Organelle Fission. Crossing the Evolutionary Divide

Katherine W. Osteryoung*

Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824

Among the products of the genome sequencing revolution are dramatic new insights into the evolutionary basis for cellular structure-function relationships. An example of this is the information beginning to accumulate regarding the key players and processes involved in the division of chloroplasts in plant cells and, more recently, in the division of mitochondria. Both types of organelles arose from prokaryotic endosymbionts related most closely to modern-day Cyanobacteria in the case of chloroplasts (McFadden, 1999), and α-proteobacteria in the case of mitochondria (Gray et al., 1999; Lang et al., 1999). Though most of the genetic functions present in the original endosymbionts have been transferred to or taken over by the nucleus (Martin and Herrmann, 1998), the prokaryotic signatures of many organellar processes are still evident in the similarities of the associated proteins to those in extant prokaryotes.

In the case of chloroplast division the discovery that a homolog of a key bacterial cell division protein was encoded in the nuclear genome of Arabidopsis and that it was targeted to the chloroplast was the first clue that plastid division in plants was evolutionarily and mechanistically related to prokaryotic cell division (Osteryoung and Vierling, 1995). Subsequent work has shown experimentally an essential role for *FtsZ* and another endosymbiotically acquired gene, *MinD*, in the division of chloroplasts in land plants (Osteryoung et al., 1998; Strepp et al., 1998; Colletti et al., 2000). Because FtsZ is encoded by an ancient gene family and was presumably present in the prokaryotic precursors of both chloroplasts and mitochondria (Erickson, 1997; Osteryoung and Pyke, 1998), it was assumed that this protein would also be a critical player in the division of mitochondria. However, this expectation was not met until a recent study by Beech and colleagues (2000) revealed the existence in a chromophyte alga of a nuclear-encoded FtsZ protein closely related to the α-proteobacterial FtsZs and localized in the mitochondrion. This commentary highlights this and other recent studies, which together indicate that the evolution of organelle division mechanisms in eukaryotes has taken at least a few different paths, all of which may be leading to the same end.

* E-mail osteryou@msu.edu; fax 517–355–1926.

FtsZ IN PROKARYOTIC CELL DIVISION

The gene encoding FtsZ was originally identified as a mutation in *Escherichia coli* that conferred a temperature-sensitive block in cell division, resulting in the formation of bacterial filaments (Yi and Lutkenhaus, 1985; Lutkenhaus, 1993). Thus, *fts* refers to the phenotype filamentation temperature-sensitive. The role of FtsZ in bacterial cell division has been reviewed extensively (Bramhill, 1997; Erickson, 1997; Lutkenhaus and Addinall, 1997; Nanninga, 1998; Rothfield et al., 1999). In vivo, FtsZ assembles into a ring at the cell midpoint on the inner surface of the cytoplasmic membrane that constricts during cytokinesis. FtsZ is now known to be structurally and evolutionary related to the eukaryotic tubulins and is presumed to be their evolutionary progenitor (Erickson, 1997; Faguy and Doolittle, 1998). The structure of the FtsZ ring in vivo still has not been characterized, but the purified protein undergoes dynamic, GTP-dependent polymerization into straight filaments and curved conformations similar to those formed by tubulins (Erickson, 1997). FtsZ ring formation is the earliest known step in assembly of the bacterial cell division complex and it is essential for the subsequent midcell localization of other cell division proteins (Margolin, 1998). Some of these latter proteins are thought to be involved specifically in cell wall in-growth at the division site, which is also essential for cell division in many bacteria (Nanninga, 1998). However, FtsZ is apparently capable of mediating constriction in the absence of a cell wall in mycoplasmas, which are wall-less bacteria with highly reduced genomes (Lutkenhaus and Addinall, 1997). Most prokaryotes have only a single FtsZ gene, but there are exceptions (Faguy and Doolittle, 1998).

