Visualization of FtsZ Rings in Plastids of the Microspore in *Lilium longiflorum*

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Accepted January 31, 2001

Summary Previous study demonstrated that the *ftsZ* gene expressed specifically in pollen generative cells of *Lilium longiflorum* was also found to be expressed during microsporogenesis before formation of them. We, therefore, proved the expression of *L. longiflorum* FtsZ (LlFtsZ) in uninucleate microspores with specific antibodies. As a result, rings were visualized in the cytoplasm of young microspores. Moreover, it was revealed that almost all rings overlapped on each of plastids by 4',6-diamidino-2-phenylindole (DAPI) staining. Each spherical and peanut-shaped plastid had one ring on its equator. These results suggest that FtsZ ring is required for plastid division.

Key words FtsZ, Plastid division, Microsporogenesis.

Plastids in all plant cells divide in the manner of constriction (Possingham and Lawrence 1983). Electron microscopy demonstrated the presence of electron-dense structures encircling the zone of constriction on a plastid membrane of various plant species (Mita *et al.* 1986, Kuroiwa *et al.* 1998). This finding strongly suggested presence of plastid-dividing apparatus. FtsZ was originally found out in *Escherichia coli* as cell division protein; it forms ring structure beneath the plasma membrane at the division site (Bi and Lutkenhaus 1991). Osteryoung *et al.* (1998) first identified nuclear-encoded homologues of FtsZ (AtFtsZ1-1, AtFtsZ2-1) in *Arabidopsis thaliana* and demonstrated that chloroplast division was depended on them by gene technological methods. Strepp *et al.* (1998) also succeeded to induce giant chloroplasts on the knock out moss (*Physcomitrella patens*). These findings suggest that FtsZ is a part of plastid-dividing apparatus. In addition, Vitha *et al.* seem to have observed the FtsZ rings at the plastid midpoint (Osteryoung 2000, data not shown). However, Kiessling *et al.* (2000) visualized a FtsZ network in the entire chloroplast with *P. patens* FtsZ-green fluorescent protein fusions recently. This weakens the direct relationship between FtsZ and the apparatus.

Mori and Tanaka (2000) found out specific accumulation of *ftsZ* transcripts in the generative cells of the bicellular pollens in *L. longiflorum*, and the same transcripts were found to be detected at higher level during microsporogenesis. It has been known that the plastids develop during this period by using fluorescence and electron microscopies (Rodriguez-Garcia and Garcia 1978, Tanaka 1991). We, therefore, purposed to observe LlFtsZ in the uninucleate microspores in expectation of plastid-encircling rings.

Material and methods

Sample preparation and fluorescence microscopy

The plant material was *L. longiflorum* cv. 'Hinomoto'. Buds 25–30 mm in length from the plants grown in a green house were collected. Sample preparation and fluorescence microscopy were performed according to the methods of Tanaka (1991) with minor modifications.

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Antibody production

The partial coding region corresponding to residues 69 through 468 (GenBank accession no. AB042101) of *LftsZ* cDNA was cloned into the expression vector pQE-30 (Qiagen, Chatsworth, CA). The plasmid was transformed into *E. coli*. LIFtsZ protein expression was induced by addition of 2 mM isopropyl β -D-thiogalactopyranoside. The recombinant protein was purified by using His-TrapTM (Amersham Pharmacia, Buckinghamshire, England) according to the manufacture's instruction. The protein was further purified by SDS polyacrylamide gel electrophoresis. The gel slices containing the protein were injected into 2 rabbits for antibody production (Sawady technology, Tokyo, Japan). The antibodies were recovered from the antisera obtained by using MabTrap G IITM (Amersham Pharmacia) according to the manufacture's instruction.

Results and discussion

We obtained pollen tetrads immediately after meiosis and young microspores with thin exine together with declined tapetal cells. Some of tetrads were separated into 4 cells by squashing. DAPI staining visualized a large nuclear DNA centered in a cytoplasm and small DNA spots derived from nucleoids of plastids (Fig. 1a). These spots were aggregated at 2 poles on long axis of the elliptic cytoplasm (Fig. 1a). Anti-LIFtsZ antibodies distributed on each of all plastids as closed rings (Fig. 1b). Each spherical (Fig. 1c) and peanut-shaped (See *Technical note* on this number.) plastid released from the cytoplasm was found to be surrounded with one ring on its putative equator. This result brought new information to previous studies described above and is correspondent to observation that Vitha *et al.* performed (Osteryoung 2000, data not shown). According to the fluorescence micrographs with phase-contrast image, these rings were observed both at non-constricting plastids and at constricting ones. This suggests that the expression of FtsZ protein is not specific to

Fig. 1. Localization of plastids and LIFtsZ proteins in young microspores with thin exine derived from a late tetrad. a) DAPI staining, large and bright spot of nuclear DNA was visualized, and a large numbers of small spots corresponding to plastid nucleoids surround it. b) Visualized LIFtsZ proteins in the same sample as a). All closed rings were detected on the loci corresponding to plastid nucleoids (arrows). Scale bar, 12.5 μ m. c) A phase-contrast image layered by fluorescence signals. Each of plastids released from a cytoplasm of a young microspore had a ring surrounding them (arrows). Scale bar, 2.5 μ m.

the dividing plastids. Henceforth, whether the FtsZ ring is belong to the plastid dividing apparatus needs to be investigated.

Acknowledgment

This research was supported by grants to T. K. (no. 12446222 and no. 12874111) from the Ministry of Education, Science, and Culture of Japan and from the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

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