Plant Biology ISSN 1435-8603

RESEARCH PAPER

A uniquely high number of *ftsZ* genes in the moss *Physcomitrella patens*

A. Martin, D. Lang, J. Heckmann, A.D. Zimmer, M. Vervliet-Scheebaum & R. Reski

Plant Biotechnology, Faculty of Biology, University of Freiburg, Freiburg, Germany

Keywords
Chloroplast division; FtsZ phylogeny; plastoskeleton; stromule.

Correspondence
R. Reski, Plant Biotechnology, Faculty of Biology, University of Freiburg, Schaenzlestrasse 1, 79104 Freiburg, Germany.
E-mail: ralf.reski@biologie.uni-freiburg.de

Editor
R. Mendel

Received: 24 October 2008; Accepted: 29 October 2008

doi:10.1111/j.1438-8677.2008.00174.x

ABSTRACT

Plant FtsZ proteins are encoded by two small nuclear gene families (FtsZ1 and FtsZ2) and are involved in chloroplast division. From the moss *Physcomitrella patens*, four FtsZ proteins, two in each nuclear gene family, have been characterised and described so far. In the recently sequenced *P. patens* genome, we have now found a fifth *ftsZ* gene. This novel gene has a genomic structure similar to Pp*ftsZ*1-1. According to phylogenetic analysis, the encoded protein is a member of the FtsZ1 family, while PpFtsZ1-2, together with an orthologue from *Selaginella moellendorffii*, forms a separate clade. Further, this new gene is expressed in different gametophytic tissues and the encoded protein forms filamentous networks in chloroplasts, is found in stromules, and acts in plastid division. Based on all these results, we have renamed the PpFtsZ proteins of family 1 and suggest the existence of a third FtsZ family. No species is known to encode more FtsZ proteins per haploid genome than *P. patens*.

INTRODUCTION

Chloroplasts are essential cellular organelles for plants and originate from an endosymbiotic event, in which a cyanobacterium was engulfed by a non-photosynthetic eukaryote. During evolution, most cyanobacterial genes were either transferred to the host nucleus or disappeared (Martin *et al.* 1998). Like bacteria, plastids multiply by fission division, relying on the protein FtsZ (Lutkenhaus *et al.* 1980; Osteryoung & Pyke 1998; Strepp *et al.* 1998; Gilson & Beech 2001). FtsZ proteins are widespread in Archaea and bacteria and share common features with tubulin, such as tertiary structure, GTPase activity and the ability to assemble protofilaments. They are therefore considered as evolutionary progenitors of this eukaryotic cytoskeletal element (Erickson 1995).

Plant nuclear genomes normally encode about three plastid-targeted FtsZ proteins in two families – FtsZ1 and FtsZ2 – suggesting that these proteins have differentiated functions (Osteryoung *et al.* 1998). So far, the moss *Physcomitrella patens* is known to harbour two FtsZ proteins in each of the two FtsZ families. Each PpFtsZ protein has a distinct subcellular localisation, and the four PpFtsZ proteins specifically interact with each other to build higher-order structures (Kiessling *et al.* 2000, 2004; Gremillon *et al.* 2007). Based on the complete sequence of the *P. patens* genome (Rensing *et al.* 2008), we here provide an update of the FtsZ gene family in this moss, including a fifth, and so far unknown, *ftsZ* gene.

MATERIAL AND METHODS

Plant material

Physcomitrella patens (Hedw.) B.S.G. was cultivated under standard conditions as described previously (Reski & Abel 1985; Frank *et al.* 2005).

RT-PCR

Total RNA from frozen protonema and gametophores was isolated from *P. patens* using Trizol reagent (Invitrogen, Karlsruhe, Germany). Two microgram of total RNA were used for cDNA synthesis, employing Superscript III reverse transcriptase (Invitrogen). cDNA derived from about 50 ng of total RNA was used as template for

RT-PCR. The following gene-specific primers were used: F1 (ATGGGCTCTACGGCGAGGTT) and R1 (CGCGATGTTGGGCTTGTG). As a control, RT-PCR was performed with primers C45fwd (GGTTGGTCATGG GTTGCG) and C45rev (GAGGTCAACTGTCTCGCC), corresponding to the constitutively expressed gene for the ribosomal protein L21.

