Cloning of two plastid division *ftsZ* genes from *Nicotiana tabacum* and their expression in *E. coli* *

WANG Dong[1,3**], KONG Dongdong[1,2**], HU Yong[1], JU Chuanli[1**], HE Yikun[1***] and SUN Jingsan[2***]

(1. Department of Biology, Capital Normal University, Beijing 100037, China; 2. Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China; 3. College of Life Sciences, Beijing Normal University, Beijing 100875, China)

Received May 20, 2002; revised June 27, 2002

Abstract Two cDNAs of plastid division gene *NtFtsZ1-1* and *NtFtsZ1-2* are isolated from *Nicotiana tabacum* by RT-PCR and rapid amplification cDNA ends (RACE) method. Analysis of the deduced amino acid sequences encoded by *NtFtsZ1-1* and *NtFtsZ1-2* indicate that these two proteins possess the typical conservative motifs and GTP binding sites existing in all FtsZ proteins. The existence of putative plastid transit peptide in their N-terminal suggests that there are at least two transit-peptide containing FtsZ proteins in higher plants. Phylogenetic analysis based on amino acid sequences of FtsZ proteins also supports this interference. These two *NtFtsZ* genes demonstrate a similar expression pattern during the plant development, detected by Northern blot. Expression of *NtFtsZ1-1* and *NtFtsZ1-2* in *E. coli* interrupts the normal division process of host cells. These results suggest the diverse functions of FtsZ proteins in higher plants.

Keywords: ***NtFtsZ* genes, plastid division, *Nicotiana tabacum*, plastid transit peptide, expression in *E. coli*.**

FtsZ is a conservative and primitive cytoskeleton protein found in nearly all prokaryotes, and it plays an important role in the prokaryotic cell division process. 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[1,2]. The limited but significant sequence similarity between FtsZ and eukaryotic cytoskeleton protein tubulin has been identified. The purified FtsZ is a GTP-binding protein with GTPase activity and undergoes GTP-dependent assembly into polymers that resembles the assembly of tubulin protofilaments under similar conditions *in vitro*[1,3~5]. Recent crystallographic data also confirmed that FtsZ and tubulins are indeed structural homologs of one another[6,7]. All of these results suggest a possibility that prokaryotic FtsZ shares a common ancestor with eukaryotic cytoskeletal protein tubulin[1,2,8]. Till now, FtsZ has been found in all studied free-living prokaryotes and an identical or similar cell division machinery is adopted by prokaryotic domain[2,9].

As a result of endosymbiosis, plastid is one of the primary features that distinguish plant cells from other eukaryotic cells. In addition to the photosynthesis carried out by chloroplasts, several major plant metabolism pathways, such as lipid biosynthesis and amino acid metabolism, occur also in plastids. Hence, it is obvious that plastid is one of the indispensable components in plant cells and plastid division is also an important part of plant cell development. However, the molecular controls underlying plastid division are largely unknown. In 1995, based on the studies of prokaryotic cell division, the homolog of prokaryotic cell division gene *ftsZ*, *AtFtsZ1-1*, was isolated firstly from higher plant *Arabidopsis thaliana*[10]. The function of *AtFtsZ1-1* in the division of plastids was also confirmed by the antisense expression of *AtFtsZ1-1* in *A. thaliana*[11]. These results not only provide direct evidence for the endosymbiosis hypothesis, but establish a basis to study the molecular mechanism of plastid division in higher plants.

Here we report the cloning and functional analysis of two plastid division genes *NtFtsZ1-1* and *NtFtsZ1-2*. The finding that the existence of a putative

* Supported by the National Natural Science Foundation of China (Grant No. 39970356), the Natural Science Foundation of Beijing (Grant No. 5992003) and the China Postdoctoral Foundation

** The authors contributed equally to this work

*** To whom correspondence should be addressed. E-mail: yhe@duke.edu or sunjs@ns.ibcas.ac.cn

transit peptide at their N-terminal of these NtFtsZs raises a possibility that multiple FtsZ proteins function inside of plastids in higher plants. It also indicates that the division mechanism of plastids might be more complicated than that of their evolutionary ancestors.

