Diversification of *fts*Z During Early Land Plant Evolution

Stefan A. Rensing, Justine Kiessling, Ralf Reski, Eva L. Decker

University of Freiburg, Plant Biotechnology, Sonnenstr. 5, D-79104 Freiburg, Germany

Abstract. The plastid division proteins FtsZ are encoded by a small nuclear gene family in land plants. Although it has been shown for some of the gene products that they are imported into plastids and function in plastid division, the evolution and function of this gene family and their products remain to be unraveled. Here we present two new *fts*Z genes from the moss *Physcomitrella patens* and compare the genomic structure of members of the two plant *fts*Z gene families. Comparison of sequence features and phylogenetic analyses confirm the presence of two clusters of paralogues in land plants and demonstrate that these genes were duplicated before the divergence of mosses, ferns and seed plants.

Key words: ftsZ — Plastid division — Phylogenetic tree — Physcomitrella patens — Moss

Introduction

One of the crucial events during the evolution of plants has been the acquisition of chloroplasts by endosymbiosis (Gray 1999; Martin and Herrmann 1998). Although plastids differ structurally from their cyanobacterial ancestors, the division process in both shares important similarities. In prokaryotes, the protein FtsZ is essential for cell division (Bi and Lutkenhaus 1991; Addinall et al. 1997). During the division process, FtsZ assembles into a ring-like

structure at the future division site and therefore forms a cytoskeletal scaffold to which other cell division proteins are recruited (Rothfield et al. 1999). Nuclear-encoded homologues of FtsZ are found in all plants and imported into plastids (Osteryoung and Vierling 1995, Kiessling et al. 2000). Even though the plastid-division apparatus consists of additional ring-forming constituents (reviewed in Hashimoto 2003) plant FtsZ homologues—like their progenitors in bacteria—are essential for plastid division (Osteryoung and Vierling 1995; Osteryoung et al. 1998; Strepp et al. 1998).

In addition, FtsZ seems to be the ancestor of tubulin (Erickson 1997). Despite the weak sequence similarity of FtsZ and tubulin, the three dimensional structures of both proteins strongly resemble each other (Löwe and Amos 1998; Nogales et al. 1998).

