J. Microbiol. Biotechnol. (2001), **11**(3), 515–523

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Cloning and Characterization of the Ribosomal RNA Gene from *Gonyaulax polyedra*

LEE, HEE-GYUN, JI-YEON LEE, AND DONG-HEE LEE*

Department of Biological Science, Ewha Womans University, Seoul 120-750, Korea

Received: April 17, 2001
Accepted: May 11, 2001

Abstract The dinoflagellates have some primitive nuclear features and are evolutionarily intermediate between prokaryotes and eukaryotes. The small subunit ribosomal RNA gene, the large subunit ribosomal RNA gene, the 5.8S ribosomal RNA gene, and the internal transcribed spacer (ITS) of *Gonyaulax polyedra* were cloned, and their sequences were analyzed to better understand their evolutionary position. The small subunit ribosomal RNA gene was 1,794 nt long, the large subunit ribosomal RNA gene was approximately 3,500 nt long, and the 5.8S ribosomal RNA gene was 159 nt long. The first internal transcribed spacer (ITS1) was 191 nt long, and the second internal transcribed spacer (ITS2) was 185 nt long. The intergenic spacer of the ribosomal RNA gene (IGS) was about 2,200 nt long, indicating that 5,800 nt of transcribed sequences were separated by roughly 2,200 nt of intergenic spacer. The ribosomal RNA genes were repeated many times and arranged in a head-to-tail, tandemly repeated manner. The repeating unit of ribosomal RNA gene of *G. polyedra* was proposed to be 8,000 nt long. Based on the lengths of ribosomal RNA, sequence alignments with representative organisms, and phylogenetic analysis on ribosomal RNA, *G. polyedra* appears to be one of the alveolates branched from the eukaryotic crown and, among dinoflagellates, it seems to not have emerged early.

Key words: rRNA gene, phylogeny, dinoflagellates, *Gonyaulax polyedra*

Gonyaulax polyedra is a marine photosynthetic dinoflagellate, one of the algae groups that are often responsible for both red tides and bioluminescence at night. Among the Protista, the phylogeny of the dinoflagellates is somewhat ambiguous. By virtue of the absence of nuclear histones and nucleosomes [14, 33, 34], the similarity of the organization of dinoflagellate chromosomes to bacterial nucleoid [38], and the substantial substitution of thymine by hydroxymethyluracil [15, 39], some investigators have regarded them as being closely affiliated to prokaryotes. However, dinoflagellates also show distinct eukaryotic features including large quantities of DNA per cell (ranging from 2.7 pg/cell in *Amphidinium carterae* to 200 pg/cell in *G. polyedra*) with 50~60% repeated sequences, a typical eukaryotic range in G+C content (37–53%), mRNA-splicing mechanisms, and the maturation of 38S rRNA precursor [32], similar to eukaryotes [17, 34]. The apparent intermediate position of the dinoflagellates, which prompted Dodge [7] to the idea of mesokaryotes, has not gained support from molecular biologists. Molecular phylogeny places the dinoflagellates firmly within the eukaryotic lineage [8, 13, 24], and lends credence to Loeblich's [26] idea that the so-called prokaryotic characters of dinoflagellates are derived, and are not a reflection of their ancestral state.

Ribosomal RNAs provide molecular markers that are particularly informative in the study of phylogeny, because their function and structure have been conserved to a large extent through the evolutionary history of organisms. Eukaryotic ribosomes contain four species of rRNAs. The 25~28S, 5.8S, and 5S rRNAs are associated with the large subunit ribosome, and 17~18S rRNA is associated with the small subunit ribosome. The ribosomal RNA genes of eukaryotes are repeated many times and arranged in tandem. Each repeating unit consists of a transcribed region and a nontranscribed spacer. The ITS (internal transcribed spacer) sequences are located between the 17~18S and 25~28S ribosomal RNA genes, and the region includes the 5.8S rRNA gene and the spacers ITS1 and ITS2. The nucleotide sequence of the IGS (intergenic spacer) region is located between the 25~28S and the 17~18S rRNA coding regions. The IGS is known to be heterogeneous, showing variation in sequence and restriction sites rather than in length, and containing sequence repeats [9]. The ribosomal RNA gene region is shown to occur in a head-to-tail, tandemly repeated manner [19].

*Corresponding author
Phone: 82-2-3277-2376; Fax: 82-2-3277-2385;
E-mail: lee@mm.ewha.ac.kr

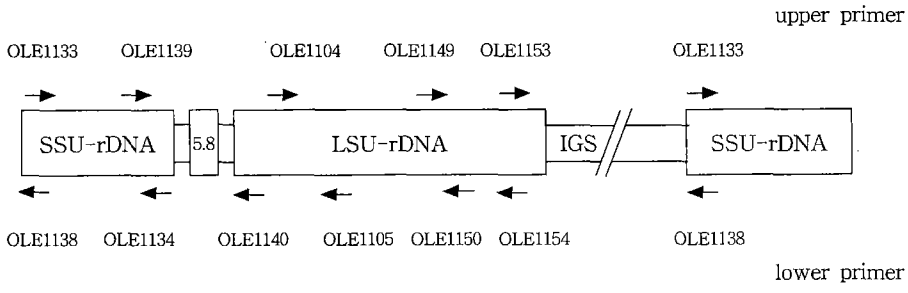
Small subunit ribosomal RNA has been the most widely used tool for studying molecular evolution of eukaryotes. Several reasons can account for the success of this molecule [16, 30]: it is universally present in all living cells; it has both slowly and rapidly evolving regions; its function has remained constant through time; many identical ribosomal RNA copies reside on the genome; horizontal gene transfers are apparently absent; and a substantial database is currently available. Unlike small subunit ribosomal RNA, large subunit ribosomal RNA is far less popular for studying phylogeny. Compared to small subunit ribosomal RNA, large subunit ribosomal RNA is roughly twice as large, and contains more variable areas, including a number of extremely variable expansion segments [12, 18]. Moreover, fewer large ribosomal RNA sequences are available. The 5.8S ribosomal RNA sequence has been conserved and used for detailed phylogenetic studies [3, 28, 43]. Ribosomal ITS sequences are known to evolve quickly and are useful for the study of intraspecific variation and biogeography in algae [2, 22, 23].