1 Materials and methods

1.1 Materials

Seeds of *Nicotiana tabacum* ver. SR1 were sowed on the 1/2 MS plates without plant hormones. Culturing conditions were 25～28℃ in a period of 16h in light and 8 h in dark per day. Rooted seedlings were transferred into pots and cultured under identical conditions. The roots used in Northern analysis were collected from the seedlings cultured in 1/2 liquid MS medium under the same conditions.

1.2 Methods

1.2.1 cDNA cloning A pair of primers were designed according to the tobacco *FtsZ* gene deposited in GenBank (accession No. AJ133453), NP1: 5′-CGGGATCCATGGCCACCATGTTAGGACTC-3′; NP2: 5′-AG GAATTCCTAAAAGAACAGCCTCCGAGT-3′. RT-PCR was performed under the instructions of TaKaRa RNA PCR Kit (AMV) Ver.2.1, except that the NP2 primer was used as the reverse transcription primer. The products of PCR were purified with Wizard® PCR preps DNA purification Kit and cloned into pBluescript II KS+ or pUCT vectors. The positive clones were verified and sequenced.

1.2.2 RACE The primers used in RACE experiments were selected from the sequence of *NtFtsZ1-2*. The reverse primers used in 5′ RACE were R1 (5′-ACGCCGACAACCTTAATCTTGGCAGA-3′) and R2 (5′-AAATGCTAAGGAATTGGAAGAAG-3′); the forward primers used in 5′ RACE were cassette primers contained in the TaKaRa LA PCR *in vitro* Cloning kit, C1 (5′-GTACATATTGTCGTTAGAACG CGTAATACGACTCA-3′) and C2 (5′-CGTTAGAACGCGTAATACGACTC ACTATAGGGAGA-3′). The genomic DNA of tobacco was used as template in 5′ RACE. The forward primers used in 3′ RACE were F1 (5′-GCATGCCAAATTGGAGAACTTCTG-3′) and F2 (5′-GGATCCACAAGCTTGGCTGATCCA-3′); the reverse primer used in 3′ RACE was M13 Primer M4 (5′-GTTTTCCCAGTCACGAC-3′) contained in the TaKaRa RNA PCR kit. The reverse transcription product of total RNA from tobacco leaves was used as template in 3′ RACE.

1.2.3 Northern and Southern analyses 20 μg tobacco total RNA isolated from root, stem, leaf and flower, respectively, was electrophoresed for 2.5 h in a 20% formaldehyde, 1% agarose gel in TAE buffer, and RNA was transferred to Hybond N + nylon membranes. Full-length *NtFtsZ1-1* and *NtFtsZ1-2* cDNAs were radiolabeled by the random-primer method and hybridized to the nylon membranes in Church buffer[12]. Furthermore, RT-PCR-Southern method was also employed to monitor the expression pattern of *NtFtsZs*. The amount of total RNA used in the reverse transcription was determined by spectrophotometry. For other molecular manipulation refer to Ref. [13].

1.2.4 Sequence and molecular phylogenetic analysis The homology searches were performed with BLAST at http://www.ncbi.nlm.nih.gov/blast/, the search of protein motifs was preformed with PROSITE at http://www.tokyocenter.genome.ad.jp/SIT/MOTIF.html, and the prediction of subcellular localization of FtsZ protein was performed with PSORT at http://psort.ims.u-tokyo.ac.jp. The evolution relationship of land plant FtsZs was analyzed by neighbor-joining method[14].

1.2.5 Expression of *NtFtsZs* in *E. coli*. *NtFtsZ1-1* and *NtFtsZ1-2* were fused in frame with pGEX-2T and expressed in *E. coli* strain JM109. Overnight growth colonies on LB plates with or without Isopropyl-β-D-thiogalactopyranoside (IPTG) were collected onto the glass slides and mixed with Rubin S dye for 1 min. The effects of expression of exogenous *ftsZ* genes in *E. coli* were observed under an OLYMPUS BH-2 microscope. Images were captured with a Leica MPS60 autographic system using Kodak ISP200 film.

