

Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga *Bigelowiella natans*

John M. Archibald, Matthew B. Rogers, Michael Toop, Ken-ichiro Ishida*, and Patrick J. Keeling†

Canadian Institute for Advanced Research, Program in Evolutionary Biology, Department of Botany, University of British Columbia, 3529-6270 University Boulevard, Vancouver, BC, Canada V6T 1Z4

Edited by Jeffrey Donald Palmer, Indiana University, Bloomington, IN, and approved April 18, 2003 (received for review February 18, 2003)

Chlorarachniophytes are amoebflagellate algae that acquired photosynthesis secondarily by engulfing a green alga and retaining its plastid (chloroplast). An important consequence of secondary endosymbiosis in chlorarachniophytes is that most of the nuclear genes encoding plastid-targeted proteins have moved from the nucleus of the endosymbiont to the host nucleus. We have sequenced and analyzed 83 cDNAs encoding 78 plastid-targeted proteins from the model chlorarachniophyte *Bigelowiella natans* (formerly *Chlorarachnion* sp. CCMP621). Phylogenies inferred from the majority of these genes are consistent with a chlorophyte green algal origin. However, a significant number of genes ($\approx 21\%$) show signs of having been acquired by lateral gene transfer from numerous other sources: streptophyte algae, red algae (or algae with red algal endosymbionts), as well as bacteria. The chlorarachniophyte plastid proteome may therefore be regarded as a mosaic derived from various organisms in addition to the ancestral chlorophyte plastid. In contrast, the homologous genes from the chlorophyte *Chlamydomonas reinhardtii* do not show any indications of lateral gene transfer. This difference is likely a reflection of the mixotrophic nature of *Bigelowiella* (i.e., it is photosynthetic and phagotrophic), whereas *Chlamydomonas* is strictly autotrophic. These results underscore the importance of lateral gene transfer in contributing foreign proteins to eukaryotic cells and their organelles, and also suggest that its impact can vary from lineage to lineage.

molecular evolution | chlorarachniophytes | chloroplast | phylogeny | secondary endosymbiosis

It is now clear that the plastids (chloroplasts) of photosynthetic eukaryotes ultimately arose from a single, ancient endosymbiosis between a heterotrophic eukaryote and a photosynthetic cyanobacterium (1–3). It is also clear that the primary plastids of red and green algae have spread laterally among distantly related organisms through the process of secondary endosymbiosis, in which a heterotrophic eukaryote engulfs a primary plastid-containing alga and retains its photosynthetic apparatus (1, 2, 4).

Secondary endosymbiosis has been a major force in eukaryotic evolution and is responsible for generating an enormous amount of algal biodiversity. Seven distinct eukaryotic groups harbor plastids derived from secondary endosymbiosis: haptophytes, heterokonts (stramenopiles), cryptomonads, dinoflagellates, apicomplexans, euglenids, and chlorarachniophytes (1, 4). The chlorarachniophytes are a relatively small group of mixotrophic marine flagellates and amoebflagellates with green plastids surrounded by four membranes (5, 6). Together with the cryptomonads, the chlorarachniophytes are distinguished by the retention of a “nucleomorph,” the reduced algal endosymbiont nucleus that resides between the plastid inner and outer membrane pairs (2, 6). The chlorarachniophyte host belongs to a large and diverse lineage referred to as Cercozoa (7–10). The chlorarachniophyte endosymbiont, on the other hand, is derived from a green alga. This finding was first suggested based on plastid pigments (5), and is now supported by numerous molec-

ular phylogenies inferred from plastid- and nucleomorph-encoded genes (11–15). More specifically, these analyses point to the chlorophyte green algae as the probable source of the endosymbiont (12, 13, 15).

All plastid genomes, both primary and secondary, encode only a small fraction of the proteins required for plastid function (16). The vast majority of plastid proteins are encoded by genes in the host nuclear genome, and are posttranslationally targeted to the plastid with N-terminal-targeting peptides (17). Protein targeting to primary plastids is mediated by a transit-peptide, whereas proteins targeted to secondary plastids are first directed to the endomembrane system with a signal peptide, and then to the plastid with a transit peptide (18–20). It is generally believed that many of these nucleus-encoded, plastid-targeted proteins were originally encoded in the cyanobacterial genome, and that the genes were transferred to the host nuclear genome during the integration of the endosymbiont. In secondary plastid-containing algae, most or all of these genes have subsequently moved from the primary algal nucleus to the secondary host nucleus. The protein complement of a plastid should thus reflect a common evolutionary ancestry.