Cloning of a PpFtsZ1-2-GFP fusion, transient transfection of *P. patens* protoplasts and microscopic analysis

For construction of full-length FtsZ1-2-GFP (aa 1–444), the coding region of *fts*Z1-2 was PCR-amplified with primers F2 (GGATCCATGGGCTCTACGGCGAGGTTGAG) and R2 (CCCGGGCAAAAACCCCTTTCGGTTAAGACC), introducing BamHI and SmaI restriction sites 5′ and 3′ of the cDNA, respectively. The PCR product was cleaved and cloned into the GFP reporter plasmid pMAV4. Isolation and transfection of protoplasts were carried out as described previously (Rother *et al.* 1994; Kiessling *et al.* 2004). Subcellular localisation of FtsZ-GFP fusion proteins was analysed by confocal laser scanning microscopy (CLSM) as described previously (Gremillon *et al.* 2007).

Computational analyses

Intron–exon boundaries were predicted using GenomeThreader (Gremme *et al.* 2005). For prediction of subcellular localisation, see Appendix S1. For pairwise global alignments of amino acid sequences and calculation of sequence identity values, the program Needle (Rice *et al.* 2000) was used. Multiple sequence alignments of all five *P. patens* FtsZ sequences were created using MAFFT L-INSI (Katoh *et al.* 2005). The alignment was curated manually with the Jalview multiple alignment editor (Clamp *et al.* 2004).

For phylogenetic analyses, sequences were aligned with six different alignment algorithms and combined into an optimal alignment. The phylogenetic tree was constructed using Bayesian inference. Synonymous substitution rates were calculated using codeml/PAML 3.14 (Yang 1997) (for details see Appendix S1).

RESULTS AND DISCUSSION

Identification of a fifth *P. patens ftsZ* gene

Prior to completion of the *P. patens* genome sequence, this moss was known to harbour four *fts*Z genes. To challenge this view, we performed TBLASTN searches (Altschul *et al.* 1997) against version 1.2 of the *Physcomitrella* genome sequence using all known *P. patens* FtsZ sequences (PpFtsZ1-1: Phypa_196781, PpFtsZ1-2: Phypa_211907, PpFtsZ2-1: Phypa_212480, PpFtsZ2-2: Phypa_185873). All BLAST searches yielded five hits: four corresponding to the known Pp*fts*Z genes, the fifth to a putative new Pp*fts*Z gene (gene model Phypa_187670) located on scaffold_109. This hit is most similar to Pp*fts*Z1-1, with 84.1% sequence identity at the amino acid level. Sequence alignments of PpFtsZ1-2, which, together with PpFtsZ1-1, was previously designated as a member of the FtsZ1 family (Rensing *et al.* 2004), show only 43.6% sequence identity to the new PpFtsZ and 38.9% to PpFtsZ1-1. With PpFtsZ2-1 and 2-2, the fifth PpFtsZ protein shares only 46.0% and 46.9% sequence identity, respectively (Fig. S1). These results indicate that the newly identified gene is most closely related to Pp*fts*Z1-1. Taken together with the results from further analyses (see below), we decided to name the new gene Pp*fts*Z1-2 and to rename the former Pp*fts*Z1-2 as Pp*fts*Z3. This altered nomenclature is used in the following parts of this publication.

Genomic organisation and expression of Pp*fts*Z1-2

The full-length *fts*Z1-2 cDNA was amplified and sequenced (1332 bp). Gene models from the http://www.cosmoss.org genome browser were curated using spliced alignments based on cDNA, genomic and protein sequences of PpFtsZ1-1 and the novel PpFtsZ1-2. While Pp*fts*Z1-2 covers 3539 bp, Pp*fts*Z1-1 spans 3606 bp. Both genes have six exons and five introns of almost equal size, except that the first intron is 249 bp longer in Pp*fts*Z1-2. Furthermore, the intron positions are almost identical. As described for Pp*fts*Z2-1 and 2-2 (Kiessling *et al.* 2000; Rensing *et al.* 2004), high sequence identity, conservation of intron position and lengths suggest that both genes originate from a rather recent duplication event. In contrast, Pp*fts*Z3 possesses only three introns that correspond to the second to fourth intron of Pp*fts*Z1-1 and 1-2, respectively (Fig. S2A).