2 Results and discussion

2.1 Cloning of tobacco *ftsZ* cDNA

The *Nicotiana ftsZ* genes were amplified by RT-PCR method from total RNA extracted from the leaves of *Nicotiana tabacum*, and a single product was obtained. The product was cloned into the *Bam*HI and *Eco*RI sites of pBluescript II KS+. After digested with *Bam*HI and *Eco*RI, two types of positive clones were identified. Then, the product of

RT-PCR was inserted directly into the pUC-T vector. The recombinants were obtained and sequenced. After sequencing, it was known that one recombinant is identical to the *NtFtsZ1-1* (GenBank accession No. AJ133453); the other one is a new tobacco *ftsZ* cDNA, designated *NtFtsZ1-2*.

2.2 RACE of *NtFtsZ1-2*

Because the initiation codon and stop codon in *NtFtsZ1-2* sequence were introduced by PCR primers, a RACE strategy was performed to obtain the detailed information about the 3′ and 5′ flanking region of *NtFtsZ1-2*, as described before. After two rounds of PCR, a 1.5 kb fragment was obtained by 5′ RACE. Sequence of this fragment showed that it is the promoter region of *NtFtsZ1-2* and also includes the 5′ flanking sequence of *NtFtsZ1-2* cDNA. A putative ATG flanked by the typical plant initiation codon sequences of NNATGGCN was identified and two stop codons upstream of this ATG were found at −32 and −51 sites, thus, this ATG should be the correct initiation codon for *NtFtsZ1-2*. After two rounds of PCR, a 400 bp fragment was obtained by 3′ RACE and it overlapped the 3′ flanking region of *NtFtsZ1-2*. A typical poly (A) sequence found at the downstream of the stop codon indicated that the fragment was the intact 3′ flanking region of *NtFtsZ1-2*. To verify the results obtained from RACE, a new pair of primers, NP3 and NP4, was used to perform the end-to-end PCR. The product of end-to-end PCR showed that the exact encoding region of *NtFtsZ1-2* is 1239 bp long (Fig. 1 (a)), which encodes for 412 amino acids (Fig. 1 (b)). This sequence has been deposited into GenBank under the accession number AF205858.

(a)

```
   1  ACCGTTGGTTTCACTTTCTTTCAGGTAAAATCCCTCATATCTCCAAAATT
  51  ATCAAAAGAAGAAATAAAAAGTAAAAAACTTTGGAGATACTAGTTGCCTG
 101  CCTCATTCTTGGAAAAGCTCCAATGGCCATGTTAGGACTCTCTTCAAACA
 151  CAGGAATCGATATATTGTCTTCTTCTTCCAATTCCTTATCATTTTATCAT
 201  AGCACTCGCTTCACACAATGCTTCTCTCCGAAAAGCCTCTGCAAACGACA
 251  ACGACGAAGATTCAGCATCTGCAGTTCCTTGTCCTCTGCCAAGATTAAGG
 301  TTGTCGGCGTCGGTGGCGGCGGCAACAATGCTGTTAACCGTATGATTGGC
 351  AGCGGCTTGCAGGGTGTTGACTTTTATGCTGTAAACACGGATGCTCAAGC
 401  ATTGCTGCAGTCTACGGTTGAGAACCCAATTCAAATTGGAGAACTTCTGA
 451  CTCGTGGACTTGGTACTGGTGGCAATCCTCTGTTGGGGGAACAGGCAGCA
 501  GAGGAGTCAAAGGAACACATTGCAAATGCTCTTAAAGGTTCGGATATGGT
 551  GTTTATAACAGCAGGCATGGGTGGCGGTACTGGATCTGGTGCTGCTCCTG
 601  TTGTTGCTCAAATAGCCAAAGAAGCAGGTTATTTGACTGTTGGTGTCGTC
 651  ACGTATCCATTCAGCTTTGAAGGACGTAAAAGATCTTTGCAGGCTTTGGA
 701  AGCAATTGAAAAACTTCAGAAAAATGTAGATACTCTTATAGTAATTCCCA
 751  ATGATCGTCTTCTGGATATTGCTGATGAGCAGACACCACTTCAAAATGCT
 801  TTTCTTCTTGCTGATGATGTACTTTGTCAAGGCGTCCAAGGAATATCTGA
 851  TATAATCACTATACCTGGGCTGGTAAATGTGGATTTTGCAGATGTTAAGG
 901  CAATCATGAAAGATTCTGGAACTGCTATGCTCGGAGTTGGGGTTTCTTCC
 951  AGTAGGAACCGTGCTGAGGAAGCAGCTGAACAAGCAACTCTGGCCCCTCT
1001  CATTGGATCATCAATTCAATCTGCAACTGGTGATGTATATAACATTACTG
1051  GAGGAAAAGATATTACTTTGCAGGAGGTGAATAAGGTGTCCCAGGTTGTC
1101  ACAAGCTTGGCTGATCCATCCGCCAACATCATATTCGGTGCTGTTGTGGA
1151  TGAGCGCTATAATGGAGAGATCCAGGTGACTCTAATTGCAACTGGTTTCG
1201  CACAGTCATTTCAGAATTCTCTTCTAACTGACCCTCGAGGAGCAAAGCTA
1251  GTTGATAAGAGCAAAGGAACCACAGAAAGAACGGTATCACCTGATACCTT
1301  GAGGTCATCAGAGTCTCCTTCCACAAAACCTCGACCAGCTGCTCGGAGGC
1351  TGTTCTTTTAGCTCCTTCTTAGTTTGTTATCTCTCCATTGTTATTTTTTA
1401  TTAATGATTCTTTTTTTTTTGGGGGATTCCTTACACTTATTTGTACCACA
1451  ACGGGTCAGGTCTGACGAGTGTACCTGATTATTTACACTTACATGTGGCT
1501  TTATAAATCATGATGAGTTTTATTAAAAAAAAAAAA
```