Despite the existence of many interesting characteristics in *G. polyedra* and the importance of ribosomal RNA in phylogeny, the ribosomal RNA gene in this organism has not been well studied so far. In this study, the ribosomal RNA gene was isolated in order to analyze its sequence and to examine the phylogenetic relationship of dinoflagellates based upon the sequence data.

PCR Cloning of the Ribosomal RNA Gene

G. polyedra (GP 70) cells were grown in a modified seawater medium (F/2) [11] at 20°C in 2.8-l Fernbach flasks under 16-h light/8-h dark cycle and were harvested at a cell density of 5,000-10,000 cells/ml by filtration in Nitex filter of 20-μm pore size. For the isolation of genomic DNA, the method described by Rogers and Bendich [35], utilizing cetyltrimethylammoniumbromide (CTAB), was

(A) The expected position and direction of the primers on ribosomal RNA gene



* The primers and directions of the primers used for the PCR reaction are shown by horizontal arrows

(B) Nucleotide sequences of primers used for PCR amplification

primer	sequences (5′ ⟶ 3′)
OLE1133	GTAGTCATATGCTTGTCTC
OLE1134	TCCGCAGGTTCACCTACGGA
OLE1139	GGAAGTAAAAGTCGTAACAAGG
OLE1140	TCCTCCGCTTATTGATATGC
OLE1104	AAGCCTCTTGGAAAAGAGCA
OLE1105	TGCATATAGTCAACGTTAGC
OLE1149	GATTGGCTCTGAGGGTTGCG
OLE1150	TGGTCGTTCACTGCACAGGG
OLE1138	GAGACAAGCATATGACTAC
OLE1153	CTGAACGCCTCTAAGTCAGAA
OLE1154	TTCTGACTTAGAGGCGTTCAG

Fig. 1. Primers used for PCR amplification.

* The abbreviations used are: SSU-rDNA: small subunit ribosomal RNA gene; 5.8S: 5.8S ribosomal RNA gene; LSU-rDNA: large subunit ribosomal RNA gene; ITS: the internal transcribed spacer; IGS: the intergenic spacer.

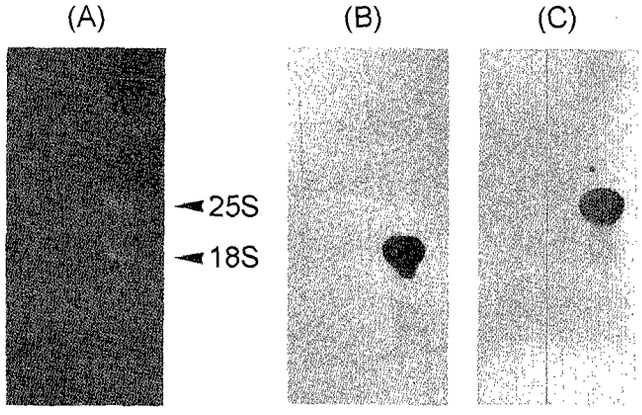


Fig. 2. Northern blot analysis with the labeled putative small subunit ribosomal RNA gene or with the labeled putative large subunit ribosomal RNA gene.

Twenty micrograms of total RNA from *G. polyedra* was electrophoresed on 1.3% formaldehyde/agarose gel and transferred to a nylon membrane. Hybridization was carried out at 55°C for 16 h with either a random-labeled probe made from the small subunit ribosomal RNA gene fragment or the large subunit ribosomal RNA gene fragment. The filter was washed and exposed to X-ray film at −80°C for 1 h. (A) Staining with ethidium bromide. (B) Hybridization with the probe of the putative small subunit ribosomal RNA gene fragment. (C) Hybridization with the probe of the putative large subunit ribosomal RNA gene fragment.

followed. For cloning and analysis of the ribosomal RNA gene fragment, we used eleven PCR primers as shown in Fig. 1, which were designed based on the information of the conserved domain of the small subunit ribosomal RNA, 5.8S RNA, and the large subunit ribosomal RNA [25, 42]. A series of PCR reactions with several different primers and genomic DNA as templates were performed. Each set of PCR reactions gave rise to a proper size of PCR products as expected (data not shown). A Northern blot analysis was performed with total RNAs to confirm PCR products of the ribosomal RNA gene. The putative ^{32}P-labeled small subunit ribosomal RNA gene fragment (PCR product from a primer pair of OLE1133 and OLE1134) or large subunit ribosomal RNA gene fragment (PCR product from a primer pair of OLE1104 and OLE1150) were used for hybridization. About 1,800 nt long small subunit ribosomal RNA (18S) and about 3,500 nt long large subunit ribosomal RNA (25S) were detected, shown in Fig. 2. The size was similar to those of the small subunit ribosomal RNA gene or the large subunit ribosomal RNA gene of typical eukaryotes.

