

# Three ORF-Containing Group I Introns in Chloroplast SSU of *Caulerpa sertularioides* (Ulvophyceae) and Their Evolutionary Implications

Jungho Lee<sup>1,2\*</sup> and James R. Manhart<sup>2</sup>

<sup>1</sup>BK21, School of Biological sciences, Seoul National University, Shillim, Kwanak, Seoul 151-742, Korea and

<sup>2</sup>Biology Department, Texas A&M University, College Station, Texas 77843, U.S.A.

Except for a group I intron in *trnL*-*uaa* occurring in eubacteria and plastids, group I introns are rarely documented in plastid genomes. Here, we report that a green alga, *Caulerpa sertularioides*, contains three group IA3 introns in the 16S gene (cpSSU), CS-cpSSU.i1, CS-cpSSU.i2 and CS-cpSSU.i3. Each intron has an open reading frame with LAGLI-DADG motifs. CS-cpSSU.i1orf and CS-cpSSU.i3orf occur at Loop 6 in the intron secondary structure and CS-cpSSU.i2orf at Loop 8. CS-cpSSU.i1orf and CS-cpSSU.i2orf contain both LAGLI-DADG motifs but CS-cpSSU.i3orf has only one. CS-cpSSU.i1 and CS-cpSSU.i2 share the insertion sites and the ORFs at Loop 6 and 8 with CpSSU-1 and CpSSU-2 introns of *Chlamydomonas pallidostigmatica* (Chlorophyceae). In contrast, CS-cpSSU.i3, containing 28 copies of GAAATAT at Loop 6, is a novel intron found only in *Caulerpa sertularioides*. Possible scenarios of the evolution of the three introns and their possible use in systematic research are discussed.

**Key Words:** 16S, *Caulerpa*, cpSSU, group IA3 intron, LAGLI-DADG motif containing ORF, Ulvophyceae

## INTRODUCTION

Group I introns are known to be widely spread in eubacteria and the nuclear and mitochondrial genomes of eukaryotes. In contrast, group I introns have been rarely found in plastid genomes. The only widely distributed group I intron in plastid genomes is the intron in *trnL*-*uaa* (Besendahl *et al.* 2000). Except for the *trnL* intron, group I introns are found only in chlorophycean algae, mostly in *Chlamydomonas*. So far, five genes in Chlorophyceae plastids are known to have group I introns. These include 23S (cpLSU), 16S (cpSSU), *chlL*, *psbA* and *rbcl*. Beyond that, the distribution of group I introns is poorly known in the plastids of plants.

The intron in *trnL*-*uaa* is the only one found in eubacteria (Kuhnel *et al.* 1990; Xu *et al.* 1990) and the plastids of most plant lineages (Besendahl *et al.* 2000). The intron is categorized as group IC on the basis of its secondary structure. It is generally accepted that the plastid *trnL*-*uaa* intron is eubacterial in origin and was vertically inherited in most lineages of plant plastids. This intron occurs in the cyanelle of *Cyanophora* (Evrard *et al.* 1988) and the plastid of *Vaucheria* and most lineages of green

plants. However, the plastids of rhodophytes, the red algae, do not contain this intron (Reith and Munholland 1995; Douglas and Penny 1999; Besendahl *et al.* 2000; Gloeckner *et al.* 2000; Ohta *et al.* 2003).

The cpLSU (23S) has attracted group I intron investigators, who have found 12 intron insertion sites in the 23S of chlorophycean algae, mostly in *Chlamydomonas* and its relatives (Thompson and Herrin 1991; Turmel *et al.* 1993). Among them, four introns have been well characterized (Lucas *et al.* 2001) at four sites (1923, 1931, 1951 and 2593). These introns are categorized as group IA3 and IB4, some of which contain open reading frames with endonuclease function (Turmel *et al.* 1995). These open reading frames (ORFs) or endonucleases are known to have LAGLI-DADG motif(s) and have been found in Loop 6 (L6) or Loop 8 (L8) of the introns.

Another relatively well surveyed plastid intron is the *rbcl* group IA2 intron in species of Chlorophyceae (Nozaki *et al.* 2002). It has been found in six genera including some members of Chlamydomonadaceae and the colonial Volvocales. The *rbcl*-462 introns, inserted between bases 462 and 463 of *rbcl*, contain an intact or degenerate ORF of various sizes except in the intron found in *Gonium multicocum*. The ORF belongs to the GIY-YIG type endonuclease and occurs at L6.

The *psbA* gene of *Chlamydomonas reinhardtii* has four

\*Corresponding author (jleegp@snu.ac.kr)

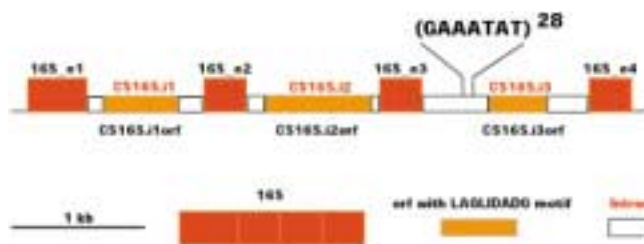
group I introns (Turmel *et al.* 1989; Herrin *et al.* 1991; Bao and Herrin 1993). The introns of Cr.psbA2 and Cr.psbA4 contain free-standing ORFs with motifs representative of the GIY-YIG and H-N-H homing endonuclease family. These two introns are also found in *Chlamydomonas moewusii*. A few other genes are known to have group I introns. Another protein coding gene, *chlL*, is known to have an ORF-free group I intron (Kapoor *et al.* 1996). This intron has been documented only in *Chlorella vulgaris* of Trebouxiophyceae (Kapoor *et al.* 1996).

