THE PLASTID DIVISION MACHINE

Katherine W Osteryoung and Rosemary S McAndrew
Department of Plant Biology, Michigan State University, East Lansing, Michigan 48824; e-mail: osteryou@msu.edu, smithr72@msu.edu

Key Words  chloroplast, FtsZ, MinD, bacterial cell division, organelle fission, Arabidopsis

■ Abstract  Plastid division is essential for the maintenance of plastid populations in cells undergoing division and for the accumulation of large chloroplast numbers in photosynthetic tissues. Although the mechanisms mediating plastid division are poorly understood, ultrastructural studies imply this process is accomplished by a dynamic macromolecular machine organized into ring structures at the plastid midpoint. A key component of the engine that powers this machine is the motor-like protein FtsZ, a cytoskeletal GTPase of endosymbiotic origin that forms a ring at the plastid division site, similar to the function of its prokaryotic relatives in bacterial cytokinesis. This review considers the phylogenetic distribution and structural properties of two recently identified plant FtsZ protein families in the context of their distinct roles in plastid division and describes current evidence regarding factors that govern their placement at the division site. Because of their evolutionary and mechanistic relationship, the process of bacterial cell division provides a valuable, though incomplete, paradigm for understanding plastid division in plants.

CONTENTS
INTRODUCTION .......................................................... 316
MORPHOLOGICAL ASPECTS OF PLASTID DIVISION .................. 316
EVOLUTIONARY ORIGIN OF THE DIVISION APPARATUS .............. 317
FTSZ FUNCTION IN PLASTID DIVISION ............................. 318
  Bacterial FtsZ .................................................. 318
  Phylogenetic Distribution of FtsZ Genes .......................... 318
  Evolutionary and Functional Diversity Among Plant FtsZ Proteins .. 318
  Proposed Functions of FtsZ1 and FtsZ2 ........................ 321
COMPARISON BETWEEN PLANT FTSZ1 AND FTSZ2 PROTEINS ....... 322
  Structural Domains .............................................. 322
  Posttranslational Modification Motifs ............................ 324
PLACEMENT OF THE PLASTID DIVISION MACHINERY .................. 324
  Selection of the Cell Division Site in Bacteria .................. 324
  Role of MinD in Plastid Division Site Selection .................. 325
ADDITIONAL COMPONENTS OF THE PLASTID DIVISION APPARATUS .... 326
FUTURE CHALLENGES ................................................. 326
INTRODUCTION

It is perhaps premature given the sparse state of our knowledge to refer to the protein components that make up the plastid division complex as a “machine.” Nevertheless, plastid division, like other cellular processes involving remodeling of organelles, is clearly orchestrated by a macromolecular complex composed of numerous proteins that coordinate the mechanical activity required to constrict the plastid. This review focuses specifically on the molecules that are known or postulated to make up the plastid division apparatus in photosynthetic eukaryotes. Because only a few components of the division complex have been identified to date, their functions are emphasized. However, information from other systems, most prominently bacteria, is included in the context of its potential relevance to understanding plastid division. For literature related to the developmental and regulatory aspects of plastid division, the reader is referred to other reviews (9, 48–50, 85a–88).

MORPHOLOGICAL ASPECTS OF PLASTID DIVISION

A brief discussion of the morphological and ultrastructural changes that accompany progression through a plastid division cycle is in order at the outset because functional analysis of the protein participants must be interpreted within the context of what is actually observed. More detailed treatments of this subject can be found in the following references: (46, 47, 51, 86, 121). Most of the information derived from light microscopy has concentrated on observations of green chloroplasts because they are easily viewed, but ultrastructural evidence and recent molecular and genetic data suggest that the division process and associated machinery are likely to be similar for all plastid types.

At the level of light microscopy, the first visible indication that chloroplast division has commenced is the formation of a constriction located at the plastid midpoint, perpendicular to the longitudinal axis (48, 51). In land plants, division is accompanied by a lengthening and narrowing of the chloroplast, and at late stages a narrow, twisted isthmus joining the two daughter plastids is sometimes observed. At the ultrastructural level, formation of the central constriction is frequently associated with the appearance of an electron-dense annular structure termed the plastid dividing (PD) ring (11–13, 23, 38, 39, 45, 47, 51, 64, 67, 68, 79, 80, 86, 114). Such structures have been described in chloroplasts from diverse plant and algal taxa, as well as in amyloplasts and proplastids from various tissues (tabulated in 47). In the red algae Cyanidoschyzon merolae and Cyanidium caldarium, in which the ultrastructure of plastid division has been investigated in detail, the PD ring is visible early in the division process and becomes thicker as the constriction narrows, but in land plants the PD ring does not become visible until constriction is well under way. In many electron micrographs, the PD ring can be resolved into two concentric rings, one associated with the stromal surface of the inner envelope membrane and
the other associated with the cytosolic surface of the outer envelope membrane. More recent work has revealed a third electron-dense component of the PD ring localized in the intermembrane space in *C. merolae* (66). In some studies, thin filaments encircling the constriction have also been described (13, 47, 79, 116). It is generally assumed that the PD ring functions in the division process by constricting the membranes, but the molecular composition of these rings and how they function remains to be established.

