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# Localization and Molecular Stoichiometry of Plastid Division Proteins FtsZ1 and FtsZ2

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Chloroplasts are power-generators and factories that produce, store, and export many biochemical processes vital for the function of the plant cell. Because chloroplasts and all plastids arise by division of existing plastids, their replication is essential for maintaining their numbers in dividing cells and for increasing their numbers to achieve maximum photosynthetic capacity. The central role in cell division of bacteria as well as in chloroplast division is played by the tubulin-like cytoskeletal protein FtsZ. FtsZ forms a dynamic ring structure at the division site providing support for multiprotein complexes that orchestrate bacterial cell or chloroplast division. While one *FtsZ* gene is sufficient for cell division in most prokaryotes, two distinct gene families, *FtsZ1* and *FtsZ2*, participate in plastid division in higher plants. Both are colocalized to a ring at the division site and physically interact with each other [1, 2]. The strict conservation of both protein families throughout the plant kingdom suggests that they both play essential and are functionally distinct roles[3]. Here, we evaluate their developmental expression, molecular levels, and stoichiometric relationships in wild type and transgenic *Arabidopsis* mutants.

*Arabidopsis thaliana* has three FtsZ genes and proteins: one of the FtsZ1 family (AtFtsZ1-1) and two FtsZ2 proteins (AtFtsZ2-1, AtFtsZ2-2). We identified knockout mutants for each of the three genes and also analyzed plant in which *AtFtsZ1-1* or *AtFtsZ2-1* expression was suppressed by antisense constructs. Compared to the wild type leaf mesophyll cells with approximately 100 chloroplasts, the *AtFtsZ1-1* and *AtFtsZ2-1* antisense and knockout mutants contain only 1 to 2 grossly enlarged chloroplasts. In contrast, the phenotype observed in mesophyll cells of *AtFtsZ2-2* knockout plants is much less severe, resulting in chloroplasts of intermediate number and size. These results suggest that AtFtsZ1-1 and AtFtsZ2-1 are equally essential for plastid division, while AtFtsZ2-2 plays a lesser role.

Immunolocalization of FtsZ was performed on tissue sections, using specific FtsZ antibodies and Alexa Fluor 488 (Invitrogen) antibody conjugates. Specimens were viewed with a Leica DMRA2 fluorecence microscope, 100x oil immersion objective, FITC (Ex. 460-500 nm, Em. 512-545 nm) and Cy3 filter sets (Ex. 510-560, Em. 572-647 nm), and a cooled CCD camera Qimaging Retiga 1350 Exi. All three FtsZ proteins were detected in ring structures in both expanded leaves and meristematic tissues of 5-week-old wild-type plants, indicating that all three proteins are coordinately expressed throughout plant cell development and are components of the FtsZ ring. Analysis of *AtFtsZ1-1* and *AtFtsZ2-1* knockout mutants showed that the respective FtsZ protein was not present and the localization of the other FtsZ species was severely perturbed. The formation of FtsZ rings was prevented in these mutants. In contrast, AtFtsZ1-1 and AtFtsZ2-1 assembled into functional rings at the chloroplast division site in AtFtsZ2-2 knockout plants.

Quantitative immunoblotting combined with confocal microscopy measurements of chloroplast volumes were used to assess the number of FtsZ molecules per chloroplast. In 3-wk-old plants, the average total molecules of FtsZ per chloroplast (± S.D.) was 101,200 (± 6000), with a molar distribution of approximately 33% AtFtsZ1-1, 47% AtFtsZ2-1, and 20% AtFtsZ2-2. Although the total amount of FtsZ was 10-fold lower in 7-wk-old plants compared to 3-wk-old plants, both the FtsZ1-to-FtsZ2 molar ratio (1:2), as well as that of AtFtsZ1-1-to-AtFtsZ2-1-to-AtFtsZ2-2, remained constant throughout leaf development. We propose that maintenance of FtsZ1 to FtsZ2 molar ratio is required for proper FtsZ ring formation and plastid division.

