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Localization of Two GFP-tagged Tobacco Plastid Division Protein NtFtsZs in Escherichia coli

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Abstract: Two plastid division genes, *NtFtsZ1* and *NtFtsZ2* isolated from *Nicotiana tabacum* L. were fused with *gfp* and expressed in *Escherichia coli*. The regular localizations of full length NtFtsZs:GFP along the filamentous bacteria indicated that the NtFtsZs could recognize the potential division sites in *E. coli* and be polymerized with heterogeneous FtsZ from bacteria. The overexpression of *NtFtsZs*:*gfp* inhibited the division of host strain cells and resulted in the long filamentous bacterial morphology. These results suggested that eukaryotic *ftsZs* have similar function to their prokaryotic homologs. Meanwhile, the different deletions of motifs of NtFtsZs are also employed to investigate the functions of these proteins in *E. coli*. The results showed that the C-terminal domains of NtFtsZs were related to the correct localization of NtFtsZs in *E. coli* and the N-terminal domains of NtFtsZs were responsible for the polymerization of homogeneous and heterogeneous FtsZ proteins. The significance of these results in understanding the functions of NtFtsZs in plastid division were discussed.
Key words: *Nicotiana tabacum*; plastid division gene; *NtFtsZ*; GFP; localization in *Escherichia coli*

FtsZ is a conservative and primitive cytoskeleton protein found from the *Escherichia coli* temperature sensitive mutant Z (filamentation temperature-sensitive Z, *ftsZ*). During the cell division cycle, FtsZ assembles into a ring structure at the division site before any other known cell division proteins, and the constriction of the ring structure results in cell division[1]. FtsZ is a GTP-binding protein with GTPase activity and also shares some considerable biochemical and structural similarities with eukaryotic cytoskeleton component tubulin, leading to the hypothesis that eukaryotic cytoskeloton might be evolved from prokaryotic cytoskeletal elements[1-3]. Compared with other known cell division proteins, FtsZ emerges in the early stage of cell division and plays an organizer to recruit the other cell division proteins forming a putative molecular apparatus dedicated to dividing the cell. Thus, FtsZ is the most important cell division protein in prokaryotic cell division cycle hereto known[1,2].

More recently, FtsZ also has been found in higher plant[4] and its role in the plastid division has been established preliminarily[5,6]. However, there are some different views about the functional patterns of plant FtsZ in control of plastids division and/or plastid shape maintaining. In a moss *Physcomitrella patens*, FtsZ not only influenced the plastid division[6], but also possibly consisted of a new subcellular structure-plastoskeleton[7]. In *Arabidopsis*, FtsZs from different families seemly form a ring-like structure located at two sides of membrane of plastid[5,8]; the ring structure is thought to be a reminiscence of prokaryotic FtsZ ring. However, recent studies on a unicellular eukaryotic red alga *Cyanidioschyzon merolae* suggested that the FtsZ may not be the component of the plastid-dividing rings, at least not be the component of outer ring[9]. All the studies make the functional patterns of plant FtsZs more confused.

Because of the importance and conservative function of FtsZs in prokaryotic cell division, it provides an efficient method to study heterogeneous FtsZ function in *E. coli*[10]. To further understand the difference between plant and prokaryotic FtsZs, here we report that two plastid division proteins NtFtsZ1 and NtFtsZ2, which are encoded by tobacco genomic genes and were tagged with green fluorescent protein (GFP), localized in living bacterial cells in a visible method. The polymerization, localization and effects of fusion proteins on bacterial morphology were worked on. Meanwhile, the effects of different deletions of NtFtsZ proteins on the polymerization and localization of fusion proteins in *E. coli* were also analysed.