Common features of FtsZ proteins are a tubulin/GTPase motif (PROSITE PS00227) and a C-terminal core domain (Ma & Margolin 1999). For PpFtsZ1-1 and 1-2, the tubulin/GTPase motif, which is involved in GTP-binding in eukaryotes (Lutkenhaus 1993), is encoded by the third exon, while it is encoded by the second exon in PpFtsZ3 (Fig. S2A). Rensing *et al.* (2004) described the existence of a C-terminal motif in PpFtsZ3, and in PpFtsZ2-1 and 2-2. Interestingly, PpFtsZ1-1 and 1-2 do not contain this motif of hydrophobic amino acids, which is probably required for interactions with other proteins of the plastid division machinery. This sets PpFtsZ3 further apart from PpFtsZ1-1 and 1-2 and thus supports renaming of the PpFtsZ1 proteins.

All four hitherto published Pp*fts*Z genes are expressed in protonema and in gametophores, the two main tissue types of mosses (Gremillon *et al.* 2007). This is also true for the novel Pp*fts*Z1-2 (Fig. S2B), indicating that all five PpFtsZ proteins are required in both tissues. Yet, the transcript level of Pp*fts*Z1-2 in gametophore tissue was considerably lower than in protonema. Notably, the same pattern was observed for Pp*fts*Z1-1 (Gremillon *et al.* 2007). In contrast, both Pp*fts*Z2 genes show considerably higher expression levels in gametophores. Thus, tissue-specific functions of PpFtsZ proteins are conceivable.

Phylogeny of FtsZ

A phylogenetic tree of FtsZ proteins was constructed using Bayesian inference (Ronquist & Huelsenbeck 2003). It includes all five *P. patens* FtsZ proteins, as well as those from nine land plants, including the recently sequenced lycophyte *Selaginella moellendorffii*, three green algae, two non-green algae, two diatoms, four cyanobacteria and four other bacteria (Eubacteria and Archaea). The tree topology confirms the two plant FtsZ families, while the sequences from the diatoms, non-green algae and cyanobacteria form monophyletic clades that are more closely related to the green FtsZ2 family (Fig. 1). Consequently, we propose that the original FtsZ, which was duplicated to form the two plant FtsZ families, was derived from the diatom/non-green algae/cyanobacteria cluster. The novel PpFtsZ1-2 is part of a bryophyte/lycophyte FtsZ1 cluster that is separate from mono- and dicotyledonous plants. A posterior probability of 1 for the PpFtsZ1-1/2 clade supports their common origin.

In contrast, PpFtsZ3 and two predicted *S. moellendorffii* proteins form a separate clade. So far, it is uncertain whether FtsZ3 proteins are restricted to mosses and lycophytes because we could not detect an orthologue in available sequences of any other bryophyte or vascular plant. Thus, one possible scenario is that FtsZ3 arose after the divergence of bryophytes (see *e.g.* Lang *et al.* 2008, for review of land plant evolution) in the lineage leading to *P. patens* and *S. moellendorffii* and was subsequently lost in spermatophytes. Availability of more sequenced bryophyte genomes, such as the upcoming *Marchantia polymorpha* genome, will help to elucidate whether the FtsZ3 family was an invention of early land plants in order to adapt to their changing environment during colonisation of the land.

To further investigate the evolutionary origin of the uniquely high number of *ftsZ* genes in *P. patens*, we assessed their evolutionary distance in terms of pairwise synonymous substitution rates (dS) (Yang 1997). A dS value of 0.6545 for the clade comprising the two PpFtsZ2 suggests an origin in the whole genome duplication (WGD) event that occurred approximately 45 million years ago (Rensing *et al.* 2007). Pairwise substitution rates comparing PpFtsZ3 to the remainder of the PpFtsZ1 clade ($dS > 7$) supports the tree topology and indicates an ancient divergence, also arguing for a distinct third FtsZ family. The pairwise dS distance between the remaining members of the PpFtsZ1 clade of 0.4067 places the duplication at the left flank of the WGD peak (dS 0.6–1.1). This indicates that either the PpFtsZ1 duplication occurred after the WGD event or that the duplication occurred during the WGD, and the two paleologs, *i.e.* duplicates derived from a large-scale duplication event, were subjected to a homogenising mechanism-like gene conversion, as has been shown recently for paleologs in rice (Wang *et al.* 2007; Xu *et al.* 2008). To test for the latter, the two Pp*fts*Z1 loci and the Pp*fts*Z3 locus where analysed for possible gene conversion tracts, revealing a 60-bp long conversion tract between Pp*fts*Z1-1 and Pp*fts*Z1-2, which overlaps with the end of the tubulin/GTPase domain (PFAM PF00091). This corroborates the finding of Wang *et al.* (2007) that functional protein domains are prone to gene conversion. Taken together, we suggest that the Pp*fts*Z1-1 and Pp*fts*Z1-2 loci are also derived from the ancient large-scale duplication event 45 million years ago, but were partly subjected to homogenising gene conversion to maintain the domain structure.

