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Skeletons in the Closet: How Do Chloroplasts Stay in Shape?

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Breakthroughs in microscopy technology provide new insights into cell biology. Early microscopes allowed Robert Hooke to see cells. Improved staining techniques enabled Camillo Golgi to see the apparatus that bears his name, and Robert Feulgen to visualize DNA in chromosomes. Similarly, EM allowed Keith Porter to visualize the endomembrane system. More recently, transgenic technology using fluorescent reporter proteins has enabled us to visualize cryptic or ephemeral processes and structures in living cells. The latest revelation with this technology is in chloroplast biology. On page 945 of this issue, Kiessling et al. show that fusion of green fluorescent protein (GFP)¹ with the FtsZ (filament temperature sensitive Z) protein targeted to chloroplasts of the moss *Physcomitrella* reveals a network of fibers within the chloroplast (Fig. 1). They dub this network the plastoskeleton, since it is reminiscent of cytoskeletons. Plant biologists have long wondered how chloroplasts (and their nongreen relatives, the plastids) maintain their specific shapes, which range from the more mundane ovoid versions of plants to the spectacular starbursts and spirals of the algae. Kiessling et al. (2000) now suggests that FtsZ could be the answer.

FtsZ is a key protein in bacterial cell division. *Escherichia coli* ftsZ mutants fail to divide at the restrictive temperature and form filaments with multiple nucleoids. In *E. coli*, FtsZ forms a ring lining the isthmus of the dividing cell (Margolin, 1998). Exactly how the cell membrane and wall are constricted during fission is not known, but FtsZ is argued to be the motor for this process (Lu et al., 2000). Ubiquity of FtsZ in the prokaryotic world attests to its core role.

Chloroplasts are derived from endosymbiotic cyanobacteria and divide by binary fission similar to bacteria (McFadden, 1999). Recently, chloroplast-targeted, nucleus-encoded versions of FtsZ have been identified in plants and algae (Beech and Gilson, 2000). Knockouts of chloroplast ftsZ in the moss *Physcomitrella* prevent chloroplasts from dividing, producing one giant chloroplast per cell. This phenotype is analogous to the ftsZ mutant phenotype in *E. coli* (Strepp et al., 1998). Similarly, antisense gene silenc-

ing of chloroplast ftsZ in higher plants (knockouts are not feasible in higher plants) also perturbs chloroplast division (Osteryoung et al., 1998). Proteins known to interact with FtsZ in bacteria also have been demonstrated to have roles in chloroplast division, thereby suggesting that plastid division retains major hallmarks of its bacterial ancestry, albeit with modifications imposed by existing within a host cell (Colletti et al., 2000). Previously, no one had visualized FtsZ in chloroplasts, so how do the FtsZ/GFP fusion structures observed by Kiessling et al. (2000) fit our notions of FtsZ structures in bacteria?

Immunolocalization and FtsZ/GFP fusion analyses reveal a ring (the so-called Z ring) at the site of constriction in dividing bacteria (Margolin, 1998). However, it has not yet been possible to visualize the Z ring by EM. Nevertheless, some insight into the ultrastructure of the ring is available. Purified bacterial FtsZ can be induced to form several structures in vitro. Depending on conditions, these structures take the form of filaments, sheets, mini-rings, and, perhaps most importantly for chloroplasts, tubules (Trusca et al., 1998; Lu et al., 2000). FtsZ tubules, which may be higher order versions of the sheets and rings (Lu et al., 2000), bear superficial resemblance to cytosolic microtubules of eukaryotes. Microtubules are composed of tubulin protein, which is probably an evolutionary derivative of FtsZ; both tubulin and FtsZ are GTPases and have similar structures (Lowe and Amos, 1998; Nogales et al., 1998). Both FtsZ tubules and eukaryotic microtubules are hollow and are composed of protofilaments arranged in helices. However, in eukaryotic microtubules there are 13 longitudinal protofilaments composed of alternating α and β subunits, whereas FtsZ tubes are helical. A pair of helical FtsZ protofilaments can form an open helical tube, or two pairs can form a solid tube (Trusca et al., 1998; Lu et al., 2000).