**EVOLUTIONARY ORIGIN OF THE DIVISION APPARATUS**

Like many plastid-associated processes, plastid division has its evolutionary origin in the cyanobacterial endosymbiont that gave rise to chloroplasts (82). The possibility that the plastid division apparatus might have components in common with those involved in prokaryotic cell division was recognized by Possingham & Lawrence (86) and more recently by Suzuki et al (114). The latter investigators specifically suggested a possible relationship between the PD rings in *C. merolae* and the bacterial cell division protein FtsZ, an essential cytoskeletal component of the bacterial cell division apparatus that forms a contractile ring during cytokinesis (described below). This relationship was confirmed with the discovery of a nuclear gene from *Arabidopsis thaliana* that encoded a protein with over 40% amino acid identity to many bacterial FtsZ proteins and over 50% identity to the cyanobacterial FtsZs. The further demonstration that the *Arabidopsis* FtsZ protein was synthesized as a precursor and posttranslationally targeted to the chloroplast by virtue of a cleavable chloroplast transit peptide strongly suggested its involvement in chloroplast division (84).

A definitive role for plant FtsZ proteins in plastid division was subsequently confirmed in the moss *Physcomitrella patens* and in *Arabidopsis*. In the moss, homologous recombination was used to create a knockout mutation in one of two FtsZ genes present in this organism (111). Instead of having multiple chloroplasts as in wild-type plants, cells in the deletion mutants appeared to contain only a single “macrochloroplast” in every tissue examined, consistent with a defect in plastid division. In *Arabidopsis*, an antisense approach was employed to generate transgenic plants in which accumulation of AtFtsZ1-1 (formerly called cpFtsZ), the chloroplast-targeted FtsZ protein described above, was greatly reduced (83, 110). In this case as well, cells from mesophyll tissue, instead of containing the wild-type complement of approximately 100 chloroplasts (Figure 1A), contained as few as one very large chloroplast (Figure 1B). These studies demonstrated an essential role for FtsZ genes in plastid division in both nonvascular and vascular land plants, indicating that a portion of the prokaryotic cell division apparatus was recruited during the evolution of chloroplasts from their cyanobacterial ancestors to function in plastid division. Subsequent studies described later in this review have revealed that this conservation extends to other prokaryotic cell division genes as well.
FTSZ FUNCTION IN PLASTID DIVISION

Bacterial FtsZ

A brief overview of the structure and function of bacterial FtsZ is important for understanding how its eukaryotic counterparts might function in plastid division. The reader is also referred to other recent reviews (7, 25, 58, 62, 76, 104). FtsZ, like numerous other genes involved in bacterial cell division, was identified genetically in a screen for temperature-sensitive mutants of *Escherichia coli* that formed long filaments at the restrictive temperature due to defects in cell division, and hence they were termed *fts* mutants (*filamentation temperature-sensitive*) (59). The protein is encoded by an ancient and highly conserved gene found in most prokaryotes, usually in a single copy. FtsZ is now known to be a structural homologue and very likely the evolutionary precursor of the eukaryotic tubulins (26, 30, 54, 77). Like tubulin, FtsZ is a self-polymerizing, filament-forming GTPase (8, 15, 28, 72–74, 99, 101, 123), and it functions during bacterial cell division by assembling into a ring structure at the division site on the interior surface of the cytoplasmic membrane (5). Mutant analysis has shown that FtsZ ring assembly is required for the subsequent midcell localization of all other components of the cell division apparatus (1, 62). The FtsZ ring remains associated with the leading edge of the division septum throughout cytokinesis, then it disassembles immediately following cell separation before rapidly reassembling at the center of the newly formed daughter cells (1, 5, 19, 113). The in vivo structure of the FtsZ ring and how it effects bacterial cytokinesis are not yet known, but recent data suggest that GTP hydrolysis triggered by FtsZ polymerization may generate the force and induce the curvature necessary for constriction (55).

Phylogenetic Distribution of FtsZ Genes

Genes encoding FtsZ have been identified in nearly all prokaryotes, including archaeal species, cyanobacteria, and the wall-less mycoplasmas as well as in diverse eukaryotes, including unicellular algae and other protists, moss, and vascular plants (reviewed recently in 2, 33). Most of the eukaryotic FtsZ genes identified to date are related to those in cyanobacteria and are presumed to be involved in chloroplast division. Recently, a role for FtsZs in mitochondrial division has also been uncovered in the chromophyte algae and in red algae, which, in addition to their cyanobacterial-like FtsZs, contain FtsZ genes closely related to those of the α-proteobacterial ancestors of mitochondria (3, 115). However, FtsZ genes have not been found in fungi or animals, organisms in which mitochondrial division is apparently accomplished by another self-assembling GTPase, dynamin (27, 81).

Evolutionary and Functional Diversity Among Plant FtsZ Proteins

A significant number of FtsZ sequences from land plants have been submitted to the gene databases in the past few years. In phylogenetic analyses, these proteins,
all encoded in the nucleus, fall into two major groups that have been designated the FtsZ1 and FtsZ2 families (83) (Figure 2). Expressed sequence tag collections suggest that both families are represented in most angiosperms and are encoded by small gene families. *Arabidopsis* has one FtsZ1 and two FtsZ2 genes; but multiple FtsZ1 genes are present in tobacco, and a partial sequence from a fern groups with the FtsZ1 family (note *Neotropterus* in Figure 2), suggesting that FtsZ1 and FtsZ2 genes are present in all vascular plants. The only two FtsZ sequences currently available from a nonvascular plant are from *Physcomitrella*, and both of these fall into the FtsZ2 family.