1 Materials and Methods

1.1 Materials

The plasmids containing full-length cDNA of *NtFtsZ1* and *NtFtsZ2*, cloning vector pBluescript II KS (+) and *Escherichia coli* strain JM109 are kept in our laboratory. The GFPmut2 plasmid, which contained $gfp_{S65A,V68L,S72A}$ mutants, was a gift from Prof. LI Jiu-Di (Institute of Botany, the Chinese Academy of Sciences). PCR primers were from Shanghai Sangon. DNAs were sequenced by TaKaRa (Dalian, China). Anti-GFP polyclonal antiserum was purchased from CLONTECH.

1.2 Construction of expression vectors

The constructions of different expression vectors refer to Fig. 1. All the constructs were made by PCR. The relative positions of primers in different constructs are shown in Fig. 1. To ensure the fidelity of PCR amplification,

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Taq was replaced by *pfu* in the PCR reaction. The fusion junctions were confirmed by DNA sequencing. To facilitate cloning and expression, all the fusion genes were inserted between the *Bam*H Ⅰ and *Sac* Ⅰ site of pBluescript Ⅱ KS (+) and under the control of *lac* promoter.

1.3 General molecular manipulations were carried out according to *Molecular Cloning*[11].

1.4 Western blotting

The preparation, separation and electroblotting of total proteins were conducted by the standard procedures[11]. The correct expressions of fusion proteins were confirmed by immunoblot analysis with anti-GFP polyclonal antiserum (Fig. 2).

1.5 Microscopic techniques

In general, the transformed cells were cultured under the conditions as described[12] and modified slightly. Overnight growth of colonies on LB plate with ampicillin were resuspended in 5 μL LB and a equal volume of warm 0.5 % low melting temperature agarose, the mixture solution was immediately dropped on a glass slide and covered with a cover glass. Microscopic observation and photography were performed under Leica DMRE microscope. Images of bacterial morphology were obtained using differential interference contrast (Nomarski) optics. For GFP fluorescence microscopy, an FITC (excitation 455 - 495 nm, emission 512 - 575 nm) filter set was used and images were captured with a Leica DC200 Digital Camera. All the images were assembled for publication using Adobe Photoshop 5.0.

2 Results

2.1 Strategy of expression vector construction

All the known FtsZ proteins contain two different domains, the conservative N-terminal GTP binding domain and the divergent C-terminal variation region (Fig. 1). There are two special motifs of FtsZ protein existed in the N-terminal conservative region, i.e. FTSZ-1: VIGVGGGGSNAVNRM (PROSITE *PS01134)* and FTSZ-2: FATAGMGGGTGS/TGAAPV/IV/IA (PROSITE *PS01135)*. The FTSZ-2 also includes a typical tubulin signature motif GGGTGS/TG (PROSITE *PS00277)* and its function had been studied in prokaryotic FtsZ proteins, indicating that it is involved in the polymerization and the GTPase activity of FtsZ. The deletion of FTSZ-2 disrupted the normal polymerization of FtsZ and the formation of Z-ring, and then the normal cell division cycle[1,2]. Site-specific mutation examinations also confirmed that FTSZ-2 is important for GTPase activity of FtsZ protein[1,2,13]. On the other hand, the function of FTSZ-1 still kept unknown. The C-terminal domain of FtsZ protein had no obvious sequence similarity among different FtsZ proteins except for the extreme C-terminus[14]. Primary studies in prokaryotic cells shows that the function of the C-terminal region may be responsible for the interaction of FtsZ with other cell division protein[14]. Based on the previous studies, we constructed a series of expression vectors (Fig. 1) and studied the functions of NtFtsZs in *E. coli*.