To further characterize PCR products, the DNA fragments were cloned onto T_7Blue pT-Vector (Novagen, Darmstadt, Germany) and transformed into DH10B cells using an

```
                                                                    .  .  .  .  .  .  .  .  .  .  .  .  .  .  .
GTAGTCATATGCTTGTCTCAAAGACTAAGCCATGCATGTCTCAGTATATGCTTGCACATAGTGAACTGCGACTGG    75

CTCATTAAAACAGTTATAGGTTTACTGGTTGACCATTTACATGGATACCTGTGGTAATTCTAGAGCTAATACATG   150

CCCACACACCTGACTTAGTGGAAGGGTTGTGCTTATTAGATTCAAAACCAATACAAGCTTCGCTTGCTCCTTTGG   225

TGATACATAGTGCTTGCATGAATTGCATGGCGCCAGCTGGCTATGCAGCTTTTAAGTTTCTGACCTATCAGCTTC   300

GGATGGTAGGGTATTGGCCTACTATGGCAATGACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCT   375

GAGAAATGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACAGGGAGGTAGTGACA   450

AGAAATAACAATACAGGGCATCCATGTCTTGTAATTGGAATGAATATATCATAAACTCCTTTATGAGTATCGATT   525

GGAGGGCAAGTCTGGTGCCAGCAGCCGTGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGCTGCGGTTAAAA   600

AGCTCGTAGTTGGACTTCTGCTTTAGATGGCCGGTCCGCCCCCTGGGTGAGCATCTGGCTTGTCTTGGGCATCTT   675

CATGGGGAACGAAACTGCACTTGACTGTGTGGGTCGGTAGCCATGATTTTACTTTGAGGAAATCAGAGTGTTTCA   750

AGCAAGCATGCGCCTTGAATACATTAGCATGGAATATTTTTATAGGGCCGTTGTTCTATTTTGTTGGTTTCTAGA   825

ATAATGGTAATGGTTGACAGGGATAGTTGGGGGTATTTGTATTTAACTGTCAGAGGTGAAATTCTTGGATTTGTT   900

AAAGACACACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTGATCAAGAACGAAAGTTAGGGGATCGAAGAC   975

GATCAGATACCGTCCTAGTCCTAACCATAAACCATGCCAACTAGAGATTGAAGGTCGTTATTCATATGACTTCTT  1050
```

Fig. 3. Nucleotide sequences of the ribosomal RNA gene fragment of *G. polyedra*.

Nucleotides are numbered in the 5' to 3' direction. The nucleotide sequence number is written on the right. The region includes the small subunit ribosomal RNA gene, the 5.8S ribosomal RNA gene, and the spacers ITS1 and ITS2 and the large subunit ribosomal RNA gene are shown. The nucleotide sequence data reported will appear in the GenBank Nucleotide Sequence Database under the accession numbers AF377944.