The FtsZ1 and FtsZ2 gene products are more closely related to the cyanobacterial FtsZs than to those in other prokaryotes, consistent with an endosymbiotic origin in both cases, yet they differ in their overall sequence relatedness and in their predicted subcellular localizations. All members of the FtsZ1 family for which full-length sequences are available are predicted by the program TargetP (24) to contain cleavable chloroplast transit peptides at their amino-terminal ends that target them to the stromal compartment (Table 1). These predictions have been verified in in vitro chloroplast import assays for *Arabidopsis* AtFtsZ1-1 (84) and for an FtsZ1 family member from *Pisum sativum* (32). In contrast, most members of the FtsZ2 family are not predicted by TargetP to bear chloroplast transit peptides, at least not in flowering plants. Consistent with the low TargetP score
TABLE 1  TargetP predictions for the presence of an N-terminal chloroplast transit peptide for all full-length FtsZ sequences from land plants currently represented in the public databases

<table>
<thead>
<tr>
<th>Organism</th>
<th>Accession number</th>
<th>Family grouping</th>
<th>TargetP score</th>
<th>Chloroplast import assay</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arabidopsis thaliana</em></td>
<td>Q43545</td>
<td>FtsZ1</td>
<td>.974</td>
<td>Imported</td>
<td>83, 84</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>AJ133453</td>
<td>FtsZ1</td>
<td>.934</td>
<td>NPD</td>
<td>—</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>AJ271749</td>
<td>FtsZ1</td>
<td>.916</td>
<td>NPD</td>
<td>—</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>AF205858</td>
<td>FtsZ1</td>
<td>.968</td>
<td>NPD</td>
<td>—</td>
</tr>
<tr>
<td><em>Pisum sativum</em></td>
<td>Y15383</td>
<td>FtsZ1</td>
<td>.945</td>
<td>Imported</td>
<td>32</td>
</tr>
<tr>
<td><em>Tagetes erecta</em></td>
<td>AF2513460</td>
<td>FtsZ1</td>
<td>.938</td>
<td>NPD</td>
<td>69</td>
</tr>
<tr>
<td><em>Arabidopsis thaliana</em></td>
<td>AAD21440</td>
<td>FtsZ2</td>
<td>.161&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Not imported&lt;sup&gt;d&lt;/sup&gt;</td>
<td>83</td>
</tr>
<tr>
<td><em>Arabidopsis thaliana</em></td>
<td>CAB89236</td>
<td>FtsZ2</td>
<td>.374</td>
<td>NPD</td>
<td>83</td>
</tr>
<tr>
<td><em>Gentiana lutea</em></td>
<td>AAF23771</td>
<td>FtsZ2</td>
<td>.555</td>
<td>NPD</td>
<td>—</td>
</tr>
<tr>
<td><em>Nicotiana tabacum</em></td>
<td>AJ271750</td>
<td>FtsZ2</td>
<td>.131</td>
<td>NPD</td>
<td>—</td>
</tr>
<tr>
<td><em>Lilium longiflorum</em></td>
<td>AB042101</td>
<td>FtsZ2</td>
<td>.712</td>
<td>NPD</td>
<td>—</td>
</tr>
<tr>
<td><em>Physcomitrella patens</em></td>
<td>AJ249140</td>
<td>FtsZ2</td>
<td>.782</td>
<td>Imported</td>
<td>43a</td>
</tr>
<tr>
<td><em>Physcomitrella patens</em></td>
<td>AJ249138</td>
<td>FtsZ2</td>
<td>.871</td>
<td>Imported</td>
<td>43a, 111</td>
</tr>
</tbody>
</table>

<sup>a</sup>(24); <sup>b</sup>http://www.cbs.dtu.dk/services/TargetP/
<sup>c</sup>Calculated using N-terminal 130 amino acids of predicted open reading frame.
<sup>d</sup>No published data.
<sup>e</sup>Based on predicted open reading frame.