(b)

```
   1  MAMLGLSSNTGIDILSSSSNSLSFYHSTRFTQCFSPKSLCKRQRRRFSIC
  51  SSLSSAKIKVVGVGGGGNNAVNRMIGSGLQGVDFYAVNTDAQALLQSTVE
 101  NPIQIGELLTRGLGTGGNPLLGEQAAEESKEHIANALKGSDMVFITAGMG
 151  GGTGSGAAPVVAQIAKEAGYLTVGVVTYPFSFEGRKRSLQALEAIEKLQK
 201  NVDTLIVIPNDRLLDIADEQTPLQNAFLLADDVLCQGVQGISDIITIPGL
 251  VNVDFADVKAIMKDSGTAMLGVGVSSSRNRAEEAAEQATLAPLIGSSIQS
 301  ATGDVYNITGGKDITLQEVNKVSQVVTSLADPSANIIFGAVVDERYNGEI
 351  QVTLIATGFAQSFQNSLLTDPRGAKLVDKSKGTTERTVSPDTLRSSESPS
 401  TKPRPAARRLFF*
```

Fig. 1. Nucleotide (a) and amino acid (b) sequences of *NtFtsZ1-2*. Bold letters in (a) indicate the initiation and stop codons; underlined bold letters indicate the two stop codons upstream ATG. Bold letters in (b) indicate the two conservative motifs in FtsZ; the italic letters in second motif indicate the common GTP binding site existing both in FtsZ and tubulin.

2.3 *NtFtsZ1-2* is a new member of *FtsZ1* family in higher plant

Fig. 2 shows that the identity between *NtFtsZ1-1* and *NtFtsZ1-2* is 84% at the amino acid level, and 88% at the nucleotide level. The difference of the two FtsZ proteins exists mainly in the N- and C-terminal regions, whereas the middle functional region is highly conservative across phyla. NtFtsZ1-1 and NtFtsZ1-2 also contain two conservative motifs that existed in known FtsZs, namely, FTSZ_1: VIGVGGGGSNAVNRM (PROSITE; PS01134) and FTSZ_2: FATAGMGGGTGS/TGAAPV/IV/IA (PROSITE; PS01135). A signature motif found both in tubulin and FtsZ is also included in the FTSZ_2: GGGTGSG (PROSITE; PS00277). The function of FTSZ_1 is still kept unknown. Because of the similarity with tubulin, the function of FTSZ_2 was postulated to relate with the GTP binding capability and GTPase activity of FtsZ proteins. This postulation has been verified in the studies of prokaryotic FtsZ proteins[2,3,15]. Furthermore, all known plant FtsZs possess an N-terminal extension, which also is an obvious feature to distinguish the eukaryotic FtsZs from prokaryotic FtsZs.