As mitochondria lack FtsZ—except those of some non-green algae (Beech et al. 2000)—it was suggested that in land plants mitochondrial FtsZ has been replaced by members of the dynamin family (Erickson 2000; Arimura and Tsutsumi 2002). However, recently a ring-forming dynamin-related protein was found in the plastids of a red alga as well (Miyagishima et al. 2003). While their cyanobacterial ancestors contain only one copy of FtsZ, the plastids of land plants possess several homologues. All *fts*Z genes found in land plants group into two small gene families, designated FtsZ1 and FtsZ2 (Osteryoung and McAndrew 2001). Whereas members of both subfamilies have been identified for several higher plants, this was not the case for any lower plant. As mosses diverged from ferns and seed plants approximately 450 million years ago (Theissen et al.
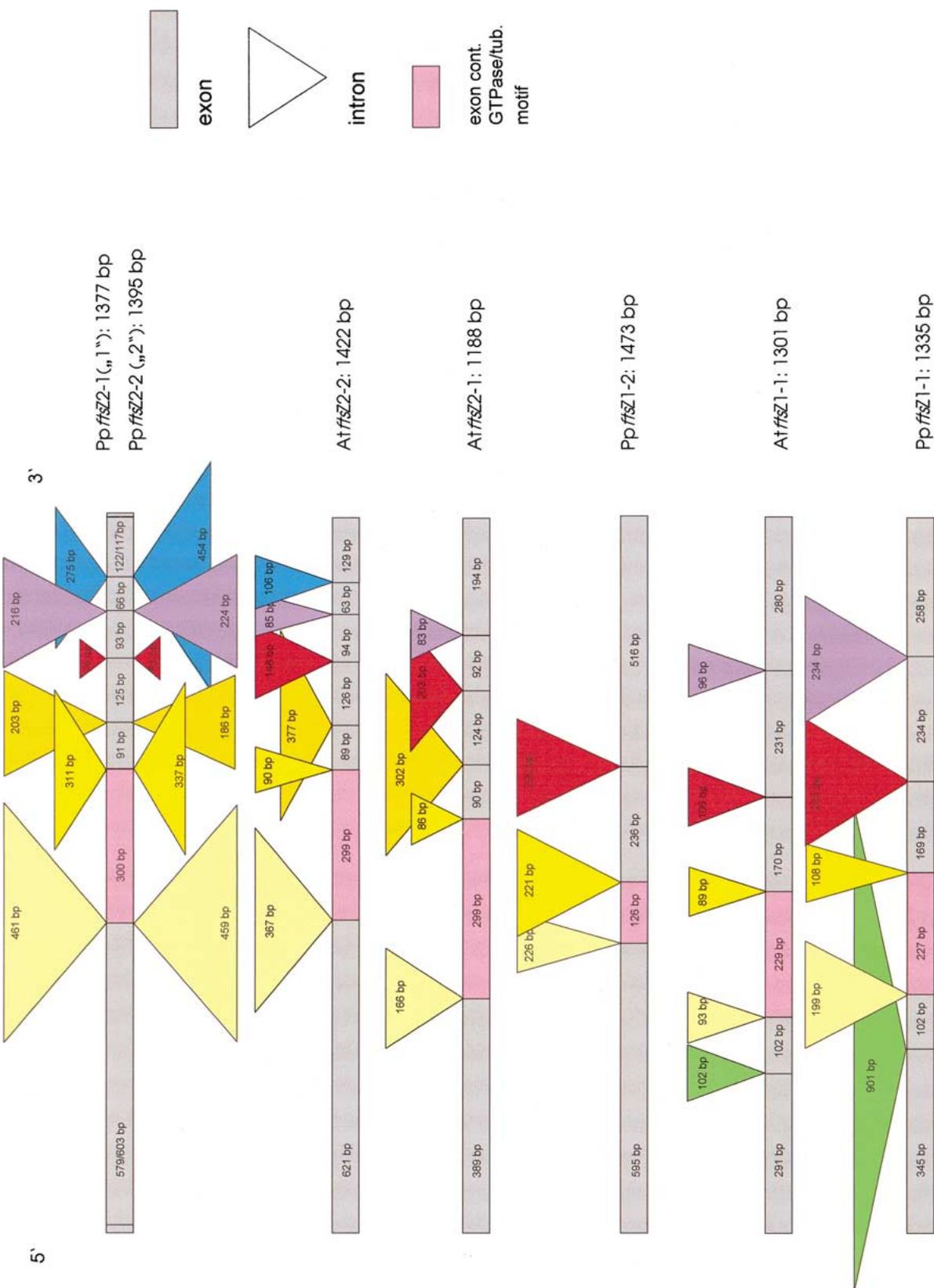
Correspondence to: Stefan A. Rensing; *email:* stefan.rensing@
biologie.uni-freiburg.de

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Fig. 1. Exon–intron structure of *Physcomitrella* and *Arabidopsis ftsZ* genes. Exons are shown as rectangular boxes: introns, as triangles. The exon containing the GTPase/tubulin motif is shown in pink. Introns that are assumed to be homologous are presented in the same color throughout the sequences.

2001), we wanted to include as many as possible moss sequences into the phylogenetic analysis to clarify when during land plant evolution the separation into two gene families took place.

We searched a clustered Physcomitrella EST database (Rensing et al., 2002) for *fts*Z homologues and subsequently cloned and sequenced the genes of two new *Physcomitrella patens fts*Z family members to enable a better understanding of the evolution of these plant gene families.

Materials and Methods

Cloning and Sequencing

Two new *Physcomitrella patens fts*Z genes are being presented in this study, *fts*Z 1-1 (AJ428993) and 1-2 (AJ428994). From a clustered EST database covering nearly the whole transcriptome (Rensing et al. 2002), clusters defining the previously unknown sequences were revealed by homology searching with members of the plant FtsZ1 family. Subsequent cloning and sequencing as well as RACE-PCR using the RLM-RACE Kit (Ambion, Germany) yielded the full-length cDNA sequences. To analyze the genomic structure of the genes, different sets of primers were synthesized. Genomic DNA was extracted as described previously (Reski et al. 1994) and used as template for PCR amplification. The resulting PCR products were subcloned in pCR 4-Topo (Invitrogen, Germany) and both strands sequenced with appropriate overlaps by primer walking.