In cpSSU, four introns (Durocher *et al.* 1989; Turmel *et al.* 1989) have been documented only in *Chlamydomonas* of Chlorophyceae. The four introns have different insertion sites. Three *Chlamydomonas* species, *C. moewusii*, *C. gelatinosa* and *C. pitschmannii*, share an ORF-free group IA3 intron. In contrast, *C. pallidostigmatica* has two introns (CpSSU-1 and CpSSU-2). These two introns belong to subgroup IA3 and contain an ORF with LAGLI-DADG like motifs. However, the ORFs are located in different regions of the intron secondary structures. CpSSU-1 has an ORF at loop 6 (L6) but CpSSU-2 has an ORF at L8. The ORF in CpSSU-1 encodes a protein (I-CpaII) with endonuclease activity but the function of CpSSU-2 ORF is unknown. The CpSSU-1 intron is not present in 13 other *Chlamydomonas* species but the CpSSU-2 intron is also found in *C. mexicana* and *Chlamydomonas* species SAG66.72. *Chlamydomonas frankii* has an ORF-containing IA3 intron but detailed information is not known.

Except for some *Chlamydomonas* species, group I introns have not been reported in the 16S gene. We determined that the cpSSU of *Caulerpa sertularioides*, a green alga of the Ulvophyceae, contains three group I introns.

## MATERIALS AND METHODS

The 5.7 kb *Cla*I fragment containing 16S, previously known from the physical map of *Caulerpa sertularioides* chloroplast DNA (Lehman & Manhart 1997), was cloned in pBlueScript II SK<sup>+</sup>. The fragment was sequenced using previously designed 16S primers (Manhart 1995) and octomers (Lee and Manhart 2002b). The 16S of *Codium fragile* was used as a reference of another Ulvophyceae algae, and was recovered by PCR from previously isolated cpDNA (Manhart *et al.* 1989). The sequencing was done by manual sequencing and automatic sequencing systems, ABI377 and ABI3100 automated sequencers, as previously described (Lee and



**Fig. 1.** The 16S of *Caulerpa sertularioides* and its three introns with ORFs. Closed boxes indicate exons and ORFs within introns, and open boxes mark introns.

Manhart 2002a, 2002b).

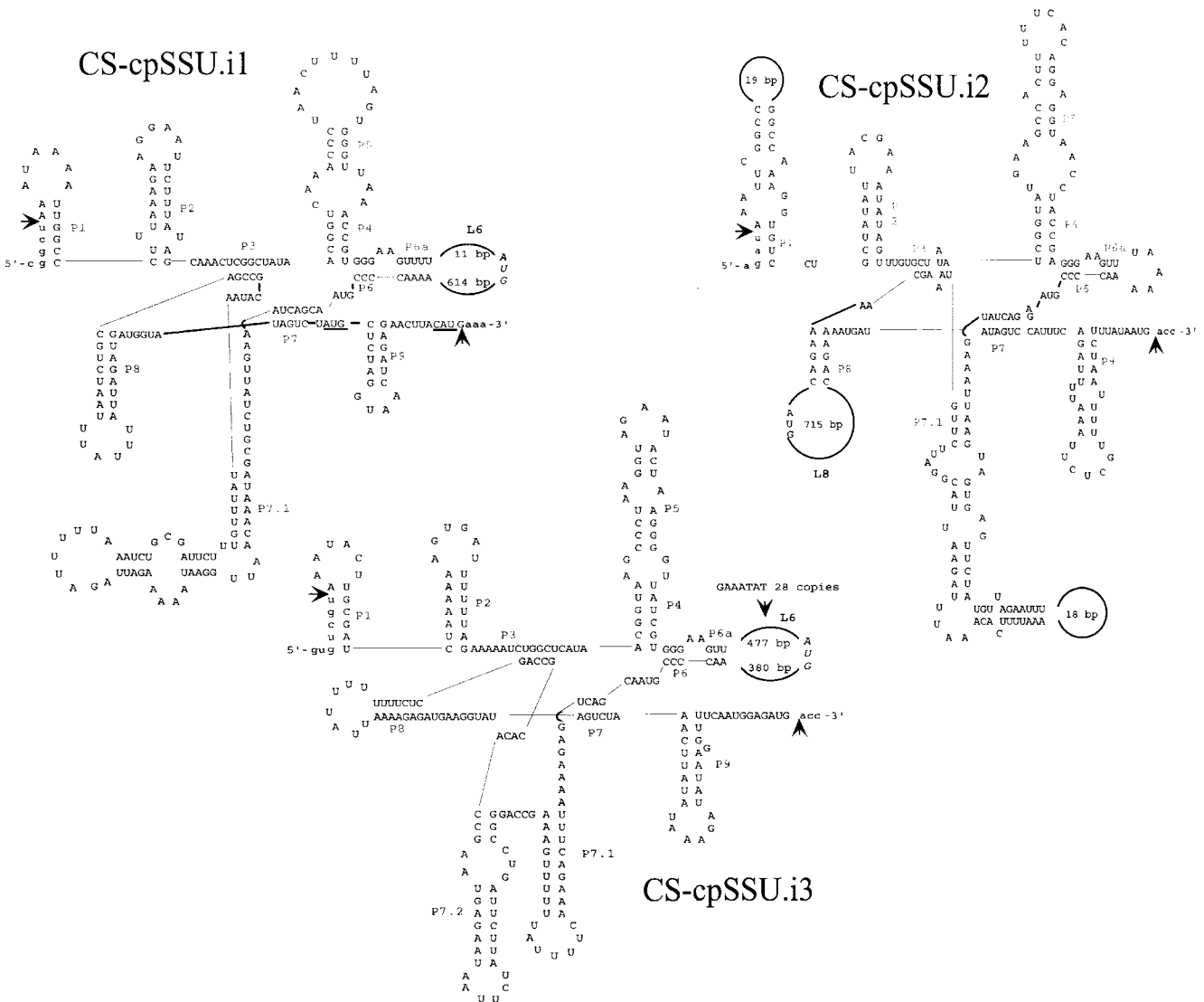
The DNA sequences were assembled using Sequencher ver. 4.0 (Gene Code Corporation) on a Macintosh Powerbook G4. The exon and introns were identified by sequence comparison using the FASTA program in the GCG package (Genetics Computer Group, Inc.). The secondary structures of the introns were constructed using FOLDRNA program referenced by Burke *et al.* (1987) and Turmel *et al.* (1995). For intron ORFs, BLAST searches through GenBank database were done. The 16S gene sequences of green algae were obtained from Genbank for phylogenetic analysis.