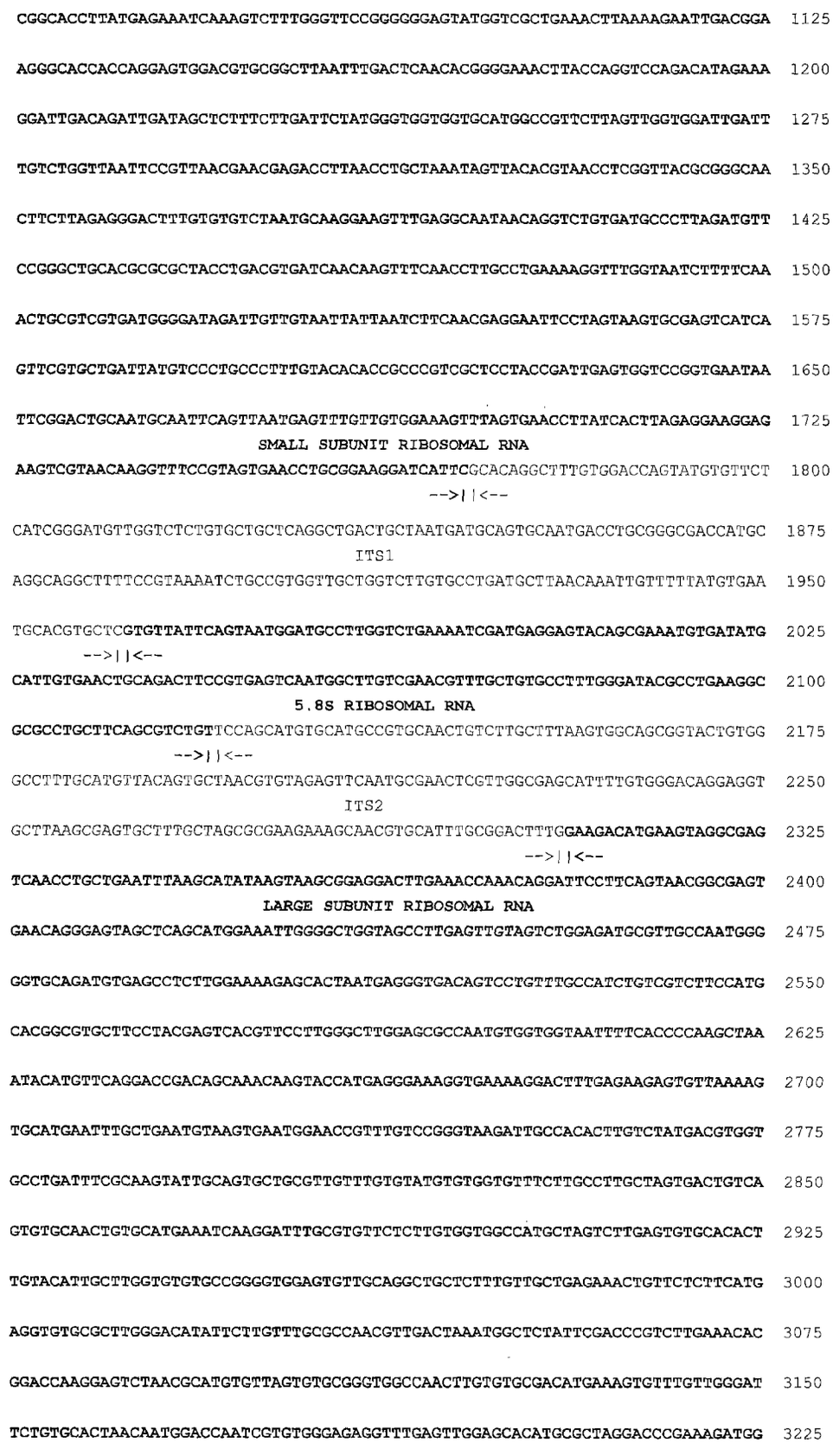


Fig. 3. Continued.

```
TGAACTATGCCTGAGGAGGACAAATTCAGGGGAAACTCTGATGGAGGCTTGTAGCGATACTGACGTGCAAATCGT  3300
TCGTCTCACTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAG  3375
GATAGCTGGAACTAAGCAGTTTTATTAGGTAAAGCAAATGTTTAGAGGAATCGGGGACGTGGTGTCCTCGACCTA  3450
TTCACAAACTTTAAATGGGTAAGATGCAGTGGTTGCTTGTGTGATCTTCTGGGGTAAATGCACTTTCTACGTGGG  3525
CCGTTTTTGGTAAGCAGAACTGGCGATGAGGGATGAACCTAACGTTGGATTAAGGTGCCGAAATGCTCGCTCATC  3600
AGATACCATAAAGGGTGCTGGTTCATTTTAACAGCAGGACGGTGGTCATGGAAGTCGAAATCCGCTAAGGAGTGT  3675
GTAACAACTCACCTGCCGAATGGACTAACCCCGAAAATGGATGGCGCTTAAGCGGGTGACTGATATCTAACCACT  3750
TGTGCAAATGTGGGCACCAGTGAGTAGGAGGGCGTGGAAGTTGCGATGAATCATCGGGTGCAAACCTTGGTGAAG  3825
CATCTTCTAGTGCAGATCTTGGTGGTAGTAGCATTTATTCAAATGGGAACTTTGAAGACCAAAGTGGAGAAGGGT  3900
TCCATGTGAACAGCAGTTGGACATGGGTTAGCCGACCCTAAGGAACAGGATATTCTGCACACAAAGGCTTTGCCT  3975
TGTCTTGCGAAAGGCAGTGGGGTTAATATTCCCCAGCCGGGACGTGGATAGTGTATGGTGACATAAGAGAATTTG  4050
GCGGTGCATTTGCGCACCTCGGGAAGCGTTGTCTTTTCTTTTTAACTCCCTTTTTACCCTGAAATCAGTTTGCCT  4125
GGAGATAGGGTTCATTGGTAGGTAAAGCACCTTTTCTTTTGAGGTGCTCGATGTGTGCGCAACTGTCCTTGAAAA  4200
GCCAAAGAAAAGATTTATTCACACCCCTGGTCGTACCCATAACCGCAGCAGGTCTCCAAGGTTAGCAGCCCTCTG  4275
GCCCTATAGAGCAAAGTAGGTAAGGGAAGTCGGCAAAACAGATCCGTATCTTTGGGAAAAGGATTGGCTCTGAGG  4350
GTTGCGTGCAGGGGTCCTTGCCCTGATGTTTGAGCTGAGCAGAAGCTACTTGTTGCCGTCTGGTGGATAGTGGAT  4425
TCATGCACGGCGAGAATTTTCAGACAAGGAGTGTCTTTATGGGCTTTTCCCTGCGCAGTGAACAGCCAACTCAGA  4500
ACTGGAGCGGACAAGGGGAATCCGACTGTTTAATTAAAACAAAGCCTTGCGATGGCCTCAGCAGGTGTTGACACA  4575
ATGTGATTTCTGCCCAATGCTCTGAATGTCAAAGTGATGAAATTCAAACAAGCGCGGGTAAACGGCGGGAGTAAC  4650
TATGACTCTCTTAAGGTAGCCAAATGCCTCGCCATCTAATTAGTGACGCGCATGAATGGATTAACGAGATTCCCA  4725
CTGTCCCTATCTACTATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGGATGATCAGCGGGGAAAGAAGACCC  4800
TGTTGAGCTTGACTCTAGTCTGACTTTGTGAAATGACTTAGGAGGTGTAGCATAAGTGGGAGTTTTGGCGATCTT  4875
GAAATACCACTACTCTTAATGTCATTTTACTTATTCTGTCAGCCTGCGGCTTGCATGTGTGTGAGTTTTGGATTT  4950
AATGCTTCTGGTTTTAGTTTCTGATCTGTTTGGGAAGCATAGTCAGGTGGGGAGTTTGGCTGGGGCGGCACATCT  5025
GTTAAATGATAACGCAGGTGTCCTAAGGTGAGCTCACTGAGGACAGAAATCTTATGTAGACCAAAAGGGGAAATG  5100
CTTGCTTGATTTTCATTTTCAGTGCGAATACAAACTGTGAAAACATGGCCTATCGATCCTCTATGTCTGCGATTT  5175
GTTCAGCTAGGGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCAGCCAAGCGTTCGTAGCGACGTTG  5250
CTTTTTGATCCTTCGATGTCGGCTCTTCCTATCATTGAGACGCAGCAGTCCCAAAGTGTCGGCTTGTTCACCCGC  5325
CAATAGGGAACGTGAGCTGGGTTTAGACCGTCGTGAGACAGGTTAGTTTTACCTTGCTGATCGGAGCGATATTGT  5400
GACAGCAATCGAAGTTAGTACGAGAGGACAACTTCGTTCAGACATTTAGTAAATGCATTTACCTGAAAAGCTATT  5475
ATTGACAAGCTACCATATGTTGGATTACGACTGAACGCCTCTAAGTCAGAA . . . . . 5526
```

Fig. 3. Continued.