Software

The GCG Wisconsin package 10.2 (Accelrys, USA) was used for sequence analysis as well as CLUSTAL W 1.81 (Thompson et al. 1994) for multiple sequence alignment. Homology searches were conducted with BLAST 2 (Altschul et al. 1997). Phylogenetic trees were created with TREECON 1.3b (Van de Peer and De Wachter 1994) and TREE-PUZZLE 5.1 (Schmidt et al. 2002). Peptide distances were calculated from the alignment using the GCG software Distances with Kimura parameters.

The GENPEPT database (release 124.0; www.ncbi.nlm.nih.gov), being a conceptual translation of GenBank, was used as a peptide database covering the known protein coding genes from all organisms. The FtsZ and tubulin motifs were extracted from PROSITE (release 16.37; www.expasy.ch).

Database Searches

BLASTP searches against GENPEPT as well as TBLASTN searches against the GENBANK EST_OTHER division (nonhuman/mouse) were run using full-length FtsZ peptide sequences known to belong to the plant FtsZ1 family (*Pisum sativum*) and FtsZ2 family (*Gentiana lutea*). From the significant hits a subset of 33 sequences was extracted for further analysis, covering all photosynthetic organisms (cyanobacteria, algae, and land plants). Redundant sequences were removed and some ESTs clustered (Table 1).

Alignment and Tree Reconstruction

The above-mentioned peptide sequences were subjected to a multiple sequence alignment using default parameters, leading to an alignment of 530 positions. Manual inspection of the alignment did not reveal any obvious errors, therefore it was not altered before further analyses took place. Neighbor-joining trees were done using the Tajima and Nei (1984) model; InDels were not taken into account. Maximum likelihood analysis was done using the Whelan and Goldman (2001) substitution model with data set frequencies, exact parameter estimation, and eight gamma distributed heterogeneity rates.

Results and Discussion

Isolation of Two New moss ftsZ Genes; Comparison with Arabidopsis

We cloned and sequenced both the full-length cDNA and the genomic loci of two novel *fts*Z genes. These new *Physcomitrella fts*Z homologues group in the land plant FtsZ1 family (see below) and therefore are designated *fts*Z 1-1 and *fts*Z 1-2. The two previously published *fts*Z homologues 1 and 2 (Kiessling et al. 2000) cluster in the land plant FtsZ2 clade and are therefore renamed *fts*Z 2-1 and *fts*Z 2-2, respectively.

The genomic organization of the *Physcomitrella* and *Arabidopsis fts*Z genes is shown in Fig. 1. Whereas the sequences of *fts*Z 2-1 ("1") and 2-2 ("2") remarkably resemble each other (Kiessling et al. 2000), *fts*Z 1-1 and 1-2 show a different organization. The genes for 2-1 and 2-2 both contain six introns in conserved positions and differ mainly in the slightly different sized exon 1 and intron 6. *Fts*Z 1-1, on the other hand, contains five introns about evenly spaced among the sequence, whereas 1-2 is host to just three introns that resemble the positions of introns 2, 3, and 4 of 1-1 or introns 1, 2, and 3 of 2-1/2-2. The close relationship of *fts*Z 2-1 and 2-2 makes it probable that these genes were duplicated only recently, in terms of evolutionary history. Whether or not 1-1 and 1-2 share a recent common ancestor, i.e., evolved by duplication of the gene and subsequent insertion or deletion of introns, cannot be answered yet. Although FtsZ 2-1 and 2-2 predictably have a low evolutionary distance (17.78 substitutions per 100 positions), they share with 1-1 (86.95/86.98) and 1-2 (93.63/94.81) about the same distance as the latter two among each other (92.36).

In comparison with the *Arabidopsis* genes it becomes evident that *Arabidopsis fts*Z 2-1 and 2-2 have a genomic structure resembling that of *Physcomitrella fts*Z 2-1 and 2-2, whereas *Arabidopsis fts*Z 1-1 is remarkably similar to *Physcomitrella fts*Z 1-1.

