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## Phragmoplastin, green algae and the evolution of cytokinesis

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Phragmoplast-mediated cell division characterizes the land plants in the streptophyte lineage and some species of the green algal orders Coleochaetales, Charales and Zygnematales that are basal to that lineage. This type of cell division is generally not found in the other green plant lineage, the chlorophyte algae. A well-developed phragmoplast-type cell division has been documented, however, in two subaerial green algae (*Cephaleuros parasiticus* and *Trentepohlia odorata*) belonging to the order Trentepohliales – an order that molecular sequence data place unequivocally within the chlorophytes rather than streptophytes. Is the phragmoplast-mediated cell division of the Trentepohliales a case of homology or non-homology? To gain more insight into this question, we are exploring the potential phylogenetic information inferred from gene sequences of phragmoplastin, a dynamin-like protein that has been associated with cell-plate formation during phragmoplast-mediated cytokinesis in land plants. Primers for green algae were designed based on an available phragmoplastin sequence from soybean and yielded PCR amplifications from the trentepohlialean green algae *Trentepohlia* and *Cephaleuros* and the leafy liverwort *Bazzania*. These are the first published data for phragmoplastins in algae and liverworts. Analysis of phragmoplastin gene sequences in chlorophyte and streptophyte green algae may help to chart the evolution of the development of the phragmoplast.

Phragmoplast-type cell division is basic to all land plants, but the evolution of this critically important cell process is still uncertain. Intriguingly, this type of cell division is not found in all algal taxa basal to the streptophyte lineage (land plants plus charophycean algae). Phragmoplast-mediated cytokinesis has been documented in some taxa within three of the five orders of charophycean algae: Coleochaetales (except *Chaetosphaeridium*) (Marchant & Pickett-Heaps, 1973), Charales (Pickett-Heaps, 1967) and Zygnematales (Fowke & Pickett-Heaps, 1969a, b; Groliig, 1992); it has not been documented in Chlorokybales or Klebsormidiales. Generally, phragmoplast-mediated cell division is not found

in taxa belonging to the other lineage of the green plant dichotomy, the chlorophytes.

Some green algae in the order Trentepohliales belie this neat dichotomy, however. Although the taxonomic affiliation of this subaerial green algal group was once problematic, ultrastructural (Sluiman, 1989) and molecular phylogenetic studies of the 18S rRNA (Zechman *et al.*, 1990; López-Bautista *et al.*, 2002) have positioned the order firmly inside the ulvophycean clade, a mainly marine algal group related to the chlorophycean (rather than charophycean) lineage of green algae. Chapman & Henk (1986), however, documented phragmoplast-type cell division in *Cephaleuros parasiticus* Karsten, and immunofluorescence cytochemical and ultrastructural studies (Chapman *et al.*, 2001) confirmed the presence of a phragmoplast-type cytokinesis in *Trentepohlia odorata* (Wiggers) Whitrock.

The presence of a phragmoplast-type cytokinesis in just a few algae of the chlorophycean lineage remains an unexplained evolutionary event, leading Chapman *et al.* (2001) to ask: is the phragmoplast-mediated cell division in the Trentepohliales the same as (i.e. homologous to) that in the streptophytes or is it a functionally similar but different (i.e. non-homologous) cellular process? Although

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The GenBank/EMBL/DDBJ accession numbers for the phragmoplastin sequences of *Bazzania trilobata*, *Cephaleuros virescens* (South Africa), *Cephaleuros virescens* (USA) and *Trentepohlia ilithus* are respectively AY146407–AY146410.

major developmental patterns in the phragmoplast-mediated cytokinesis of the Trentepohliales and the land plants are similar, important differences have been reported (Chapman *et al.*, 2001). Based on the particular temporal separation of karyokinesis and cytokinesis, the double cone microtubular structure and the centripetal coalescence of phragmoplast vesicles found in the Trentepohliales and not shared by the streptophytes, one could suggest parallel evolution of phragmoplast-mediated cytokinesis in the Trentepohliales.

Gu & Verma (1996) reported that a plant dynamin-like (PDL) protein isolated from soybean was associated with cell-plate formation during cytokinesis. They termed this PDL protein 'phragmoplastin' and concluded that it is associated with exocytic vesicles that deposit cell-plate materials during cytokinesis. Later studies (Gu & Verma, 1997) suggested that phragmoplastin was concentrated in the area where membrane fusion is active and that phragmoplastin participates in an early membrane fusion event during cell-plate formation. Zhang *et al.* (2000) demonstrated that phragmoplastin polymerizes into spiral coiled structures, suggesting that it may assemble into helical arrays wrapping around and squeezing vesicles into vesicle-tubule-vesicle structures during cell-plate formation. In a recent review, Verma (2001) named phragmoplastin as a marker for the phragmoplast and initiation of the cell plate.

Therefore, the key to understanding the evolutionary history of the phragmoplast cytokinetic system may lie in the study of phragmoplastin. Previous research on phragmoplastin has only involved higher land plants, so the important first step is to determine whether the phragmoplastin gene is also found in more basal taxa. In this research note, we report the results of preliminary research designing primers and amplifying the phragmoplastin gene from three algal taxa and one liverwort.