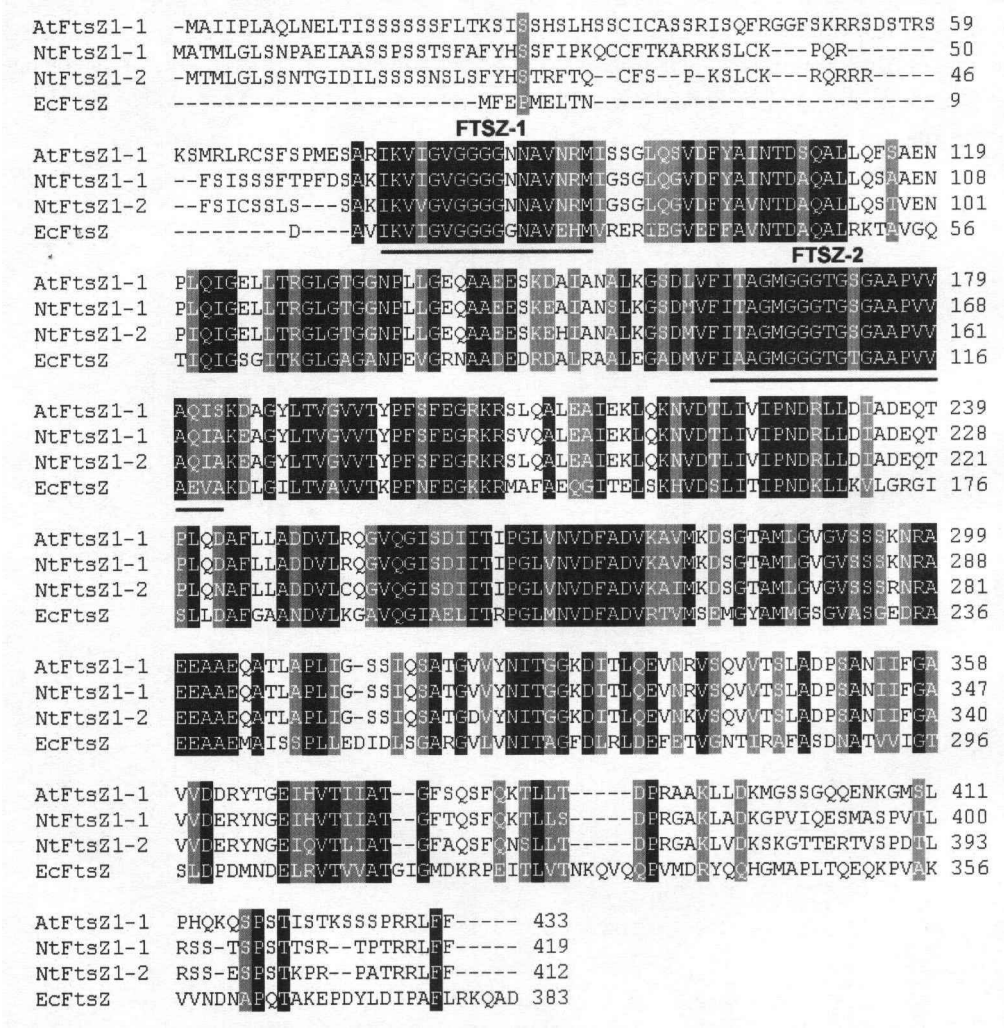

Fig. 2. Alignment of amino acid sequences of AtFtsZ1-1(*Arabidopsis*), EcFtsZ(*E. coli*), NtFtsZ1-1(tobacco) and NtFtsZ1-2(tobacco). The identical residues are shown in black; the similar residues in gray; the two conservative motifs of FtsZ proteins are underlined.