Figure 1 also displays a structural feature of the FtsZ proteins: the tubulin/GTPase motif-containing exon (pink). Because of the fundamental importance of this motif for FtsZ function, we assume that the exon harboring the tubulin/GTPase motif is homologous throughout the genes, as is also the case for the two introns bordering this exon. There are in

Table 1. Sequences used for analyses

Organism	Nomenclature	Family	Acc. No.[a]	EST	Identical to/cluster with
Arabidopsis thaliana	1-1	1	AAA82068		BAB08597, cDNA:U39877, gen.: AB010071
Ceratopteris richardii		1	BE643351	X	
Medicacgo truncatula		1	AW775962	X	
Neottopteris nidus		1	AF275720	X	
Nicotiana tabacum	1-1	1	CAB89286		
Nicotiana tabacum	1-2	1	AAF23770		
Nicotiana tabacum	1-3	1	CABS9287		
Nicotiana tabacum	1-4	1	CAB41987		
Oryza sativa		1	AAK64282		
Physcomitrella patens	1-1	1	AJ428993		
Physcomitrella patens	1-2	1	AJ428994		
Pisum sativum		1	CAA75603		
Sorghum propinquum		1	BF588200	X	
Tagetes erecta		1	AAF81220		
Triticum aestivum		1	BE444075	X	BE498211
Arabidopsis thaliana	2-1	2	AAK92779		AAC35987, BAB68127, AAD21440, cDNA: AB052757, gen.: AC006921
Arabidopsis thaliana	2-2	2	AAL07180		CAB89236, AAK63846, cDNA: AF384167, gen.: AL353912
Chlamydomonas reinhardtii		2	BE441845	X	BG855721, AV624700
Gentiana lutea		2	AAF23771		
Lilium longiflorum		2	BAA96782		
Nicotiana tabacum	2-1	2	CAB89288		
Nicotiana tabacum	2-2	2	CAC44257		
Physcomitrella patens	2-1 ("1")	2	CAB54558		
Physcomitrella patens	2-2 ("2")	2	CAB76387		
Anabaena sp.		cy-ng	AAA85526		
Cyanidium caldarium	1	cy-ng	BAA82871		
Cyanidium caldarium	2	cy-ng	BAA82090		
Cyanidioschyzon merolae		cy-ng	BAA85116		
Guillardia theta		cy-ng	CAA07676		CAB40398
Mallomonas splendens		cy-ng	AAF35433		
Nostoc sp. PCC 7120		cy-ng	CAA83241		
Synechococcus sp. PCC 7942		cy-ng	AAC26227		
Synechocystis sp. PCC 6803		cy-ng	BAA17496		

[a] The accession numbers are GENPEPT except for the ESTs (EST_OTHER) and those mentioned as cDNA/genomic (GenBank). In the case of *Chlamydomonas reinhardtii* and *Triticum aestivum*, three and two EST sequences, respectively, have been used to build longer ORFs.

total three introns shared by all the genes, the two above-mentioned ones (marked light and dark yellow) as well as another intron 3′ of those (marked in red). Whereas the FtsZ2 family contains an additional intron in the extreme 3′ end (blue; lacking from *Arabidopsis ftsZ* 2-1) and between the dark-yellow and the red introns (orange), the FtsZ1 family exhibits an additional intron in the 5′ end of the gene (green; lacking from *Physcomitrella* 1-2). The intron shown in purple is common to both families as well but absent in *Physcomitrella ftsZ* 1-2.

In terms of genomic organization, *Physcomitrella ftsZ* 2-1 and 2-2 and *Arabidopsis ftsZ* 2-2 strongly resemble each other. The same is the case for the *Physcomitrella ftsZ* 1-1 and the *Arabidopsis ftsZ* 1-1 genes. The *Arabidopsis ftsZ* 2-1 gene, however, lacks one of the family 2 introns. The *Physcomitrella ftsZ* 1-2 gene lacks two of the family 1 introns and therefore seems to be quite atypical for its subfamily.

