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LINKAGE OF 5S RIBOSOMAL DNA TO OTHER rDNAS IN THE CHROMOPHYTIC ALGAE AND RELATED TAXA[1]

Hiroshi Kawai[2]

Kobe University Research Center for Inland Seas, Rokkodai, Kobe 657, Japan

Takeshi Nakayama, Isao Inouye

Institute of Biological Sciences, University of Tsukuba, Tsukuba 305, Japan

and

Atsushi Kato

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan

ABSTRACT

Gene organization within the nuclear ribosomal DNA cistron and linkage of the 5S rDNA gene to the cistron were surveyed in 20 taxa of protists representing most of the Chromophyta (stramenopiles) and representatives of the Dinophyceae (alveolata) and Euglenophyceae. The intergenic spacer, which separates adjacent cistrons, was first PCR-amplified from total DNA using primers anchored in the 3′ end of the large subunit and the 5′ end of the small subunit in the next downstream cistron. Presence of the 5S gene in the cistron was determined by a second round of PCR using primers anchored in the large subunit and the 5S gene. Where 5S-linked rDNA was not detected in the cistron, the presence of 5S tandem repeating units were confirmed by the PCR of 5S-5S fragments from the total DNA. Results show that most of the Chromophyta, as well as Opalina, Proteromonas *(colorless stramenopiles), Dinophyceae, and Euglenophyceae have a 5S-linked type of rDNA organization. In contrast, only tandem repeats of 5S rDNA were detected in Bacillariophyceae and Synurophyceae. The occurrence of 5S-unlinked rDNA is hypothesized to be the result of secondary transfer from an ancestral, linked 5S type. The 5S-linked type of rDNA organization is apparently common in protists. Given the fact that most of these protists have mitochondria with tubular or discoid cristae, as compared with flattened cristae common in higher plants and animals, we conclude that the 5S-linked type of rDNA diverged at a very early stage in the evolution of eukaryotes.*

Key index words: 5S-linked rDNA; 5S rRNA; Chromophyta; Euglenophyceae; Dinophyceae; Opalina; *phylogeny;* Proteromonas; *stramenopiles*

Virtually all organisms have multiple copies of the ribosomal genes (rDNA) which in turn code for ribosomal RNA (rRNA) (Gerbi 1985, Appels and Honeycutt 1986). In most prokaryotes (eubacteria), the rDNA operon consists of three genes arranged in the order 5′-16S-23S-5S-3′ and transcribed by a single RNA polymerase (RNA polymerase I). If multiple copies are present in a genome, the cistrons are repeated in tandem arrays separated by a nontranscribed, intergenic spacer (IGS). In most eukaryotes (N.B. surveys based almost exclusively on angiosperms and animals), the operon consists of three genes arranged in the order 5′-18S-5.8S-25S-3′ in which the 18S is homologous to the prokaryotic 16S, the 5.8S to a part of the prokaryotic 23S, and the 25S to the rest of the prokaryotic 23S, respectively. These, too, are tandemly repeated and separated by IGS sequences. The 5S rDNAs are repeated on separate loci and transcribed by a different RNA polymerase (RNA polymerase III; Gerbi 1985, Appels and Honeycutt 1986). Exceptions to the above generalizations have been found in the archaebacteria in which the cistron may be interrupted. This results in variable cistronic compositions and repeat patterns (Larsen et al. 1986, Bensaadi-Merchermek et al. 1995).

In some eukaryotes the 5S gene has been found in the same repeating unit as the other rDNAs (so-called "linked 5S"; Rubin and Sulston 1973, Maizels 1976, Kramer et al. 1978, Drouin et al. 1987, 1992, Vahidi et al. 1988, Belkhiri et al. 1992, Hofmann et al. 1993, Gilson et al. 1995, Kawai et al. 1995, Belkhiri and Klassen 1996) but is thought to be transcribed separately by a different RNA polymerase. Many of the 5S-linked rDNA organizations reported in eukaryotes are from taxa considered to be phylogenetically "primitive" as compared with 5S-unlinked types associated with phylogenetically "advanced" animals and plants.

It has been hypothesized that the eukaryotic types of rDNA organization, either of the 5S-linked or 5S-unlinked, have their evolutionary origins in the prokaryotic type of rDNA, and that the eubacterial type of rDNA with a 16S-23S-5S operon is more primitive than the split type found in the archaebacteria (Gerbi 1985). However, putative evolutionary linkage between the prokaryotic and eukaryotic types of rDNAs remains controversial because of conflicting distributional observations in different

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[2] Author for correspondence.