of 0.161 calculated for AtFsZ2-1, an *Arabidopsis* FtsZ2 family member (Table 1, accession number AAD21440), this protein failed to undergo import into isolated chloroplasts under the same conditions in which AtFsZ1-1 was imported (83). However, the higher TargetP score for an FtsZ2 protein from *Lilium longiflorum* suggest that some higher plant FtsZ2 family members could be imported into the chloroplast, as recently noted for the two FtsZ proteins from *Physcomitrella* (43a) (Table 1). This ambiguity underscores the limitations of the available computational resources for predicting the presence or absence of chloroplast transit peptides, and it emphasizes the need for verifying the localization predictions experimentally, particularly in cases where the scores are equivocal. A further caveat to use of TargetP and related programs is that the input sequences used in the analyses are often taken from the open reading frames (ORFs) identified by gene prediction programs, which may not accurately predict the true translational initiation sites. For example, recent database submissions have revealed that the ORF predicted from the genomic and cDNA sequences of *AtFtsZ2-1* (Table 1, accession number AAD21440) is shorter at its 5′ end than the ORFs predicted for several other FtsZ genes. However, inspection of the *AtFtsZ2-1* gene sequence reveals a second potential in-frame start codon 243 nucleotides (81 codons)
upstream of the predicted start site that corresponds closely to the position of the predicted start codon for a second Arabidopsis FtsZ2 gene, AtFtsZ2-2 (Table 1, accession number CAB89263). This suggests the possibility of misidentified or perhaps multiple translational initiation sites among FtsZ2 genes. Analysis of the longer AtFtsZ2-1 ORF by TargetP changes the chloroplast targeting score from 0.161 to a more ambiguous 0.555 and suggests that the corresponding polypeptide might be imported into chloroplasts if tested in an in vitro import assay, contrary to the results obtained using the shorter ORF (Table 1) (83). In addition, targeting prediction programs may be less reliable for proteins from taxonomic groups that are not well represented in the datasets used in their construction. Nevertheless, these programs have been invaluable for revealing a potentially important distinction between FtsZ1 and FtsZ2 proteins in flowering plants, though whether both groups are represented in all land plants, and if so whether the apparent correlation between family grouping and presence or absence of a transit peptide will apply in all cases, remains unknown.

Although the localization predictions suggest that many FtsZ2 proteins are not targeted to the stromal compartment, an essential role for them in plastid division was established by the finding that Arabidopsis plants expressing an AtFtsZ2-1 antisense transgene exhibited defects in chloroplast division identical to those observed in the AtFtsZ1-1 antisense plants (83) (Figure 1C). The demonstration that AtFtsZ1-1 protein levels were unaffected in the AtFtsZ2-1 antisense plants and vice versa (110) confirmed that FtsZ1 and FtsZ2 proteins, though postulated to be localized in different subcellular compartments, each played essential but functionally distinct roles in plastid division.

Proposed Functions of FtsZ1 and FtsZ2

A consistent feature of recent models depicting the macromolecular configuration of the plastid division apparatus is inclusion of the stromal and cytosolic PD rings observed in ultrastructural studies (47, 65, 79). Osteryoung et al (83) incorporated the results of the AtFtsZ1-1 and AtFtsZ2-1 chloroplast import and antisense experiments described above into these models by postulating that, in higher plants, FtsZ1 and FtsZ2 proteins are components of the stromal and cytosolic PD rings, respectively, functioning together on the two envelope surfaces to constrict the organelle. In partial support of this model, recent immunofluorescence localization studies have confirmed that FtsZ1 and FtsZ2 proteins colocalize to rings at the plastid midpoint in Arabidopsis and other plants (Figure 3) (S Vitha, RS McAndrew, & KW Osteryoung, submitted). Based on the combination of targeting predictions and in vitro chloroplast import experiments (32, 84), it can be reasonably concluded that the FtsZ1-containing ring is localized inside the chloroplast, though whether it represents the same structure as the stromal PD ring remains to be established. However, higher resolution techniques are needed to determine precisely where with respect to the envelope membranes the FtsZ2-containing ring is situated. Though its proposed localization on the cytosolic surface of the outer
envelope is consistent with the position of the cytosolic PD ring, these two rings may represent distinct structures, and the FtsZ2 ring may be positioned along with FtsZ1 inside the stromal compartment. If this were true, it would suggest the possibility that FtsZ1 and FtsZ2 form heterodimers in vivo, as do α- and β-tubulin (78). Alternatively, FtsZ2 could be localized in the intermembrane space, a possibility suggested by the recent description of a third PD ring positioned between the envelope membranes in the red alga *C. merolae* (66). Complicating this issue further is the fact that it has not yet been determined whether there is functional overlap between AtFtsZ2-1 and AtFtsZ2-2. The arrangement of the FtsZ1 and FtsZ2 rings remains a major outstanding issue with important implications for understanding how the plastid division complex as a whole is organized and how its component proteins function together to achieve organelle constriction.

**COMPARISON BETWEEN PLANT FTSZ1 AND FTSZ2 PROTEINS**

Although the predicted presence or absence of a chloroplast transit peptide in higher plant FtsZ proteins is correlated with their assignment to the FtsZ1 or FtsZ2 family, respectively, the phylogenetic analyses responsible for these assignments (Figure 2) are based on sequence alignments that exclude the amino-terminal portions of the proteins, which are highly variable and thus phylogenetically uninformative. It is therefore instructive to consider structural properties in addition to the transit peptides that distinguish FtsZ1 and FtsZ2 proteins from one another. This is best accomplished by comparing the plant proteins with their well-studied bacterial counterparts.