Here, we have characterized 83 genes for plastid-targeted proteins in the chlorarachniophyte *Bigelowiella natans*. Although the majority of nucleus-encoded, plastid-targeted proteins in chlorarachniophytes are derived from the chlorophyte endosymbiont, a significant number are not. Twenty-one percent of the *B. natans* genes encoding plastid-targeted proteins show signs of lateral gene transfer from a variety of exogenous sources, including streptophyte algae, red algae and algae with red algal endosymbionts, and bacteria. In stark contrast, however, the homologous plastid proteins in *Chlamydomonas* show no indications of lateral gene transfer. Lateral gene transfer seems to have played a significant role in shaping the complement of plastid proteins in chlorarachniophytes, and perhaps other mixotrophic secondary plastid-containing algae.

Materials and Methods

Identification of *B. natans* Plastid-Targeted Proteins. ESTs from *B. natans* (CCMP621) were analyzed in the course of an ongoing EST sequencing project of a unialgal cDNA library. cDNAs encoding putative plastid-targeted proteins were identified according to three criteria: their phylogenetic relationship to other plastid homologs, their involvement in a process known to be

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: ML, maximum likelihood; RuBisCO, ribulose 1,5-bisphosphate carboxylase/oxygenase.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AY267621–AY267704).

See commentary on page 7419.

*Present address: Department of Biology, Faculty of Science, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan.

†To whom correspondence should be addressed. E-mail: pkeeling@interchange.ubc.ca.

localized in the plastid, and/or their possession of distinctive bipartite plastid-targeting leader sequences consisting of a signal peptide and a transit peptide. cDNAs encoding plastid-targeted proteins were assembled to form contiguous ORFs, and unique clones were completely sequenced by primer walking. The N-terminal regions of conceptual amino acid sequences were analyzed for the presence of signal peptides by using the neural network-based tool SIGNALP (21) and IPSORT (22). IPSORT was also used to detect putative transit peptides.

Dataset Assembly. The nonredundant (NR), EST, and genomic sequence survey (GSS) public databases were searched by BLAST (23) using *B. natans* EST sequences as queries. ESTs and GSSs were downloaded and assembled to form contiguous ORFs encoding plastid-targeted proteins from the following organisms: *Porphyra yezoensis*, *Phaeodactylum tricorutum*, *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Marchantia polymorpha*, *Lycopersicon esculentum*, *Hordeum vulgare*, *Glycine max*, and *Physcomitrella patens*. Amino acid sequences inferred from these genes and the *B. natans* ESTs were aligned with homologs obtained from the NR database using CLUSTALX (24). Alignments were adjusted manually, and ambiguously aligned regions were removed before phylogenetic analysis. Alignments are available in Data Set 1, which is published as supporting information on the PNAS web site, www.pnas.org.

Phylogenetic Analysis. Phylogenetic trees were inferred from amino acid sequence alignments by using maximum likelihood (ML) and ML-distance methods. ML trees were constructed by using the program PROML in PHYLIP 3.6 (<http://evolution.genetics.washington.edu/phylip.html>) using the Dayhoff (PAM 001) amino acid substitution matrix, the global rearrangements option, one randomized sequence-input order, and an among-site rate variation model using a six rate category discrete approximation to the Γ distribution plus an invariable rate category. The relative rates for each category were estimated using TREE-PUZZLE 5.0 (25). ML-distance trees were inferred from Γ -corrected distance matrices (constructed with TREE-PUZZLE 5.0 with eight rate categories plus an invariable rate category) using BIONJ (26), weighted neighbor-joining (27), and Fitch–Margoliash (in PHYLIP 3.6). For Fitch analyses, the global rearrangements option was used, and the sequence-input order was randomized (with 5 or 10 jumbles). Support for ProML trees was obtained by bootstrapping 100 datasets. Due to the computational intensity of ML analyses, a uniform-rates model was used for bootstrapping ML trees, with the global rearrangements option and one randomized sequence input order. Support for ML-distance trees was obtained by bootstrapping (500 replicates) with PUZZLEBOOT (A. Roger and M. Holder; www.tree-puzzle.de) as described above. ML-distance trees were constructed (and bootstrapped) for all 78 datasets, and representative datasets were chosen for more comprehensive ML and ML-distance analysis.

Results

Plastid-Targeted Proteins in *B. natans*. From a survey of 3,995 *B. natans* EST sequences, 83 distinct genes encoding 78 putative plastid-targeted proteins were identified. Eighty-six percent were full-length or nearly full-length (possessing obvious N-terminal extensions), and 84% of these were predicted to function as signal peptides by IPSORT (22) and/or SIGNALP (21). Fourteen percent of the genes were 5' truncated; some were truncated within N-terminal leaders with characteristics of transit peptides. Others were truncated within the mature protein, and their N-terminal extensions were thus not available for analysis. Nevertheless, they are assumed to encode proteins targeted to the endosymbiont compartment and plastid by virtue