TABLE 1. *Systematic position, taxon, and source of the DNA used in the present study.*

Systematic position	Taxon	Source
Phaeophyceae		
Scytosiphonales	*Scytosiphon lomentaria* (Lingb.) Link	field material, Muroran
Chrysophyceae		
Chrysomeridales	*Phaeosaccion collinsii* Farlow	culture, H. Kawai
Synurophyceae		
Synurales	*Synura petersenii* Korshikov	culture, NIES233
Xanthophyceae		
Vaucheriales	*Vaucheria* (sect. *Corniculatae*) *terrestris* Götz	culture, H. Kataoka
Vaucheriales	*Vaucheria* (sect. *Woroninia*) *dichotoma* (L.) Agardh	culture, H. Kataoka
Mischococcales	*Ophiocytium* sp.	culture, T. Nakayama
Raphidophyceae		
Raphidomonadales	*Heterosigma akashiwo* (Hada) Hada	culture, NIES146
Raphidomonadales	*Olithodiscus luteus* Carter	culture, NIES15
Eustigmatophyceae		
Eustigmatales	*Eustigmatos vischeri* Hibberd	culture, UTEX310
Bacillariophyceae		
Centrales	*Ditylum brightwellii* (T. West) Grunow et Heurck	culture, NIES350
Pennales	*Nitzschia palea* (Kützing) W. Smith	culture, NIES487
Dictyochophyceae		
Pedinellales	*Pseudopedinella pyriforme* Carter	culture, H. Sekiguchi
Incertae cedis	*Sulcochrysis biplastida* Honda, Kawachi et Inouye	culture, D. Honda
	Phaeomonas parva Honda et Inouye *nom. nud.*	culture, D. Honda
Slopalinids	*Opalina* sp.	from intestine of *Rana* sp.
	Proteromonas lacertae (Grassé) Grassé	culture, ATCC 30270
Haptophyceae		
Isochrysidales	*Emiliania huxlery* (Lohmann) Hay et Mohler	culture, M. Kawachi
Dinophyceae		
Prorocentrales	*Prorocentrum micans* Ehrenberg	culture, T. Nakayama
Gymnodiniales	*Amphidinium* sp.	culture, T. Nakayama
Euglenophyceae		
Eutreptiales	*Eutreptiella* sp.	culture, T. Nakayama

higher order lineages; that is, the 5S-linked type is found in phylogenetic groups as different as protists, fungi, and arthropods. Some of this may be attributed to secondary introductions of unlinked 5S into 18S-5.8S-25S rDNA repeating units (Drouin et al. 1987, 1992, Drouin and Moniz de Sá 1995)

The division Chromophyta (Christensen 1962), or the kingdom Chromista (Cavalier-Smith 1986, 1989), is a large heterogeneous group that includes more than 10 class-level or higher lineages. To date, rRNA gene organization has only been investigated in two of the lineages, the Phaeophyceae (Kawai et al. 1995) and oomycetes (Rozek and Timberlake 1979). The present paper surveys rDNA organization in representative members of the remaining lineages in order to explore the phylogenetic implications of these data.

MATERIALS AND METHODS

Sixteen species in 11 classes and four species of uncertain taxonomic position, representing many members of the Chromophyta (stramenopiles), Dinophyceae (alveolata), and Euglenophyceae, were examined (Table 1).

DNA extraction. Total DNA was extracted from *Scytosiphon lomentaria* (Lyngb.) Link according to Kawai et al. (1995). For the remaining samples the following protocols were used.

Organisms without cell walls were harvested by centrifugation; suspended in 400 μL of extraction buffer containing HTE buffer [(50 mM Tris-HCl, 20 mM EDTA, pH 8), *N*-lauroylsarcosine (20 $mg \cdot mL^{-1}$) and proteinase K (200 $\mu g \cdot mL^{-1}$)], and incubated at room temperature for 1 h; followed by the addition of an equal volume of phenol saturated with 1 M Tris-HCl (pH 8) and gentle mixing for 10 min; followed by centrifugation at 7,000 × *g* for 10 min. The upper aqueous phase was transferred to a new tube, and an equal volume of phenol/CIA (chloroform : isoamyl alcohol = 24:1) was added and mixed gently for 10 min and followed by centrifugation as before. This step was repeated a second time. The upper phase was transferred to a new tube, and 20 μL of 4 M NaCl was added and mixed, followed by 2.5 volumes of cold ethanol. Precipitation of DNA was allowed to proceed at −20 °C (1 h to overnight) followed by centrifugation at ca. 14,000 × *g* for 10 min. The pellet was rinsed in 70% ethanol, air-dried and dissolved in 0.1–1 mL TE buffer (10 mM Tris-HCl, 1 mM EDTA).

For the organisms with cell walls, UNSET buffer (Garriga et al. 1984) was used for extraction of total DNA. Algal tissue was ground to a fine powder in liquid nitrogen or harvested by centrifugation and then lysed in 400 μL UNSET buffer (8 M urea, 2% SDS, 0.15% NaCl, 1 mM EDTA, 100 mM Tris-HCl, pH 7.5). The lysate was mixed gently for 30 min, followed by the addition of an equal volume of phenol/CIA mixture, and followed the same procedure as described above.