The sequences of ORFs with LAGLI-DADG motifs were collected from GenBank on the basis of Lucas *et al.* (2001) and the acronym of the ORFs were used as described by Lucas *et al.* (2001), except for *Caulerpa sertularioides* and *Chlamydomonas pallidostigmatica*. For sequence alignments, Maclade, Clustal X, and PILEUP program of GCG package were used in combination and, for phylogenetic analyses, Paup4.0b10 (Swofford 2000) was used. The sequences generated from this study were deposited in GenBank (AY389514-AY389515).

## RESULTS AND DISCUSSION

We determined the sequence of 4,785 bp from the cloned 5.7 kb *Cla*I fragment of *Caulerpa sertularioides* cpDNA (GenBank accession no. AY389514). The 16S gene contains three introns (Fig. 1), which are 865 (CS-cpSSU.i1), 1016 (CS-cpSSU.i2) and 1086 (CS-cpSSU.i3) bps in size, respectively. The three introns belong to group IA3 with an ORF. The sizes of the ORFs are 204 (CS-cpSSU.i1orf), 266 (CS-cpSSU.i2orf) and 130 amino acids (CS-cpSSU.i3orf). CS-cpSSU.i1 and CS-cpSSU.i3 have an ORF at L6 in the secondary structure, but CS-cpSSU.i2 has the CS-cpSSU.i2orf at L8 (Fig. 2).

The first two introns, CS-cpSSU.i1 and CS-cpSSU.i2,



**Fig. 2.** Secondary structure model of three *Caulerpa* introns. Splice sites between exon (lower case) and introns (upper case) residues are denoted by arrows. The start and stop codons of the ORFs are denoted by italicized triangles. The microsatellite sequence, which has 28 repeats of GAAATAT, is marked at Loop 6 of the third intron.

Caulerpa	AAGCATCGCGCTAAATAAAA- 850BP-CTTACATGAACCTCGT
Chlamydomonas_p	....CCC.....GGTT-1396BP-GA.T.....CT..
Closteriopsis	.....TT- 596BP-T.AT.....CT..
Mesostigma	..... ..T.C.T..T
Caulerpa	TAGGATTAGATAAAATTCGG-1001BP-TTATAATGACCCTAGT
Chlamydomonas_p	.....C....-1275BP-GGT.TT.....
Closteriopsis	.G..... ..C.....
Mesostigma	.G..... ..
Caulerpa	GCTCGTGTGCTAAATACTT-1070BP-GTTCATGGAGATGTT
Chlamydomonas_p	.....ATT. ....AT....
Closteriopsis	.....T. ....
Mesostigma	..... ..

**Fig. 3.** The insertion sites of *Caulerpa sertularioides* 16S introns. *Caulerpa*: *Caulerpa sertularioides* of Ulvophyceae, *Chlamydomonas\_p*: *Chlamydomonas pallidostigmatica* (L39865) of Chlorophyceae, *Closteriopsis*: *Closteriopsis acicularis* of Trebouxiophyceae (Y17632); *Mesostigma*: *Mesostigma viride* of Prasinophyceae (NC\_002186).

share insertion sites with two introns of *Chlamydomonas pallidostigmatica* of Chlorophyceae (Turmel *et al.* 1995). The 16S intron of *Closteriopsis acicularis* of Trebouxiophyceae (Ustinova *et al.* 2001) shares the insertion site with CS-cpSSU.i1 (Fig. 3). Both *C. pallidostigmatica* introns have ORFs with LAGLI-DADG like motifs, though *Closteriopsis* has an ORF-free intron. The ORFs of *Caulerpa sertularioides* and *Chlamydomonas pallidostigmatica* have LAGLI-DADG like motifs (Fig. 4). Both taxa share two LAGLI-DADG like motifs in the first two ORFs. In contrast, CS-cpSSU.i3orf, the ORF of the third intron in *Caulerpa sertularioides*, has only one LAGLI-DADG like motif.