<i>Bigelowiella natans</i> protein	Phylogenetic origin	Support
Expected		
■ ATP synthase beta	Chlorophyte	Fig. 2
■ ATP synthase gamma	Chlorophyte	94
■ Chlorophyll a/b binding protein II	Chlorophyte	<50
■ Chlorophyll a/b binding protein CP29 (lhcb4)	Chlorophyte	96
■ Cytochrome c6	Chlorophyte	90
■ Enoyl-ACP reductase	Chlorophyte	92
■ Ferredoxin	Chlorophyte	<50
■ Ferredoxin nitrite reductase	Chlorophyte	100 *
■ Ferredoxin-NADP oxidoreductase	Chlorophyte	74
■ Fructose biphosphate aldolase	Chlorophyte	<50
■ Peptide deformylase	Chlorophyte	61
■ PsalD	Chlorophyte	<50
■ PsalE	Chlorophyte	51
■ PsbO (OEE1)	Chlorophyte	Fig. 2
■ Ribosomal protein L3	Chlorophyte	96
■ Ribosomal protein L4	Chlorophyte	96
■ Ribosomal protein L9	Chlorophyte	<50
■ Ribosomal protein L11	Chlorophyte	<50
■ Ribosomal protein L12	Chlorophyte	<50
■ Ribosomal protein L13	Chlorophyte	<50 *
■ Ribosomal protein L17	Chlorophyte	72
■ Ribosomal protein L21	Chlorophyte	81
■ Ribosomal protein L28	Chlorophyte	57 *
■ Ribosomal protein S9	Chlorophyte	62
■ Ribosomal protein S10	Chlorophyte	89
■ Tha4	Chlorophyte	54
□ Thylakoid lumen rotamase	Chlorophyte	75
■ Ycf24	Chlorophyte	<50
■ Ycf65	Chlorophyte	<50
■ Albino 3	Green algal (basal, no reds)	77
■ ATP synthase delta	Green algal (basal)	70
■ Coproporphyrinogen III oxidase	Green algal (no reds)	75
□ Chaperonin 60 (beta subunit)	Green algal (basal)	93
■ FE-S subunit of cytochrome b6f	Green algal (basal, no reds)	92
□ Heat shock protein 70	Green algal (basal)	92
□ L-ascorbate peroxidase	Green algal (paraphyly)	89
□ Mbb1	Green algal (basal)	<50
■ NADPH protochlorophyllide reductase	Green algal (basal, no reds)	100
■ Phytoene dehydrogenase	Green algal (basal)	80
□ Phytoene synthase	Green algal (no reds)	<50 *
■ PsalF	Green algal (basal)	<50
■ Quinol-to-oxygen oxidoreductase	Green algal (no reds)	69
■ Ribosomal protein L10	Green algal (no reds)	95
■ Ribosomal protein L35	Green algal (no chlorophyte)	<50
■ Ribosomal protein S1	Green algal (no chlorophyte)	73
■ Ribosomal protein S5	Green algal (no chlorophyte)	98
■ Ribosomal protein S20	Green algal (basal)	79
■ Sedoheptulose-1,7 biphosphatase	Green algal (basal, no reds)	97
■ SufE	Green algal (basal, no reds)	<50
Other algae		
■ Chlorophyll synthetase	Streptophyte	60
■ Ribosomal protein L31	Streptophyte	67
■ Ribulose 1,5-biphosphate carboxylase (SSU)	Streptophyte	Fig. 3 *
■ Acyl carrier protein	Red algal (or derivative)	61
■ Delta-aminolevulinic acid dehydratase	Red algal (or derivative)	Fig. 3 *
■ Fructose-1,6 biphosphatase	Red algal (or derivative)	56
■ Geranylgeranyl reductase	Red algal (or derivative)	Fig. 3
■ Glutamate-1-semialdehyde 2,1-aminomutase	Red algal (or derivative)	100
■ Phosphoglycerate kinase	Red algal (or derivative)	Fig. 3
■ Ribosomal protein L1	Red algal (or derivative)	<50
□ Ribosomal protein S22	Red algal (or derivative)	60
Bacteria		
■ Glyceraldehyde-3-phosphate dehydrogenase	Proteobacteria / Gram +ve	100
■ Ribulose-5-phosphate 3-epimerase	Pseudomonad	Fig. 5
Ambiguous		
■ Chlorophyll a/b binding protein LI818	Unresolved phylogeny	N/A
□ ClpB protease	Divergent	N/A
■ ClpP protease (NM)	Divergent	N/A
□ mRNA binding protein	Poor sampling	N/A
□ Nitrate reductase	Complex protein family	N/A
■ OEE3	Poor sampling	N/A
■ Phosphoribulokinase	Divergent	N/A
■ PsbP (OEE2)	Poor sampling	N/A
■ PsbW	Poor sampling	N/A
■ PSII10	Poor sampling	N/A
■ Ribosome-binding factor A	Poor sampling, divergent	N/A
■ Ribosomal protein L15	Unresolved phylogeny	N/A
■ Ribosomal protein L24	Unresolved phylogeny	N/A
■ Ribosomal protein L33	Unresolved phylogeny	N/A
□ Threonyl-tRNA synthetase	Poor sampling	N/A
■ Ycf16	Divergent	N/A

