

## ***Arabidopsis* Plastid Division Proteins FtsZ1 and FtsZ2: Macromolecular Assembly and Subunit Exchange Dynamics**

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The bacterial cell division protein FtsZ (Filamentous temperature sensitive Z) plays an essential role in prokaryotic division locating mid-cell and forming a contractile ring. Unlike bacteria, chloroplasts harbor two distinct families of FtsZ (1 and 2) that are essential, non-redundant and localize to a mid-plastid ring [1,2]. The division components in chloroplasts are a mixture of proteins displaying homology to bacterial counterparts and novel proteins that consequently demand a strikingly different division mechanism as compared to bacteria. Understanding the mechanism of chloroplast division may have commercial applications that could improve wet-milling efficiency and produce a domestic savings of \$280 million annually [3].

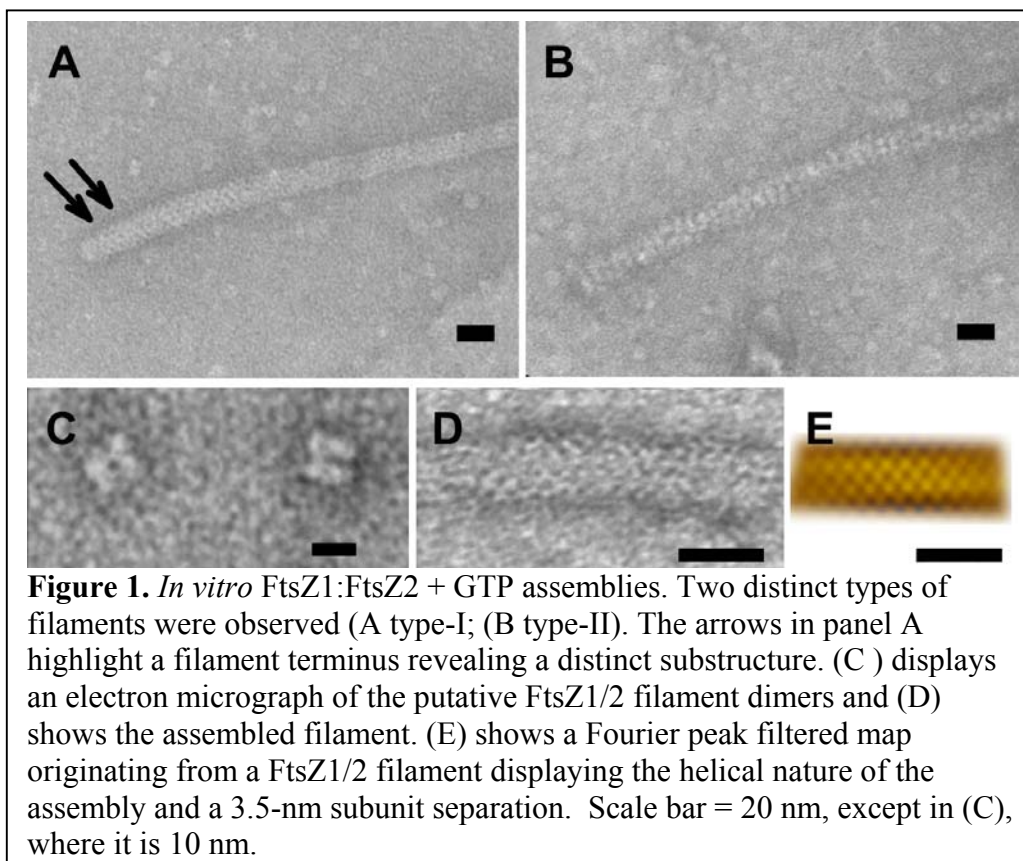
The nature of the macromolecular mechanism and assembly of the mid-plastid ring remains an unresolved question. This report characterizes *in vitro* FtsZ assemblies and addresses FtsZ turnover rates in an *in vivo* model system. A commercially available expression system was recently optimized to yield adequate amounts of active FtsZ1 and FtsZ2 for *in vitro* studies [4]. FtsZ-GFP proteins from *Arabidopsis* were used to generate the *in vivo* data.