References

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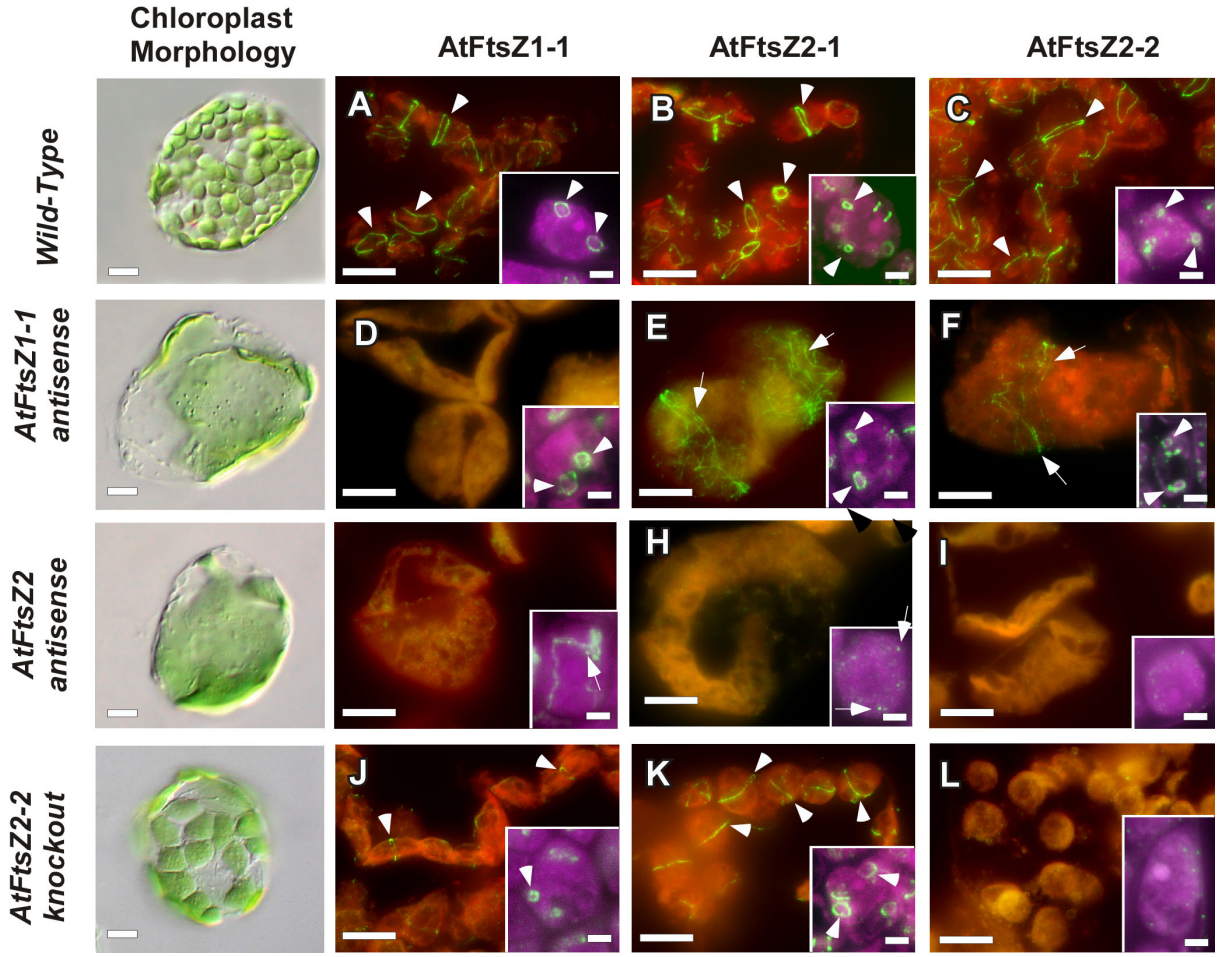


Figure 1: Leaf mesophyll chloroplast phenotypes and immunofluorescence localization of FtsZ proteins in wild type and mutant plants. Immunolabeling was performed on sections from expanding leaves and from very young apical tissue (insets). The FtsZ immunofluorescence signal is shown in green and indicated by arrowheads. The *AtFtsZ1-1* and *AtFtsZ 2-1* knockout mutants showed FtsZ localization identical to that in the respective antisense plants. Bars = 10 µm and 2 µm (insets).

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