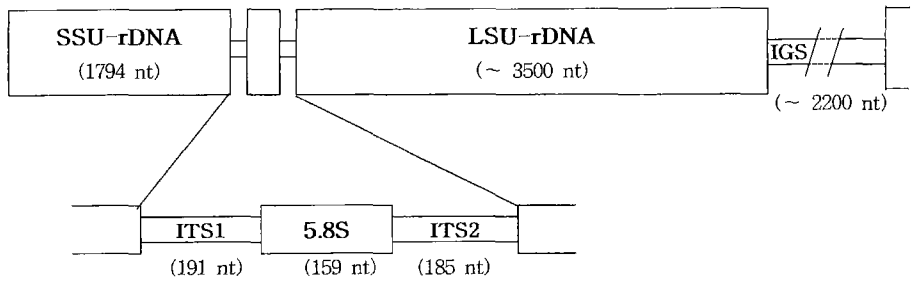


Fig. 4. A schematic representation of the proposed organization of the ribosomal RNA gene region from *G. polyedra*.
* The abbreviations used are: SSU-rDNA: small subunit ribosomal RNA gene; 5.8S: 5.8S ribosomal RNA gene; LSU-rDNA: large subunit ribosomal RNA gene; ITS: the internal transcribed spacer; IGS: the intergenic spacer.

electroporator. The plasmids of white colonies on X-gal plates were isolated by alkaline methods and their insert size was determined by restriction mapping [36]. Several clones from each PCR product were selected and the sequences of each insert were determined by an automated sequencing system (Long-Read IR 4200, LI-COR). The sequences were analyzed using the BLAST [1], Clustal W sequence alignment [41], and GeneDoc [29] programs.

Sequence Analysis of Ribosomal RNA Gene

As shown in the upper part of Fig. 3, by combining sequence information from the PCR products of OLE1133 and OLE1134 with those of OLE1139 and OLE1140, we obtained a nearly complete sequence of the small subunit ribosomal RNA gene, except for about 20 nt at the 5'-end. The fragment obtained by the PCR reaction using OLE1139 and OLE1140 was cloned on pT_7Blue T-vector and its sequence was analyzed. The sequenced genomic DNA had 535 nt, containing the entire ITS1, ITS2, and the entire 5.8S ribosomal RNA gene. The coding region termini were determined by comparison with the ribosomal RNA gene sequence of dinoflagellates. Sequence comparison of these regions from *G. polyedra* and other organisms including yeast suggested that the first internal transcribed spacer (ITS1) between the small subunit ribosomal RNA gene and the 5.8S ribosomal RNA gene was 191 nt long, as shown in the middle part of Fig. 3. The second internal transcribed spacer (ITS2) between the 5.8S ribosomal RNA gene and the large subunit ribosomal RNA gene was 185 nt long. The 5.8S ribosomal RNA gene was 159 nt long. The fragment obtained by the PCR reaction using OLE1104 and OLE1150 was cloned on the pT_7Blue T-vector and its sequence was analyzed. By combining sequence information from the PCR products of OLE1104 and OLE1150 with those of OLE1139 and OLE1140, we obtained the nearly complete sequence of the large subunit ribosomal RNA gene, except for a small part of sequences at the 3'-end, as shown in the lower part of Fig. 3. Prokaryotic and eukaryotic large subunit ribosomal RNA genes display a common, largely conserved structural core which in eukaryotes is interspersed with 12 divergent, more rapidly evolving domains (D1~D12) [12, 27].

Structure of Ribosomal RNA Gene Region of *G. polyedra*

The small subunit ribosomal RNA gene was estimated to be 1,800 nt long, the large subunit ribosomal RNA gene was estimated to be 3,500 nt long, and the nucleotide sequence of the entire internal transcribed spacer between the small subunit ribosomal RNA and the large subunit ribosomal RNA was estimated to be 500 nt long. The intergenic spacer (IGS) was 2,200 nt long. Therefore, 5,800 nt transcribed sequences were separated by roughly 2,200 nt of intergenic space and the repeating units of ribosomal RNA gene was proposed to be 8,000 nt long by PCR amplification. The ribosomal RNA gene contained units repeated many times, which were arranged in a head-to tail, tandemly repeated manner. A schematic representation of the proposed organization of the ribosomal RNA gene region from *G. polyedra* is shown in Fig. 4.

Phylogenetic Analysis

In order to obtain a detailed picture of the evolution of ribosomal genes, phylogenetic analyses were performed using sequences from a broad array of taxa. Sequences from a wide range of species used were obtained from GenBank by the accession numbers (Table 1 and Table 2). The ribosomal RNA sequence alignment was converted to arrays of unordered, character state data in agreement with DNA-type option of PAUP (phylogenetic analysis using parsimony, version 3.1.1., for the Macintosh [40]) for Maximum Parsomony (MP) analysis. All nucleotide characters were weighted equally in the character matrix, and the alignment gaps were considered as a fifth base. Variable characters were subjected to cladistic analysis under the parsimony criterion with the PAUP computer package. Most-parsimonious cladograms were sought by random (50 replicates) sequential addition of taxa in the heuristic search option. The TBR algorithm (tree bisection-reconnection branch swapping) was used to search for most-parsimonious cladograms. The in-group taxa subsequently were rooted with reference to out-groups.

Table 1. Sources of the small subunit ribosomal RNA sequences from prokaryote and eukaryote genera used in this study.