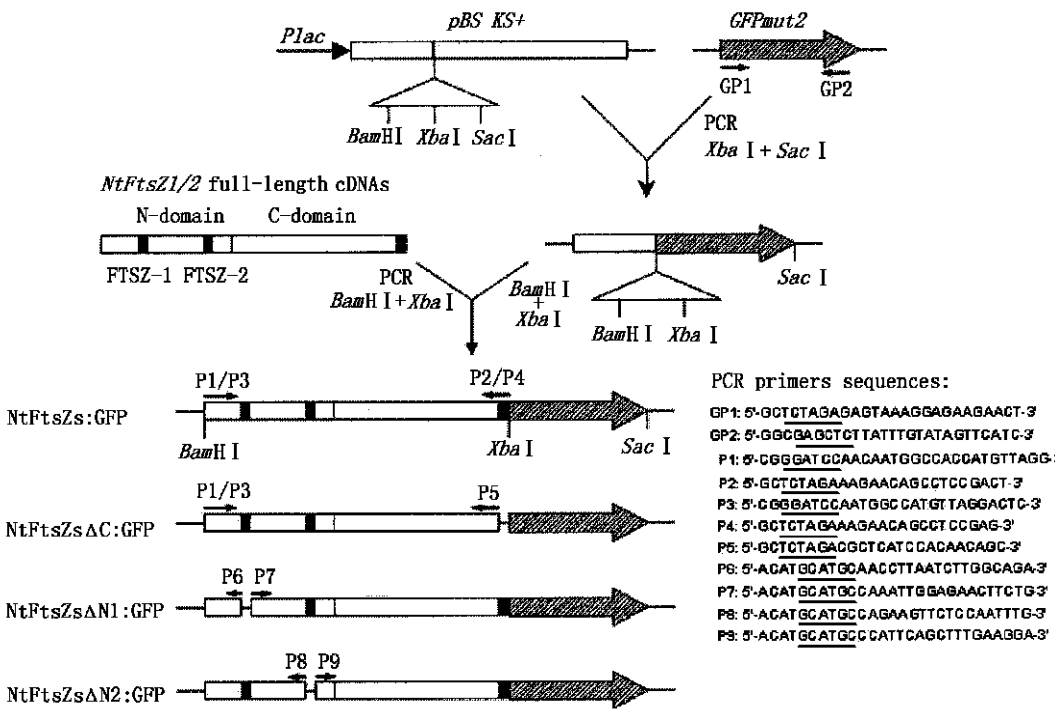

Fig. 1. Schematic representation of the construction strategy of *NtFtsZs : gfp* fusion expression vectors.
Pairs of PCR primers were specific for *NtFtsZ1* full-length cDNA (P1 and P2), *NtFtsZ2* full-length cDNA (P3 and P4), C-terminal deletion of NtFtsZs (P1 and P5 for *NtFtsZ1*, P3 and P5 for *NtFtsZ2*), N-terminal deletion of NtFtsZs (P6 and P7 for *NtFtsZsΔN1*, P8 and P9 for *NtFtsZsΔN2*), respectively. Purified plasmids containing *NtFtsZ1* or *NtFtsZ2* cDNA were used as the templates for PCR amplification.

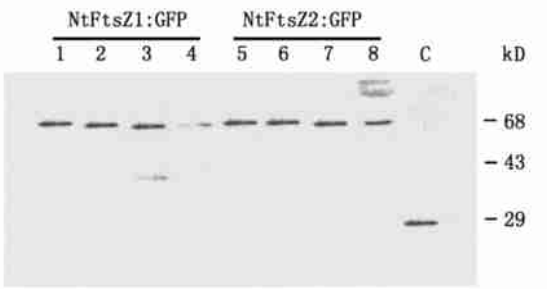

Fig. 2. Western blotting analyses of fusion proteins expression. Analyzed were NtFtsZs:GFP (lanes 1 and 5), NtFtsZsΔC:GFP (lanes 2 and 6), NtFtsZsΔN1:GFP (lanes 3 and 7), NtFtsZsΔN2:GFP (lanes 4 and 8) and GFP only (lane C), respectively. The calculated molecular weight of fusion protein was 73, 70, 69 kD for full-length *NtFtsZs*, C-terminal deletions and N-terminal deletions, respectively.