**Structural Domains**

In general, plant FtsZ proteins share most of the structural features common to the bacterial proteins (26, 53, 54). These features are highlighted in Figures 4, 5, and 6, which represent the primary (excluding the extreme amino-terminal end), secondary, and tertiary structures, respectively, of FtsZ1 and FtsZ2. All FtsZs can be divided into two major structural domains: a highly conserved N-terminal domain and a less well-conserved C-terminal domain. The N-terminal domain forms a Rossmann fold (53, 103), typical of the GTPase domain of Ras and other G-proteins. This structure contains the “tubulin signature motif,” GGGTG(T/S)G, required for GTP binding in FtsZs and tubulins (15, 25, 56, 73, 99) (note double underline in Figure 4) as well as residues that contact the guanine nucleotide (black bars) and are essential for GTP-hydrolysis (red diamonds) (120). These residues are completely conserved in the plant sequences with one exception. In FtsZ1 proteins, an alanine residue essential for GTP-binding and hydrolysis in the bacterial proteins has been replaced by a threonine residue (circled red diamond). This may be important for its function in the chloroplast stroma, as just prior to this substitution there is a helical structure (H3a) (Figure 5B) not present in FtsZ2 proteins that corresponds to the only region in the N-terminal
domain in which FtsZ1 and FtsZ2 differ significantly in their hydrophobicity (Figure 5A). Otherwise, the secondary (Figure 5B) and tertiary (Figure 6) structures of the N-terminal domains in FtsZ1, FtsZ2, and the bacterial FtsZ proteins (26, 53) are nearly identical. In addition, there is a stretch of amino acids at the amino-terminal end of E. coli FtsZ, protruding from the otherwise globular structure, that forms a small helix and is required for FtsZ ring assembly in vivo (120). This helix is not evident in the plant structures (Figure 5B), but the residues at the amino-terminal ends of the FtsZ1 and FtsZ2 proteins are also predicted to protrude (Figure 6) and may be involved in assembly of the chloroplast FtsZ rings in plants.

The C-terminal domain of FtsZ is more variable among proteins from different organisms and appears to play a more regulatory role. However, some conserved features can be distinguished. Among these are three highly conserved “synergy” residues that regulate GTP hydrolysis and are essential for cell division in E. coli, although the C-terminal domain is dispensable for GTPase activity and FtsZ polymerization per se (120). These synergy residues are completely conserved in the plant FtsZ1 and FtsZ2 families (Figure 4, red dots; Figure 6, stars). Also present in the C-terminal domain are loop structures that have been implicated in the binding of calcium (53), which is thought to stabilize FtsZ polymer networks formed in vitro (29, 75, 109, 113, 123). Similar loop structures are predicted in FtsZ1 and FtsZ2, and they may participate in the assembly of plant FtsZs into multimers, which has been reported for an FtsZ1 protein from pea (32). The carboxyl end of the C-terminal domain contains a high proportion of hydrophilic residues exposed at the surface of the protein (Figure 5A), and it resembles the microtubule-associated protein (MAP) binding regions of tubulin (20). Within this region is a small, highly conserved sequence, D/E-I/V-P-X-F/Y-L, termed the C-terminal core domain (60), which is required for direct interactions between FtsZ and two other essential cell division proteins in E. coli, ZipA and FtsA (21, 37, 52, 60, 70, 71, 120, 122). ZipA is an integral membrane protein with homology to MAPs that stabilizes the FtsZ ring structure and may be involved in anchoring it to the bacterial cell membrane (35–37, 98). FtsA is a peripheral membrane protein related to the actin/HSP70 superfamily of ATPases and also functions in part to support the FtsZ ring structure (4, 35, 61, 106). Interestingly, the C-terminal core domain and associated secondary structure are conserved in the plant FtsZ2 proteins (Figure 4, red box; Figure 5B, H11; Figure 6, H11), but they are missing from FtsZ1. This suggests that proteins similar to ZipA and/or FtsA may interact specifically with FtsZ2, and it reveals a structural distinction between the two protein families that may be relevant to their distinct functions in plastid division.

The N-terminal and C-terminal domains are connected and clearly delimited by a long central helix (Figure 5B, H7; Figure 6, H7). In bacteria, this helix contains additional residues that contact the guanine nucleotide (53). The linker helix and associated GTP-binding residues are also conserved in both FtsZ1 and FtsZ2 (Figure 4, black bars in unboxed region). The presence in both plant proteins of all structural elements required for FtsZ activity in bacteria implies that the FtsZ1 and FtsZ2 proteins catalyze similar reactions even though they have distinct functions and may be localized in different subcellular compartments.
Posttranslational Modification Motifs

Posttranslational modifications of FtsZ proteins have not been documented so far, but they occur for tubulins. In animal systems, phosphorylation by various kinases mediates tubulin assembly and stability, microtubule localization, and binding of tubulin and microtubules to other proteins such as MAPs (61a). Related modifications of tubulins also occur in higher plants (108a). Analysis of FtsZ1 and FtsZ2 for posttranslational modification consensus motifs using various online resources reveals a number of potential phosphorylation sites that are unique to, but conserved within, each family (Figure 5B). For example, FtsZ2 proteins share one tyrosine kinase recognition site (Figure 5B, blue box) and one cGMP-dependent kinase recognition site (red box) that are not present in FtsZ1 proteins. Likewise, FtsZ1 proteins may be uniquely phosphorylated by casein kinase II (Figure 5B, red diamond).

Although the consensus phosphorylation sites indicated in Figure 5B are derived primarily from studies of kinases from nonplant systems, kinases with comparable activities exist in plants (34a, 52a, 114a, 119a). Other kinds of modifications that are not easily predicted are also possible, such as palmitoylation, which mediates attachment of tubulin to membranes (10, 124). Although the predicted posttranslational modifications shown in Figure 5B would have to be tested experimentally to determine whether they occur in vivo, they nevertheless provide potential clues to mechanisms that may be important for regulating FtsZ1 and FtsZ2 function.