The pairwise comparison between NtFtsZs and other known plant FtsZs was performed (Table 1). The results showed that the identity between NtFtsZs and AtFtsZ1-1[11] or between NtFtsZs and PsFtsZ[16] was above 80% at the amino acid level, whereas the identity between NtFtsZs and AtFtsZ2-1[11] or between NtFtsZs and PpFtsZ1[17] was under 62% at the amino acid level. This finding suggested that the FtsZ proteins in plants could be classified into two different groups based on the sequence similarity, and the difference of the two FtsZ groups might be decided by the existence of plastid transit peptide at their

N-terminal extension. According to the criteria, Osteryoung had identified two small *FtsZ* gene families in plants[11]. The antisense expressions of different members from two families demonstrated the inhibition of normal plastid division in sequential experiments[11]. Thus, a model was put forward to describe the functional pattern of FtsZ proteins in the division of plastids: the FtsZ with plastid transit peptide could be targeted into plastids, the FtsZ without plastid transit peptide stayed in cytoplasm, the coincident constriction of two group FtsZs resulted in the division of plastids[11]. So far, only one transit-peptide containing FtsZ has been identified from different species in higher plants[11,16]. However, *NtFtsZ1-1* and *NtFtsZ1-2* presented here should be grouped in *FtsZ1* family based on the sequence identity. To further verify this assumption, the N-terminal sequences of NtFtsZ1-1, NtFtsZ1-2 and AtFtsZ1-1 were analyzed. About 60 amino acids at the N-terminal of these FtsZ proteins displayed some typical plastid transit-peptide features[18]: rich in hydrophilic amino acid Ser and Thr (30%), almost no acidic amino acids (<2%). Subcellular localization analysis with PSORT also suggested that these FtsZ proteins stay in the stroma of plastids. Meanwhile, molecular phylogenetic analysis based on the FtsZ amino acid sequences identified *NtFtsZ1-1* and *NtFtsZ1-2* as the members of *FtsZ1* family (Fig. 3). The topology of phylogenetic tree is consistent with the results of Beech et al.[19] and Kiessling et al.[20] Hence, the *NtFtsZ1-2* should be a new member of land plant *FtsZ1* family.

Table 1. Pairwise comparisons of partial land plant FtsZ sequences

	NtFtsZ1-1	NtFtsZ1-2	PsFtsZ	AtFtsZ1-1	AtFtsZ2-1	PpFtsZ
NtFtsZ1-1	—	88	77.2	75.5	63.4	60.3
NtFtsZ1-2	83.5	—	84.2	80	62.9	60.2
PsFtsZ	86.7	85	—	78	61	59
AtFtsZ1-1	84.9	80.8	86	—	60	59
AtFtsZ2-1	60.1	61.1	59	59	—	68
PpFtsZ	57.2	56.5	57	57	77	—

Shadows indicate the amino acid identity (%) between different FtsZs; non-shadows indicate that the nucleotide identity (%) between different *FtsZs*. GenBank accession numbers for the comparisons are: *NtFtsZ1-1*, AJ133453; *NtFtsZ1-2*, AF205858; *PsFtsZ*, Y15383; *AtFtsZ1-1*, U39877; *AtFtsZ2-1*, AF089738; *PpFtsZ*, AJ001586.

2.4 Expression of *NtFtsZ1-1* and *NtFtsZ1-2*

Result of Northern blot (Fig. 4 (a)) showed that both of *NtFtsZ1-1* and *NtFtsZ1-2* expressed strongly in leaves, a pattern consistent with their plastid division function. *NtFtsZ1-2* also displayed a strong signal in flower. But whether *NtFtsZ1-1* and *NtFtsZ1-2* possess different function in different developmental stages remains unknown. Weak signals displayed in root and stem for both sequences indicated that plastid division event rarely happened in these organs. RT-PCR Southern analysis displayed a similar result to Northern blot, although there were differences in the expression level. All of these results indicate that the expression patterns of *NtFtsZ1-1* and *NtFtsZ1-2* are consistent with the development and differentiation of plastids.