CS-cpSSU.i1, the first intron of *Caulerpa sertularioides*, is similar to the *Closteriopsis* intron and the first intron of

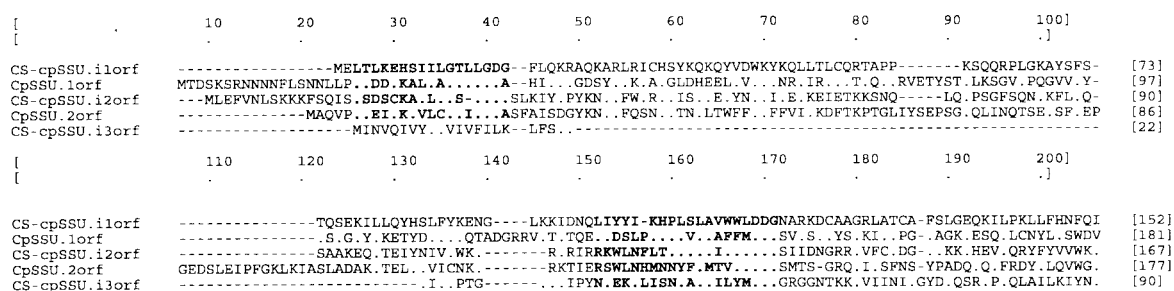


Fig. 4. The 16S intron ORF alignment from *Caulerpa sertularioides* and *Chlamydomonas pallidostigmatica*. LAGLI-DADG-like motifs are in bold.

*C. pallidostigmatica*, CpSSU.1. First, they share all the insertion site (Fig. 3). Second, the introns all belong to the same class, Group IA3. Third, the introns in *Caulerpa* and *Chlamydomonas pallidostigmatica* have an ORF at L6. Fourth, the ORF have both LAGLI-DADG like motifs. These results indicate that the introns in these three taxa had a common origin. CS-cpSSU.i2, the second intron of *Caulerpa sertularioides*, shares many characteristics with the *Chlamydomonas pallidostigmatica* CpSSU.2 intron. They share the same insertion site, are subgroup IA3 and contain an ORF with both LAGLI-DADG like motifs at L8, again indicating a common origin with the loss of the ORF in *Closteriopsis*.

The third intron of *Caulerpa sertularioides*, CS-cpSSU.i3, has not been found in any other plastid at that insertion site. This intron has an interesting feature in L6, in which the ORF occurs. Loop 6 contains 28 tandem repeats of GAAATAT, which is rare in introns. The repeat was likely generated by repeated slippage during replication.

In order to check the presence/absence of these three introns in other cpSSUs, Genbank was searched, and sequences were found from three classes of green algae. These include 4 species of Chlorophyceae, 2 species of Trebouxiophyceae, 5 species of Prasinophyceae. *Caulerpa sertularioides* is a member of the Ulvophyceae and none of the introns were found in other members of this class. We also sequenced *Codium fragile* 16S (AY389515) as an additional member of the Ulvophyceae. Except for the three taxa mentioned above, none of the 11 taxa contain any of these introns. A 16S gene phylogeny (Fig. 5) supported the designation of the four classes. The CS-cpSSU.i1, the first intron of *Caulerpa sertularioides*, occurs in the three classes including Ulvophyceae, Chlorophyceae and Trebouxiophyceae. However, the intron is not present in Prasinophyceae, the group suggested to be the basal most lineage of green plants (Mattox and Stewart 1983).