PLACEMENT OF THE PLASTID DIVISION MACHINERY

The many images of intact, dividing plastids present in the literature, whether obtained by conventional light microscopy (51, 68, 92, 100, 102), three-dimensional reconstruction (31, 67), or scanning electron microscopy (12, 65), invariably show the constriction positioned near the center of the organelle, perpendicular to the longitudinal axis. This implies the existence of a mechanism for ensuring proper placement of the division machinery. Recent data have revealed that this process also has a prokaryotic origin, having evolved from the mechanism controlling the site of cell division in many bacteria.

Selection of the Cell Division Site in Bacteria

In E. coli, placement of the FtsZ ring is governed by the minB operon, which encodes three gene products: MinC, MinD, and MinE (reviewed in 57, 104, 105, 112). Genetic analysis has shown that the activities of these proteins exhibit a complicated pattern of interdependency. In wild-type cells, MinC acts as a division inhibitor by suppressing formation of the FtsZ ring at all sites except the cell center (6, 17, 18) in part by destabilizing FtsZ polymerization at inappropriate sites (41). This activity is dependent on MinD, which is thought to form a heterodimer with MinC (42). Localization studies in E. coli that are based on fusions to green fluorescent protein have revealed that MinD is associated with the cell membrane but only on one side of the cell at a time. MinD oscillates between the two cell poles.
with a periodicity of less than one minute and is thought to carry along as cargo the MinC division inhibitor, which exhibits a similar membrane association and oscillatory behavior (40, 95, 96). The oscillation of MinD requires MinE, which localizes to a ring at the midcell independently of FtsZ and in turn requires MinD for its activity (97). The MinE ring prevents MinCD from acting at the cell center, thereby allowing FtsZ ring assembly, and hence cell division, only at that position. The min locus is so-named because mutations in \textit{minC} or \textit{minD} allow the FtsZ ring to assemble at aberrant sites near the cell poles, resulting in the formation of mini-cells that lack chromosomes and cannot expand (16). Mutations in \textit{minE}, on the other hand, permit MinCD to act ectopically at the midcell, thereby preventing FtsZ ring assembly at all sites and resulting in the formation of bacterial filaments. The mechanisms responsible for this complex situation are not yet fully understood.

Genes encoding MinC, MinD, and MinE are also found in numerous other prokaryotes, including the cyanobacterium \textit{Synechocystis} PCC6803 (43). MinE is absent in \textit{Bacillus subtilis}, however, and exclusion of the MinCD inhibitor from the cell center is accomplished by a different protein (104).

**Role of MinD in Plastid Division Site Selection**

That placement of the plastid division machinery in photosynthetic eukaryotes is mediated by a mechanism related to that in bacteria was strongly suggested by the discovery that homologues of MinD and MinE are encoded in the plastid genomes of the unicellular algae \textit{Chlorella vulgaris} (119) and \textit{Guillardia theta} (22). MinD is also encoded in the plastid genomes of the algae \textit{Nephroselmis olivacea} (118) and \textit{Prototheca wickerhamii} (accession number CAB53105) as well as the nuclear genomes of diverse higher plants, including \textit{Arabidopsis}, rice, and marigold (14, 69). The MinD proteins from \textit{Arabidopsis} and marigold bear cleavable chloroplast transit peptides that direct their import into isolated chloroplasts (14; J Froehlich & KW Osteryoung, unpublished results). \textit{MinC} genes have not been identified in any photosynthetic eukaryote.

A role for MinD in positioning of the chloroplast division apparatus has recently been confirmed. Using an antisense approach, Colletti et al (14) demonstrated that reduced expression of \textit{AtMinD1}, which encodes the chloroplast-targeted MinD homologue from \textit{Arabidopsis}, yielded plants that had asymmetrically constricted plastids, indicating misplacement of the division apparatus and an abnormally high degree of heterogeneity in chloroplast size and number, resembling the bacterial minicell phenotype (Figure 1D). Similar phenotypes have been described in the \textit{Arabidopsis} mutant \textit{arc11} (63), which may be allelic with \textit{AtMinD1} (14). The antisense phenotypes also had implications for the order in which components of the plastid division apparatus are assembled. The asymmetric constriction suggested that reduced levels of plastid-targeted MinD allows misplacement of the plastid-localized FtsZ1 ring, which in turn leads to misplacement of other division components and hence to asymmetric division. Therefore, these results suggested that placement of the entire plastid division apparatus is normally determined from inside the chloroplast by the position of the FtsZ1 ring (14).
ADDITIONAL COMPONENTS OF THE PLASTID DIVISION APPARATUS

Currently, \textit{FtsZ}, \textit{MinD}, and \textit{MinE} are the only obvious homologues of bacterial cell division genes known to exist in photosynthetic eukaryotes, and a role for \textit{MinE} in plastid division has yet to be demonstrated. Because the functions of most of the other bacterial cell division proteins are unknown, they provide few clues regarding the likelihood that functional counterparts might participate in plastid division. However, at least nine proteins localize to the division septum in \textit{E. coli} (62, 104), and the plastid division apparatus is likely to be at least as complex (82).