FtsZ Peptide Sequence Features and Patterns

A schematic representation of the FtsZ peptide sequence alignment is given in Fig. 2a. It depicts the location of the PROSITE FtsZ motifs 1 and 2 as well as the C-terminal core domain (Ma and Margolin 1999) and the highly conserved central region of the protein. Both the land plant FtsZ1 and 2 families (according to Osteryoung and McAndrew [2001]; see Table 1 for family assignment) and the nongreen organisms (cyanobacteria and nongreen algae) are shown as independent groups. A detailed description of the features present in plant FtsZ sequences is given by Osteryoung and McAndrew (2001). Here we want to emphasize the differences existing within the three subfamilies, plant FtsZ1 and FtsZ2 and cyanobacteria/nongreen algae (cy-ng). The existing PROSITE pattern PS01134 is able to find FtsZ proteins from SWISS-PROT with a high accuracy (no false positives, low number of false negatives). However, the pattern
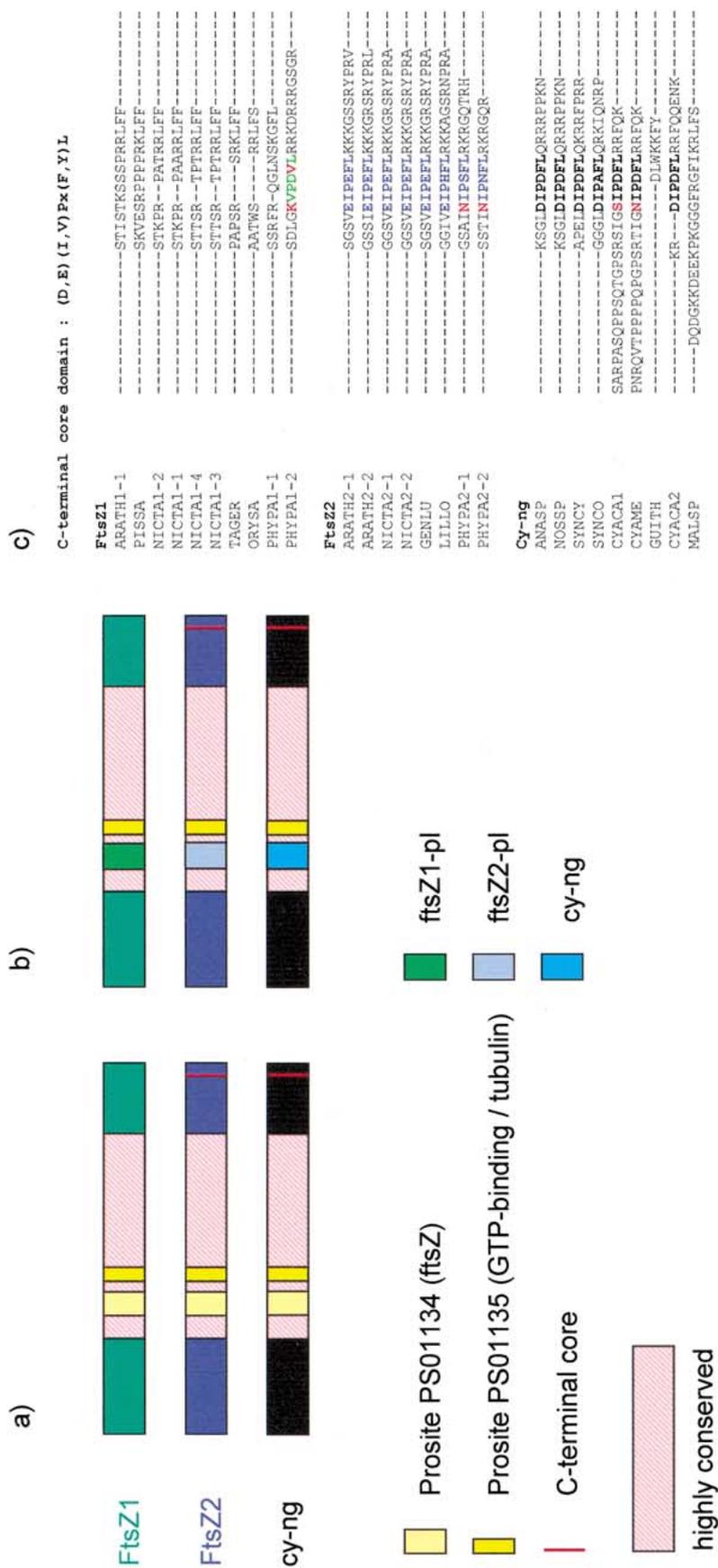
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Fig. 2. Schematic representation of FtsZ subfamilies and patterns, based on an alignment (580 aa) of chlorobiont FtsZ proteins, **a** The location of the two FtsZ PROSITE motives is shown, as well as the C-terminal core domain and the highly conserved region of the protein together with **b** the new motives. **c** Presence or absence of the C-terminal core domain including mismatches. See text for details.