Species	Taxon	GenBank	Size
Homo sapiens	Animal	X03205	1869
Arabidopsis thaliana	Land plant	X16077	1803
Saccharomyces cerevisiae	Fungi	M27607	1798
Characium saccatum	Green algae	M84319	2233
Gracilaria verrucosa	Red algae	M33638	1771
Bacillaria paxillifer	Heterokont	M87325	1790
Tetrahymena thermophila	Ciliate	M10932	1792
Gonyaulax polyedra	Dinoflagellate	AF377944	1775
Sarcocystis muris	Apicomplexa	M64244	1809
Escherichia coli	Eubacteria	M24833	1541
Archaeoglobus fulgidus	Archaebacteria	X05567	1492

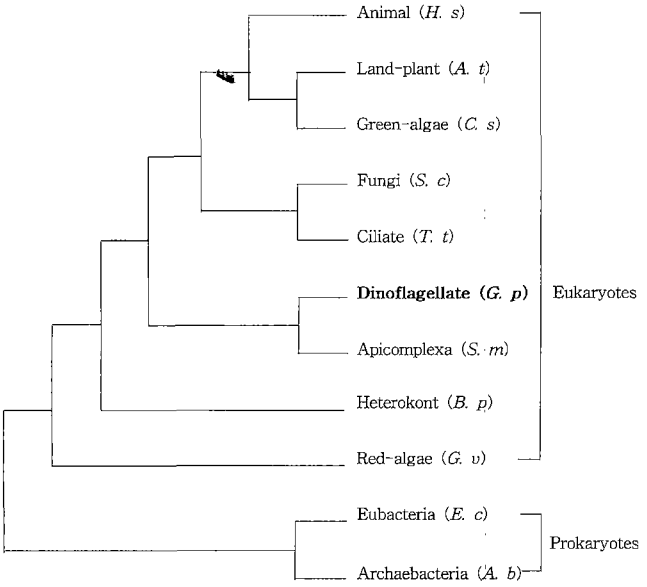


Fig. 5. Evolutionary relationships among the prokaryotes and eukaryotes based on small subunit ribosomal RNA sequences. Statistics for the most-parsimonius tree: length=3232, consistency index=0.700, homoplasy index=0.300, retention index=0.397.

The inference of evolutionary trees of 11 different organisms belonging to the prokaryotes and eukaryotes using parsimony analysis showed that the origin of dinoflagellates is closely related to eukaryotes as one of the alveolates. As shown in Fig. 5, the combined assemblage, termed the alveolates (an alliance of the dinoflagellates with the ciliates and apicomplexans), branches from within the eukaryotic crown. Within the alveolates, dinoflagellates and apicomplexans share a common ancestor, a possibility that dinoflagellates are derived from apicomplexa, as suggested by Goggin and Bakker [10], while in turn they both share a common origin with the ciliate. Therefore, this data rejects the Mesokaryote hypothesis. Early molecular studies of dinoflagellates were largely concerned with the origins of this group relative to other protistan lines. Dodge [6, 7] considered the unusual chromosomes and mitosis of dinoflagellates, and proposed that this group of protists are intermediate between the prokaryotes and eukaryotes, hence the designation Mesokaryote. Phylogenetic trees inferred from the ribosomal RNA sequence, in previous studies with partial small subunit ribosomal RNA sequences [5, 20, 21] and with partial large subunit ribosomal RNA sequences [4, 24, 25, 31], pointed to a monophyletic group, including the apicomplexans, ciliates, and dinoflagellates, with this combined assemblage from the crown rather than the base of the eukaryotic line.

Table 2. Sources of the small subunit ribosomal RNA sequences from the dinoflagellates used in this study.

Species	Order	GenBank	Size
Alexandrium minutum	Gonyaulacales	U27499	1800
Alexandrium tamarense	Gonyaulacales	AF022153	1800
Amphidinium belauense	Gymnodiniales	L13719	1798
Cachonina hallii	Peridiniales	AF33865	1752
Ceratium fusus	Gonyaulacales	AF022153	1744
Ceratocorys horrida	Gonyaulacales	AF022154	1801
Crypthecodinium cohnii	Gonyaulacales	M64245	1796
Gloeodinium viscum	Phytodiniales	L13716	1797
Gonyaulax spinifera	Gonyaulacales	AF022155	1790
Gonyaulax polyedra	Gonyaulacales	AF377944	1775
Gymnodinium beii	Gymnodiniales	U41087	1799
Gymnodinium sanguinium	Gymnodiniales	U41085	1798
Gyrodinium impudicum	Gymnodiniales	AF022190	1804
Heterocapsa triquetra	Peridiniales	AF022198	1801
Lepidodinium viride	Gymnodiniales	AF022199	1803
Noctiluca scintillans	Noctilucales	AF022200	1787
Prorocentrum micans	Prorocentrales	M14649	1801
Prorocentrum minimum	Prorocentrales	Y16238	1789
Pyrocystis noctiluca	Pyrocystales	AF022156	1792
Symbiodinium microadriaticum	Gymnodiniales	M188521	1790
Sarcocystis muris	Apicomplexa	M64244	1809
Perkinsus sp.	Apicomplexa	L07375	1798

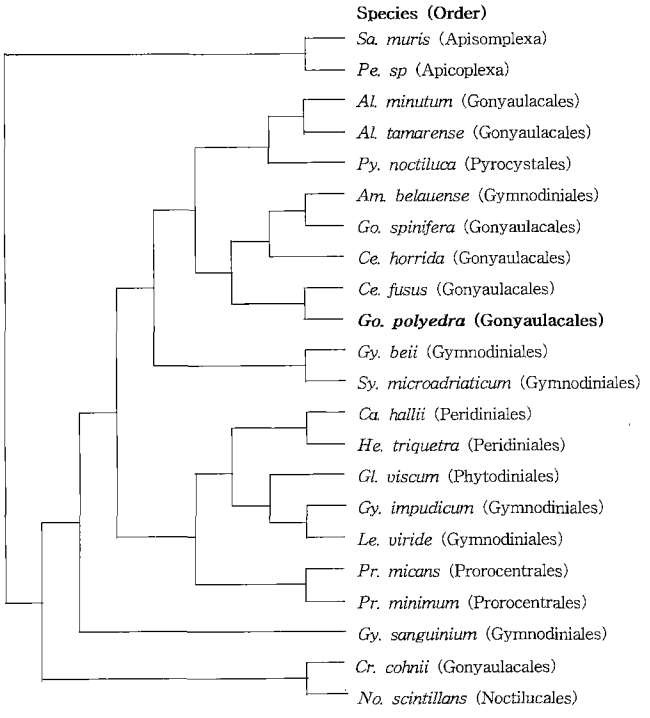


Fig. 6. Molecular phylogeny of the dinoflagellates based on small subunit ribosomal RNA sequences.
Statistics for the most-parsimonius tree: length=2191, consistency index= 0.529, homoplasy index=0.471, retention index=0.341.