Fig. 1. Plastid-targeted proteins in the chlorarachniophyte alga *B. natans* grouped according to their inferred evolutionary origin. The left column indicates the protein, the center column describes its phylogenetic placement, and the right column indicates the phylogenetic support for its position (percentage bootstrap support out of 500 replicates) or gives the relevant figure number. Proteins with N-terminal extensions predicted to contain plastid-targeting information (see text) are highlighted by filled boxes, those with N-terminal extensions lacking obvious plastid-targeting information are indicated by half-filled boxes, and proteins encoded by 5'-truncated cDNAs are indicated by open boxes. Sequences containing insertions/deletions consistent with the placement of the *B. natans* protein in phylogenetic analysis are highlighted with asterisks. Proteins were designated "green algal" or of ambiguous origin according to criteria described in the text. The *B. natans* ClpP protease seems to be nucleomorph-encoded (NM). Phylogenies of all genes putatively derived by lateral transfer are available in Figs. 2, 3, 5, and 6.

of their significant similarity to cyanobacterial homologs and/or proteins known to function in the plastids of plants and algae.

The 78 *B. natans* plastid-targeted proteins determined in this study (Fig. 1) encompass a wide range of functions associated with plastid biochemistry and molecular biology. Included among these proteins are a large number with basic housekeeping functions, such as mRNA processing (mRNA binding protein), translation (22 ribosomal proteins, threonyl-tRNA synthetase), posttranslational protein modification (peptide deformylase), and protein folding and import (cpn60, hsp70). As expected, a large number of the proteins play a role in photosynthesis. Components of both photosystem I (e.g., PsaD, PsaE, PsaF) and II (e.g., PsbO, PsbP, PsbW) were identified, as were three subunits of plastid ATP synthase (β' , γ , and δ), ferredoxin, ferredoxin-NADP oxidoreductase, the iron/sulfur subunit of the cytochrome *b6f* complex, and the small subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO). A variety of enzymes associated with other metabolic processes were also identified, such as enoyl-ACP reductase (fatty acid biosynthesis), phytoene synthase, and phytoene dehydrogenase (carotenoid biosynthesis). No ESTs were found encoding enzymes involved in the shikimate (aromatic amino acid biosynthesis) or the non-mevalonate isopentyl diphosphate (isoprenoid biosynthesis) pathways.

B. natans Plastid-Targeted Proteins Have Diverse Evolutionary Origins.

To infer the evolutionary histories of the 78 *B. natans* plastid-targeted proteins, amino acid sequences were aligned with homologs from other organisms, and phylogenetic analyses were performed. ML-distance trees were inferred, and bootstrap analyses (500 replicates) were performed for all 78 datasets, and the results tabulated in Fig. 1. Twenty percent of the proteins yielded phylogenies that were ambiguous with respect to the origin of the *B. natans* sequence, due to the small size of the alignment, poor conservation of sequences, and/or insufficient taxonomic sampling. These proteins were not considered further. For the remaining 62 proteins, the expectation is that each would share a close relationship with chlorophyte homologs, because the *B. natans* endosymbiont was likely a chlorophyte. Accordingly, we conservatively considered plastid-targeted proteins to be chlorophyte-derived if they met any of several criteria. Most obviously, if a protein branched with a chlorophyte sequence in phylogenetic analyses and if the dataset contained at least one sequence from streptophytes, red algae, and cyanobacteria for appropriate comparison, it was considered chlorophyte in origin. ML phylogenies of two such examples are presented in Fig. 2. The phylogeny of PsbO (Fig. 2A) shows the *B. natans* homolog branching robustly with the chlorophytes *C. reinhardtii*, *Volvox carteri*, and *D. salina*, with this clade forming a sister group to the streptophytes. The phylogeny of the β' subunit of ATP synthase shows the same pattern (Fig. 2B), consistent with previous analyses of plastid- and nucleomorph-encoded genes (12, 13, 15). Other *B. natans* proteins (32%) produced phylogenies that were consistent with a green algal origin, but for reasons of taxonomic sampling (e.g., no chlorophyte, red algal, or cyanobacterial sequence available) or phylogenetic ambiguity (e.g., the sequence branched basal to chlorophytes and streptophytes), this finding could not be confirmed unambiguously. Without evidence to the contrary, these proteins are most likely derived from the endosymbiont, and are labeled “green algal” in Fig. 1. Altogether, these results indicate that the majority of plastid-targeted proteins in *B. natans* are probably of chlorophyte ancestry, and that the genes encoding them were presumably inherited from the chlorophyte endosymbiont, and transferred to the nucleus of the secondary host, as expected.