fails to detect a number of plant FtsZ1/2 family members from GENPEPT (data not shown). The PROSITE pattern PS01135 covers the FtsZ GTP-binding domain that includes the tubulin pattern PS00227 and can detect FtsZ family members in SWISS-PROT with about the same accuracy as the above-mentioned pattern. It fails, however, in detecting a number of both FtsZ2 and cy-ng family members because of a mismatch (data not shown). As the region covered by PS01134 allows us to distinguish between the subfamilies FtsZ1/2 and cy-ng, we created patterns that enable this task. The pattern ftsz1-pl, (A,V)(I,V)NTDxQALxx(F,S)x(A,V)x{21, 21} (L,T)GE(Q,E), is able to find all current FtsZ1 family members in GENPEPT (without false positives/ negatives), as is the case for the pattern called ftsz2-pl, I(V,A)NTD(I,V,A)QA(M,I,L)(R,K,A)xSPVx{23,23} IG(M,C,A)(N,S,K) for FtsZ2 and (I,V,L)NTD (A,S,V)QALxxxx(A,T)x{17,17}G(N,K)P(A,E)(I,V) Gx(K,Q) for cy-ng; the position of these new patterns is shown in Fig. 2b. The patterns will allow us to scan for new FtsZ sequences belonging to those groups in further releases of the international databases. Of course they might have to be subject to refinement as more sequence information becomes available.

Phylogenetic Trees

From the intron/exon structure of the four Physcomitrella *fts*Z genes and from the phylogenetic trees is evident that an ancestral *fts*Z gene was duplicated during the evolution of photosynthetic organisms. The phylogenetic tree including all photosynthetic organisms (Fig. 3) shows a clear separation of the chlorobiont (i.e., chlorophyll a/b-bearing organisms) FtsZ families 1 and 2, designated after the nomenclature for the Arabidopsis FtsZ proteins (see e.g., Osteryoung and McAndrew 2001; Beech et al. 2000 for other phylogenetic trees).

The sequences from the cyanobacteria and nongreen algae both form strongly-supported monophyletic clades that cluster between the FtsZ1 and the FtsZ2 families. The cy-ng cluster appears to be more closely related to the FtsZ2 family, which also is the case in maximum likelihood analyses of this data set (not shown). Because cyanobacteria and nongreen algae, which appear generally to possess just one "plastid"-type FtsZ, cluster with the chlorobiont FtsZ2 family, it seems likely that the "original" (ancestral) gene was the one that evolved to become this subfamily. The duplication giving rise to the FtsZ1 family must have taken place somewhere in the evolution of the green lineage, probably in the green algae before they occupied the land. Maximum likelihood analysis of the same data set (data not shown) confirms the overall topology of the neighbor-joining tree, while introducing some multifurcating branch points instead of bifurcation.

Whereas the Physcomitrella FtsZ 2-1 and 2-2 sequences cluster within the FtsZ2 family, FtsZ 1-1 and 1-2 are found within the FtsZ1 family, albeit the latter sequence as a long-branch outgroup to the clade. The long branch of the 1-2 sequence might point to a pseudogene, however, transcription of the gene has been shown using RT-PCR (data not shown).

As can be seen from the sequences of Arabidopsis, Physcomitrella, and tobacco, the typical plant genome seems to possess a small *fts*Z gene family with at least one member to both the FtsZ1 and 2 subfamilies. This hypothesis is supported by our new Physcomitrella FtsZ sequences. Although there is no single fern or monocot plant present in the tree of which two FtsZ sequences are available, this hypothesis seems to be a valid assumption for all land plants and probably even green algae, given the fact that there are monocot sequences in both subfamilies and sequences from the moss Physcomitrella— representing the oldest living branch of land plant evolution—show up in both clades as well.

The tree is shown as unrooted. However, a phylogenetic analysis using additional sequences from eubacteria, mitochondria and Archaea (used as an outgroup root, shown as an asterisk [*] below) significantly placed the root at the branch separating the FtsZ2 clade from the FtsZ1/cy-ng clade in both neighbor-joining and maximum likelihood analyses (data not shown). In the maximum likelihood analysis of the above mentioned large data set, however, the three families were placed into a trifurcating branch point. In order to further investigate this, we calculated the likelihood of four different branching orders. Besides the determined trifurcating maximum likelihood topology [* ((cy, ng), ftsZ1, ftsZ2)] we tested the topology of the neighbour-joining tree [* ftsZ1, (ftsZ2, (cy, ng))] as well as an early branching of the whole cy-ng clade [* (cy, ng), (FtsZ1, FtsZ2)] and of the cyanobacteria alone [* cy, (ng, FtsZ1, FtsZ2)] (the latter making the most sense from an evolutionary point of view). All three topologies had a slightly worse (−10.63, −7.82 and −8.45) likelihood than the original tree and did not prove to be significantly worse in terms of the Kishino-Hasegawa (1989) test than the maximum likelihood tree. Therefore, detailed placement of the root was not possible.