Subcellular localisation of PpFtsZ1-2

The subcellular localisation of all published PpFtsZ proteins has been investigated employing *ftsZ::gfp* fusions. PpFtsZ1-1 builds organised filamentous scaffolds within the chloroplasts and cytosolic, tubular extensions emanating from the plastid membrane (stromules) (Gremillon *et al.* 2007). PpFtsZ3 is dual-targeted, assembles into rings in the plastids as well as in the cytosol, and acts in cell and plastid division (Kiessling *et al.* 2004). PpFtsZ2-1 and 2-2 exclusively form network-like structures in the plastids. As these filamentous networks resemble the eukaryotic cytoskeleton, the term 'plastoskeleton' was coined: a novel structure to ensure chloroplast integrity (Kiessling *et al.* 2000; Reski 2002).

Arabidopsis thaliana encodes three FtsZ proteins that are all localised to plastids, forming coaligned rings at the organelle midpoint (Vitha *et al.* 2001). Similar rings have been observed in several other organisms (*e.g.* Miyagishima *et al.* 2001; Mori *et al.* 2001; Kuroiwa *et al.* 2002; Klint *et al.* 2007).

To analyse the subcellular localisation of the novel PpFtsZ1-2, C-terminal GFP fusions were generated and used for transient transfection of *P. patens* protoplasts. GFP fluorescence was visualised by CLSM. Three days after transfection, GFP signals forming filamentous structures within and surrounding the plastids were observed (Fig. 2A), confirming the *in silico* predictions of plastidic localisation for PpFtsZ1-2 (Table S1). The structural pattern of PpFtsZ1-2 resembled that of PpFtsZ1-1 and was distinct from the networks of the PpFtsZ2 proteins and the ring structures built by PpFtsZ3. Moreover, PpFtsZ1-2, like PpFtsZ1-1, was found in stromules, indicating that only the PpFtsZ proteins of family 1 are localised to these structures. Similarly, in *A. thaliana* only the sole FtsZ1 protein, but not the two FtsZ2 proteins, is found in stromules (for a review see Hanson & Sattarzadeh 2008).

Functional analysis of PpFtsZ1-2

During bacterial cytokinesis, the FtsZ ring serves as a scaffold for the specific and interdependent recruitment of more than ten other cell division proteins. Especially, the molar ratios of FtsZ, FtsA and ZipA, which are the first divisome components to localise at mid-cell, are critical for divisome functionality (Dai & Lutkenhaus 1992; Hale