Tubules are not routinely visualized in bacteria (Bermudes et al., 1994), which made it difficult to assess the physiological importance of the in vitro assembled FtsZ tubules (Lu et al., 2000). However, a skeleton in the chloroplast cell biology closet might be the answer to this conundrum. Tubules resembling cytoplasmic (eukaryotic) microtubules are common in chloroplasts of plants and green algae, where they are proposed to function in division and maintenance of shape (Lawrence and Possingham, 1984). Are these chloroplast tubules composed of chloroplast FtsZ?

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¹Abbreviations used in this paper: ftsZ, filament temperature-sensitive Z; GFP, green fluorescent protein.

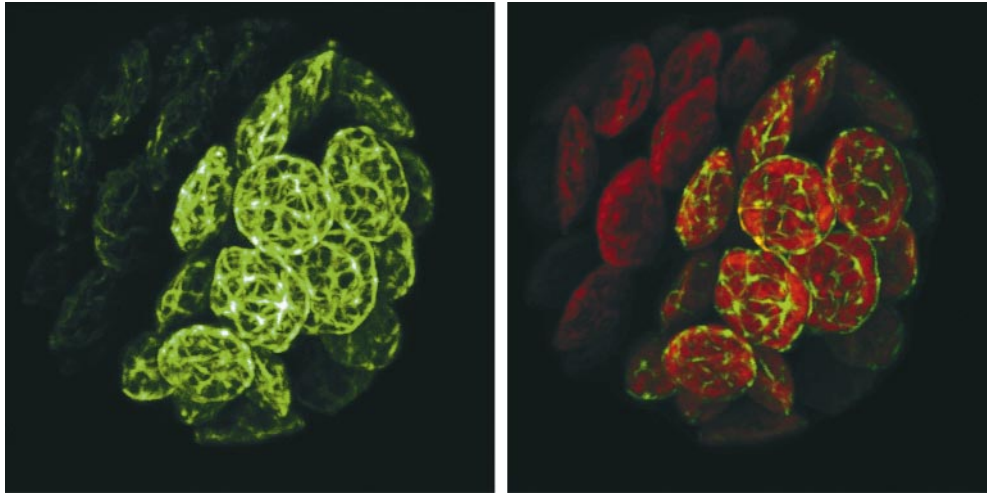


Figure 1. Confocal image of *Physcomitrella patens* protoplast expressing PpFtsZ1/GFP fusion protein. GFP fluorescence forms a reticulated fibrillar network (a plasto-skeleton) within the plastid (left). Red autofluorescence defines the individual chloroplasts (right). Micrograph courtesy of Justine Kiessling.

The cytoskeleton-like structures observed in *Physcomitrella* chloroplasts with FtsZ/GFP fusions (Fig. 1) correlate well with the tubules observed in other chloroplasts, which are described as forming anastomosing or reticulated networks ramifying throughout the entire chloroplast (Hoffman, 1967; Pickett-Heaps, 1968; Rivera and Arnott, 1982; Lawrence and Possingham, 1984). These tubules are relatively long (up to 2.5 μm) and often associate into orderly bundles incorporating between 2 and 30 tubules (Hoffman, 1967). The different sized bundles of tubules could explain the bramble-like fluorescence images observed in *Physcomitrella* (Fig. 1). But it is the structure of the chlo-

roplast tubules (Fig. 2) that provides the most compelling evidence for them being composed of FtsZ. Hoffman (1976) examined chloroplast tubules of the green alga *Oedogonium* and proposed a detailed model for their substructure. Hoffman's model describes a two helix, hollow tube with an outer diameter of 25–27 nm and a wall thickness of 5.5 nm (Hoffman, 1967). The two helical gyres ascend at a pitch of between 17° and 27° (Hoffman, 1967). This model for chloroplast tubules bears an extraordinary resemblance to the models for in vitro polymerized bacterial FtsZ tubules (Trusca et al., 1998; Lu et al., 2000). FtsZ tubules have an outer diameter of 23 nm, a wall thickness of 5.4 nm, and comprise a twin helix with a pitch of either 18° or 24°, depending on whether the tubes are four or five start helices (Lu et al., 2000). The prominent helical element of the plastid tubules (Fig. 2) would be more closely matched by the open, two-protofilament FtsZ helix than the four-protofilament solid tube.