The peculiar predicted position of the FtsZ1 clade might be due to a long-branch phenomenon, as has been demonstrated in other cases as well (e.g. Figge et al. 1999). On the other hand, if the FtsZ2 family has evolved to exhibit a different function than FtsZ1 (and the FtsZ of cyanobacteria and non-green algae) in the course of evolution, this might also explain the problems to resolve the correct branching order.
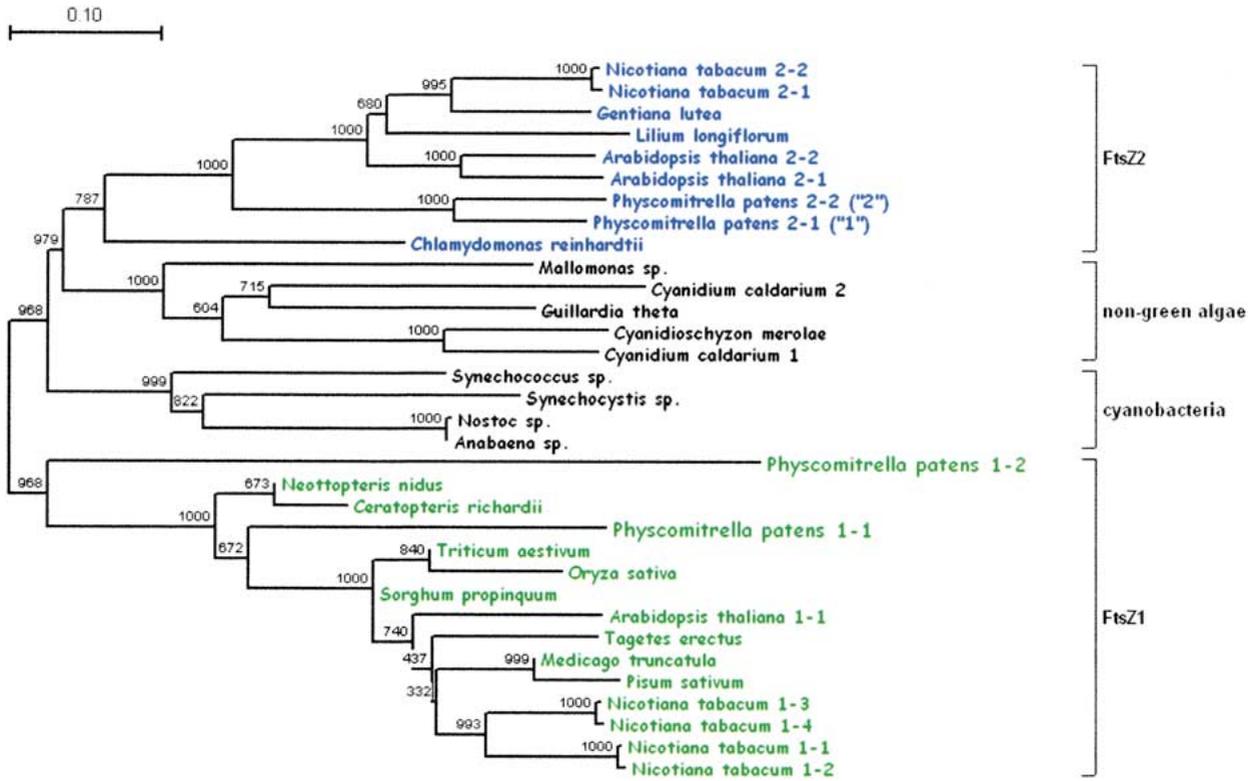
Fig. 3. Neighbor-joining phylogenetic tree based on 530 amino acid positions. Numbers at the branches result from 1000 bootstrap resamplings. See text for details.

Because of a change in function, it is to be expected that the region- or site-specific rate of acquired mutational differences will vary, potentially compromising the outcome of phylogenetic analyses.