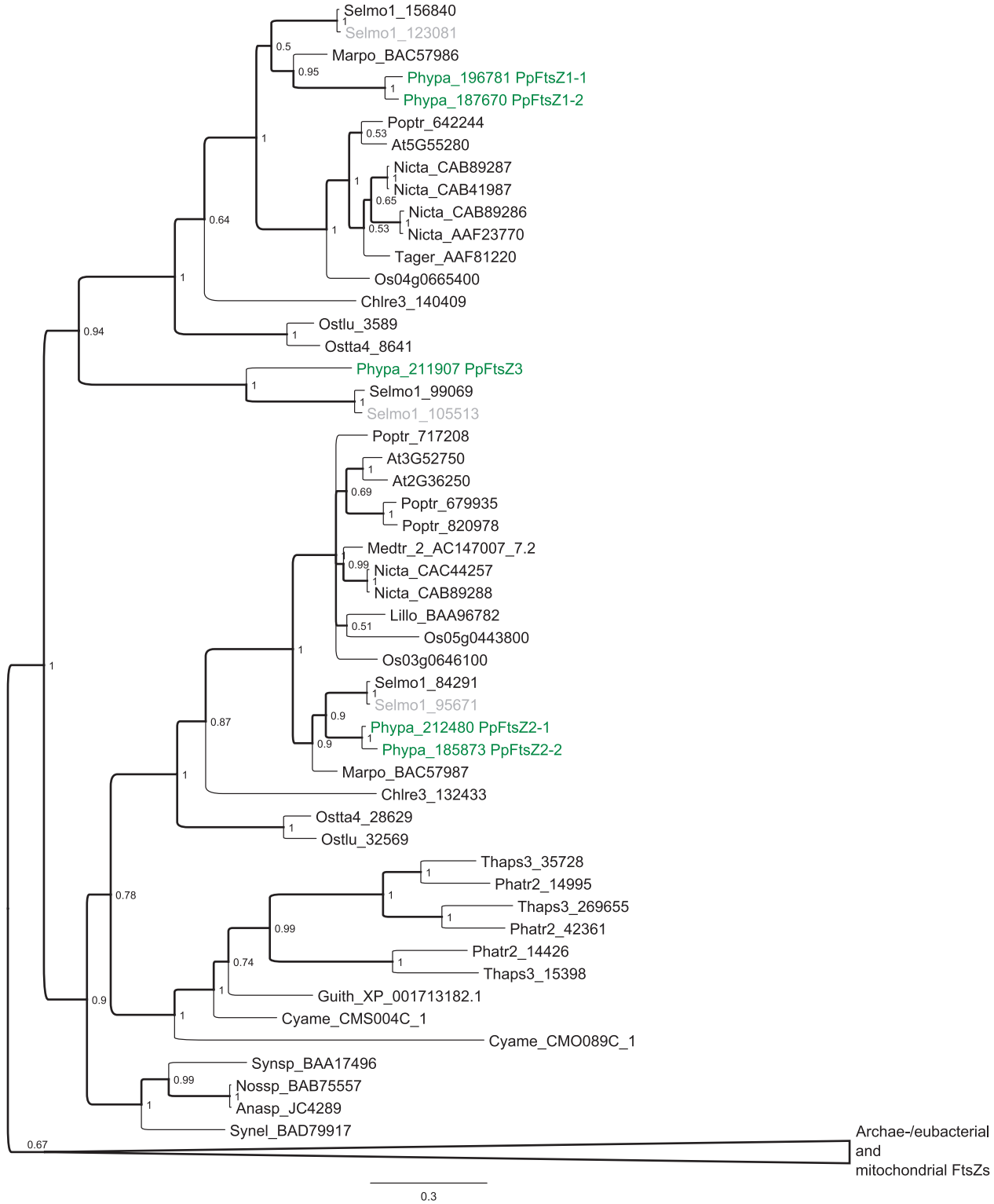

Fig. 1. Phylogenetic relationship of eukaryotic FtsZ proteins based on 366 amino acid positions from 58 taxa. The Bayesian phylogeny is shown. Branch thickness indicates posterior probabilities. *Physcomitrella* FtsZ proteins are marked in green. *Selaginella moellendorffii* (Selmo) accessions in grey indicate probably redundant sequences due to the two different haplotypes that have been sequenced. This is supported by the fact that, in all three cases, one of the gene models was removed from the haplotype-filtered release of the gene predictions (FM3). The tree is rooted using the archae-/eubacterial and mitochondrial FtsZs clade as an outgroup (shown collapsed). The bar gives the distance parameter for each tree. For species name abbreviations, see Appendix S1.