One potentially rich source of new plastid division genes is represented by the \textit{Arabidopsis arc} mutants (accumulation and replication of chloroplasts). These mutants, which exhibit various abnormalities in chloroplast number and size, define at least 11 loci that are important in the control of plastid division and expansion in plants (63, 87, 89–91, 93). The \textit{arc6} mutation, which causes drastically reduced numbers of enlarged chloroplasts and proplastids (93, 94) resembling those in plants expressing the \textit{AtFtsZ1-1} and \textit{AtFtsZ2-1} antisense transgenes (83; see also Figure 1B & 1C), is not allelic with any of the \textit{FtsZ} genes in \textit{Arabidopsis} (KA Pyke, personal communication) and may represent a regulatory step in plastid division (63). In most of the other \textit{arc} mutants, the nature of the affected locus is not obvious and could be either structural or regulatory. The phenotype of \textit{arc5}, however, in which the chloroplasts fail to separate completely and remain permanently constricted (102), strongly suggests that the \textit{ARC5} gene product is a late-acting structural component of the division machinery. Cloning of this and other \textit{arc} loci should reveal additional plastid division proteins and provide new insights as to how plastid division in plants is regulated.

Other approaches likely to yield new plastid division components include identification of proteins that interact directly with plant \textit{FtsZ} proteins and proteomics-based strategies that rely on isolation of plastid division complexes. Progress toward the latter goal is represented by the recent isolation of intact chloroplasts from synchronized cultures of \textit{C. merolae} to which PD rings remain visibly attached (65).

FUTURE CHALLENGES

As molecular dissection of the plastid division machinery moves forward, many of the questions alluded to throughout this review will begin to find answers. These include: \textit{(a)} What is the composition of the plastid division apparatus? \textit{(b)} How and in what order are its component parts assembled? \textit{(c)} How are they topologically organized? \textit{(d)} What are their biochemical activities and how do they affect constriction of the two envelope membranes? \textit{(e)} How are these activities coordinated? \textit{(f)} How is the division site specified and recognized? Because of the evolutionary relationship between plastid and prokaryotic cell division, bacterial models
will no doubt continue to inform investigation of these questions, though the finding that multiple FtsZ genes participate in plastid division already suggests some fundamental differences between the plastid and bacterial cell division processes (82). It will also be of interest to learn whether there are differences between the plastid division machineries in algae and land plants that might shed light on the evolutionary steps that gave rise to the FtsZ1 and FtsZ2 gene families during land plant evolution (33).

Related questions that remain largely unexplored include the following: 

(a) Do active mechanisms exist for controlling plastid DNA segregation and thylakoid membrane partitioning to daughter plastids during division? The former possibility has been suggested by the discovery of a plastid DNA binding protein associated with the envelope membranes (107, 108). 

(b) Is there an active mechanism governing plastid segregation during cell division? The fact that aplastidic cells are not observed (except in guard cells) (94) suggests this may be the case. 

(c) How is total plastid compartment volume perceived and regulated? That such processes exist is implied by the finding that decreases in plastid number are almost always compensated for by corresponding increases in plastid size and vice versa (87, 90). 

(d) How are plastid division and expansion integrated into the plant’s developmental program? A full understanding of plastid division will ultimately require investigation of all these issues.

LITERATURE CITED

17. de Boer PAJ, Crossley RE, Rothfield LI. 1989. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli*. *Cell* 56:641–49


50. Leech RM, Pyke KA. 1988. Chloroplast division in higher plants with particular reference to wheat. See Ref. 6a, pp. 39–62

85a. Possingham JV, Hashimoto H, Oross J. 1988. Factors that influence plastid division in higher plants. See Ref. 6a, pp. 1–20
113. Sun Q, Margolin W. 1998. FtsZ dynamics during the division cycle of


121. Whatley JM. 1988. Mechanisms and morphology of plastid division. See Ref. 6a, pp. 63–84


**NOTE ADDED IN PROOF**

Recent experiments based on cDNAs for the longer *AtFtsZ2-1* ORF referred to on page 321, as well as for *AtFtsZ2-2*, show both gene products are translocated into isolated chloroplasts and processed upon import. These results rule out the hypothesis that FtsZ2 proteins in *Arabidopsis*, and probably other plants, are components of the cytosolic PD ring.
Figure 1  Phenotypes of Arabidopsis leaf mesophyll cells from wild type (A) or transgenic plants expressing AtFtsZ1-1 (B), AtFtsZ2-1 (C) or AtMinD1 (D) antisense transgenes. The inset in D shows an asymmetrically dividing petal plastid from an AtMinD1 antisense plant. Bar = 5 µm.