Surprisingly, however, 21% of the 62 informative proteins show phylogenetic affinities for lineages other than chlorophytes or green algae in general (Fig. 1), suggesting that they come from

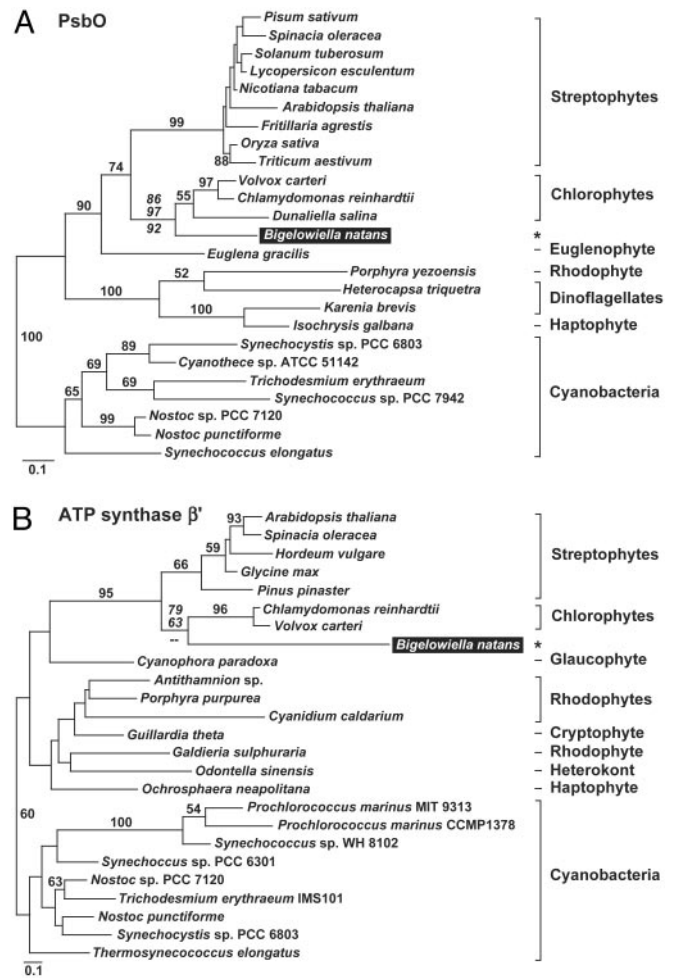


Fig. 2. Protein ML phylogenies showing examples of *B. natans* plastid-targeted proteins with the expected relationship to chlorophyte green algae. (A) Oxygen-evolving enhancer 1 (PsbO) phylogeny ($-\ln L = 5399.95518$). (B) ATP synthase β' subunit phylogeny ($-\ln L = 5134.21803$). *B. natans* plastid-targeted sequences are shaded black and highlighted with asterisks. ML bootstrap values are provided for all nodes $>50\%$. Additional support values (italicized) are provided for the node specifically showing the placement of the *B. natans* sequences and are, from top to bottom, ML, weighted neighbor-joining, and Fitch–Margoliash. Scale bars indicate the expected number of amino acid substitutions (corrected) per site.

a source other than the endosymbiont. Interestingly, the majority of these are derived from some other algal plastid. Fig. 3 shows ML phylogenetic trees of four examples. In phosphoglycerate kinase (PGK) phylogenies, the *B. natans* homolog, which possesses a full-length leader, branches strongly within a clade containing the red alga *P. yezoensis*, as well as *P. tricorutum* and *Laminaria digitata*, two heterokont algae with red secondary plastids (Fig. 3A). The same pattern is seen in phylogenies of two enzymes involved in chlorophyll biosynthesis, delta-aminolevulinic acid dehydratase and geranylgeranyl reductase. In the delta-aminolevulinic acid dehydratase tree, the *B. natans* sequence forms a highly supported clade with the red alga *Gracilaria gracilis*, which, as expected, branches with the red secondary plastid-containing heterokonts (Fig. 3B). In this case, the red algal origin of the *B. natans* protein is further supported by a putatively homologous insertion shared between the *B. natans* and *G. gracilis* sequences (Fig. 4A). Interestingly, the plastid sequences in this phylogeny form a well-supported clade with a specific subset of cyanobacteria, unlike most phylogenies,