2.2 Localization of the full length NtFtsZs:gfp in E. coli

The full length *N. tabacum NtFtsZ1* and *NtFtsZ2* cDNAs lacking stop codons were fused in a frame with $gfp_{S65A,V68L,S72A}$ gene and expressed in *E. coli* strain JM109. The *E. coli* cells cultured on plates without IPTG had no obvious changes (Fig. 3A), and displayed a similar morphology with that of the control cells (Fig. 3B). When the cells were cultured on plates with IPTG, the morphology of the cells changed sharply, Fig. 3 C and D show two extreme phenotypes. Although the division of host bacteria were inhibited, the NtFtsZs:GFP fusion proteins still could be regularly distributed in the cells. This distribution pattern was similar to the localization of GFP tagged endogeneous FtsZ protein in *E. coli*[12], and indicated that NtFtsZs could recognize the potential cell division site in *E. coli* and interact with the bacterial cell division proteins. On the other hand, the overexpression of the fusion proteins in *E. coli* also blocked the host cell division and resulted in a filamentous phenotype of the cells, which is similar to the overproduction of endogenous FtsZ in *E. coli*[12,15]. These results suggested that NtFtsZs had the functions similar to those of bacterial.

In *E. coli*, FtsZ could form an obvious ring structure at the division site[12], but in our experiments we could not find any ring or band structures around the bacteria. The fusion protein was distributed mainly on the side of cells (Fig. 3E, arrowheads). Thus, it is undefined whether NtFtsZs are involved in the formation of prokaryotic division ring. Although the overexpression of NtFtsZs blocked host cell division, no any ring structure was observed. It is suggtsted that the inhibition of cell division possibly came from the right cell division site which might be occupied by the polymerization of NtFtsZs with endogeneous FtsZ, and finally, the formation of division ring could be interrupted. In addition to some regular dots polymerized along the filamentous cells, it is noteworthy that fusion proteins also could form some special structures (Fig. 3, F, G, arrowheads). As above mentioned, FtsZ is a GTPase and shares some similar structural and biochemical characters with tubulin, we speculate these special structures similar to the tubulin-like polymers formed by polymerization of prokaryotic FtsZs *in vitro* or *in vivo*[12,16]. These structures should be, to our knowledge, the first demonstration of eukaryotic FtsZ polymerization *in vivo* in *E. coli*. However, whether these structures represented the polymerization patterns of NtFtsZs in plastids remained to be seen.

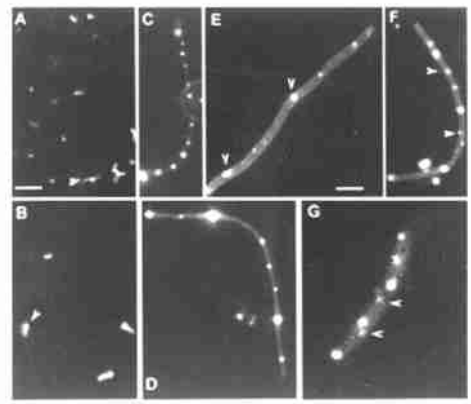

Fig. 3. Localizations of full-length NtFtsZs:GFP in *E. coli*. A. JM109/NtFtsZ1:GFP cells were from colonies on plates without IPTG. B. JM109/GFPmut cells were from colonies on plates without IPTG. C, D. The typical filamental cell with NtFtsZ1:GFP or NtFtsZ2:GFP vector, showing the regularly spaced dots, respectively. E. Filamentous cell showing the distribution of fusion protein. F, G. Filamentous cells showing the speculated spiral fluorescences. Bars: A and B, 5 μm; C, D, E, F and G, 2 μm.