PCR strategy for detection of presence or absence 5S rDNA linkage. PCR

TABLE 2. *List of primers and their sequences.*

Name of primers	Sequences
18S-L	TGGATGTGGTAGCCGCTCTCAGGCTCCCTCTC
25S-L	AGCGGGGAAAGAAGACCCTGTTGAGCTTGACTC
25S-S	GATTCTTCGATGTCGGCTCT
5S-3	TTCCACGTGGTCCCCCACC
5S-P	TCGATCGCACCACATCCCGT
5S-H	TAAACTGCACCCGGTCTCGT
5S-B	ATGCCAATACCGCTTCCCGT

(polymerase chain reaction) was performed with three sets of primers. Primers were designed from the conserved sequences based on the comparisons of the reported rDNA sequences of *Scytosiphon lomentaria* and several land plants (Takaiwa et al. 1984, 1985a, b, Kato et al. 1990, Kawai et al. 1995). Primer set-I consisted of 18S-L and 25S-L (Table 2, Fig. 1). Primer set-II consisted of 25S-S and 5S-3 or 25S-S with either the 5S-P (Phaeophyceae), 5S-B (Bacillariophyceae), or 5S-H (Chrysophyceae) reverse primer (Table 2, Fig. 2). Primer set-III (for taxa in which no 5S link was detected) consisted of 5S-3 with 5S-P, 5S-H, or 5S-B. The primer 25S-S was designed as a downstream 25S-L primer sequence (i.e. inside the PCR product sequences) based on known sequences. The transcriptional orientation of the linked 5S rDNA can be of either direction, so 5S primers of both transcriptional directions were examined (5S-3 and the others, Fig. 2).

PCR reactions were performed using a TaKaRa LA PCR Kit and a TaKaRa PCR Amplification Kit (Takara Shuzo Co. Ltd., Ohtsu, Shiga, Japan). The reaction conditions followed the manufacturer's recommendation. PCR products were separated by electrophoresis in 1.0% or 1.5% agarose including Tris-acetate buffer (40 mM Tris, 10 mM NaOAC, 1 mM NaEDTA, pH8) and 0.5 $\mu g \cdot mL^{-1}$ ethidium bromide. For the detection of bands, gels were illuminated with UV light and photographed using Polaroid Type T667 films (Polaroid, Cambridge, Massachusetts, USA).

Direct sequence from PCR products. In order to confirm the identity of the PCR products obtained, amplified fragments were partially sequenced from both directions using the ABI PRISM Dye Terminator Cycle Sequencing Kit (Perkin Elmer Cetus, Norwalk, Connecticut, USA) and an ABI 373A automated sequencer (Applied Biosystems, Foster City, California, USA) following the manufacturers' recommendations. PCR products were purified for sequencing by low-melting-point agarose gel electrophoresis. Excised gel pieces were melted at 67 °C, and the fragments were purified by phenol treatment followed by ethanol precipitation. The same primers used in the PCR reactions were used for sequencing.

RESULTS

25S-18S PCR. The PCR using the primers 25S-L and 18S-L for the extracted total DNA of the 20 taxa produced products ranging from ca. 1.5–12 kb (Fig. 3). Bands of less than 1 kb were found in *Sulcochrysis, Olithodiscus,* and *Phaeomonas* and considered to be nonspecific products. Some samples produced multiple bands at regular intervals (e.g. *Sulcochrysis, Synura,* and *Phaeomonas*), whereas others produced bands at irregular intervals (e.g. *Olithodiscus, Phaeosaccion, Opalina,* and *Pseudopedinella*). All of the distinct bands, excluding ones that were apparently too

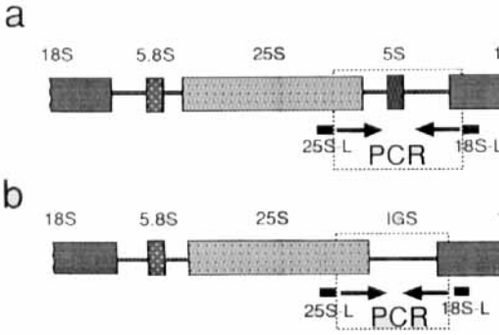

FIG. 1. Cistron organization of rDNA. a. 5S-linked type, b. 5S-unlinked type. Sites of primary PCR amplifications shown.

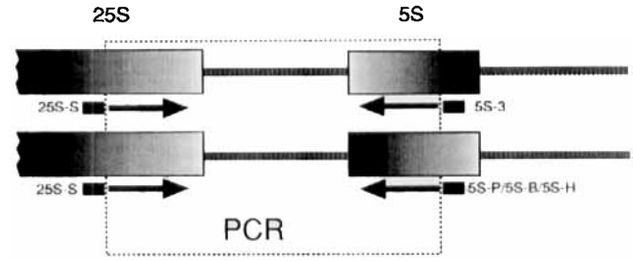

FIG. 2. Secondary 25S-5S PCR indicating the 5S primers for different transcriptional directions of the 5S rDNA. Gradation across rectangles indicates the direction of transcription.