Phragmoplastin sequences were obtained from GenBank for two isolates of *Glycine max* (U25547 and U36430), *Arabidopsis thaliana* (L36939) and *Nicotiana tabacum* (AJ244024). DNA samples were obtained from *Bazzania trilobata* (L.) S. Gray (liverwort) (provided by Yin-Long Qui; collected by Karen Renzaglia, s.n.) and *Chlamydomonas* sp. D66. Plant samples were *Trentepohlia iolithus* (L.) Wallroth from Ireland and two isolates of *Cephaleuros virescens* Kunze in Fries; one from the LSU campus, Baton Rouge, USA, and isolate SAG 119.80 from Natal, South Africa.

Total cellular DNA was extracted from *Trentepohlia iolithus* and *Cephaleuros virescens* samples as described in the handbook for DNA isolation from plant tissue from the DNeasy Plant Mini kit (Qiagen). The nucleic acid pellet was dissolved in sterile distilled water. DNA samples were stored at  $-20^{\circ}\text{C}$ . The integrity of DNA preparations was checked by electrophoresis in agarose gels stained with ethidium bromide.

DNA amplification by PCR of the phragmoplastin gene was accomplished by using primers designed from the conserved regions of the phragmoplastin gene of *Glycine max* (Gu & Verma, 1996). Template DNA was amplified in 0.2 ml thin-wall PCR tubes in a total reaction volume of 12  $\mu\text{l}$ . Each amplification reaction consisted of 2  $\mu\text{l}$  template DNA, 0.625  $\mu\text{l}$  of two flanking primers, 1.25  $\mu\text{l}$  each of  $\text{MgCl}_2$  (25 mM), Thermophilic DNA polymerase 10  $\times$  buffer and 1 mM dNTPs, 0.1  $\mu\text{l}$  *Taq* DNA polymerase (New England Biolabs) and sterile distilled water. Negative controls without DNA templates were included.

Amplification cycles were controlled in a GeneAmp PCR System 2400 (Perkin-Elmer) thermocycler. An initial denaturation step at  $95^{\circ}\text{C}$  for 3 min was followed by five cycles, each consisting of 1 min at  $95^{\circ}\text{C}$ , primer annealing at  $40^{\circ}\text{C}$  for 1 min and an extension step at  $68^{\circ}\text{C}$  for 2 min. Thirty-five cycles followed consisting of 1 min at  $95^{\circ}\text{C}$ , primer annealing at  $42^{\circ}\text{C}$  for 1 min and an extension step at  $70^{\circ}\text{C}$  for 2 min.

Amplified products and a standard 1 kb DNA ladder (Life Technologies) were subjected to electrophoresis on 0.8% agarose gels stained with ethidium bromide and analysed for correct length, yield and purity. PCR products were purified through a low-melting-point agarose gel (SeaPlaque agarose BMA). Embedded PCR products were viewed under UV illumination, separated with sterile razor blades and transferred to 0.5 ml centrifuge tubes. Agarose digestion was performed in a thermocycler for 5 min at  $70^{\circ}\text{C}$  followed by  $42^{\circ}\text{C}$  with 0.5  $\mu\text{l}$  Gelase for 2 h. PCR products were checked for concentration in an agarose gel against a DNA ladder (see above).

Purified PCR amplification products were sequenced with the same PCR primers using Big-Dye terminator cycle-sequencing ready reactions (Applied Biosystems) in an ABI 3100 Prism automated sequencer. DNA sequences were captured as both text and colour-coded electrophoregrams. Sequence data were visualized using Sequencher and as NBRF files. An alignment was performed using Sequencher and visually inspected using MacClade 4 (Maddison & Maddison, 2002); the alignment is available upon request from the authors. Phylogenetic analyses were performed using the computer program PAUP\* 4.0b10 (Swofford, 2002) for phylogenetic reconstruction. Given the number of taxa under analysis (Swofford *et al.*, 1996), an exhaustive search from the parsimony criterion was selected (Nei & Kumar, 2000). As a measure of internal support, a bootstrap resampling technique was used (Graur & Li, 2000) based on 10 000 resampling replications with a 50% majority-rule consensus tree selected. Tree length and consistency (CI) and retention (RI) indexes were also calculated.

The successfully designed primers were based on conserved regions of the phragmoplastin sequence of *Glycine max* (GenBank accession no. U25547). The forward primer (FRAF0R; 5'-CCTAATCTCTTTGGTCAACAAAAT-3', 24

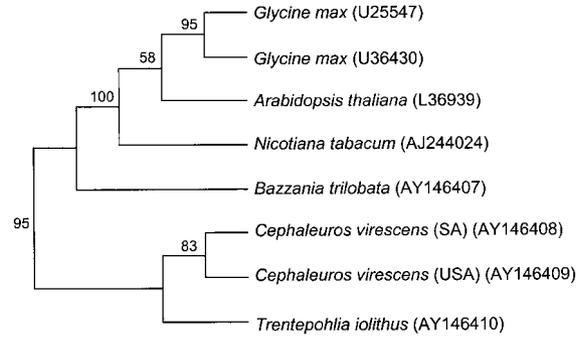
bases) anneals to *Glycine max* positions 183–206. The reverse primer (FRAREV; 5'-GGTTGACCTCTACAGCT-ACT-3', 21 bases) anneals to *Glycine max* positions 636–616.