2.3 Effects of C-terminal deleted NtFtsZs on the fusion protein localization in E. coli

There were two mainly localization patterns of NtFtsZsΔC:GFP in *E. coli*: 1) Fusion proteins was distributed at a special region, but the distribution space was not similar to that of full-length NtFtsZs:GFP fusion proteins which had obviously regular distribution (Fig. 4, B, C). 2) In addition to the fluorescence dots distributed along bacterial cells, C-terminal deletion fusion proteins also could be polymerized into some uncontinuous spiral fluorescence throughout the filamentous cells (Fig. 4, D, E, F, G). Compared with the localization of full-length NtFtsZs:GFP, the fusion proteins with C-terminal deletion lost the ability to be located correctly and distributed randomly in cells. Meanwhile, the fusion proteins also could be polymerized in cells, suggesting that the polymerization function of NtFtsZs could not be affected by C-terminal deletion. Based on the above observations, we speculated that the C-terminal domain of NtFtsZs might be involved in the selection of correct division site. In *E. coli*, the function of FtsZ C-terminus was responsible for the interaction of FtsZA with ZipA[14]. However, there is no FtsZA or ZipA homolog found in higher plants till now, thus the function of eukaryotic FtsZ C-terminal domain might be different from that of prokaryotic homolog. Furthermore, it also provides a possible explain to the localization observations of full-length NtFtsZs:GFP in which we could not find any ring structures, i.e., the C-terminal domains of NtFtsZs were incompetent for the formation of prokaryotic division ring. In addition, the spiral

fluorescence observed in C-terminal deletion experiments further showed that the eukaryotic FtsZ also could be polymerized into tubulin-like protofilaments as that of its prokaryotic counterpart. This may also provides the important clues for understanding the role of FtsZ in division of chloroplast and its morphology maintained.

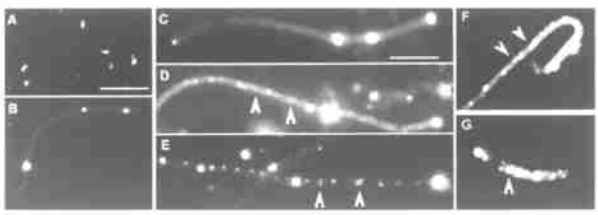

Fig. 4. Localizations of C-terminal deleted NtFtsZs:GFP in *E. coli*.
A. JM109/NtFtsZ2ΔC:GFP cells were from the colonies on plates without IPTG. B, C. Filamentous cells showing the localization pattern of NtFtsZ1ΔC:GFP or NtFtsZ2ΔC:GFP, respectively. D, E, F, G. Arrowheads showing the speculated uncontinuous, spiral structures in filamentous cells. Bars: A, B, F and G, 10 μm; C, D and E, 5 μm.

2.4 Localization of N-terminal deleted NtFtsZs:GFP in E. coli

The deletion of motif FTSZ-1 had no obvious effects on the polymerization of fusion proteins, their fluorescence dots still could be observed in cells (Fig. 5, A, B, C, D). This result indicted that the deletion of FTSZ-1 did not affect the polymerization of fusion proteins. It is worth to note that the NtFtsZsΔN1:GFP fusion proteins exhibited a similar localization pattern in cells as the NtFtsZsΔC:GFP did, but it remained to be seen if these observations meant the FTSZ-1 motif was also responsible for the function of selecting division site. The fusion proteins with deletion of motif FTSZ-2 did not display any specific localization patterns such as dots or spiral fluorescence. The green fluorescence could be seen throughout the whole cells (Fig. 5, E, F, G, H), which appears to be the case as that in control cells. Thus, the function of the FTSZ-2 motif of NtFtsZs is likely similar to their prokaryotic homologs, that is responsible for the polymerization of homogeneous/heterogeneous FtsZ proteins. The bright fluorescence dots at the poles of cells were thought as inclusion bodies produced by overexpression of exogeneous proteins[12], because it also could be found in control cells without NtFtsZs:GFP plasmids (Fig. 3, B, arrowhead). Thus, these fluorescence dots at poles might represent a non-specific aggregation of proteins in cells.