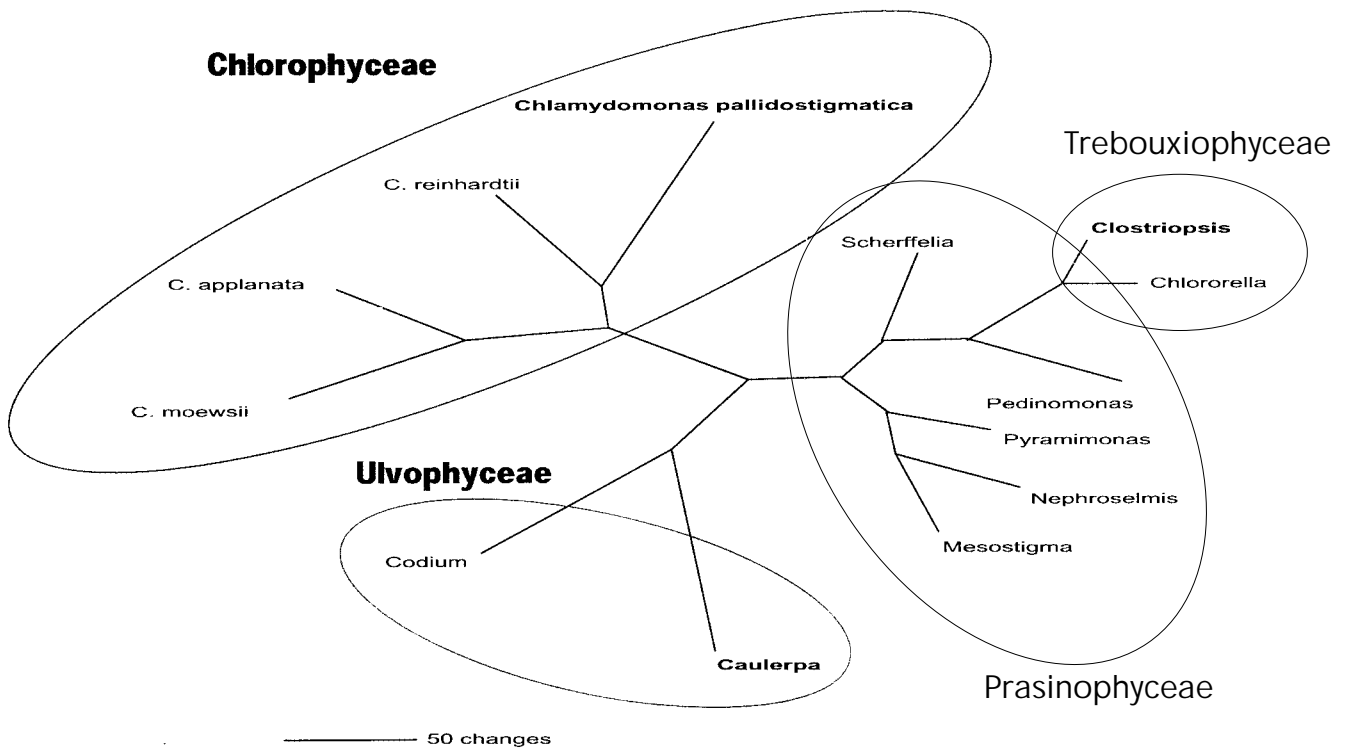
Introns with endonuclease activity are known to have

homing capacity and lateral transfer capacity among genomes within organisms, sometimes between organisms. To test possible modes of intron movement, a phylogenetic analysis of known group I intron ORFs with LAGLI-DADG motifs in chlorophyte plastids and their suggested relatives in mitochondria was done (Fig. 6). As suggested by Lucas *et al.* (2001), the four groups of ORFs from chloroplasts and mitochondria based on subgroups and the position of ORFs formed separate lineages. However, the three ORFs of *Caulerpa sertularioides* do not belong to any of the suggested lineages. Instead, the first ORF (CS-cpSSU.i1orf and CpSSU.1ORF) and the second ORF (CS-cpSSU.i2orf and CpSSU.2ORF (*CpaII*)) were members of separate lineages. In addition, CS-cpSSU.i3orf was isolated from all other lineages. These results suggest that the three ORFs of *Caulerpa sertularioides*, along with those in *Chlamydomonas pallidostigmatica*, had separate origins from other known LAGLI-DADG ORF lineages, and that the three ORFs from *Caulerpa sertularioides* might have had separate origins each other.

#### The possible origin of the *Caulerpa* 16S group I introns

The plastid group I introns are categorized into three classes including group IA, IB, and IC. Group IA introns occur widely in *rbcL*, 16S (cpSSU) and 23S (cpLSU) in green algal plastids. Group 1B occurs in cpLSU. Group IC introns occur in *trnL*, which occurs in most lineages of plants except for the red algae. On the basis of their distribution, the group IC intron of plastid *trnL* is likely eubacterial in origin, and possibly lost in the rhodophyte lineage.

Group IB introns are limited to the cpLSU of green algal chloroplasts, but are not found in non-green algal plastids and streptophyte plastids. The green algae with Group IB introns in cpLSU include *Chlamydomonas* spp. of Chlorophyceae, and *Pedidomonas*, *Pterosperma* and *Monomastrix* spp. of Prasinophyceae (Lucas *et al.* 2001). Prasinophycean algae are generally accepted as the basal



**Fig. 5.** The phylogenetic position of three taxa containing *Caulerpa* cpSSU intron in the most parsimonious unrooted tree from 16S sequences. The intron containing taxa are in bold. In the phylogenetic analysis, indels were deleted. *Chlamydomonas applanata* (AF394204); *Chlamydomonas moewusii* (X15850); *Chlamydomonas pallidostigmatica* (L36815); *Chlamydomonas reinhardtii* (BK000554); *Codium fragile* (this study AY389515); *Caulerpa sertularioides* (this study AY389514); *Mesostigma viride* (NC\_002186); *Nephroselmis olivacea* (NC\_000927); *Pedinomonas minor* (AF393605); *Pyramimonas parkeae* (AF393608); *Scherffelia dubia* (AF393609); *Closteriopsis acicularis* (Y17632); *Chlorella vulgaris* (NC\_001865).

green plants. In contrast, the chloroplasts of streptophytes, a sister lineage of chlorophytes, do not contain any group IB introns. On the basis of the distribution of group IB introns, the intron was probably introduced into chloroplast genomes early in the chlorophyte lineage because the basal green plants and the basal streptophytes do not contain the intron. The intron may have been introduced from bacteria or mitochondrial genomes, or from both. The question is still open.