PCR products were obtained for *Trentepohlia iolithus*, two isolates of *Cephaleuros virescens* and *Bazzania trilobata*. PCR products, when compared to a standard DNA ladder, showed a size of ~500 bp, the expected size for the section of the phragmoplastin gene under amplification. All sequences obtained were submitted to BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>), and the results confirmed the similarity to phragmoplastin sequences previously deposited in GenBank.

Efforts to amplify the phragmoplastin gene from *Chlamydomonas* sp. failed, despite attempts using many different PCR conditions. Although there are other possible reasons for the negative results, they may be related to the mode of cell division in *Chlamydomonas*, which is characterized by phycoplast-mediated cytokinesis. *Chlamydomonas* is assigned to the Volvocales, an order that molecular phylogenetic studies (Buchheim *et al.*, 1990) have positioned in the class Chlorophyceae (chlorophyte lineage rather than streptophyte lineage). This result in *Chlamydomonas* is interesting; if phragmoplastin is functioning mainly in the coalescence of vesicles during phragmoplast-mediated cytokinesis, its presence would not be necessary during cytokinesis in *Chlamydomonas*, which does not involve vesicle coalescence. It would be appropriate to look for this gene in phycoplast-containing algae that do form cell plates (such as *Stigeoclonium* and *Oedogonium*) and particularly in *Spirogyra*, a genus in the streptophyte lineage that undergoes cleavage, cell-plate formation and vesicle fusion (McIntosh *et al.*, 1995), as well as all those related members of the Zygnematales (desmids and filamentous forms).

Phragmoplastin sequences from the green algae *Trentepohlia iolithus* and *Cephaleuros virescens* and the liverwort *Bazzania trilobata* as well as the higher land plant sequences from GenBank (*Glycine*, *Arabidopsis* and *Nicotiana*) were aligned and visually inspected. Land plants, represented by *Glycine*, *Arabidopsis*, *Nicotiana* and *Bazzania*, formed a group that was aligned relatively easily. The green algal sequences showed more variation, and they aligned better within the group than with the land plant sequences. Two *Cephaleuros virescens* isolates, one from the USA and another from South Africa, showed substantial differences in their phragmoplastin sequences despite belonging to the same species. This interesting result is not surprising. Analysis of the 18S rDNA sequences of the two samples, as well as another set of isolates from Taiwan and South Africa, indicates that this taxon may consist of different species sharing a convergent morphology (López-Bautista *et al.*, 2002).

The parsimony analysis performed on this preliminary database is shown in Fig. 1. Although distance methods (neighbour-joining) with bootstrap resampling were also



**Fig. 1.** Maximum-parsimony tree based on partial phragmoplastin sequences, estimated from exhaustive search. A total of 584 positions are represented in the phylogram. GenBank accession numbers are given in parentheses. Tree length = 1090; CI = 0.868; RI = 0.636. Bootstrap values represent percentages derived from 10 000 replicates.

applied (data not shown), they did not resolve the tree; in contrast, parsimony methods did resolve the phylogenetic tree fully. As expected, the isolates of *Glycine max* form a group closely related to *Arabidopsis thaliana* and *Nicotiana tabacum*. *Cephaleuros virescens* from Baton Rouge, LA, USA, and from South Africa form another group closely related to *Trentepohlia iolithus*. The liverwort *Bazzania trilobata* forms an intermediate clade between the higher land plants and the green algal group.

These preliminary results indicate that comparative study of the phragmoplastin genes of green plants is feasible. More taxon samples, especially from both charophycean and chlorophycean algae, as well as complete sequences of the phragmoplastin gene are needed in order to evaluate the usefulness of this gene in clarifying the evolutionary history of the phragmoplast. Brown & Lemmon (1993) stated that clues to the evolution of the mitotic and cytokinetic apparatus in higher plants are found in simple land plants. We believe, however, that the solution to this puzzle resides further down the taxonomic scale, in the charophycean green algae that are basal to the streptophyte lineage and in the green algae of the chlorophyte lineage. The charophycean taxa *Spirogyra* and *Mougeotia*, for example, have shown a perhaps 'primitive' phragmoplast-mediated cytokinesis different from that of land plants; *Coleochaete* has even been reported to exhibit two types of cytokinesis, the typical phragmoplast and a variant, in the same thallus (Marchant & Pickett-Heaps, 1973; Brown *et al.*, 1994; but see also comments on polarized cytokinesis in Doty *et al.*, 2002). The chlorophycean Trentepohliales, with their phragmoplast-mediated cytokinesis, exhibit the 'wrong' type of cell division for their phylogenetic lineage! Exploration of the phragmoplastin gene may unveil the evolutionary history of the development of the phragmoplast and shed some light on the early events of colonization of the land by the green algae.

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