FtsZ IN CHLOROPLAST DIVISION

Genes encoding FtsZ are now known to be present in the nuclear genomes of divergent photosynthetic eukaryotes, and most are highly conserved with their cyanobacterial counterparts. In phylogenetic analyses, FtsZ proteins from higher plants group into two distinct families, FtsZ1 and FtsZ2, both of which are essential for plastid division (Osteryoung and Pyke, 1998; Osteryoung et al., 1998). All members of the FtsZ1 family for which full-length sequences are available are predicted with high confidence to be synthesized as precursors in the cytosol and targeted to the chloroplast by virtue of a cleavable transit

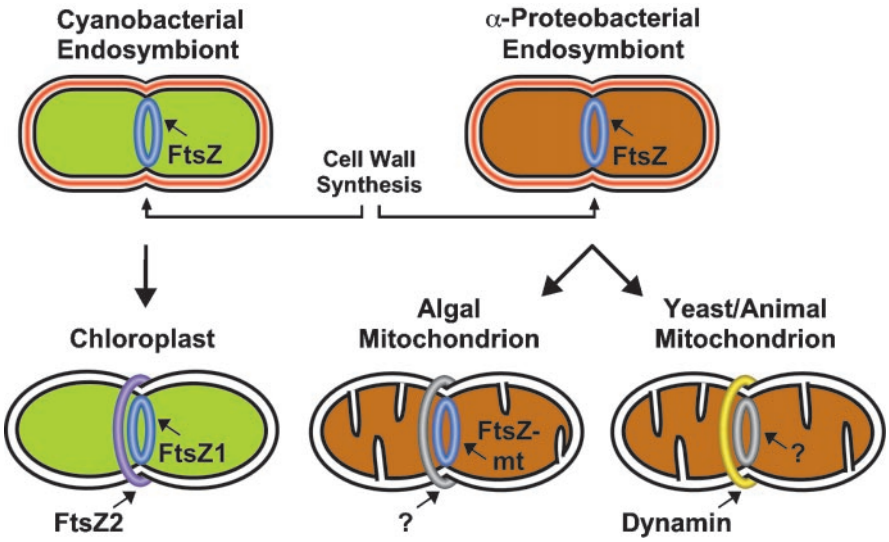

Figure 1. Postulated roles of FtsZ and dynamin in organelle constriction. In the cyanobacterial and α-proteobacterial ancestors of chloroplasts and mitochondria, FtsZ ring formation and cell wall synthesis at midcell were presumably both essential for cell division. During organelle evolution, FtsZ was recruited to function in constriction in the lineages leading to plants and algae, but not fungi or animals. In higher plant chloroplasts and algal mitochondria, FtsZ1 and FtsZ-mt are localized inside their respective organelles and may be functionally analogous to bacterial FtsZ. FtsZ2 in chloroplasts, and dynamin in yeast and animal mitochondria, are proposed to function on the cytosolic surface in organelle restriction, each perhaps having replaced the requirement for cell wall synthesis in division of the endosymbionts. The potential participation of cytosolic and organellar partners, respectively, of FtsZ-mt in algae, and dynamin in yeast and animals, is indicated by question marks.

peptide. FtsZ2 proteins lack obvious subcellular sorting signals and are postulated to be cytosolic, though this conclusion is still tentative, as there are only three full-length FtsZ2 genes from higher plants currently represented in the public databases. Nevertheless, the predicted localizations of FtsZ1 and FsZ2 correspond with electron micrographs of dividing chloroplasts in which dense deposits termed "plastid dividing rings" are clearly visible on both the stromal and cytosolic surfaces of the envelope membranes at the site of constriction (Kuroiwa et al., 1998). FtsZ1 and FtsZ2 are hypothesized to be components of the stromal and cytosolic plastid-dividing rings, respectively, and to function together in constricting the organelle (Osteryoung et al., 1998).

In support of this model, immunofluorescence microscopy has revealed that FtsZ1 and FtsZ2 colocalize to rings at the plastid midpoint (S. Vitha, R. McAndrew, and K.W. Osteryoung, manuscript in preparation), though definitive proof that the two rings are on opposite sides of the envelope awaits higher resolution imaging studies. At this writing full-length FtsZ1 and FtsZ2 sequences from a single species are only available for Arabidopsis, but expressed sequence tag collections provide evidence that both gene families are represented in other dicots, as well as in monocots (K. Stokes and K. Osteryoung, unpublished data). This suggests that plastidic and cytosolic forms of FtsZ are required for plastid division in all angiosperms.