To link the FtsZ/GFP structures observed in moss chloroplasts to the tubule bundles and networks in plant and algal chloroplasts, and then further to the in vitro assembled bacterial FtsZ tubules, is drawing a very long bow, but the models are compellingly similar. Intriguingly, chloroplast tubules were described long before the discovery of FtsZ. At this time, eukaryotic cytosolic microtubules were only beginning to be characterized, and Hoffman (1967) and Pickett-Heaps (1968) related the chloroplast tubules to cytoplasmic microtubules, although at the same time they recognized key differences in the substructure of the two types of tubules. Chloroplast tubules have not yet been observed in *Physcomitrella*, so there is no validation of the extraordinary structures observed in the FtsZ/GFP fusion transfected moss (Fig. 1). It remains possible that they are artefacts similar to those observed in mammalian cells expressing bacterial FtsZ (Yu et al., 1999). The key test will be to demonstrate that the structures observed by Kiessling et al. (2000) are comprised of tubules similar to those observed in other chloroplast, and that these are indeed composed of FtsZ.

What of chloroplast division and the Z ring? Kiessling et al. (2000) also observed possible plastid division rings at the constriction between two nascent daughter chloroplasts, and these could be plastid division rings or chloroplast Z rings. Interestingly, plants have multiple chloroplast FtsZ

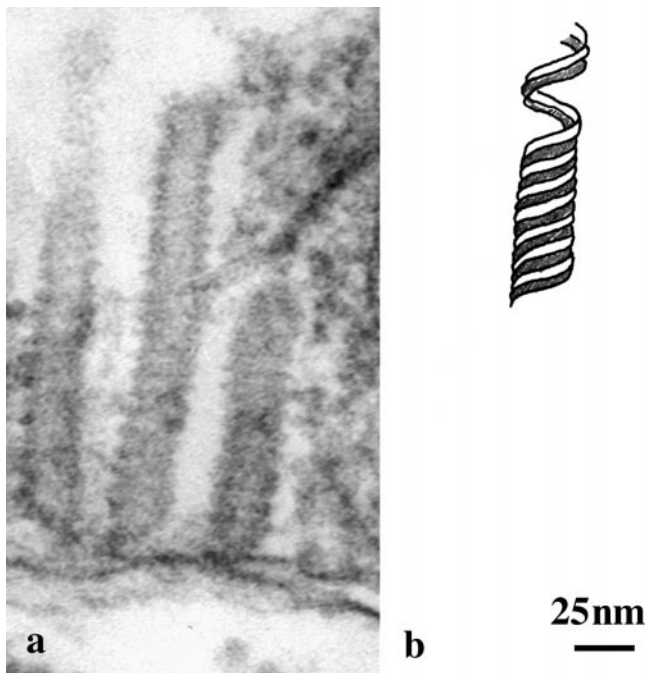


Figure 2. (a) Transmission electron micrograph of tubules from chloroplast of *Volvox* sp. showing longitudinal section through three tubules with spiral substructure. Micrograph courtesy of J. Pickett-Heaps. (b) Model for tubules redrawn from Hoffman (1967). The architecture and dimensions of the model are remarkably similar (see text) to models for in vitro polymerized tubules of bacterial FtsZ (Trusca et al., 1998; Lu et al., 2000).

genes, so the protein may have multiple functions in chloroplasts and plastids, perhaps being responsible not only for division, but also for maintenance of plastid shape, just as tubulin has roles in mitosis and cell shape in the eukaryotic cytoplasm. Kiessling et al. (2000) propose that the plastoskeleton evolved to compensate for the loss of the peptidoglycan wall during integration of the cyanobacterial endosymbiont. This hypothesis predicts that plastids of the alga *Cyanophora*, which retain a peptidoglycan wall, will lack a plastoskeleton. It will also be interesting to learn whether FtsZ tubules have skeletal roles in wall-less bacteria, or even mitochondria. FtsZ was recently implicated as having a role in mitochondrial division of certain algae (Beech and Gilson, 2000; Beech et al., 2000), but dynamins appear to have taken over the division function in animal and yeast mitochondria (Erickson, 2000). There is no evidence of cytoskeletal structures within mitochondria as yet, so how is their shape maintained?

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