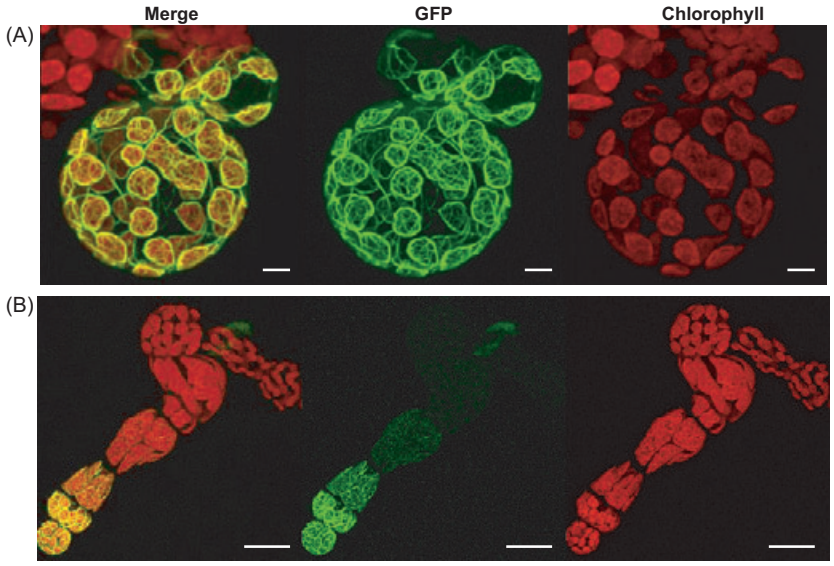

Fig. 2. A: Subcellular localisation of PpFtsZ1-2. Moss protoplasts were transiently transfected with the full-length Pp*fts*Z1-2 cDNA fused to the *gfp* gene. Three days after transfection, GFP fluorescence was visualised *via* CLSM. GFP and chlorophyll channel and the merging of both channels of the same protoplast are shown (Scale bar: 5 μm). B: Overexpression of Pp*fts*Z1-2-*gfp* has a dose-dependent effect on chloroplast division. Seventeen days after transfection with the Pp*fts*Z1-2-*gfp* construct, regenerated protoplasts were analysed *via* CLSM. GFP and chlorophyll channel and the merging of both channels of the same protoplast are shown (Scale bar: 20 μm).

& de Boer 1997). Changed dosage of FtsZ leads to aberrant phenotypes: slightly elevated FtsZ levels lead to enhanced division frequency resulting in minicell formation, while high levels of FtsZ inhibit cell division (Ward & Lutkenhaus 1985). Such dose-dependent effects were also observed for PpFtsZ1-1, 2-1 and 2-2: in transient protoplast transfection assays, low FtsZ-GFP levels led to the formation of small plastids, indicating enhanced division processes. In contrast, high FtsZ levels inhibited plastid division, leading to the accumulation of undivided macrochloroplasts (Kiessling *et al.* 2000; Gremillon *et al.* 2007).

This functional test was also applied for the novel PpFtsZ1-2. On day 17 after transfection, the first cell of protonema filaments exhibited strong GFP fluorescence and wild-type plastids (Fig. 2B). In the second to fifth cell, a drastically changed plastid shape occurred: instead of being roundish and globular, chloroplasts became enlarged and elongated. In the subsequent cells, macrochloroplasts divided until they acquired the wild-type shape and the GFP label disappeared (Fig. 2B). The gradual decrease in fluorescence and alteration in plastid shape result from dilution of the fusion protein along the axis of the growing protonema filament. The switch from macrochloroplasts to regular plastid shape points to the existence of an inhibitory threshold of fusion protein concentration with regard to plastid division. Taken together, our results indicate a role for PpFtsZ1-2 in chloroplast division.

CONCLUSION

Although *P. patens* is morphologically less complex than *A. thaliana*, the high number of *fts*Z genes in this moss may be interpreted in light of approximately 500 million years of divergent evolution (Lang *et al.* 2008). We here propose that non-seed plants like *P. patens* and *S. moellendorffii* encode a third FtsZ family that was lost in seed plants. Notably, PpFtsZ3 builds a ring structure, which, according to interaction data (Gremillon *et al.* 2007) and pharmacological evidence (Suppanz *et al.* 2007), is the backbone of a complex plastoskeleton in *P. patens* built by the four members of the PpFtsZ families 1 and 2. In addition, both members of the PpFtsZ1 family are located to stromules. The latter is in accordance with the localisation of AtFtsZ1-1 in the stromules of *A. thaliana* (Vitha *et al.* 2001). In this species, all three FtsZ proteins build ring structures similar to those in bacteria, but do not form filamentous networks. Although it is conceivable that the plastids of all species contain a plastoskeleton, it has to be determined in seed plants if this function has been taken over by proteins originating from the eukaryotic host and not by the bacterial endosymbiont. Thus, although *P. patens* and *A. thaliana* share features such as plastid fission–division and stromules, the underlying role of FtsZ isoforms differs between these two species. The phylogenetic implications described here suggest the FtsZ family as phylogenetic marker for further studies of land plant evolution.

ACKNOWLEDGEMENTS

Funding from the Deutsche Forschungsgemeinschaft (SFB388 and Re837/10) is gratefully acknowledged. We thank Stefan Rensing for helpful comments on the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Multiple amino acid sequence alignment of all *Physcomitrella patens* FtsZ proteins.

Fig. S2. Gene structure and expression analysis of *fts*Z1-2.

Table S1. Prediction of subcellular localisation using different computational methods.

Appendix S1. Prediction of subcellular localisation, phylogenetic inference and evolutionary rates and gene conversion.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

REFERENCES

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Miller W., Lipman D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*, **25**, 3389–3402.