Interaction and Possible Function

The fact that two divergent gene families with usually more than one member per family are present in plants suggests more than one function for FtsZ. The C terminus of bacterial FtsZ is described to interact with FtsA and ZipA (Ma and Margolin 1999; Yan et al. 2000; Haney et al. 2001). This short peptide pattern (Osteryoung and McAndrew 2001) can be found unambiguously in the extreme C terminus of most of the FtsZ2 peptide sequences (Fig. 2c), in Physcomitrella 2-1 and 2-2 it contains a mismatch. The pattern can also be found in most of the cy-ng family members, in some containing a single mismatch as well. The C termini of the *Guillardia theta* and *Mallomonas splendens* sequences do not contain this motif, as is the case for the FtsZ1 family with one noteworthy exception: Physcomitrella 1-2 does contain it with two mismatches. As the C-terminal domain is not tightly defined by a pattern, it remains speculative to conclude protein–protein interactions from the presence or absence of a short amino acid pattern. However, the nearly ubiquitous presence in the ftsZ2 and cy-ng family members may suggest an interaction with other members of the plastid division machinery (e.g., putative ZipA/FtsA homologues or analogues).

Import experiments performed in Physcomitrella and other organisms demonstrated that FtsZ proteins encoded by both gene families are subject to plastid localisation (Osteryoung and Vierling 1995; Gaikwad et al. 2000; Kiessling et al. 2000). The function of the gene products should be found mainly in plastids for this reason. On the other hand, when predicting cleavage sites of putative plastid transit signals, FtsZ1 family members generally exhibit a much higher score than members of the FtsZ2 family (Osteryoung and McAndrew 2001), pointing to a possible difference in localization or import patterns.

Although involvement in plastid division by means of a ring-like structure has been shown (Vitha et al. 2001), additional functions seem possible, especially in the light of the obviously paralogous (compared with the cyanobacterial gene) FtsZ1 family. Members of the FtsZ1 family have quite a different genomic organization compared to the FtsZ2 group in terms of intron/exon structure and they also apparently do not contain the C-terminal core domain thought to be involved in interaction with proteins of the plastid division machinery.

As the correct function of FtsZ1 in vivo may be dependent on tight control of stochiometry of the

interaction partners—probably including interaction of ftsZ variants with each other—it seems quite likely that particular protein levels of both FtsZ families are needed for the precise action of FtsZ in land plants.

New functions of plant FtsZ proteins have to be assumed (Reski, 2002) because of changing demands on the plastids during land plant evolution. In green algae, plastids are shaped into curious forms (the so-called chromatophores) quite often, i.e., they do not use the standard pattern of globular-shaped plastids. In both green algae and plants, plastids possess the ability to move, e.g., in dependence of light conditions. Whereas in single cellular algae the division of the sole plastid is coordinated with the division of the cell, in the cells of multicellular green algae and plants, with their higher number of plastids per cell, cell cycle-independent plastid division and its coordination becomes a much more difficult process. Whereas in lower plants plastids are always green chloroplasts, in angiosperms proplastids have the capability to develop into specialized forms like chromoplasts, amyloplasts, or chloroplasts. Members of the two FtsZ families might be part of processes that yield those differences between lower and higher plants.

Conclusions

Our phylogenetic analyses confirm the partitioning of the "plastid-type" ftsZ genes into two clearly separated groups. It is evident that a common ancestor was duplicated during the evolution of the photosynthetic organisms. By characterization of two new Physcomitrella ftsZ genes, we prove that the duplication was already established in the bryophytes as the oldest living group of land plant evolution. Therefore, it preceeded the evolution of dominating sporophytes, vascular tissue, plastid differentiation, massive RNA-editing and other features common to ferns and higher plants, but not to mosses (Reski 1998).

The divisional function of FtsZ within the cyanobacterial progenitors has apparently been conserved in both plant FtsZ families (Osteryoung et al. 1998; Strepp et al. 1998; Kiessling et al. 2000). However, the appearance of the new FtsZ1 family in the early evolution of land plants points toward an additional function for these proteins necessitated by changes to the plastids during land plant evolution. Phylogenetic development started with one big oddly shaped plastid and proceeded via fragmentation into multiple small plastids to the formation of several plastid types corresponding to tissue and environmental conditions. These changes generated organizational requirements which perhaps could be met by the fine-tuning of the primary FtsZ proteins by means of a newly arising land plant-specific FtsZ family.

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