Rings and networks: the amazing complexity of FtsZ in chloroplasts

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Bacteria have proteins that can form filaments and rings, and these are thought to be the evolutionary progenitors of actin and tubulin. Plant homologues of the most intensively studied bacterial FtsZ protein are nuclear-encoded by a small gene family, are plastid-bound and participate in the plastid division process. The hypothesis is put forward that FtsZ and other proteins form a filamentous network in plastids, a plastoskeleton, which keeps these organelles in shape and helps them to divide.

Temperature-sensitive *E. coli* mutants, designated *fts* (filamentous temperature-sensitive), show a filamentous phenotype because they are unable to divide under non-permissive temperature. One of these mutants, *ftsZ*, has a mutated gene that codes for a protein that polymerizes to a ring (Z-ring) during constriction division of bacterial cells [1]. Because the FtsZ protein is likely to represent the evolutionary ancestor of the eukaryotic tubulins, some essential proteins of the eukaryotic cytoskeleton might be inherited from the bacterial kingdom [2–5].

Plant ftsZ genes

In plants, FtsZ homologues are nuclear-encoded by a small gene family, transported into chloroplasts, and are essential for chloroplast division. Plant FtsZ was the first protein identified as essential for the division of any eukaryotic organelle [6–8]. Thus, bacterial cell division and eukaryotic plastid division, which both proceed by constriction, share, at least, the essential role of the FtsZ protein in this process. Although all eubacteria seem to possess only one *fts*Z gene, plants seem to possess at least four different *fts*Z genes encoded by two different gene families, indicating that FtsZ in plants has a more complex role than in bacteria [9,10].

Rings and networks?

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According to various models, FtsZ monomers polymerize in bacteria to form filaments in the same way as tubulin does in the eukaryotic cytoplasm. These filaments are thought to be part of the transient bacteria-dividing Z-ring [1]. Because similar transient plastid-dividing rings (PD rings) had been described for many years in plants [11], the next question was, who will be the first person to identify plant FtsZ as part of the plastid-dividing ring? This question has been addressed mainly by two different approaches: (i) labelled FtsZantibodies have been used to highlight the protein in wild-type cells using light and electron microscopy, and (ii) FtsZ–green fluorescent protein (GFP) fusions have been used to highlight the protein in transgenic cells using confocal laser scanning microscopy.

The first results for FtsZ-localization in plastids were a complete surprise: in transiently transformed *Physcomitrella* cells, FtsZ–GFP monomers polymerized to highly organized networks within the plastids (Fig. 1). Decoration of a ring structure could be detected only rarely [9]. Subsequently, FtsZ was found to build a ring structure within the chloroplasts of *Lilium longiflorum* and *Arabidopsis* using the antibodyapproach (Figs 2 and 3), but filaments could be seen only rarely [12,13].

Although it was tempting to believe that these FtsZ-rings represent the electron-dense plastid dividing ring, obviously these are different structures [14], representing bacterial-based and eukaryotic-based division rings [15]. Because these FtsZ-rings are exactly what were expected from the data achieved from bacteria, the previously observed FtsZ-networks can be regarded as artefacts, possibly the result of overexpression or caused by the GFP-part of the fusion protein.



Fig. 1. *In vivo* filamentation of PpFtsZ1. Transiently FtsZ1–green fluorescent protein-expressing *Physcomitrella patens* cells were analysed by confocal laser scanning microscopy two days after transfection. They show a filamentous network within the plastids termed the 'plastoskeleton'. Photograph courtesy of Justine Kiessling. Scale bar = 10 μ m.

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Opinion



Fig. 2. Phase-contrast image combined with fluorescent microscopy image. A typical FtsZ2 ring from plastids of *Lilium longiflorum* is visualized by fluorescein isothiocyanate (FITC). Photograph courtesy of Toshiyuki Mori. Scale bar = $2 \mu m$.

Re-discovery of the plastoskeleton?

In experiments conducted in my laboratory,

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FtsZ–GFP-fusions were biologically active: like in bacteria [16], these proteins influenced the division process in a dose-dependent manner [9]. Moreover, based on transmission electron micrographs (TEMs) of genetically unmanipulated plant tissue, filamentous structures were found in plastids several years ago by different authors and were described as microtubule-like-structures (MTLS) [17–19]. Most strikingly, the models drawn from the older TEM-results match the novel data obtained for filamentous FtsZ–GFP proteins in plastids [20]. Therefore, it is likely that in intact, unmanipulated plastids a filamentous protein network persists. Based on these observations and bearing in mind that FtsZ resembles an ancient tubulin, the term



Fig. 3. Fluorescent microscopy of immunofluorescence labelling of FtsZ2 in chloroplasts from wild-type *Arabidopsis thaliana*. Photograph courtesy of Stanislav Vitha and Katherine W. Osteryoung. Scale bar = $10 \,\mu$ m.

'plastoskeleton' was suggested for these filamentous networks within the chloroplasts [9].

Why has this network not been identified with the antibody-approach? Two of many possible reasons are that it might need confocal laser scanning microscopy to detect these tiny structures, or that the existing antibodies might only detect FtsZ-filaments in a specific conformation.

What is this complexity good for?

The intriguing question is why have plants evolved at least four different ftsZ genes in at least two different families from one eubacterial ancestor? Because all FtsZ proteins in green plants are plastid-related (and only additionally correlated with mitochondria in non-green algae [21–23]), the most probable answer is that the different proteins achieved additional functions in plastids. One of these additional functions might be the participation in a persistent plastoskeleton that helps chloroplasts to keep their shape and to change their shape in different tissues. Without a plastoskeleton, what other mechanism could ensure plastid integrity and flexibility after the loss of the bacterial cell wall during the establishment of endosymbiosis?

Amazingly, microbiologists again set the pace. Not only did bacteria invent tubulin and other cytoskeletal elements [24], but also actin [25]. It is likely that they

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also invented a persistent cytoskeleton [26] that was inherited by the plastids during the establishment of endosymbiosis. Therefore, plastids might be more highly organized than previously suspected. The existence of a persistent plastoskeleton still has to be proven by a combination of molecular and cell biological tools. But the door is now wide open for a new chapter in cell biology.

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