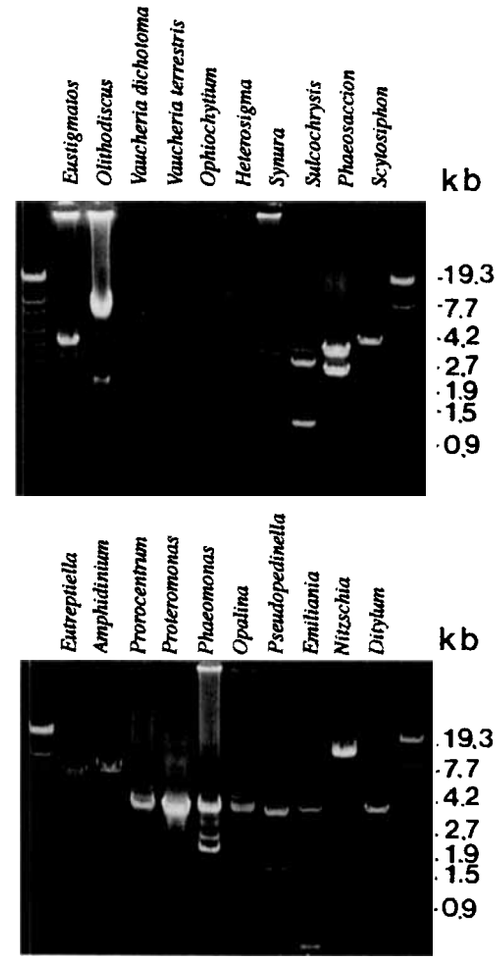

FIG. 3. Separated PCR products on agarose gels of the primary 25S-IGS-18S amplifications.

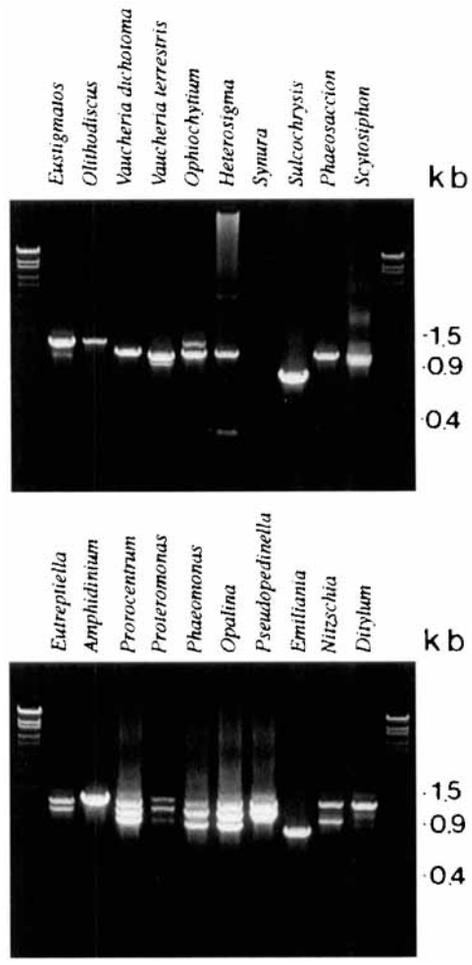

FIG. 4. Separated PCR products on agarose gels of secondary 25S-5S amplifications on isolated DNA from the products of the primary PCR.

short, were excised from the gel, DNA extracted, and used for the second round of PCR.

25S-5S PCR. The PCR using the primers 25S-S and 5S-3, 5S-P/5S-H/5S-B for the first PCR products of 25S-18S PCR resulted in products ranging from ca. 0.8–1.3 kb (Fig. 4). Figure 5 shows the partial 5S rDNA sequences of the 25S-5S PCR products. Similarly the presence of the sequences encoding the 25S rDNA in the PCR products was also examined (data not shown).

No distinct band was detected in *Synura,* and many other samples included multiple bands. Most of these bands were excised from the gels, and the DNA was extracted from the gels and used for the subsequent sequencing. Among these samples, as shown in Figure 4, the sequences encoding 5S rDNA sequences were detected in 17 taxa from at least one of the bands examined in each sample. Although multiple bands were seen in the PCR products of *Ditylum* and *Nitzschia* (Fig. 3), 5S rDNA sequences were detected in none of them. 5S rDNA was not detected in the faint band from *Synura.*

5S-5S PCR. PCR products for *Synura* (650* bp), *Ditylum* (450 and 480* bp) and *Nitzschia* (220, 350*, and 450 bp) are shown in Figure 6. Sequences homologous with 5S rDNA in the above fragments are indicated with an asterisk. Figure 5 shows the 5S rDNA sequences in the 5S-5S PCR products in *Synura, Ditylum,* and *Nitzschia.*

DISCUSSION

In the present study most of the taxa representing the Chromophyta (Phaeophyceae, Chrysophyceae, Xanthophyceae, Raphidophyceae, Eustigmatophyceae, Haptophyceae, Dictyochophyceae) and their presumptive relatives *Opalina* and *Proteromonas* (slopalinids; Patterson 1989, Silberman et al. 1996), as well as Dinophyceae and Euglenophyceae, were shown to have the 5S-linked type of rDNAs, with