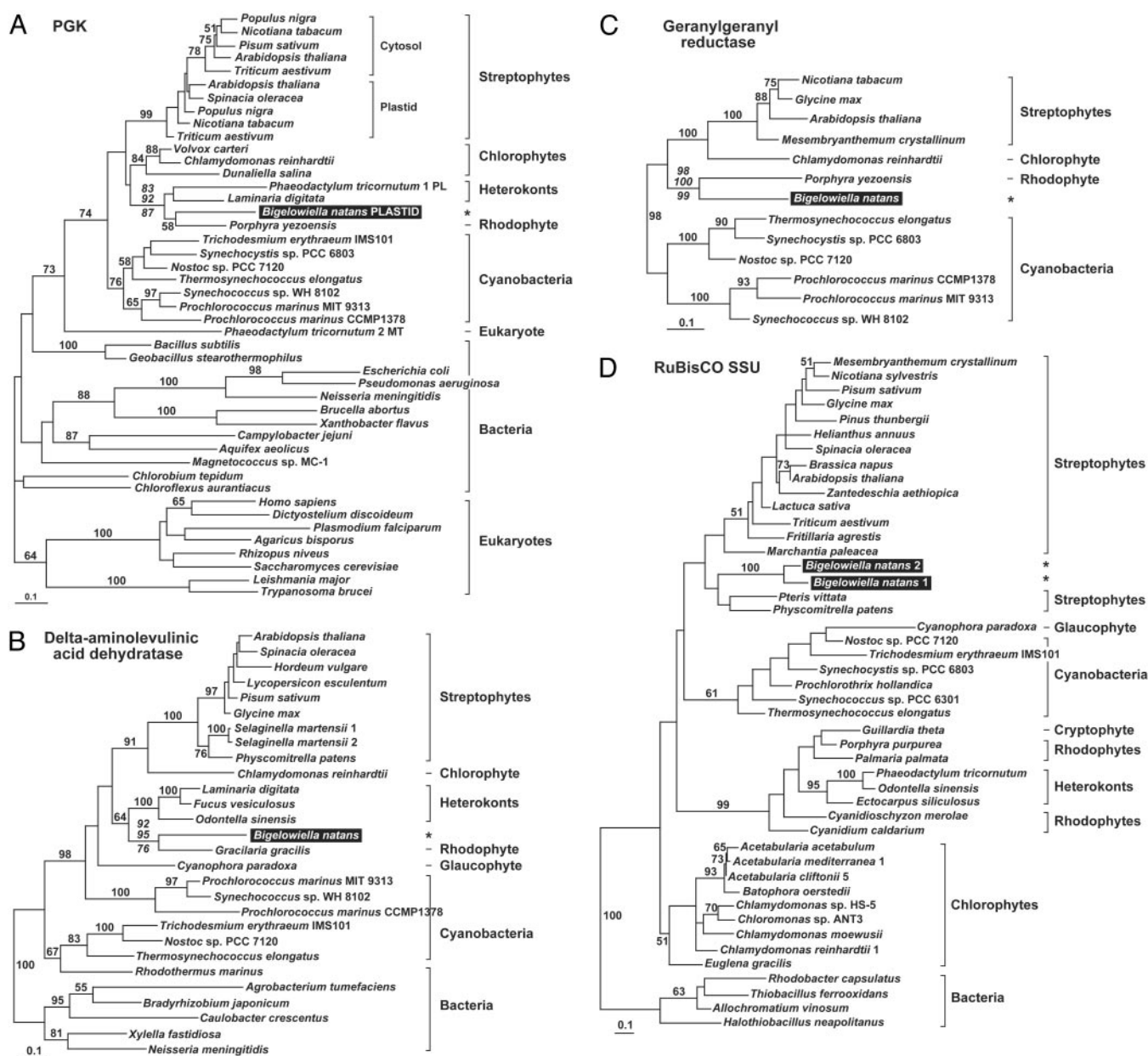


Fig. 3. Protein ML phylogenies showing *B. natans* plastid-targeted proteins derived from other algae. (A) Phosphoglycerate kinase (PGK) ($-\ln L = 16275.24733$). (B) Delta-aminolevulinic acid dehydratase ($-\ln L = 8636.18318$). (C) Geranylgeranyl reductase ($-\ln L = 4490.47915$). (D) Small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) ($-\ln L = 4103.65322$). Bootstrap values and scale bars are as in Fig. 2. PL, plastid; MT, mitochondrial.

which place plastids as a sister group to cyanobacteria as a whole. The taxonomic sampling available for geranylgeranyl reductase is relatively poor, but phylogenetic analysis reveals a very strongly supported relationship between *B. natans* and the red alga *P. yezoensis*, a clade well-separated from the streptophytes and the chlorophyte *C. reinhardtii* (Fig. 3C). We also identified two *B. natans* paralogs of the small subunit of RuBisCO that consistently branched near the base of the streptophyte algae (Fig. 3D), although this position was not well supported and the placement of the *B. natans*/streptophyte clade relative to other groups varied with different methods. The lack of resolution in these phylogenies is likely due to the fact that the RuBisCO small subunit is a very short protein and provides few sites for analysis (only 89 positions). However, independent evidence in support of the phylogeny comes from a variable region of the enzyme in which the *B. natans* sequences are distinctly similar to strepto-

phyte homologs in length and sequence (Fig. 4B). In addition to these genes, seven others seem to be related to streptophytes, red algae, or algae with red algal secondary plastids (Fig. 1; and Fig. 6, which is published as supporting information on the PNAS web site).

