Molecular evolution of 5S ribosomal RNA from
red and brown algae

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ABSTRACT

The cytoplasmic 5S ribosomal RNA (rRNA) sequences from seven red algae, a golden-yellow alga, a diatom and five brown algae have been determined. Using these sequences, phylogenic tree of these organisms have been constructed.

Three types of algae, i.e., Rhodophyta (red algae), green algae (members of green plants), and Chromophyta (brown algae, the diatom and the golden-yellow alga) are only remotely related to one another phylogenically as deduced from the 5S rRNA sequences. Red algae emerged first in the eukaryotic evolution (1.3 to 1.4 billion years ago), and thereafter various fungi, green plants including green algae and Chromophyta emerged in this order. In the red algal evolution, the so-called "primitive group" of the class Bangiophyceae and "more advanced group" of the class Florideophyceae separated first from each other in an early stage of their evolution. The tree also indicates that various red algae, such as *Batrachospermum*, *Gelidium*, *Gloiopeltis* and *Carpopeltis* are phylogenically rather remote from one another. Thus, red algae are very widely and anciently separated organisms in the evolution of eukaryotes.

Chromophyta contains golden-yellow algae, diatoms and brown algae. These three groups are related to one another. During the Chromophyta evolution, the golden-yellow alga separated first, and thereafter the diatom and brown algae separated from each other. Although brown algae are very different in their morphology and life cycle, the 5S rRNA sequences clearly indicate that the brown algae were diversified quite recently within a short period.

1. INTRODUCTION

With development of molecular evolution studies, the 5S rRNA sequences from over 330 different organisms throughout prokaryotes and eukaryotes have been reported, and successfully used to study evolutionary relationships of various groups of organisms, such as eubacteria (Dekio *et al.* 1984), metabacteria (Hori *et al.* 1982), fungi (Gottschalk and Blantz, 1984), green plants including green algae (Hori *et al.* 1985), protozoans (Kumazaki *et al.* 1983),

and meso- and metazoans (Ohama *et al.* 1983).

However, the 5S rRNA phylogenic trees of various algae groups other than green algae have been wanting. We have thus determined the 5S rRNA sequences of seven red algae, a golden-yellow alga, a diatom and five brown algae, and have constructed phylogenic trees for these algal groups.

2. MATERIALS AND METHODS

Eisenia bicyclis, Sargassum fulvellum, Gracilaria compressa, Carpopeltis crispata, Gloiopeltis complanata, and *Gelidium amansii* were collected at the seashore in an area of Chita Peninsula near Nagoya. *Porphyra tenera* was furnished by Messrs. K. Sato and T. Hamamoto of the Hiroshima Fisher's Promotion Center. *Hydrurus foetidus* was collected in freshwater at Jozankei near Sapporo. *Batrachospermum ectocarpum* and *Diatoma tenue* were collected at Chitose River near Sapporo. *Acinetospora crinita* was collected at Oshoro near Otaru. *Chordaria flagelliformis f. chordaeformis* and *Akkesiphycus lubricum* were collected at Akkeishi, Hokkaido. *Palmaria palmata* was collected near the Institute of Algological Research, Hokkaido University, Muroran, Hokkaido.

The cytoplasmic 5S rRNAs were isolated from whole organisms by the phenol method and purified by polyacrylamide gel electrophoresis as previously described (Kumazaki *et al.* 1982). The sequences were determined by both the chemical and enzymatic methods (Peattie 1979; Donis-Keller 1980). The sequence alignment was done as previously described (Hori and Osawa, 1985).

Phylogenic trees were constructed by the WPGMA average clustering method (Sneath and Sokal 1973) using *Knuc* values (relative evolutionary distances) of 5S rRNAs calculated according to Kimura (1980).
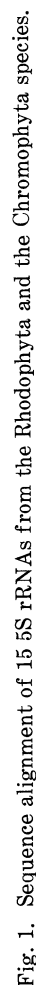
3. RESULTS AND DISCUSSION

The 5S rRNA sequences from Rhodophyta and Chromophyta

The 5S rRNA sequences from seven red algae, a golden-yellow alga, a diatom and five brown algae were determined and aligned in Fig. 1. Some 5S rRNA sequences in Fig. 1 were reported as short communications (Lim *et al.* 1983; 1984).