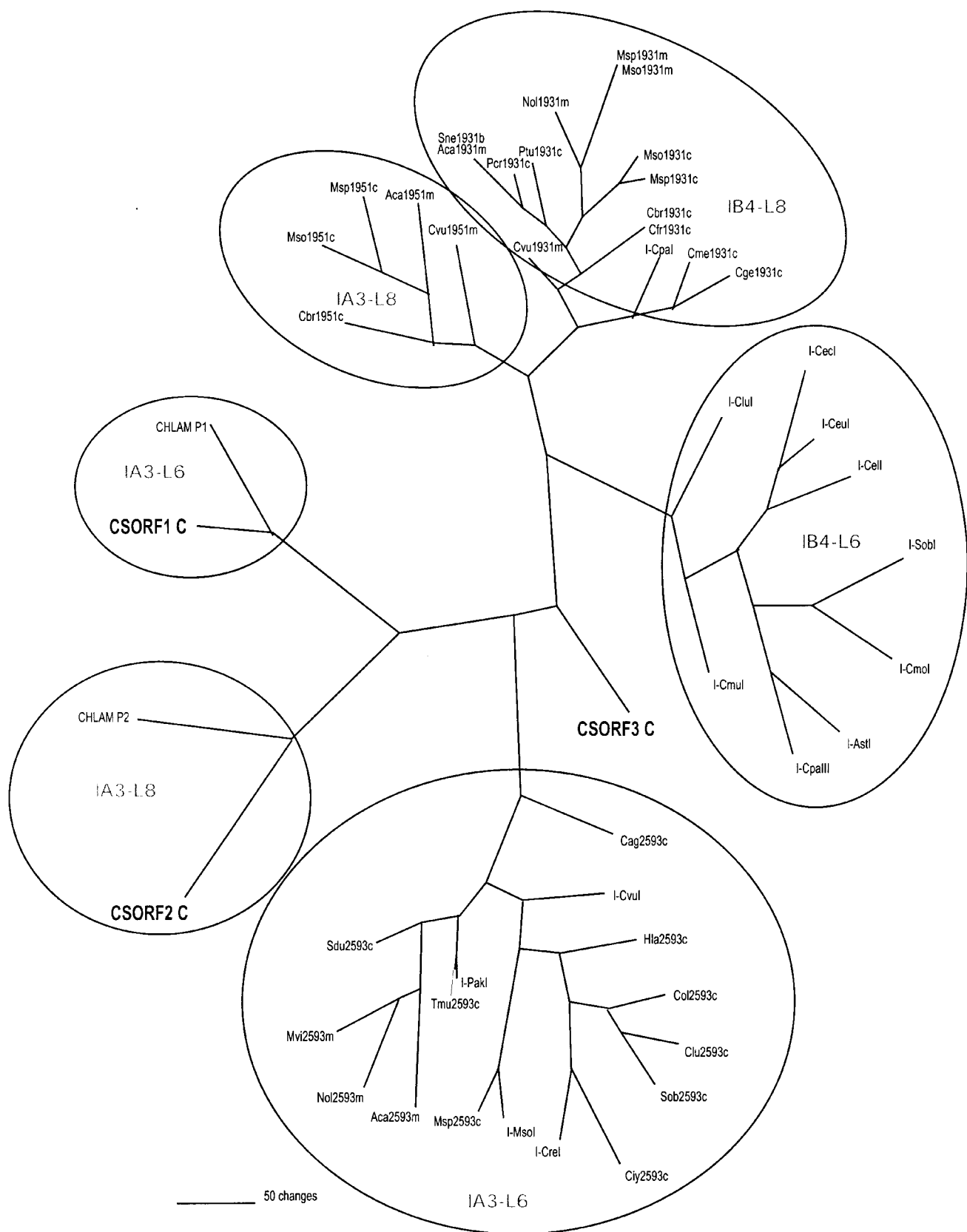
Group IA introns have been found in *rbcL*, 16S and 23S. The Group IA2 intron, which has an ORF with a GIY-YIG motif, is found in *rbcL*. In contrast, group IA3 introns are found in 16S and 23S, which have ORFs with LAGLI-DADG motifs. On the basis of the ORF motif, group IA2 and IA3 introns had different origins. The ORFs of group IA3 introns are located at Loop 6 or Loop 8 as found in three introns of *Caulerpa*. The known 23S group IA3 introns with ORFs at the same position were phylogenetically not related (Fig. 5) to any of three *Caulerpa* 16S introns.

Instead, the introns group with 16S introns of

*Chlamydomonas pallidostigmatica* (Fig. 6). In addition, the ORFs from *Caulerpa* do not have direct phylogenetic relationships to each other (Fig. 6), indicating that they are different in origin. In other words, the introns are not the evolutionary results of within genome intron homing.