Related *FtsZ* genes have also been identified in more primitive photosynthetic eukaryotes. The moss *Physcomitrella patens* has two *FtsZ* genes, at least one of which is essential for plastid division (Strepp et al., 1998). The proteins encoded by both moss genes are most similar to the FtsZ2 sequences from higher plants (Osteryoung et al., 1998; K. Stokes and K. Osteryoung, unpublished data), but contain amino-terminal extensions that may function as chloroplast transit peptides, though this has not been shown experimentally. Similar sequences have been uncovered in the nuclear genomes of several unicellular organisms that harbor plastids, including the red algae *Cyanidium caldarium* and *Cyanidioschyzon merolae* (Takahara et al., 1999; Beech and Gilson, 2000), the chromophyte alga *Mallomonas splendens* (Beech et al., 2000), and the cryptomonad alga *Guillardia theta* (Zauner et al., 2000). In the latter case the FtsZ protein is encoded in the nucleomorph, a remnant of a nuclear genome acquired by secondary endosymbiosis of a red alga. All of these proteins are most similar to plant and cyanobacterial FtsZs, and though their subcellular localizations have not been clearly established, their involvement in chloroplast division can reasonably be assumed.

Stromal and cytosolic plastid-dividing rings have been observed in unicellular algae as well as in land plants (Kuroiwa et al., 1998; Osteryoung and Pyke, 1998; Beech and Gilson, 2000), and in the red algae, have been characterized in considerable ultrastructural detail (Kuroiwa et al., 1998; Miyagishima et al., 1999). However, whether plastid division in these organisms involves both plastidic and cytosolic forms of FtsZ, as appears to be the case in higher

plants, remains to be seen. Nevertheless, the occurrence of closely related genes in such divergent photosynthetic organisms suggests that FtsZ was enlisted early during the evolutionary transformation of endosymbiont to chloroplast to continue its function in the division process and that it has been retained for this purpose in all lineages in which chloroplasts are present.

MITOCHONDRIAL DIVISION

A very different story is emerging in the case of mitochondrial division. Given the near-universal involvement of FtsZ in cell division in prokaryotes, including in the α-proteobacterial relatives of mitochondria (Lang et al., 1999; Rothfield et al., 1999), it came as a surprise when release of the complete sequence of the *Saccharomyces cerevisiae* nuclear genome (Goffeau et al., 1996) failed to reveal an obvious FtsZ homolog in yeast. FtsZ is also missing from the *Caenorhabditis elegans* nuclear genome (*C. elegans* Sequencing Consortium, 1998) and the mitochondrial genomes of both organisms, indicating that a different mechanism for mitochondrial division has evolved in fungi and animals that is no longer based on FtsZ.

Recent studies reveal that the role of FtsZ in yeast and *C. elegans* has been taken over at least partially by another type of GTPase, dynamin. Dynamins were originally shown to be required for endocytosis, forming a collar on the outer surface of budding vesicles that acts to pinch the vesicles off the plasma membrane (Hinshaw and Schmid, 1995; Takel et al., 1995; McNiven et al., 2000). In vitro, dynamin can form rings and spirals resembling those formed by FtsZ and tubulins (Erickson, 2000). Mutations in the genes encoding the dynamins Dnm1p in yeast (Bleazard et al., 1999; Sesaki and Jensen, 1999) and Drp-1 in *C. elegans* (Labrousse et al., 1999) cause morphological abnormalities in the mitochondria consistent with defective severing of the outer, but not the inner, mitochondrial membrane. Immunogold-labeling studies with Dnm1p and fusions of Drp-1 to green fluorescent protein indicate that both proteins are localized on the cytosolic surface of the mitochondria at points corresponding with sites of constriction (Bleazard et al., 1999; Labrousse et al., 1999). These data indicate a critical role for dynamins in mitochondrial fission, providing an important insight as to how mitochondria divide without FtsZ. It is intriguing that they also suggest that distinct molecules may be involved in severing of the inner and outer mitochondrial membranes during division.