More surprising still, two *B. natans* plastid-targeted proteins are not related to plastid sequences at all, but are instead related to bacterial proteins. The most striking example is the *B. natans* Calvin cycle enzyme ribulose-5-phosphate 3-epimerase, which branches very strongly within the Pseudomonadaceae and within the proteobacteria (Fig. 5), suggesting it was acquired recently by lateral gene transfer from a pseudomonad. The possibility that this gene represents a bacterial contaminant is extremely unlikely for two reasons. First, the *B. natans* gene encodes an N-terminal extension resembling other plastid-targeting peptides. Second, we used PCR to amplify the genomic copy of the



Fig. 4. Representative alignments showing insertions/deletions supporting the phylogenetic position of *B. natans* plastid-targeted protein sequences. (A) Insertion shared between delta-aminolevulinic acid dehydratase sequences of *B. natans* and the red alga *G. gracilis*. (B) Variable region of RuBisCO small subunit proteins. The *B. natans* sequence shares length and sequence conservation with streptophyte homologs. Numbers for both correspond to coordinates within the *Arabidopsis thaliana* homolog.

gene and found it to possess several canonical spliceosomal introns, the hallmark of eukaryotic nuclear genes (data not shown). An equally compelling case is the *B. natans* GAPDH, a full-length gene that encodes a distinctive plastid-targeting leader sequence. This plastid-targeted GAPDH is not related to other plastid-targeted homologs, but instead branches with 100% support with genes from proteobacteria and Gram-positive bacteria (Fig. 6). In summary, many plastid-targeted proteins from *B. natans* are not derived directly from the endosymbiont, but instead come from the plastids of other algae or from bacteria.

The support for the position of the *B. natans* sequences in individual gene trees varies considerably, as does the diversity of taxa available for comparison. It is therefore possible that some of the unusual phylogenies are due to tree reconstruction artifacts. Nevertheless, even if some of the trees are misleading, there is still a large proportion of *B. natans* genes that were acquired by lateral transfer. This result is most apparent when the *B. natans* data are compared with the phylogenetic pattern

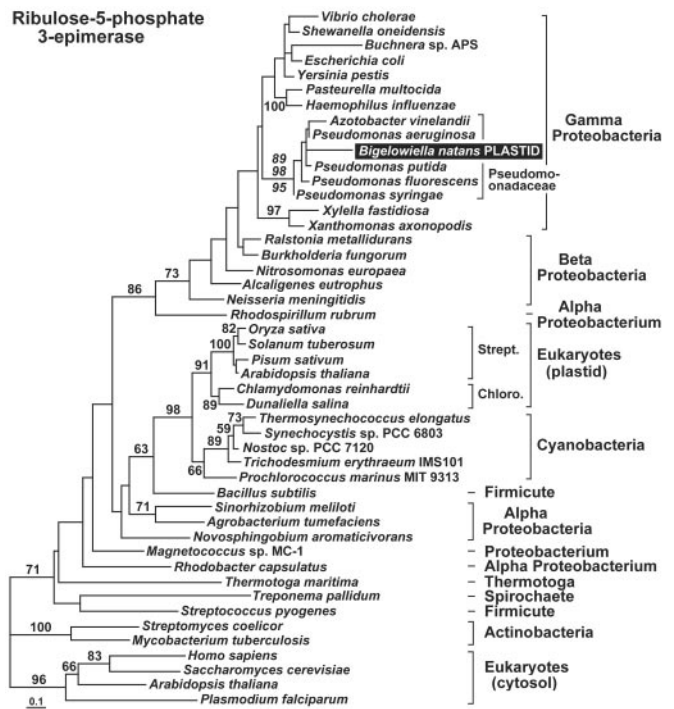


Fig. 5. Protein ML phylogeny ($-\ln L = 8618.55993$) of ribulose 5-phosphate 3-epimerase. The *B. natans* plastid-targeted sequence is highlighted black. Bootstrap values and scale bars are as in Fig. 2. The *B. natans* protein is nested within the Pseudomonadaceae, indicative of lateral transfer from a pseudomonad.

observed for plastid-targeted proteins in the chlorophyte, *C. reinhardtii*. Ninety-one percent of our datasets contained a *C. reinhardtii* homolog, and these proteins typically branched as the sister to the streptophytes, as expected (e.g., Figs. 2, 3, and 5). In the few exceptions, it virtually always branched with other chlorophyte sequences (if present) and with the *B. natans* homolog, suggesting that the anomalous placement was a feature of the chlorophyte sequences as a whole. Therefore, in contrast to *B. natans*, there is no evidence for plastid-targeted proteins derived from exogenous sources in *C. reinhardtii*.

