

Elucidating the pathway of plant FtsZ assembly

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Chloroplast division is an essential process that involves proteins that are conserved from prokaryotic fission and proteins evolved in eukaryotes. The FtsZ (Filamentous temperature sensitive Z) proteins are the first to localize to mid-plastid/cell prior to a division event and form a contractile ring. Constant remodeling of the contractile ring occurs by subunit exchange with a monomeric or oligomeric pool [1]. Importantly, bacteria and archaea possess one FtsZ protein species while *Arabidopsis* chloroplasts contain two (FtsZ1 and FtsZ2) [2, 3]. Interaction of FtsZ and other chloroplast division proteins (e.g. ARC3, ARC6) have been demonstrated and may play a role in the contractile ring assembly or disassembly [4].

Assembly reactions in the presence of GTP have shown considerable heterogeneity with regards to particle types observed (Fig.1) [5]. The most common particle population observed in FtsZ1 assembly reactions was selected for single particle analysis. This population most likely represents an assembly intermediate and not the initial precursor. Particles were adsorbed to carbon-coated grids and electron micrographs recorded in a JEOL 1200X electron microscope. Images were processed with CRISP to evaluate initial image quality [6]. Suitable micrographs (absence of drift, astigmatism etc.) were further processed within the framework of the EMAN software package [7]. Images of selected particles ($n > 500$) were bandpass-filtered and aligned by reference-free alignment [8]. Classes of particles representing identical orientations were determined by multivariate statistical analysis and hierarchical ascendant classification using complete linkage. Resolution was assessed by using the standard Fourier shell correlation (FSC) criterion (Fig. 2) [9,10]. The angular refinement process was stopped when no further increase of resolution was observed. The 3-D reconstruction was visualized using the Chimera software package [11]. Surface rendering was carried out at different threshold levels to assess the significance of the observed structure. The final reconstruction has C4 symmetry and dimensions of 12x12x12 nm with an approximately 5-nm protein deficit at the base of the structure (Fig. 3). Four protomers are connected at the base from which they extend 6 nm. At the distal end opposite the base, the protomers are well separated and measure ~4 nm across.

To further these studies, FtsZ1 was purified and subjected to size-exclusion gel chromatography using a Superdex S-200 column demonstrating that both proteins are homogeneous as they are eluted in a single peak and occur as a homodimer in solution (Fig. 4). Samples were adsorbed to carbon-coated grids and the previously described filament types were observed in the presence of GTP as expected. Filament assembly was not promoted in samples lacking GTP and these particles were selected for single particle analysis. The 3-D reconstruction for these unassembled particles is currently underway and data is currently being collected for FtsZ2-1 preparations with the view to examine whether FtsZ2 assembly intermediates differ from the FtsZ1 reconstruction presented here.

References:

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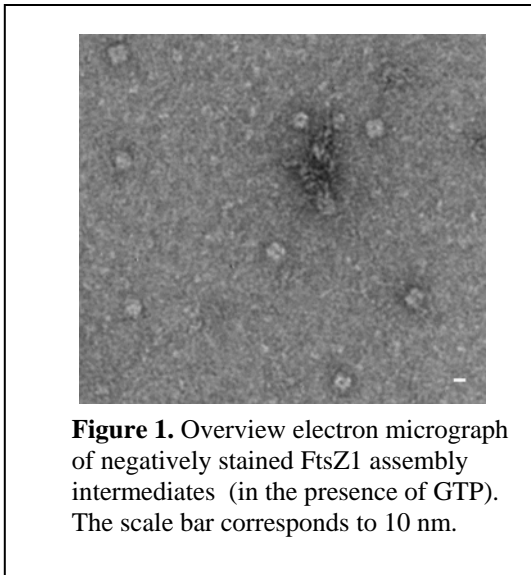


Figure 1. Overview electron micrograph of negatively stained FtsZ1 assembly intermediates (in the presence of GTP). The scale bar corresponds to 10 nm.

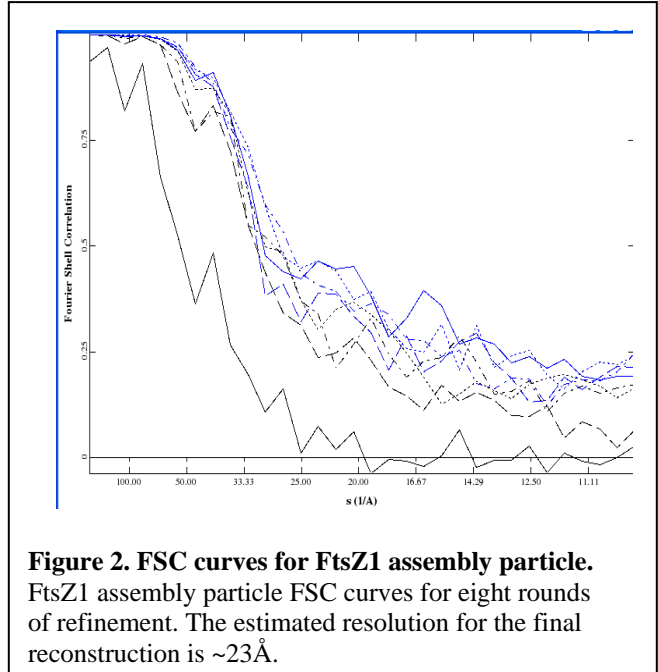


Figure 2. FSC curves for FtsZ1 assembly particle. FtsZ1 assembly particle FSC curves for eight rounds of refinement. The estimated resolution for the final reconstruction is $\sim 23\text{\AA}$.

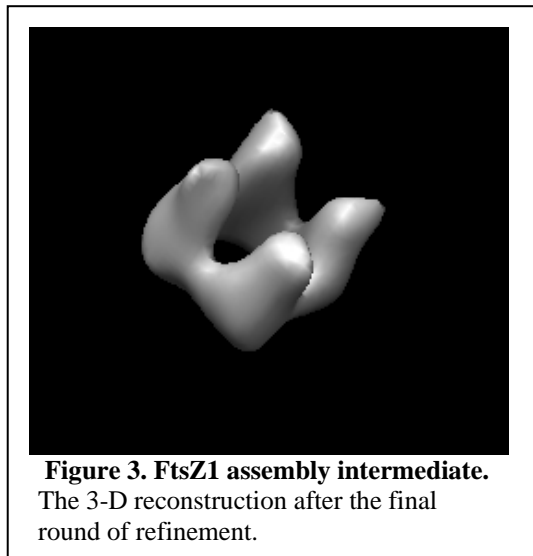


Figure 3. FtsZ1 assembly intermediate. The 3-D reconstruction after the final round of refinement.