5S-linked

Scytosiphon	AGGAACGGCCATACCACGCCGATCGCACCACATCCCGTCCGCTCTGTGAAGTTAAGCGGCGTCGGGCCAGGCTAGTA
Phaeosaccion	ATCCACGGCCACAGGACTCAGAAAGCACCGCATCCCGTCCGATCTGCGAAGTTAAGCAGAGTATCGCCTAGTTAAT-
Sulcochrysis	ATCCACGGCCATAGGACTTCGAAAGCACCGCATCCCGTCCGATCTGCGCAGTTAACCGGAGTGCCGCCTAGTTAGT-
Heterosigma	ATCCACGGCCATAGGACTCAGAAAATACCGCATCCCGTCCGATCTGCGAAGTCAAGCTGAGTACCGCCTAGTTA---
Ophiocytium	ATCTGCGGCCACAGAACCGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCACGGTATCGACTAGTTAG--
Vauch. terrestris	ATCCACGGCCATAGGACTCTGAAAGCACCGCATCCCGTCCGATCTGCGCAGTTAACCAGAGTACCGCCTAGTTAGA-
Vauch. dichotoma	ATCCACGGCCATAGGACTCAGAAAATACCGCATCCCGTCCGATCTGCGAAGTCAAGCTGAGTACCGCCTAGTTAGA-
Olithodiscus	ATCTGCGGCCACAGAACCGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCACGGTATCGACTAGTTAG--
Eustigmatos	TACTGCGGCCACAGAACCGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCACGGTATCGACTAGTTAGA-
Emiliania	ATCCACGGCCATAGGACCCAGAAAATACCGCATCCCGTCCGATCTGCGAAGTCAAGCTGGGTACCGCCTCGTTAGT-
Pseudopedinella	ATCTGCGGCCACAGAACCGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCACGGTATCGACTAGTTAGT-
Opalina	ATCTGCGGCCACAGAACCGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCACGGTATCGACTAGTTAGT-
Phaeomonas	ATCTGCGGCCACAGNACTGNGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCANGGTATCGTCTAGTTAGT-
Proteromonas	ATCTGCGGCCACAGAACCGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCACGGTATCGTNTAGTTA---
Prorocentrum	ATCTGCGGCCACAGGACTGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCAGGGTATCGATTAGTTAG--
Amphidinium	GTTGGCGGCCATACCGAGTTGAAAGCACCAGATCTCTTCTGACCTCTGAAGTTAAACAGCTCAGGGCCTGGTTAGT-
Eutreptiella	ATCTGCGGCCACAGAACCGTGAAAGCACCGTATCCCGTCCGATCTGCGAAGTTAAGCACGGTATCGACTAGTTAGT-

5S-unlinked

Synura	ATGAACGGTCATATCAAGGATACTGCACCCGCTCTCGTCTGATCGCGGAAGTTAAACTCCTTCGAGCACACGGGGGGGGACCAACGGGGANGCCCGGGGCNGTTCGTTTAGGACTCC-
Ditylum	AGGAACGACCATACAACATNGATAGCACCACNTCCCGTCTGCTCAGCGAAGTTAAGCATTGTCTGGCCTGGTTANTACTACGGTGGGGGACCAACGTGGAATCCCAGCTGTTGTTCTT
Nitzschia	GGGAACGACCATACCAGCTTGATAGCACCACATCCTGTCCGCTCTGTGAAGTTAAGCATGTTCGGGCTCGGTTANTANTACGGTGGGGGACCACGNGGGNATCCNGAGTGTTNTTCTC

FIG. 5. Detected partial 5S rDNA sequences in the 25S-5S PCR products in the 17 taxa examined indicating taxa with 5S-linked rDNA organization, and those in the 5S-5S PCR products in *Synura, Ditylum,* and *Nitzschia* indicating taxa with 5S-unlinked rDNA organization.

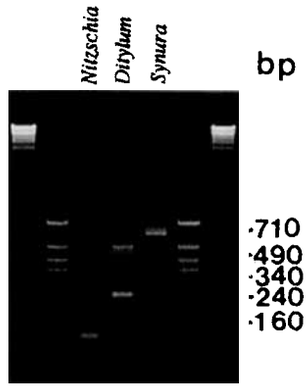

FIG. 6. Electrophoresis of the PCR amplification products of the 5S-5S region on the extracted total DNA.

linked 5S sequences arranged in the same transcriptional direction as the other rDNAs [i.e. as in the prokaryote (eubacteria) type of rDNA]. Within the Chromophyta, 5S-linked rDNA was not detected in *Synura* (Synurophyceae) nor in *Ditylum* and *Nitzschia* (both Bacillariophyceae). Here, only tandemly arranged repeat units of 5S rDNA were found. Curtis and Rawson (1981) determined that *Euglena* has a 5S-linked rDNA but the transcriptional direction of the sequence was not specified. In the present study, *Eutreptiella* sp., a close relative of *Euglena,* is shown to have 5S-linked rDNA arranged in the same transcriptional direction as the other rDNAs.

The trans-spliced leader (TSL) was recently found to be linked with 5S rDNA in some protozoa (trypanosomatids, Aksoy et al. 1992; *Bodo,* Campbell 1992; *Toxoplasma,* Guay et al. 1992; *Euglena,* Keller et al. 1992). On the other hand, in the crustacean *Artemia,* a histone gene is reported to be linked with 5S rDNA (Cruces et al. 1989). Such linkages may be explained by the fact that the 5S rDNA is transcribed by RNA polymerase III separately from the other rDNAs in eukaryotes (by RNA polymerase I). This could result in a translocation of the gene to another locus relatively easily. Although it is possible that taxa containing 5S-linked rDNA also possess tandemly repeated 5S, attempts to amplify 5S-5S repeating units in the primary PCR experiments were unsuccessful. From this we concluded that only a single rDNA organization per genome is characteristic.