The first intron of *Caulerpa*, CS-cpSSU.i1, occurs in two other different classes of chlorophytes. They are *Closteriopsis* of Trebouxiophyceae and *Chlamydomonas pallidostigmatica* of Chlorophyceae. Some might suggest the possible lateral transfer of the intron by other organism(s) because of the absence of 11 taxa from those classes and of the presence of an ORF with homing capacity, LAGLI-DADG motif. However, the completely different habitat of these three taxa does not support this interpretation. *Caulerpa* occurs in ocean, salty water, while *Closteriopsis* inhabits in the soil, and *Chlamydomonas pallidostigmatica* occurs in fresh water. Therefore, lateral transfer between these group is not plausible.

One possible explanation of CS-cpSSU.i1 evolution is that the intron was introduced after chlorophytes



**Fig. 6.** The most parsimonious unrooted tree of intron ORFs with LAGLI-DADG like motifs in green algae. Deduced amino acid sequences were used for phylogenetic analyses. The known LSU intron ORFs sequences with LAGLI-DADG like motifs (Lucas *et al.* 2001) were used in this analysis. The acronyms used by Lucas *et al.* (2001) are used here except for ORF in *Chlamydomonas pallidostigmatica* (Turmel *et al.* 1995) and *Caulerpa* in this study.

diverged from other green plants, and has been lost in many chlorophytes lineages independently. In this scenario, CS-cpSSU.i2, only found in *Caulerpa* and *C. pallidostigmatica*, might have been introduced later than CS-cpSSU.i1. CS-cpSSU.i3, found only in *Caulerpa* of Ulvophyceae, might have been introduced early in the Ulvophyceae.

### The possible use of the group I intron and ORF in phylogenetic studies

As discussed above, the three introns are probably vertically transmitted at least in limited lineages of Ulvophyceae. The three introns have potential in phylogenetic studies, especially at the population level, in which fast evolving genes are required. First, *Caulerpa* has three ORFs with LAGLI-DADG motifs that are fast evolving (Lucas *et al.* 2001). Second, group I introns are variable compared to Group II introns (Manhart & Palmer 1990; Qiu *et al.* 1998; Qiu and Lee 2000; Lee and Manhart 2002a, b) and *Caulerpa* has three introns rather than one intron. CS-cpSSU.i2 has proven to be useful in population studies of *Caulerpa* (Meusnier *et al.* 2002). Third, CS-cpSSU.i3, the third intron of *Caulerpa*, has repeats of GAAATAT, which are rarely found in group I introns. Besides, the presence of the 28 repeats should provide enough variation in population level research.

### ACKNOWLEDGEMENTS

This project was supported in part by NSF Grant 9306462 to JRM and by KRF-2003-003-C00135 to J.L. J. Lee is supported by BK21 from the Ministry of Education and Human Resources.

### REFERENCES

- Bao Y. and Herrin D.L. 1993. Nucleotide sequence and secondary structure of the chloroplast group I intron Cr.psbA-2: novel features of this self-splicing ribozyme. *Nucleic Acids Res.* **21**: 1667.
- Besendahl A., Qiu Y.-L., Lee J., Palmer J.D. and Bhattacharya D. 2000. The cyanobacterial origin and vertical transmission of the plastid tRNA(Leu) group-I intron. *Curr. Genet.* **37**: 12-23.
- Burke J.M., Belfort M., Cech T.R., Davies R.W., Schweyen R.J., Shub D.A., Szostak J.W. and Tabak H.F. 1987. Structural conventions for group I introns. *Nucleic Acids Res.* **15**: 7217-7221.
- Douglas S.E. and Penny S.L. 1999. The plastid genome of the cryptophyte alga, *Guillardia theta*: Complete sequence and conserved synteny groups confirm its common ancestry with red algae. *J. Mol. Evol.* **48**: 236-244.
- Durocher V., Gauthier A., Bellemare G. and Lemieux C. 1989. An optional group I intron between the chloroplast small subunit rRNA genes of *Chlamydomonas moewusii* and *C. eugametos*. *Curr. Genet.* **15**: 277-282.
- Evrard J.-L., Kuntz M., Straus N.A. and Weil J.-H. 1988. A class-I intron in a cyanelle tRNA gene from *Cyanophora paradoxa*: phylogenetic relationship between cyanelles and plant chloroplasts. *Gene* **71**: 115-122.
- Genetic Computer Group 1991. *Program Manual for the GCG Package, Version 7*. Madison, Wisconsin.
- Gloeckner G., Rosenthal A. and Valentin K. 2000. The structure and gene repertoire of an ancient red algal plastid genome. *J. Mol. Evol.* **51**: 382-390.
- Herrin D.L., Bao Y., Thompson A.J. and Chen Y.F. 1991. Self-splicing of the *Chlamydomonas* chloroplast *psbA* introns. *Plant Cell* **3**: 1095-1107.
- Kapoor M., Wakasugi T., Yoshinaga K. and Sugiura M. 1996. The chloroplast *chlL* gene of the green alga *Chlorella vulgaris* C-27 contains a self-splicing group I intron. *Mol. Gen. Genet.* **250**: 655-664.
- Kuhse M.G., Strickland R. and Palmer J.D. 1990. An ancient group I intron shared by eubacteria and chloroplasts. *Nature* **250**: 1570-1573.
- Lee J. and Manhart J.R. 2002a. Four embryophyte introns and *psbB* operon indicate *Chlorokybus* as a basal Streptophyte lineage. *Algae* **17**: 53-58.
- Lee J. and Manhart J.R. 2002b. The chloroplast *rpl23* gene cluster of *Spirogyra maxima* (Charophyceae) shares many similarities with the angiosperm *rpl23* Operon. *Algae* **17**: 59-68.
- Lehman R. and Manhart J.R. 1997. A preliminary comparison of restriction fragment patterns in the genus *Caulerpa* (Chlorophyta) and the unique structure of the chloroplast genome of *Caulerpa sertularioides*. *J. Phycol.* **33**: 1055-1062.
- Lucas P., Otis C., Mercier J.P., Turmel M. and Lemieux C. 2001. Rapid evolution of the DNA-binding site in LAGLIDADG homing endonucleases. *Nucleic Acids Res.* **29**: 960-969.
- Manhart J.R., Kelly, K. Dudock B.S. and Palmer J.D. 1989. Unusual characteristics of *Codium fragile* chloroplast DNA revealed by physical and gene mapping. *Mol. Gen. Genet.* **216**: 417-21.
- Manhart J.R. and Palmer J.D. 1990. The gain of two chloroplast tRNA introns marks the green algal ancestors of land plants. *Nature* **345**: 500-505.
- Manhart J.R. 1995. Chloroplast 16S rDNA sequences and phylogenetic relationships of fern allies and ferns. *Am. Fern. J.* **85**: 182-192.
- Mattox K.R. and Stewart K.D. 1983. Classification of the green algae: A concept based on comparative cytology. In: Irvine D.E.G. and John D.M. (eds). *Systematics of the Green Algae*. Academic Press. London. pp. 29-72.
- Meusnier I., Valero M., Destombe C., Gode C., Desmarais E., Bonhomme F., Stam W. T. and Olsen, J. L. 2002. Polymerase chain reaction-single strand conformation polymorphism analyses of nuclear and chloroplast DNA provide evidence for recombination, multiple introduc-