Clamp M., Cuff J., Searle S.M., Barton G.J. (2004) The Jalview Java alignment editor. *Bioinformatics*, **20**, 426–427.

Dai K., Lutkenhaus J. (1992) The proper ratio of FtsZ to FtsA is required for cell division to occur in *Escherichia coli*. *Journal of Bacteriology*, **174**, 6145–6151.

Erickson H.P. (1995) FtsZ, a prokaryotic homolog of tubulin? *Cell*, **80**, 367–370.

Frank W., Decker E.L., Reski R. (2005) Molecular tools to study *Physcomitrella patens*. *Plant Biology*, **7**, 220–227.

Gilson P.R., Beech P.L. (2001) Cell division protein FtsZ: running rings around bacteria, chloroplasts and mitochondria. *Research in Microbiology*, **152**, 3–10.

Gremillon L., Kiessling J., Hause B., Decker E.L., Reski R., Sarnighausen E. (2007) Filamentous temperature-sensitive Z (FtsZ) isoforms specifically interact in the chloroplasts and in the cytosol of *Physcomitrella patens*. *New Phytologist*, **176**, 299–310.

Gremme G., Brendel V., Sparks M.E., Kurtz S. (2005) Engineering a software tool for gene structure prediction in higher organisms. *Information and Software Technology*, **47**, 965–978.

Hale C.A., de Boer P.A. (1997) Direct binding of FtsZ to ZipA, an essential component of the septal ring structure that mediates cell division in *E. coli*. *Cell*, **88**, 175–185.

Hanson M.R., Sattarzadeh A. (2008) Dynamic morphology of plastids and stromules in angiosperm plants. *Plant, Cell and Environment*, **31**, 646–657.

Katoh K., Kuma K., Miyata T., Toh H. (2005) Improvement in the Accuracy of Multiple Sequence Alignment Program MAFFT. *Genome Informatics*, **16**, 22–33.

Kiessling J., Kruse S., Rensing S.A., Harter K., Decker E.L., Reski R. (2000) Visualization of a cytoskeleton-like FtsZ network in chloroplasts. *Journal of Cell Biology*, **151**, 945–950.

Kiessling J., Martin A., Gremillon L., Rensing S.A., Nick P., Sarnighausen E., Decker E.L., Reski R. (2004) Dual targeting of plastid division protein FtsZ to chloroplasts and the cytoplasm. *EMBO Reports*, **5**, 889–894.

Klint J., Rasmussen U., Bergman B. (2007) FtsZ may have dual roles in the filamentous cyanobacterium *Nostoc/Anabaena* sp. strain PCC 7120. *Journal of Plant Physiology*, **164**, 11–18.

Kuroiwa H., Mori T., Takahara M., Miyagishima S.Y., Kuroiwa T. (2002) Chloroplast division machinery as revealed by immunofluorescence and electron microscopy. *Planta*, **215**, 185–190.

Lang D., Zimmer A.D., Rensing S.A., Reski R. (2008) Exploring plant biodiversity: the *Physcomitrella* genome and beyond. *Trends in Plant Science*, **13**, 542–549.

Lutkenhaus J. (1993) FtsZ ring in bacterial cytokinesis. *Molecular Microbiology*, **9**, 403–409.

Lutkenhaus J.F., Wolf-Watz H., Donachie W.D. (1980) Organization of genes in the ftsA-envA region of the *Escherichia coli* genetic map and identification of a new *fts* locus (ftsZ). *Journal of Bacteriology*, **142**, 615–620.

Ma X., Margolin W. (1999) Genetic and functional analyses of the conserved C-terminal core domain of *Escherichia coli* FtsZ. *Journal of Bacteriology*, **181**, 7531–7544.

Martin W., Stoebe B., Goremykin V., Hapsmann S., Hasegawa M., Kowallik K.V. (1998) Gene transfer to the nucleus and the evolution of chloroplasts. *Nature*, **393**, 162–165.

Miyagishima S., Kuroiwa H., Kuroiwa T. (2001) The timing and manner of disassembly of the apparatuses for chloroplast and mitochondrial division in the red alga *Cyanidioschyzon merolae*. *Planta*, **212**, 517–528.

Mori T., Kuroiwa H., Takahara M., Miyagishima S.Y., Kuroiwa T. (2001) Visualization of an FtsZ ring in chloroplasts of *Lilium longiflorum* leaves. *Plant and Cell Physiology*, **42**, 555–559.