Maslov et al. (1993) discussed the linkage of TSL and 5S rDNA in the euglenozoa, *Trypanosoma, Herpetomonas, Bodo,* and *Euglena.* They considered that the linked stage represents an evolutionary primitive condition within the phylogenetic lineage. In contrast, Drouin and Moniz de Sá (1995) suggested that linkage of 5S rDNA with other genes is a secondary feature in evolution. According to their hypothesis, segregation of 5S rDNA from the other rDNAs occurred early followed by 5S rDNA duplication in some other loci isolated from the 18S-5.8S-25S repeating unit. From there, secondary linkages with other genes (e.g. 18S-5.8S-25S rDNA, histone genes) occurred in various phylogenetic groups of eukaryotes. Support for their hypothesis comes from the fact that: 1) in 5S rDNA genes linked with the other rDNAs, both transcriptional directions are observed depending on the taxa; 2) linkages are observed not only in phylogenetically primitive groups but also in putatively advanced groups such as arthropods; and 3) linkages are found not only with 18S-5.8S-25S rDNA but also with some other genes (TSL, histone). However, it is misleading to discuss the linkages of different types of genes at the same time. They are not equivalent. If we focus only on the linkage of 5S rDNA and other rDNAs, it appears that linkages and loss of linkages have occurred multiple times in the evolution of eukaryotes within various phylogenetic groups. This means that 5S-linked status is not necessarily a more derived condition, as suggested by Drouin and Moniz de Sá (1995). This is represented in Figure 7. The 5S-linked type of rDNA (rectangles) is distributed widely among Chromophyta, and the 5S-unlinked type of rDNA of Synurophyceae and Bacillariophyceae (round-cornered rectangles) is more likely to be caused by the secondary separation of 5S from the 5S-linked type. The fact that all of the 5S-linked rDNA had the same transcriptional orientations also suggests that the 5S-linkage is of the same origin. Although information on rDNA organization is still lacking in a number of major phylogenetic lineages referred to in Figure 7 (notably the choanoflagellates, bicosoechids, labyrinthulids, Ciliophora, Rhodophyceae), many other protists that are considered to have diverged in the early stages of protist evolution (e.g. Euglenophyceae, Curtis and Rawson 1981; *Toxoplasma,* Guay et al. 1992; *Dictyostelium,* Hofmann et al. 1993; Cryptophyceae, Gilson et al. 1995) have the 5S-linked type of rDNA.

Moreover, most of the Chromophyta, as well as *Opalina, Proteromonas, Dictyostelium,* and Dinophyta, that have the 5S-linked type of rDNA also have tubular cristae in the mitochondria. These are morphologically distinct from the flat cristae common to higher plants and animals (Leipe et al. 1994, Patterson 1994). *Euglena* also has a unique type of mitochondrial cristae (discoid or radiate) and differs from the other groups. In the phylogenetic relationship schemes of Cavalier-Smith (1993), phylogenetic groups that include such mitochondria (discoid or tubular) represent more primitive groups that those with the flat type of cristae. This feature might further support the notion that 5S-linked rDNA organization is the primitive condition in protists.

In conclusion, the present survey (as well as some other recent reports) shows that the 5S-linked type of rDNA is common in a large proportion of protistin lineages (e.g. stramenopiles, alveolata, euglenoids) and likely to be more primitive than 5S-unlinked rDNA. However, it is still premature to draw definitive conclusions about the most primitive