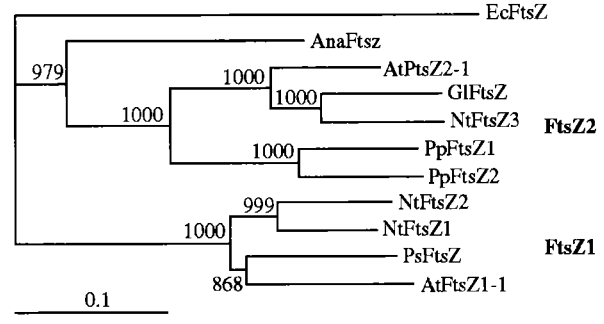

Fig. 3. Molecular phylogeny tree of land plant FtsZs. The values at branches indicate the bootstrap values of 1000 replicates. The EcFtsZ of *E. coli* and AnaFtsZ of *Anabaena* spp were used as the outgroup.

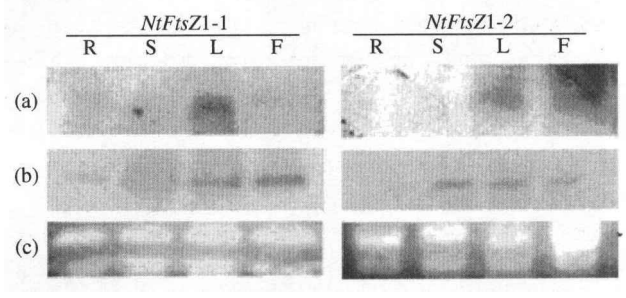

Fig. 4. Expression of *NtFtsZ1-1* and *NtFtsZ1-2*. (a) The result of Northern blot; (b) the result of RT-PCR Southern analysis; (c) the amount of total RNA. R, S, L, F indicate the RNA from root, stem leaf, and flower, respectively.

2.5 Expression of *NtFtsZ1-1* and *NtFtsZ1-2* in *E. coli*

Because of its conservative sequence and function, it is of interest to know if the eukaryotic FtsZ protein is involved or interferes with the prokaryotic cell division. Compared with the control strain (Fig. 5(a) and (d)) that contains the pGEX-2T plasmid only, the morphology of host cells containing *NtFtsZ1-1* or *NtFtsZ1-2* recombinant changed obviously (Fig. 5(b), (c), (e) and (f)). The expression product of empty pGEX-2T plasmid did not influence the growth of host cells, which could divide normally and maintained normal morphology no matter whether induced by IPTG or not. Whereas, the

morphology of cells containing recombinant plasmids changed significantly, especially when they were induced by 1 mmol/L IPTG overnight. The host cells appeared extended obviously and showed a filamentous phenotype (Fig. 5(e) and (f)), which is similar to that found in *ftsZ* mutants[21], and to the overexpression of exogenous *ftsZ* gene in *E. coli*[22]. Therefore, these results indicated that the expressions of *NtFtsZ1-1* and *NtFtsZ1-2* could block the division of *E. coli* cells, and also suggested that NtFtsZs not only possess the sequence similarity, but also possess the similar functions as their prokaryotic counterpart. Furthermore, these results provided direct evidence for the endosymbiosis hypothesis of plastid origin.

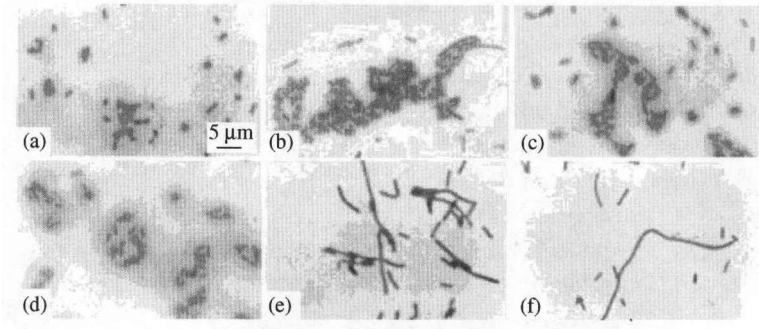

Fig. 5. Expressions of *NtFtsZ1-1* and *NtFtsZ1-2* in *E. coli* and their effects on the morphology of host cells. (a) and (d), controls; (b) and (e), cells containing *NtFtsZ1-1* plasmids; (c) and (f), cells containing *NtFtsZ1*-plasmid. (a~c), without IPTG induction; (d~f), treated with 1 mmol/L IPTG.