Figure 3  Immunofluorescence localization of FtsZ1 (top) and FtsZ2 (bottom) in Arabidopsis (left) and pea (right) leaf sections. The right side of each panel is a bright field image of the immunostained section taken with DIC optics.
Figure 4  Comparison of FtsZ1 and FtsZ2 proteins from higher plants. Each sequence shown is a consensus derived from several full-length FtsZ1 or FtsZ2 sequences using the Clustal W program (117) at the Biology WorkBench 3.2 website (http://workbench.sdsc.edu). Sequences used to generate the FtsZ1 consensus were from Arabidopsis thaliana (U39877), Pisum sativum (Y15383), Tagetes erecta (AF251346) and Nicotiana tabacum (AJ133453, AJ271749, and AF205858). Sequences used to generate the FtsZ2 consensus were from A. thaliana (AF089738 and CAB89236), G. lutea (AAF23771), N. tabacum (AJ271750), and Lilium longiflorum (AB042101). The highly variable extreme amino termini are not included in the alignment. The N-terminal and C-terminal domains are enclosed in boxes. Residues identical in FtsZ1 and FtsZ2 are highlighted in gray. Residues in FtsZ1 that differ from FtsZ2, and vice versa, are highlighted in green and blue, respectively. Similar residues, underscored by a colon, were as specified by the default setting of the Boxshade program at the Biology WorkBench 3.2 website. Upper case letters indicate residues that are identical in all FtsZ1 or FtsZ2 family members indicated above. Lower case letters indicate amino acids that are similar within FtsZ1 or FtsZ2 family, but not identical. Residues that are not similar and not conserved within protein family are indicated by a dash. Thick black lines above the alignment show residues known to contact the guanine nucleotide in M. jannaschii FtsZ, (53) that are also conserved in the plant FtsZ sequences. Red diamonds indicate residues essential for GTPase activity in bacterial FtsZ, and a change in one of these specific to FtsZ1 is circled in black (26). Red dots indicate “synergy” residues that do not contact GTP, but are required for the regulation of GTP hydrolysis in the bacterial protein. The “tubulin signature motif” is underscored by a double black line. The “C-terminal core domain” sequence (60), specific to FtsZ2, is enclosed by a red box. Further details are provided in the text.
Figure 5  Comparison of structural properties and sequence motifs predicted for FtsZ1 and FtsZ2 proteins from higher plants. (A) Hydropathy profiles of FtsZ1 from *Arabidopsis* (U39877) (red) and FtsZ2 from *Gentiana lutea* (AAF23771) (blue), were determined using the Kyte-Doolittle x-1 method at the Weizmann Institute Bioinformatics website (http://bioinfo.weizmann.ac.il/). Regions described in the text containing the N-terminal and C-terminal domains, and the linker region connecting them (L), are separated by vertical black lines. The difference in hydrophobicity from FtsZ2 seen in the helical region H3a of FtsZ1 also is shown. (B) Alignment between FtsZ1 and FtsZ2 consensus sequences (described in legend to Figure 4) showing predicted motifs and secondary structural features. Numbers at the right of each line indicate the approximate residue numbers within the FtsZ1 and FtsZ2 proteins. The top two lines in the alignment indicate various phosphorylation sites predicted for FtsZ1 or FtsZ2 proteins that are conserved within each family. Included are those recognized by protein kinase C (yellow ovals), cGMP-dependent kinases (red box), casein kinase II (red diamond), tyrosine-dependent kinase (blue box), and those that may be phosphorylated by either cGMP-, cAMP-, or calmodulin-dependent kinases (green boxes). Predictions were made using the programs PPSEARCH (http://expasy.cbr.nrc.ca, and http://www.ebi.ac.uk), PhosphoBase (44) and NetPhos 2.0 (http://www.cbs.dtu.dk). Consensus sites were included in the figure only if they were indicated by all three programs, were conserved in all members of the FtsZ1 or FtsZ2 family listed in Figure 4, and received a NetPhos 2.0 score of at least 0.9. Secondary structural predictions made by
Figure 5  Continued
PSIPRED (http://globin.bio.warwick.ac.uk) are shown in the bottom two lines of the align-
ment. Regions predicted to be non-structured random coils, or loops, are indicated by wavy
black lines; α-helices or β-strands that are conserved in each protein family are indicated by
black or white boxes and labeled H or S, respectively. Hatched boxes indicate two helices,
H3a and H11, that are found only in FtsZ1 or FtsZ2 proteins, respectively. The strands and
helices, more fully described in the text, are numbered according to their relative positions
in the primary sequences of FtsZ1 or FtsZ2.
Figure 6  Comparison of hypothetical three-dimensional structures of FtsZ1 and FtsZ2. Ribbon diagrams of FtsZ1 (top) and FtsZ2 (bottom) were modeled using the program RasMol 2.6 from structural coordinates determined by the Swiss-Model protein modeling server (34, 85) (http://www.expasy.ch/) using the AtFtsZ1-1 (U39877) and AtFtsZ2-1 (AF089738) protein sequences, respectively, and the crystal structure of FtsZ from Methanococcus jannaschii (ID1FSZ) (54). α-Helices and β-strands of the N-terminal domain are green and blue, respectively, and those of the C-terminal domain are orange and red. The N- and C-terminal ends, and the central helices connecting the two domains (yellow) are labeled N, C, and H7, respectively; the helix containing the “C-terminal core domain” (60) in the FtsZ2 structure (light blue) is labeled H11. The Rossmann fold GTP-binding structures in the N-terminal domains of FtsZ1 and FtsZ2 are composed of alternating β-strands (S1-S6 in Figure 5B) and α-helices (H1-H5 in Figure 5B), which together form parallel β-sheets (53, 103). The loops in the C-terminal domains contain the “synergy” residues (stars). The FtsZ2 structure is shown from a slightly different angle than that of FtsZ1 to emphasize the presence of the C-terminal core domain, which is not found in FtsZ1 proteins. Otherwise, the structures are nearly superimposable. The figure is further described in the text.