Osteryoung K.W., Pyke K.A. (1998) Plastid division: evidence for a prokaryotically derived mechanism. *Current Opinion in Plant Biology*, **1**, 475–479.

Osteryoung K.W., Stokes K.D., Rutherford S.M., Percival A.L., Lee W.Y. (1998) Chloroplast division in higher plants requires members of two functionally divergent gene families with homology to bacterial *ftsZ*. *The Plant Cell*, **10**, 1991–2004.

Rensing S.A., Kiessling J., Reski R., Decker E.L. (2004) Diversification of ftsZ during early land plant evolution. *Journal of Molecular Evolution*, **58**, 154–162.

Rensing S.A., Ick J., Fawcett J.A., Lang D., Zimmer A., Van de Peer Y., Reski R. (2007) An ancient genome duplication contributed to the abundance of metabolic genes in the moss *Physcomitrella patens*. *BMC Evolutionary Biology*, **7**, 130.

Rensing S.A., Lang D., Zimmer A.D., Terry A., Salamov A., Shapiro H., Nishiyama T., Perroud P.F., Lindquist E.A., Kamisugi Y., Tanahashi T., Sakakibara K., Fujita T., Oishi K., Shin I.T., Kuroki Y., Toyoda A., Suzuki Y., Hashimoto S., Yamaguchi K., Sugano S., Kohara Y., Fujiyama A., Anterola A., Aoki S., Ashton N., Barbazuk W.B., Barker E., Bennetzen J.L., Blankenship R., Cho S.H., Dutcher S.K., Estelle M., Fawcett J.A., Gundlach H., Hanada K., Heyl A., Hicks K.A., Hughes J., Lohr M., Mayer K., Melkozernov A., Murata T., Nelson D.R., Pils B., Prigge M., Reiss B., Renner T.,

Rombauts S., Rushton P.J., Sanderfoot A., Schween G., Shiu S.H., Stueber K., Theodoulou F.L., Tu H., Van de Peer Y., Verrier P.J., Waters E., Wood A., Yang L., Cove D., Cuming A.C., Hasebe M., Lucas S., Mishler B.D., Reski R., Grigoriev I.V., Quatrano R.S., Boore J.L. (2008) The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants. *Science*, **319**, 64–69.

Reski R. (2002) Rings and networks: the amazing complexity of FtsZ in chloroplasts. *Trends in Plant Science*, **7**, 103–105.

Reski R., Abel W.O. (1985) Induction of budding on chloronemata and caulonemata of the moss, *Physcomitrella patens*, using Isopentenyladenine. *Planta*, **165**, 354–358.

Rice P., Longden I., Bleasby A. (2000) EMBOSS: the European Molecular Biology Open Software Suite. *Trends in Genetics*, **16**, 276–277.

Ronquist F., Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Rother S., Hadeler B., Orsini J.M., Abel W.O., Reski R. (1994) Fate of a mutant macrochloroplast in somatic hybrids. *Journal of Plant Physiology*, **143**, 72–77.

Strepp R., Scholz S., Kruse S., Speth V., Reski R. (1998) Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin. *Proceedings of the National Academy of Sciences United States of America*, **95**, 4368–4373.

Suppanz I., Sarnighausen E., Reski R. (2007) An integrated physiological and genetic approach to the dynamics of FtsZ targeting and organisation in a moss, *Physcomitrella patens*. *Protoplasma*, **232**, 1–9.

Vitha S., McAndrew R.S., Osteryoung K.W. (2001) FtsZ ring formation at the chloroplast division site in plants. *Journal of Cell Biology*, **153**, 111–120.

Wang X., Tang H., Bowers J.E., Feltus F.A., Paterson A.H. (2007) Extensive concerted evolution of rice paralogs and the road to regaining independence. *Genetics*, **177**, 1753–1763.

Ward J.E. Jr, Lutkenhaus J. (1985) Overproduction of FtsZ induces minicell formation in *E. coli*. *Cell*, **42**, 941–949.

Xu S., Clark T., Zheng H., Vang S., Li R., Wong G.K., Wang J., Zheng X. (2008) Gene conversion in the rice genome. *BMC Genomics*, **9**, 93.

Yang Z. (1997) PAML: a program package for phylogenetic analysis by maximum likelihood. *Computer Applications in the Biosciences*, **13**, 555–556.