Although FtsZ is not involved in mitochondrial division in yeast or *C. elegans*, it was almost certainly present in the endosymbiotic progenitor of mitochondria. A recent study by Beech et al. (2000) has provided evidence for what might be considered a missing link in the evolution of mitochondrial division mechanisms. As noted above, the single-celled alga *M. splendens* contains a nuclear-encoded FtsZ protein, FtsZ-cp, which is presumed to be involved in plastid division. But it also contains a second FtsZ, FtsZ-mt, which shares a high degree of similarity with the FtsZ sequences from the α-proteobacterial ancestors of mitochondria. Two experimental lines of evidence support the involvement of FtsZ-mt in mitochondrial division. First, when FtsZ-mt is expressed as a fusion to green fluorescent protein in yeast cells, the fluorescence colocalizes with mitochondria, indicating that FtsZ-mt is targeted to the mitochondrion. Second, immunofluorescence microscopy using antibodies directed against FtsZ-mt shows localization of the protein to the mitochondrial midpoint in *M. splendens*. Together, these two findings suggest that FtsZ-mt, like FtsZ1 in chloroplasts, works from inside the organelle, forming a ring on the inner membrane surface in a position analogous to that of the FtsZ ring in bacteria. Thus, bacterial FtsZ, chloroplast FtsZ1, and FtsZ-mt appear to play functionally equivalent roles in constriction.

A putative mitochondrial FtsZ has also been identified in the unicellular red alga *C. merolae* (Beech and Gilson, 2000). Ultrastructural studies of mitochondrial division in this organism have shown the presence of "mitochondrial dividing rings" both inside and outside the mitochondrion (Kuroiwa et al., 1998; Miyagishima et al., 1998). These observations suggest that constriction of mitochondria, like constriction of chloroplasts, probably involves a complex of proteins containing both organellar and cytosolic components. The fact that Dnm1p and Drp-1 appear to be involved in severing only the outer mitochondrial membrane in yeast and *C. elegans* further suggests that proper fission of the organelle requires separate structures on both membranes surfaces.

WHAT DOES IT TAKE TO CLEAVE AN ORGANELLE?

An obvious conclusion from the collective data is that there is more than one way to sever an organelle. Chloroplast division, at least in higher plants, appears to be accomplished by FtsZ-containing structures on both envelope surfaces. Mitochondrial division can involve either FtsZ from the inside or dynamin from the outside. The inference is that these two distinct types of GTPases may have evolved cognate functions in their capacities as organelle division proteins. This conjecture raises the broader question of what the structural and energetic prerequisites might be for cleaving organelles surrounded by single versus double membranes. For the former it may be sufficient that the single membranes be brought together either by pinching from the outside, as performed by dynamin during vesicle budding, or by pulling from the inside, as is apparently carried out by FtsZ in wall-less mycoplasmsas. Cleavage of

double-membrane structures, on the other hand, such as those present in chloroplasts and mitochondria, may require both pinching from one surface and pulling from the other, perhaps because more force is needed for membrane invagination, or possibly as a means of facilitating fusion of the four membrane layers as they draw together at the very latest stages of constriction. With loss of the cell wall as the organelles evolved from their endosymbiotic progenitors, this process might have necessitated the evolution of a dynamic complex with cytoskeletal properties that could assemble on both membrane surfaces to drive fission (Fig. 1). If so, we can expect that a cytosolic partner of FtsZ-mt (which could even be a dynamin) and organellar partners of Dnm1p and Drp-1 will eventually be identified. It is interesting that a mitochondrial form of dynamin that localizes to the matrix surface of the inner membrane has recently been described in *Schizosaccharomyces pombe* (Pelloquin et al., 1999). In chloroplasts FtsZ appears to operate on both membrane surfaces, though this does not preclude the involvement of dynamins or other cytoskeletal proteins as well.

Regardless of whether dividing organelles have single or double membranes, the molecules involved in constriction must also be able to undergo conformational changes associated with contractile movement, i.e. a progressive increase in curvature. Dynamins have been shown to exhibit dramatic GTP-dependent conformational changes leading to constriction and vesicle budding in vitro (Sweitzer and Hinshaw, 1998; Stowell et al., 1999), and recent work on FtsZ suggests that GTP hydrolysis may produce the force and induce the curvature needed for FtsZ ring constriction during bacterial cytokinesis (Lu et al., 2000). Whatever the biophysical and enzymatic requirements are for fission of either chloroplasts or mitochondria, it would appear that both FtsZs and dynamins may be equally up to the task.