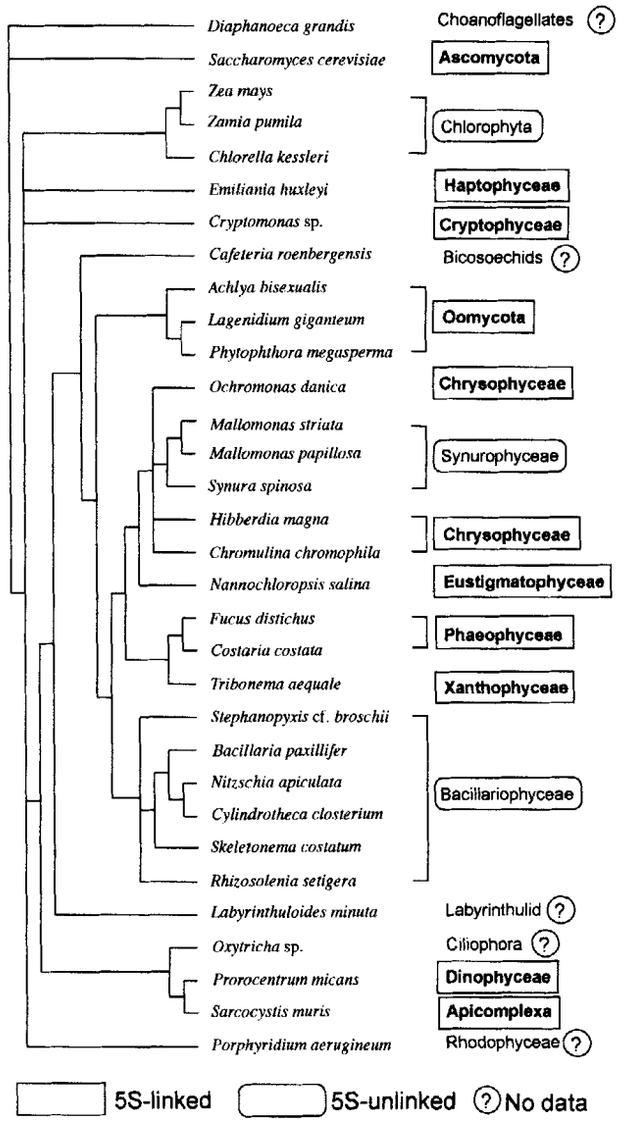

FIG. 7. Phylogenetic tree of protists deduced from 18S rDNA sequences (Leipe et al. 1994) and the distribution of rDNA organization types (this study and previous reports). Taxa within rectangles indicate 5S-linked rDNA; taxa within round-cornered rectangles indicate 5S-unlinked rDNA; ? indicates no data obtained. The species shown in the 18S rDNA tree and the species for which rDNA organizations were studied were not always identical with respect to species, but only of genus. Data for Ascomycetes, Chlorophyta, Cryptophyceae, and Oomycetes follow Gerbi (1985), Gilson et al. (1995), and Kawai et al. (1995).

rDNA organizations in eukaryotes because our knowledge in the most primitive eukaryotes is still very scanty.

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Aksoy, S., Shay, G. L., Villanueva, M. S., Beard, C. B. & Richards, F. F. 1992. Spliced leader RNA sequences of *Trypanosoma rangeli* are organized within the 5S rRNA-encoding genes. *Gene* 113:239–43.

Appels, R. & Honeycutt, R. L. 1986. rDNA: evolution over a billion years. *In* Dutta, S. K. [Ed.] *DNA Systematics.* CRC Press, Boca Raton, Florida, pp. 81–135.

Belkhiri, A., Buchko, J. & Klasson, G. R. 1992. The 5S ribosomal RNA gene in *Pythium* species: two different genomic locations. *Mol. Biol. Evol.* 9:1089–102.

Belkhiri, A. & Klassen, G. R. 1996. Diverged 5S rRNA sequences adjacent to 5S rRNA genes in the rDNA of *Pythium pachycaule. Curr. Genet.* 29:287–92.

Bensaadi-Merchermek, N., Salvado, J. C., Cagnon, C., Karama, S. & Mouches, C. 1995. Characterization of the unlinked 16S rDNA and 23S-5S rRNA operon of *Wolbachia pipientis,* a prokaryotic parasite of insect gonads. *Gene* 165:81–6.

Campbell, D. A. 1992. *Bodo caudatus* medRNA and 5S rRNA genes; tandem arrangement and phylogenetic analysis. *Biochem. Biophys. Res. Com.* 182:1053–8.

Cavalier-Smith, T. 1986. The kingdom Chromista: origin and systematics. *In* Round, F. E. & Chapman, D. J. [Eds.] *Progress in Phycological Research,* Vol. 4. BioPress, Bristol, pp. 309–47.

——— 1989. The kingdom Chromista. *In* Green, J. C, Leadbeater, B. S. C. & Diver, W. I. [Eds.] *The Chromophyte Algae: Problems and Perspective.* Clarendon Press, Oxford, pp. 381–407.

——— 1993. Kingdom Protozoa and its 18 phyla. *Microbiol. Rev.* 57:953–94.

Christensen, T. 1962. Alger. *In* Böcher, T. W., Lange, M. & Sørensen, T. [Eds.] *Systematisk Botanik,* Vol. 2, No. 2. Munksgaard, Copenhagen, 178 pp.

Cruces, J., Diaz-Guerra, M., Gil, I. & Renart, J. 1989. The 5S rRNA-histone repeat in the crustacean *Artemia:* structure, polymorphism and variation of the 5S rRNA segment in different populations. *Nucl. Acids Res.* 17:6283–97.

Curtis, S. E. & Rawson, J. R. Y. 1981. Characterization of the nuclear ribosomal DNA of *Euglena gracilis. Gene* 15:237–47.

Drouin, G., Hofman, J. D. & Doolittle, W. F. 1987. Unusual ribosomal RNA gene organization in copepods of the genus *Calanus. J. Mol. Biol.* 196:943–6.

Drouin, G. & Moniz de Sá, M. 1995. The concerted evolution of 5S ribosomal genes linked to the repeat units of other multigene families. *Mol. Biol. Evol.* 12:481–93.

Drouin, G., Sévigny, J-M., McLaren, I. A., Hofman, J. D. & Doolittle, W. F. 1992. Variable arrangements of 5S ribosomal genes within the ribosomal DNA repeats of arthropods. *Mol. Biol. Evol.* 9:825–35.

Garriga, G. H., Bertrandt, H. & Lambowitz, A. 1984. RNA splicing in Neurospora mitochondria: nuclear mutants defective in both splicing and 3′ end synthesis of larger rRNA. *Cell* 36: 623–34.

