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# *In Situ* FtsZ Mini-Ring Structure revealed by TEM Tomography and STEM

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Both bacteria and chloroplasts divide by the process of binary fission that is initiated by assembly of a cytoskeletal protein FtsZ into a ring structure (Z-ring) at the division site. FtsZ assembly and Z-ring structure depend on the balance between stabilizing and destabilizing agents [1]. In chloroplasts, the absence of stabilizing agents causes the formation of additional types of assemblies, termed mini-rings [2,3]. While *in vitro* studies of these mini-rings revealed a diameter of ~200 nm and proposed that they could represent an energy-minimized state of FtsZ [4], their *in situ* structure remained elusive. Here we describe the structure of FtsZ mini-rings in *Arabidopsis thaliana* leaf chloroplasts using TEM tomography and STEM after high pressure freezing and freeze substitution (HPF-FS).

*Arabidopsis* plants expressing FtsZ-YFP in wild-type background were used in the experiments. For fluorescence imaging, leaf tissue was fixed with 3% formaldehyde in phosphate-buffered saline (PBS) and gradually infiltrated with 80% v/v glycerol in PBS mixture. Structured illumination microscopy (SIM) was then performed on an OMX microscope (GE Healthcare, Issaquah, WA) using a 100×/1.4 oil immersion objective, voxel size 0.04×0.04×0.12 µm. HPF of 2-mm leaf disks immersed in 1-hexadecene was performed using a Wohlwend Compact 01 (Technotrade, Manchester, NH). The FS solution used on the HFP samples was 4% (w/v) uranyl acetate (UA) dissolved in acetone. The FS procedure was a modification of the protocol by McDonald and Webb [5] raising the temperature over the course of 5 h from - 169 °C to 23 °C. Infiltration with LR White/acetone was performed in 10% steps with 3 exchanges in 100% resin under rotation at room temperature. Polymerization was for 24 h at 60 °C. Thin sections (80 nm-100 nm) were picked up on uncoated nickel grids and viewed in a FEI Tecnai™ G2 F20 equipped with a Fischione HAADF detector and operated at 200 kV. Tomography data at 67k × (0.32 nm/pixel) were acquired at 1° increments between +73°/-75° tilt using a Fischione 2020 holder in conjunction with the FEI programs Xplore3D™ and Inspect 3D™ for acquisition and processing, respectively. Reconstruction, segmentation and rendering were performed in EM3D [6,7]. Segmented protofilaments were colored and rendered with about 40% saturation of isosurface value. Image alignment was aided by the use of fiducial markers.

Whole-leaf SIM imaging revealed mini-rings ranging in diameter from 90 to 500 nm with a mean of 206 ± 68 nm ($n$=250) (Fig. 1A). Using semi-thin (300 nm) sections, fluorescence microscopy confirmed the presence of FtsZ-YFP in these rings. TEM imaging of thin sections from the same block showed both mini-rings and linear structures. For mini-rings, the overall diameter was 183 ± 50 nm ($n$= 21) which was in excellent agreement with diameter determined by SIM imaging and control samples from *Arabidopsis* plants lacking FtsZ did not show any circular or linear assemblies. As judged by TEM bright-field and STEM dark-field images, the rings appeared to be made up of multiple non-contiguous strands and bundles. To gain a better understanding of FtsZ structure within the mini-rings, TEM tomography was employed. Following alignment and manual segmentation, individual filaments comprising the mini-ring could be discerned and traced (Fig. 1B). Comparing the left-hand side (LHS)

with the right-hand side (RHS) of the ring, different degrees of order were observed (Fig. 1C). The RHS shows a higher degree of unraveling and entanglement, while the LHS consists of parallel layers of protofilaments. The curvature on the LHS of the filament was on average three-fold higher than that on the RHS. Individual protofilaments were also observed to form higher order structures, such as helices and superhelices (Fig. 1C). In conclusion, two states appear to be amalgamated in this *in situ* structure: a closed, stable state which maintains the LHS of the ring and a perhaps more dynamic (open) state on the RHS laying the foundations for the proposed spooling mechanism [3].

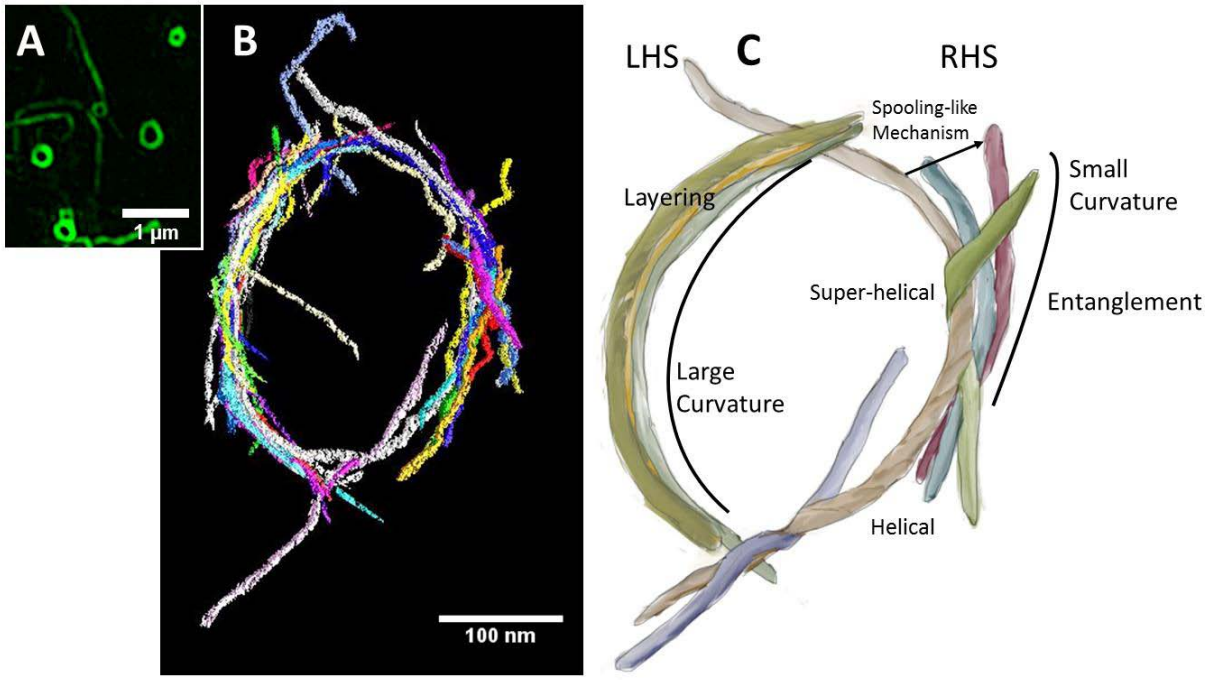


**Figure 1.** FtsZ mini-ring structure. A) SIM imaging of intact leaf tissue. B) Rendered 3-D TEM tomography reconstruction from a thin resin section. C) Cartoon of the reconstruction highlighting structural features of the mini-ring. LHS = Left-hand side; RHS = Right-hand side.

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