Discussion

Biochemical and molecular data suggest that the chlorarachniophyte endosymbiont is derived from a chlorophyte green alga (5, 11–15). Our analysis of 78 plastid-targeted proteins in *B. natans* shows that the evolutionary history of most such proteins is consistent with this conclusion. However, our analyses also suggest that a significant proportion (about 21%) of the nuclear genes encoding plastid-targeted proteins in *B. natans* are not endosymbiont-derived, but have been acquired by lateral gene transfer from a variety of sources. At face value, the evolutionary history of *B. natans* plastid-targeted proteins should be similar to homologs in the chlorophyte *C. reinhardtii*; however, we found no evidence for lateral transfer in this organism. This contrast is interesting in light of differences in the trophic behaviors of the two species. Unlike *C. reinhardtii*, chlorarachniophytes are capable of photosynthesis and phagocytosis. *Chlorarachnion reptans* has been shown to ingest a wide variety of algae (5), and partially digested and undigested bacteria have been observed inside food vacuoles in *B. natans* (28). The presence of a lateral transfer ratchet mechanism in phagotrophic eukaryotes has been suggested previously (29); this mechanism seems a likely explanation for the foreign plastid-targeted proteins in *B. natans* and the apparent lack of such proteins in *C. reinhardtii*.

The fact that most of the foreign genes identified in *B. natans* come from other algae may be explained by two factors. First, plastids use a restricted set of proteins, and other algae are the most likely source of genes encoding proteins that could conceivably function in the *B. natans* plastid. Second, plastid-targeted proteins must encode both a signal peptide and a transit peptide. Homologs from other algae would already possess transit peptides and perhaps also signal peptides, which may facilitate the integration of such a gene if the endogenous targeting machinery can recognize these leaders. That said, *de novo* acquisition of signal and transit peptides has occurred for at least some of the laterally transferred proteins identified in *B. natans*. This finding is most obvious in the case of transfers involving bacterial genes (ribulose-5-phosphate 3-epimerase and GAPDH), but also applies to genes transferred from plastid genomes. For example, the *B. natans* gene encoding ribosomal protein L1 seems to have been acquired by lateral gene transfer from a red alga or red algal plastid-containing organism (Figs. 1 and 6), and, in such organisms, rpl1 is encoded in the plastid genome, not the nucleus.

At present, lateral transfers involving plastid proteins are more easily recognized than those involving cytosolic ones, simply because of the increased taxonomic sampling of plant and algal plastid proteins. There is, however, no obvious reason to assume that the acquisition of foreign genes by chlorarachniophytes is limited to those involved in plastid function. Indeed, it has already been shown that the chlorarachniophyte cytosolic enolase is derived from a streptophyte (30). It is interesting to note that a recent analysis of the *Arabidopsis* genome showed that the plant cytosol contains a significant number of proteins

derived from the ancestral plastid (31). Our work extends this observation to the plastid itself by showing that the *B. natans* organelle is a mosaic of proteins with different evolutionary histories.

Analyses of bacterial and archaeal genomes have made it abundantly clear that lateral gene transfer is an important force in prokaryotic evolution (32), but whether this extends to eukaryotes is less obvious. Although a handful of gene transfers involving eukaryotes have been documented (e.g., refs. 33 and 34), the full impact of lateral gene transfer in eukaryotic genomes is largely unexplored. Recently, however, a survey of diplomonad genes identified numerous instances of lateral gene transfer (35), and a comprehensive study of flowering plants has revealed rampant transfer between their mitochondrial genomes (36). Whether lateral transfer will prove to be as important in eukaryotes as in prokaryotes remains to be seen, but the wealth of eukaryotic genome sequence data on the horizon should soon provide an answer. At present, it seems that lateral gene transfer has been a factor in the evolution of eukaryotic genomes, but that its impact may vary from lineage to lineage.

We thank G. I. McFadden and P. R. Gilson for a *B. natans* cDNA library and N. M. Fast and B. S. Leander for helpful comments on the manuscript. This work was supported by Grant 227301-00 from the Natural Sciences and Engineering Research Council of Canada (to P.J.K.). J.M.A. is supported by postdoctoral fellowships from the Canadian Institutes of Health Research and the Killam Foundation. P.J.K. is a scholar of the Canadian Institute for Advanced Research, the Canadian Institutes of Health Research, and the Michael Smith Foundation for Health Research.

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