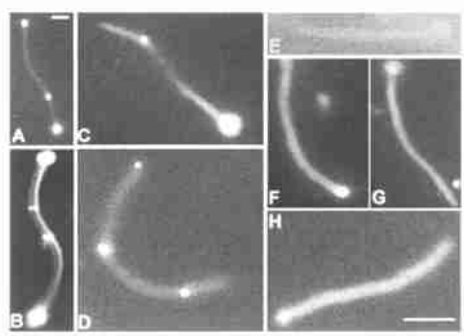

Fig. 5. Localizations of N-terminal deleted NtFtsZs:GFP in *E. coli*.
A, C. Localizations of NtFtsZ1ΔN1:GFP showing the irregularly distribution of fusion proteins. B, D. Localizations of NtFtsZ2ΔN1:GFP showing the irregularly distribution of fusion proteins. Distribution patterns of NtFtsZsΔN2:GFP, E and F for NtFtsZ1ΔN2:GFP, G and H for NtFtsZ2ΔN2:GFP. Bars: A and B, 5 μm; C, D, E, F, G, H, 5 μm.

3 Discussion

Plastids are a group of important organelles in plant cells and involved in the whole growth and development process of plant cells. Plastid division is an indispensible stage for development and differentiation of plant cells. Recent studies have revealed the role of eukaryotic FtsZ in plastid division process. However, what patterns of FtsZ play in controlling plastid division is still a matter of debate. In this paper, we first report that the *in vivo* localization and polymerization of eukaryotic FtsZ in *E. coli*, and provide a direct evidence to support the view that NtFtsZs also have the typical characters of FtsZ protein in prokaryote and can form some special structures by self-polymerization. It was given that there were no any ring structure formed by NtFtsZs observed in our experiments, whether they also act as a ring pattern in tobacco chloroplasts, still needs to be further studied. Moreover, all of these results will help us to further understand which pattern could represent the function of eukaryotic FtsZ, i.e. it is a simple succession of prokaryotic cell division mechinery (plastid division ring)[8] or a novel subcellular structure (plastoskeleton)[7], or both, in higher plants. In addition, these results not only provide the direct evidence to support the endosymbiosis hypothesis, but also establish a feasible foundation for further studies of *in vitro* polymerization and *in vivo* subcellular localization of eukaryotic FtsZ. Certainly, there are many obvious differences exist between chloroplasts and their prokaryotic evolutionary ancestors in morphology, structure of chloroplasts and their situated enviroments. The experiments that test the functions of NtFtsZs in plastid division and shape maintenance in tobacco plants are under way.

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烟草质体分裂蛋白 NtFtsZs 在大肠杆菌中的定位分析

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摘要: 分别构建了两个烟草(*Nicotiana tabacum* L.)质体分裂基因 *NtFtsZ1* 和 *NtFtsZ2* 与编码绿色荧光蛋白的 $gfp_{S65A、V68L、S72A}$ 基因相融合的原核表达载体,并导入大肠杆菌(*Escherichia coli*) JM109 菌株中进行表达。全长 NtFtsZs∶GFP 融合蛋白在菌体中有规律地定位,暗示 NtFtsZs 能识别大肠杆菌潜在的分裂位点,并能与大肠杆菌的内源 FtsZ 发生聚合作用;融合蛋白的诱导表达抑制了宿主菌的分裂,形成了明显的丝状菌体,证明真核生物的 *ftsZ* 基因与大肠杆菌的 *ftsZ* 基因有相似的作用。同时构建了 NtFtsZs 不同缺失的原核表达载体,对这两个基因所编码蛋白不同结构域的功能做了初步分析。实验结果表明,烟草 FtsZ 蛋白的 C 端结构域与其在大肠杆菌细胞中的正确定位有关;而 N 端结构域与 NtFtsZs∶GFP 融合蛋白的聚合有关。

关键词: 烟草; 质体分裂基因; *NtFtsZ*; 绿色荧光蛋白; 缺失表达; 原核定位
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