Phylogeny of various groups of organisms and emergence of algal groups

First, using the 330 5S rRNA sequences available to date, we have constructed a phylogenic tree to see the phylogenic relationships of representative groups of organisms especially to settle the emergence point of various algal groups (the tree not shown; see Hori *et al.* 1985). First, the eubacteria separated from the metabacteria/eukaryotes branch. After the emergence of the eubacteria, the metabacteria and the eukaryotes separated from each

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              1         2         3          4          5         6         7         8
   1234567890123456789012301234567890123 4567890123456789 90123456789012345678901234567890123456789012345
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              1          1   1
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   678901234567890123456789012345678 90123456789012345678
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14 AGGGAUCACUCGGGAAUCCCCGGUGCCGAAUGU
15 AGGGAUCACACAGGCGAACCCCCAAUGCUGUACGUC
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 1 Eisenia bicyclis (Brown alga)
 2 Sargassum fulvellum (Brown alga)
 3 Chordaria flagelliformis (Brown alga)
 4 Acinetospora crinita (Brown alga)
 5 Akkesiphycus lubricum (Brown alga)
 6 Diatoma tenue (Diatom)
 7 Hydrurus foetidus (Golden-yellow alga)
 8 Grarcilaria compressa (Red alga)
 9 Palmaria palmata (Red alga)
10 Gleiopeltis complanata (Red alga)
11 Carpopeltis crispata (Red alga)
12 Batrachospermum ectocarpum (Red alga)
13 Gelidium amansii L (Red alga)
14 Gelidium amansii S (Red alga)
15 Porphyra tenera (Red alga)

Fig. 1. Sequence alignment of 15 5S rRNAs from the Rhodophyta and the Chromophyta species.

other from their common ancestor. In early eukaryotic evolution, the red algae evolved first and were followed by the emergence of various fungi (Ascomycetes and Basidiomycetes). Green plants including green algae, brown algae and various protozoan groups then emerged at nearly the same time but probably in that order. In any case, these four groups seem to have emerged one by one within a relatively short time. The occurrence of the Mesozoa and Metazoa followed. Thus, the red algae seem to be the most anciently emerged group among the eukaryotes. This is also suggested by the lowest similarity of the red algal 5S rRNA sequences to other eukaryotic groups (Table 1). The three types of algae (i.e., red algae, brown algae, and green algae) are only remotely related from one another phylogenically.

Table 1. *Similarity matrix of 5S rRNA sequences from eukaryotes (%)*

	a	b	c	d	e	f	g	h	i
a. *Animals**	—								
b. *Protozoa**	67	—							
c. *Dinoflagellata*	71	71	—						
d. *Euglenophyta*	71	72	68	—					
e. *Cryptophyta*	63	64	65	63	—				
f. *Chromophyta**	66	66	69	66	65	—			
g. *Green plants**	65	64	67	64	62	64	—		
h. *Fungi**	62	61	63	61	60	64	58	—	
i. *Red algae**	57	62	61	61	59	60	58	56	—

* The mean similarity values calculated from the 5S rRNA sequences of 73 animals, 13 protozoans, 7 Chromophyta species (including a golden-yellow alga, a diatom and brown algae), 28 green plants, 55 fungi (basidiomycetes and ascomycetes) and 7 red algae. The sequences were taken from Fig. 1 of this paper and a collection of 5S rRNA sequences (Erdmann *et al.* 1985).

Phylogeny of red algae (*Rhodophyta*)

Rhodophyta has been usually classified into two classes, the "primitive" Bangiophyceae and the "more advanced" Florideophyceae (Dixon 1973). The 5S rRNA data indicated that all the red algae examined here belong to one branch derived from the eukaryotic stem, and diverged as shown in Fig. 2. The tree shows that the *Porphyra* species (Bangiophyceae) emerged from the ancestor common to the Florideophyceae species in an early stage of their evolution, and thereafter the species that are included in Florideophyceae emerged one by one. Thus, the 5S rRNA data is consistent with the view that Rhodophyta is divided into two classes, "primitive" Bangiophyceae and "more advanced" Florideophyceae.