In order to understand the evolutional position of dinoflagellates, a phylogenic analysis with the sequences of the small subunit ribosomal RNA gene from several dinoflagellates including *G. polyedra* was performed with the same method used in the evolutionary relationship among prokaryotes and eukaryotes. The dinoflagellates are a marvelously diverse group of protists. However, these sequences were partially determined to establish a molecular system for understanding relationships with this taxonomically complex group. *C. cohnii* has emerged early and *G. polyedra* seems to have evolved recently as shown in Fig. 6, consistent with a previous report [37]. A strongly supported molecular phylogeny of the major lineages of dinoflagellates is currently not available, and relationships among groups are uncertain. This is due largely to the partial sequence data and a relatively small number of sequence data currently available. These results imply that reexamination of the taxonomic position of family may therefore be justified and molecular data may help refine the taxonomy of these dinoflagellates.

Acknowledgment

This research was partly supported by a grant for 1999 BSRI of the National R&D and by a grant for the Brain Korea 21 Project in 2001.

REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410.
2. Bakker, F. T., J. L. Olsen, W. T. Stam, and C. Vad Den Hoek. 1992. Nuclear ribosomal DNA internal transcribed spacer regions (ITS 1 and ITS 2) define discrete biogeographic groups in *Cladophora albida (Chlorophyta)*. *J. Phycol.* **28:** 839–845.
3. Baldwin, B., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *An. Missouri Botanical Garden* **82:** 247–277.
4. Baroin, A., R. Perasso, L. H. Qu, G. Brugerolle, J. P. Bachellerie, and A. Adoutte. 1988. Partial phylogeny of the unicellular eukaryotes based on rapid sequencing of a portion of 28S ribosomal RNA. *Proc. Natl. Acad. Sci. USA* **85:** 3474–3478.
5. Cedergren, R., M. W. Gray, Y. Abel, and D. Sankoff. 1988. The evolutionary relationships among known life forms. *J. Mol. Evol.* **28:** 98–112.
6. Dodge, J. D. 1965. Chromosome structure in the dinoflagellates and the problem of the mesokaryotic cell. *Excerpta Medica International Congress Series* **91:** 339–341.
7. Dodge, J. D. 1966. The Dinophyceae, pp. 96–115. *In* Godward MBE (ed.), *The Chromosomes of the Algae.* London, Arnold, U.K.
8. Gajadhar, A. A., W. C. Marquardt, R. Hall, J. Gunderson, E. V. Ariztia-Carmona, and M. L. Sogin. 1991. Ribosomal RNA sequences of *Sarcocystis muris, Theileria annulata* and *Crypthecodinium cohnii* reveal evolutionary relationships among apicomplexans, dinoflagellates and ciliates. *Mol. Biochem. Parasit.* **45:** 147–154.
9. Gatehouse, H. S. and L. A. Malone. 1998. The ribosomal RNA gene region of *Nosema apis (Microspora)*: DNA sequence for small and large subunit rRNA genes and evidence of a large tandem repeat unit size. *J. Invertebr. Pathol.* **71:** 97–105.
10. Goggin, C. L. and S. C. Barker. 1993. Phylogenetic position of the genus *Perkinsus* (Protista, Apicomplexa) based on small subunit ribosomal RNA. *Mol. Biochem. Parasit.* **60:** 65–70.
11. Guillard, R. and J. Ryther. 1962. Studies of marine planktonic diatoms I. *Cyclosella mana* Hustedt and *Detonula confervacea* (Cleve). *Gran. Canad. J. Microbiol.* **8:** 229–239.
12. Hassouna, N., B. Michot, and J. P. Bachellerie. 1984. The complete nucleotide sequence of mouse 28S rRNA gene. Implications for the process of size increase of the large subunit rRNA in higher eukaryotes. *Nucleic Acids Res.* **12:** 3563–3583.
13. Hendriks, L., R. De Baere, Y. Van de Peer, J. Neefs, A. Goris, and R. De Wachter. 1991. The evolutionary position of the rhodophyte *Porphyra umbilicalis* and the basidiomycete *Leucosporidium scottii* among other eukaryotes as deduced from complete sequences of small ribosomal subunit RNA. *J. Mol. Evol.* **32:** 167–177.

