference volumes, or resolution of the reconstruction. A comparison of 3D volumes obtained using the different starting models revealed no major differences at
the final resolution of 27 Å. This structure was highly reproducible when the entire algorithm was repeated on multiple occasions. This is the first 3D reconstruction of the native septin assembly and appears compatible with the hypothesis that the septin complex is a hexamer consisting of dimers or heterotrimeres.

**Publications**

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