Gerbi, S. A. 1985. Evolution of ribosomal DNA. *In* MacIntyre, R. J. [Ed.] *Molecular Evolutionary Genetics.* Plenum Press, New York, pp. 419–517.

Gilson, P. R., Adcock, G. J., Howlett, B. J. & McFadden, G. I. 1995. Organisation and sequence analysis of nuclear-encoded 5S ribosomal RNA genes in cryptomonad algae. *Curr. Gen.* 27: 239–42.

Guay, J. M., Huot, A., Gagnon, S., Tremblay, A. & Levesque, R. C. 1992. Physical and genetic mapping of cloned ribosomal DNA from *Toxoplasma gondii:* primary and secondary structure of the 5S gene. *Gene* 114:165–71.

Hofmann, J. T., Winckler, A., Hanenkamp, M., Bukenberger, G., Schumann, G., Marscalek, R. & Dingermann, T. 1993. The *Dictyostelium discoideum* 5S rDNA is organized in the same transcriptional orientation as the other rDNAs. *Biochem. Biophys. Res. Commun.* 191:558–64.

Kato, A., Nakajima, T., Yamashita, J., Yakura, K. & Tanifuji, S. 1990. The structure of the large spacer region of the rDNA in *Vicia faba* and *Pisum sativum. Plant Mol. Biol.* 14:983–93.

Kawai, H., Muto, H., Fujii, T. & Kato, A. 1995. A linked 5S rRNA gene in *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *J. Phycology* 31:306–11.

Keller, M., Tessier, L. H., Chan, R. L., Weil, J. H. & Imbault, P. 1992. In *Euglena,* spliced-leader RNA (SL-RNA) and 5S rRNA genes are tandemly repeated. *Nucl. Acids Res.* 20:1711–5.

Kramer, R. A., Phylippsen, A. P. & Davis, R. W. 1978. Divergent transcription in the yeast ribosomal RNA coding region as shown by hybridization to separated strands and sequence analysis of cloned DNA. *J. Mol. Biol.* 123:405–16.

Larsen, N., Leffers, J. K. & Garrett, R. A. 1986. Evolutionary divergence between the ribosomal RNA operons of *Halococcus morrhuae* and *Desulfurococcus mobilis. Syst. Appl. Microbiol.* 7:49–57.

Leipe, D. D., Wainright, P. O., Gunderson, J. H., Porter, D., Patterson, D. J., Valois, F., Himmerich, S. & Sogin, M. L. 1994. The stramenopiles from a molecular perspective: 16S-like rRNA sequences from *Labyrinthuloides minuta* and *Cafeteria roenbergensis. Phycologia* 33:369–77.

Maizels, N. 1976. *Dictyostelium* 17S, 25S, and 5S rDNAs lie within a 38,000 base pair repeated unit. *Cell* 9:431–8.

Maslov, D. A., Elgort, M. G., Wong, S., Peckova, H., Lom, J., Simpson, L. & Campbell, D. A. 1993. Organization of mini-exon and 5S rRNA genes in the kinetoplastid *Trypanoplasma borreli. Mol. Biochem. Parasitol.* 61:127–36.

Patterson, D. J. 1989. Stramenopiles: chromophytes from a protistan perspective. *In* Green, J. C., Leadbeater, B. S. C. & Diver, W. L. [Eds.] *The Chromophyte Algae. Problems and Perspectives.* Clarendon Press, Oxford, pp. 357–79.

——— 1994. Protozoa: evolution and systematics. *In* Hausmann, K. & Hülsmann, N. [Eds.] *Progress in Protozoology.* Gustav Fischer Verlag, Stuttgart, pp. 1–14.

Rozek, C. E. & Timberlake, W. E. 1979. Restriction endonuclease mapping by crossed contact hybridization: the ribosomal RNA genes of *Achlya ambisexualis. Nucl. Acids Res.* 7:1567–8.

Rubin, G. M. & Sulston, J. E. 1973. Physical linkage of the 5S cistrons to the 18S and 28S ribosomal RNA cistrons in *Saccharomyces cerevisiae. J. Mol. Biol.* 79:521–30.

Silberman, J. D., Sogin, M. L., Leipe, D. D. & Clark, C. G. 1996. Human parasite finds taxonomic home. *Nature* 380:398.

Takaiwa, F., Oono, K. & Sugiura, M. 1984. The complete nucleotide sequence of a rice 17S rRNA gene. *Nucleic Acids Res.* 12: 5441–8.

——— 1985a. Nucleotide sequence of the 17-25S spacer region from rice rDNA. *Plant Mol. Biol.* 4:355–64.

Takaiwa, F., Oono, K., Iida, Y. & Sugiura, M. 1985b. The complete nucleotide sequence of a rice 25S rRNA gene. *Gene* 37: 255–9.

Vahidi, H., Curran, J., Nelson, D. W., Webster, J. M., McClure, M. A. & Honda, B. M. 1988. Unusual sequences, homologous to 5S RNA, in ribosomal DNA repeats of the nematode *Meloidogyne arenaria. J. Mol. Evol.* 27:222–7.