Received March 27, 2000; accepted April 15, 2000.

LITERATURE CITED

Beech PL, Gilson PR (2000) Protist **151:** 11–16
Beech PL, Nheu T, Schultz T, Herbert S, Lithgow T, Gilson PR, McFadden GI (2000) Science **287:** 1276–1279
Bleazard W, McCaffery JM, King EJ, Bale S, Mozdy A, Tieu Q, Nunnari J, Shaw JM (1999) Nat Cell Biol **1:** 298–304
Bramhill D (1997) Annu Rev Cell Dev Biol **13:** 395–424
***C. elegans* Sequencing Consortium** (1998) Science **282:** 2012–2018
Colletti KS, Tattersall EA, Pyke KA, Froelich JE, Stokes KD, Osteryoung KW (2000) Curr Biol **10:** 507–516
Erickson HP (1997) Trends Cell Biol **7:** 362–367
Erickson HP (2000) J Cell Biol **148:** 1103–1105
Faguy DM, Doolittle WF (1998) Curr Biol **8**: R338–R341
Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Science **274:** 546, 563–567
Gray MW, Burger G, Lang BF (1999) Science **283:** 1476–1481
Hinshaw JE, Schmid SL (1995) Nature **374:** 190–192
Kuroiwa T, Kuroiwa H, Sakai A, Takahashi H, Toda K, Itoh R (1998) Int Rev Cytol **181:** 1–41
Labrousse AM, Zappaterra MD, Rube DA, van der Bliek AM (1999) Mol Cell **4:** 815–826
Lang BF, Gray MW, Burger G (1999) Annu Rev Genet **33:** 351–397
Lu C, Reedy M, Erickson HP (2000) J Bacteriol **182:** 164–170
Lutkenhaus J (1993) Mol Microbiol **9:** 403–409
Lutkenhaus J, Addinall SG (1997) Annu Rev Biochem **66:** 93–116
Margolin W (1998) Trends Microbiol **6:** 233–238
Martin W, Herrmann RG (1998) Plant Physiol **118:** 9–17
McFadden GI (1999) Curr Opin Plant Biol **2:** 513–519
McNiven MA, Cao I, Pitts KR, Yoon I (2000) Trends Biochem Sci **25:** 115–120
Miyagishima S, Itoh R, Aita S, Kuroiwa H, Kuroiwa T (1999) Planta **209:** 371–375
Miyagishima S, Itoh R, Toda K, Takahashi H, Kuroiwa H, Kuroiwa T (1998) Planta **206:** 551–560
Nanninga N (1998) Microbiol Mol Biol Rev **62:** 110–129
Osteryoung KW, Pyke KA (1998) Curr Opin Plant Biol **1:** 475–479
Osteryoung KW, Stokes KD, Rutherford SM, Percival AL, Lee WY (1998) Plant Cell **10:** 1991–2004
Osteryoung KW, Vierling E (1995) Nature **376:** 473–474
Pelloquin L, Belenguer P, Menon Y, Gas N, Ducommun B (1999) J Cell Sci **112:** 4151–4161
Rothfield L, Justice S, Gracia-Lara J (1999) Annu Rev Genet **33:** 423–428
Sesaki H, Jensen RE (1999) J Cell Biol **147:** 699–706
Stowell MH, Marks B, Wigge P, McMahon HT (1999) Nat Cell Biol **1:** 27–32
Strepp R, Scholz S, Kruse S, Speth V, Reski R (1998) Proc Natl Acad Sci USA **95:** 4368–4373
Sweitzer SM, Hinshaw JE (1998) Cell **93:** 1021–1029
Takahara M, Takahashi H, Matsunaga S, Sakai A, Kawano S, Kuroiwa T (1999) Plant Cell Physiol **40:** 784–791
Takel K, McPherson PS, Schmid SL, De Camilli P (1995) Nature **374:** 186–190
Yi Q-M, Lutkenhaus J (1985) Gene **36:** 241–247
Zauner S, Fraunholz M, Wastl J, Penny S, Beaton M, Cavalier-Smith T, Maier UG, Douglas S (2000) Proc Natl Acad Sci USA **97:** 200–205