- tions and nascent speciation in the *Caulerpa taxifolia* complex. *Mol. Ecol.* **11**: 2317-2325.
- Nozaki H., Takahara M., Nakazawa A., Kita Y., Yamada T., Takano H., Kawano S. and Kato M. 2002. Evolution of *rbcl* group IA introns and intron open reading frames within the colonial Volvocales (Chlorophyceae). *Mol. Phylogenet. Evol.* **23**: 326-338.
- Ohta N., Matsuzaki M., Misumi O., Miyagishima S., Nozaki H., Tanaka K., Shin-i T., Kohara Y. and Kuroiwa T. 2003. Complete sequence and analysis of the plastid genome of the unicellular red alga *Cyanidioschyzon merolae*. *DNA Res.* **10**: 67-77.
- Qiu Y.-L., Cho Y., Cox J.C. and Palmer J.D. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature* **394**: 671-674.
- Qiu Y.-L. and Lee J. 2000. Transition to a land flora: a molecular phylogenetic perspective. *J. Phycol.* **36**: 799-802.
- Reith M.E. and Munholland J. 1995. Complete nucleotide sequence of the *Porphyra purpurea* chloroplast genome. *Plant Mol. Biol. Rep.* **13**: 333-335
- Swofford D.L. 2000. *Phylogenetic analysis using parsimony, version 4.0 beta.: User's manual*. Illinois Natural History Survey, Champaign, IL.
- Thompson A.J. and Herrin D.L. 1991. In vitro self-splicing reactions of the chloroplast group I intron Cr.LSU from *Chlamydomonas reinhardtii* and in vivo manipulation via gene-replacement. *Nucleic Acids Res.* **19**: 6611-6618.
- Turmel M., Boulanger J. and Lemieux C. 1989. Two group I introns with long internal open reading frames in the chloroplast psbA gene of *Chlamydomonas moewusii*. *Nucleic Acids Res.* **17**: 3875-3887.
- Turmel M., Gutell R.R., Mercier J.P., Otis C. and Lemieux C. 1993. Analysis of the chloroplast large subunit ribosomal RNA gene from 17 *Chlamydomonas* taxa. Three internal transcribed spacers and 12 group I intron insertion sites. *J. Mol. Biol.* **232**: 446-467.
- Turmel M., Mercier J.P., Cote V., Otis C. and Lemieux C. 1995. The site-specific DNA endonuclease encoded by a group I intron in the *Chlamydomonas pallidostigmatica* chloroplast small subunit rRNA gene introduces a single-strand break at low concentrations of Mg<sup>2+</sup>. *Nucleic Acids Res.* **23**: 2519-2525.
- Ustinova I., Krienitz L. and Huss V.A.R. 2001. *Closteriopsis acicularis* (G.M. Smith) Belcher et Swale is a fusiform alga closely related to *Chlorella kessleri* Fott et Novakova (Chlorophyta, Trebouxiophyceae). *Eur. J. Phycol.* **36**: 341-351.
- Xu M.-Q., Kathe S.D., Goodrich-Blair H., Nierzwicki-Bauer S.A. and Shub D.A. 1990. Bacterial origin of a chloroplast intron: conserved self-splicing group I introns in Cyanobacteria. *Science* **250**: 1566-1570.

---

Received 25 July 2003

Accepted 5 August 2003