14. Herzog, M. and M. O. Soyer. 1981. Distinctive features of dinoflagellate chromatin. Absence of nucleosomes in a primitive species *Prorocentrum micans*. *Eur. J. Cell. Biol.* **23:** 295–302.
15. Herzog, M., M. O. Soyer, and G. Daney de Marcillac. 1982. A high level of thymine replacement by 5-hydroxymethyluracil in nuclear DNA of the primitive dinoflagellate *Prorocentrum micans*. *Eur. J. Cell. Biol.* **27:** 151–155.
16. Hills, D. M. and M. T. Dixon. 1991. Ribosomal RNA: Molecular evolution and phylogenetic inference. *Q. Rev. Biol.* **66:** 411–453.
17. Hinnebusch, A. G., L. C. Klotz, R. L. Blanken, and A. R. Loerlich III. 1981. An evaluation of the phylogenetic position of the dinoflagellate *Crypthecodinium cohnii* based on 5S rRNA characterization. *J. Mol. Evol.* **17:** 334–347.
18. Hong, S. G., J. S. Chun, J. S. Nam, Y. D. Park, and K. S. Bae. 2000. Phylogenetic analysis of genus *Sporobolomyces* based on partial sequences of 26S r DNA. *J. Microbiol. Biotechnol.* **10:** 363–366.
19. Hwang, S. K. and J. G. Kim. 1995. Cloning and characterization of the ribosomal RNA genes of the mushroom *Tricholoma matsutake*. *J. Microbiol. Biotechnol.* **5:** 194–199.
20. Johnson, A. M., S. Illana, P. Hakendorf, and P. R. Baverstock. 1988. Phylogenetic relationships of the apicomplexan protist *Sarcocystis* as determined by small subunit ribosomal RNA comparison. *J. Parasitol.* **74:** 847–860.
21. Johnson, A. M. and P. R. Baverstock. 1989. Rapid ribosomal RNA sequencing and the phylogenetic analysis of protists. *Parasitol. Today* **5:** 102–105.
22. Kim, K. S. and Y. S. Lee. 2000. Rapid and accurate species-specific detection of *Phytophthora infestans* through analysis of ITS regions in its rDNA. *J. Microbiol. Biotechnol.* **10:** 651–655.
23. Kooistra, W. H. C., W. T. Stam, J. L. Olsen, and C. Van Den Hoek. 1992. Biogeography of *Cladophoropsis membranacea (Chlorophyta)* based on comparisons of nuclear rDNA ITS sequences. *J. Phycol.* **28:** 660–668.
24. Lenaers, G., L. Maroteaux, B. Michot, and M. Herzog. 1989. Dinoflagellates in evolution: A molecular phylogenetic analysis of large subunit ribosomal RNA. *J. Mol. Evol.* **29:** 40–51.
25. Lenaers, G., C. Scholin, Y. Bhaud, D. Saint-Hilaire, and M. Herzog. 1991. A molecular phylogeny of dinoflagellate protists (*Pyrrophyta*) inferred from the sequence of 24S rRNA divergent domains D1 and D8. *J. Mol. Evol.* **32:** 53–63.
26. Loeblich, A. R. 1984. Dinoflagellate evolution, pp. 481–522. *In* D. L. Spector (ed.), *Dinoflagellates*. Academic Press, Inc., Orlando, U.S.A.
27. Michot, B., N. Hassouna, and J. P. Bachellerie. 1984. Secondary structure of mouse 28S rRNA and general model for the folding of the large rRNA in eukaryotes. *Nucleic Acids Res.* **25:** 4259–4279.
28. Nickrent, D. L., K. P. Schuette, and E. M. Starr. 1994. A molecular phylogeny of *Arceuthobium* (*Viscaceae*) based on nuclear ribosomal DNA internal transcribed spacer sequences. *Am. J. Bot.* **81:** 1149–1150.
29. Nicholas, K. B., H. B. Nicholas Jr., and D. W. Deerfield II. 1997. GeneDoc: Analysis and visualization of genetic variation. *EMBNEW. News* **4:** 14.
30. Olsen, G. J. 1988. Phylogenetic analysis using ribosomal RNA, pp. 793–812. *In* H. F. Noller and K. Moldave (eds.), *Ribosomes - Method. Enzymol.*, vol. **164** Academic Press, Inc., London. U.K.
31. Qu, L. H., R. Perasso, A. Baroin, G. Brugerolle, J. P. Bachellerie, and A. Adoutte. 1988. Molecular evolution of the 5'-terminal domain of large subunit rRNA from lower eukaryotes. A broad phylogeny covering photosynthetic and non-photosynthetic protists. *Biosystems* **21:** 203–208.
32. Rae, P. M. 1970. The nature and processing of ribosomal ribonucleic acid in a dinoflagellate. *J. Cell. Biol.* **46:** 106–113.
33. Rizzo, P. J. 1981. Comparative aspects of basic chromatin proteins in dinoflagellates. *Biosystems* **14:** 433–444.
34. Rizzo, P. J. 1987. Biochemistry of the dinoflagellates nucleus, pp. 143–173. *In* F. J. R. Taylor (ed.), *The Biology of Dinoflagellates*. (Botanical monographs, vol. 21) Blackwell Scientific Publications, Boston, U.S.A.
35. Rogers, S. O. and A. J. Bendich. 1988. Extraction of DNA from plant tissues, pp. 1–10. *In* S. B. Gelvin, R. F. Schilperoort, and D. S. Verma (eds.), *Plant Molecular Biology Manual*, Vol. **1**, A6. Kluwer Academic Publisher, Belgium.
36. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning, A Laboratory Manual*, 2nd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, U.S.A.
37. Saunders, G. W., D. R. A. Hill, J. P. Sexton, and R. A. Andersen. 1997. Small subunit ribosomal RNA sequences from selected dinoflagellates: Testing classical evolutionary hypotheses with molecular systematic methods. *Plant Syst. Evol.* **11:** 237–259.
38. Soyer, M. O. and M. Herzog. 1985. The native structure of dinoflagellate chromosomes. Involvement of structural RNA. *Eur. J. Cell. Biol.* **36:** 334–342.
39. Steele, R. E. and P. M. Rae. 1980. Ordered distribution of modified bases in the DNA of a dinoflagellate. *Nucleic Acids Res.* **24:** 4709–4725.
40. Swofford, D. L. 1993. *PAUP: Phylogenetic Analysis Using Parsimony*, version 3.1.1., Illinois Natural History Survey, Champaign, Illinois, U.S.A.
41. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.
42. White, T. J., T. D. Burns, S. B. Lee, and J. W. Taylor. 1990. Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal genes, pp. 315–322. *In: PCR Protocols*, Academic Press, Inc., New York, U.S.A.
43. Wojciechowski, M. F., M. J. Sauderson, B. G. Baldwin, and M. J. Donoghue. 1993. Monophyletics of aneuploid *Astragalus* (*Fabaceae*): Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Am. J. Bot.* **80:** 711–722.