From the results presented here, we conclude that there are two or more transit-peptide containing FtsZ in higher plants. Thus, the plastid division mechanism in higher plants might not be as simple as indicated by Osteryoung et al.[11] The members in the same family or members from different families might play important roles in plastid division in a more complicated way than previously thought. Because of its endosymbiotic origin, the mechanism of plastid division is similar to its prokaryotic counterpart. However, the difference between the mechanisms of prokaryotic cell and plastid division is distinct due to the changes of living condition and the gene transfer from plastids to nucleus during the long evolutionary process. Further studies will no doubt reveal additional similarities and differences between the mechanisms of plastid and prokaryotic cell division.

References

1 Bramhill, D. Bacteria cell division. Annu. Rev. Cell Dev. Biol., 1997, 13: 395.
2 Lutkenhaus, J. F. et al. Bacterial cell division and the Z ring. Annu. Rev. Biochem., 1997, 66: 93.
3 de Boer, P. et al. The essential bacterial cell-division protein FtsZ is a GTPase. Nature, 1992, 359: 254.
4 RayChaudhuri, D. et al. *Escherichia coli* cell-division gene *ftsZ* encodes a novel GTP-binding protein. Nature, 1992, 359: 251.
5 Mukherjee, A. et al. Guanine nucleotide-dependent assembly of FtsZ into filaments. J. Bacteriol., 1994, 176: 2754.
6 Nogales, E. et al. Tubulin and FtsZ form a distinct family of GTPases. Natl. Struc. Biol., 1998, 5: 451.
7 Lowe, J. et al. Crystal structure of the bacterial cell-division protein FtsZ. Nature, 1998, 391: 203.
8 Erickson, H. P. FtsZ, a prokaryotic homolog of tubulin? Cell, 1995, 80: 367.
9 Wang, X. et al. FtsZ ring: the eubacterial division apparatus conserved in archaebacteria. Mol. Microbiol., 1996, 21: 313.
10 Osteryoung, K. W. et al. Conserved cell and organelle division. Nature, 1995, 376: 473.
11 Osteryoung, K. W. et al. Chloroplast division in higher plants requires members of two functionally divergent families with homology to bacterial ftsZ. Plant Cell, 1998, 10: 1991.
12 Church, G. et al. Genomic sequencing. Proc. Natl. Acad. Sci. USA, 1984, 81: 1991.
13 Sambrook, J. et al. Molecular Cloning: A Laboratory Manual, 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
14 Saitou, N. et al. The neighbor-joining method: A new method for reconstruction phylogenetic trees. Mol. Biol. Evol., 1987, 4: 406.
15 RayChaudhuri, D. et al. A point mutation converts *Escherichia coli* FtsZ septation GTPase to an ATPase. J. Biol. Chem., 1994, 269: 22941.
16 Gaikwad, A. et al. Pea chloroplast FtsZ can form multimers and correct the thermosensitive defect of an *Escherichia coli ftsZ* mutant. Mol. Gen. Genet., 2000, 263(2): 213.
17 Strepp, R. et al. Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin. Proc. Natl. Acad. Sci. USA, 1998, 95: 4368.
18 von Heijne, G. et al. Domain structure of mitochondrial and chloroplast targeting peptides. Eur. J. Biochem., 1989, 180: 535.
19 Beech, P. L. et al. Mitochondrial FtsZ in a chromophyte alga. Science, 2000, 287: 1276.
20 Kiessling, J. et al. Visualization of a cytoskeleton-like FtsZ network in chloroplasts. J. Cell Biol., 2000, 151: 945.
21 Ward, J. E. et al. Overproduction of FtsZ induces minicell formation in *E. coli*. Cell, 1985, 42: 941.
22 Baumann, L. et al. Characterization of *ftsZ*, the cell division gene of *Buchnera aphidcola* (endosymbiont of aphids) and detection of the product. Current Microbiology, 1998, 36(2): 85.