*In vitro* assembly reactions revealed two types of filaments termed type-I and type-II (Fig. 1A,B) and unincorporated precursor molecules (Fig. 1C) during routine negative staining with 2% uranyl acetate or 2% phosphotungstic acid [4]. In support of the added complexity of chloroplast FtsZ division mechanisms, the assembly properties differed from bacterial FtsZ [4,5]. Image analysis suggests that type-I filaments have a helical nature at the ends of filaments and a 3.5 nm subunit separation (Fig. 1D,E). Type-I filaments were found to be slightly thicker (140Å) in terms of diameter measurements as compared to type-II filaments (120Å). Volume measurements of incorporated molecules in type-II filaments show that the smallest subunits are monomers; it should be noted that these measurements are in agreement with an earlier X-ray crystallographic study of bacterial FtsZ [6]. These results provide a basis for a more rigorous structural analysis aimed at fully understanding the differences between the types of filaments.

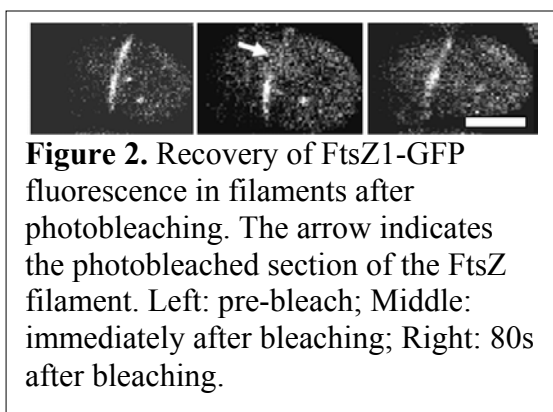
*In vivo* fluorescence recovery after photobleaching (FRAP) was used to investigate the subunit exchange dynamics in filaments in *Arabidopsis thaliana* chloroplasts tagged with GFP (Figs. 2,3). The half time to recovery ( $t_{1/2}$ ) value for FtsZ1-GFP was  $58.76 \pm 30.8$  sec. This value is higher than the  $t_{1/2}$  value of  $\sim 7$  sec reported for bacterial FtsZ-GFP in *E. coli* and *B. subtilis* [7]. The recovery time of FtsZ2-GFP is currently under investigation. This data may help elucidate the non-redundant functional roles of FtsZ1 and FtsZ2.

## References:

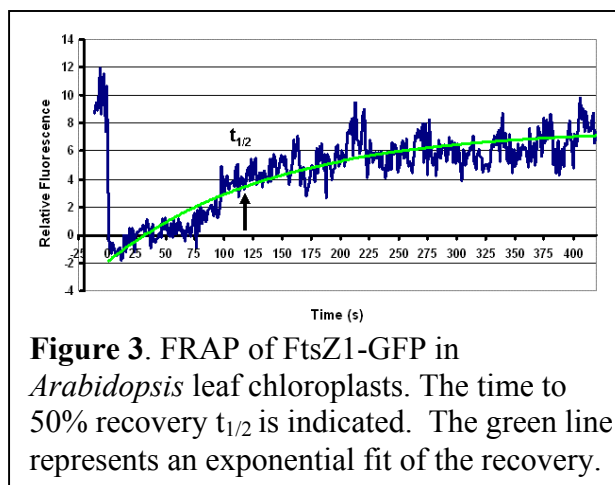
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**Figure 1.** *In vitro* FtsZ1:FtsZ2 + GTP assemblies. Two distinct types of filaments were observed (A type-I; B type-II). The arrows in panel A highlight a filament terminus revealing a distinct substructure. (C) displays an electron micrograph of the putative FtsZ1/2 filament dimers and (D) shows the assembled filament. (E) shows a Fourier peak filtered map originating from a FtsZ1/2 filament displaying the helical nature of the assembly and a 3.5-nm subunit separation. Scale bar = 20 nm, except in (C), where it is 10 nm.



**Figure 2.** Recovery of FtsZ1-GFP fluorescence in filaments after photobleaching. The arrow indicates the photobleached section of the FtsZ filament. Left: pre-bleach; Middle: immediately after bleaching; Right: 80s after bleaching.



**Figure 3.** FRAP of FtsZ1-GFP in *Arabidopsis* leaf chloroplasts. The time to 50% recovery  $t_{1/2}$  is indicated. The green line represents an exponential fit of the recovery.