According to the 5S rRNA tree, *Batrachospermum/Palmaria* separated first from other species of Florideophycidae, and long after, they separated from
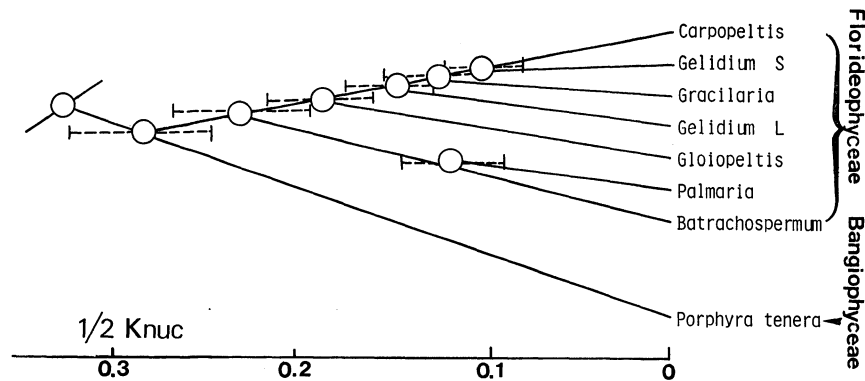
Fig. 2. Phylogenic tree of eight 5S rRNAs from seven red algal species (Rhodophyta); 1/2 *Knuc*: relative evolutionary distance; |---O---|: σ_k (range of standard error of 1/2 *Knuc*; see Kimura 1980).

each other. The tree also shows that *Gloiopeltis*, *Gelidium* L (see later), *Carpopeltis* and *Glacilaria/Gelidium* S (see later) branched one by one in that order after the emergence of *Batrachosperm/Palmaria*.

The two *Gelidium* L and S sequences derived from the same population are considerably different (Figs. 1 and 2). This suggests that the ancestor common to *Gelidium*, *Carpopeltis* and *Gracilaria* possessed at least two kinds of 5S rRNA genes, L and S, and these two evolved independently. The L-type gene might have disappeared or have been repressed in *Carpopeltis* and *Gracilaria*, while the L- and the S-type genes have been functioning in *Gelidium*.

In any case, diversification of the Rhodophyta species examined here took place very anciently as compared with any other algal groups. In fact, Table 2 shows that the similarity of the 5S rRNA sequences among most of the red algae is much lower as compared with the similarity among brown algal sequences (see Table 3).

Table 2. *Similarity matrix of 85S rRNA sequences from red algae* (%)

	a	b	c	d	e	f	g	h
a. *Porphyra*	—							
b. *Batrachospermum*	57	—						
c. *Palmaria*	65	80	—					
d. *Gloiopeltis*	61	64	67	—				
e. *Carpopeltis*	63	69	63	69	—			
f. *Gelidium* L	57	64	64	64	74	—		
g. *Gelidium* S	68	69	67	79	81	74	—	
h. *Gracilaria*	64	65	65	71	73	73	79	—

The emergence of Rhodophyta has been estimated to be about 1.3 to 1.4 billion years ago, when the yeast-animal divergence time is taken to be 1.2 billion years ago (Kimura and Ohta 1973).

Phylogeny of Chromophyta

The brown algae, the diatoms and the golden-yellow algae are sometimes grouped together in Chromophyta, because of their having common characteristics of chlorophylls a and c, and of unique storage substances, laminarin or chrysolaminarin (Corliss 1984).

The comparison of 5S rRNA sequences from the Chromophyta species indicated that brown algae, the diatom and the golden-yellow alga are more closely related to one another (68-81% identity; 74% on average) than to other eukaryotic groups (52-68%; 63% on average). Supporting the view of Corliss (1984), all these Chromophyta species examined here are closely related as shown in the tree of Fig. 3.
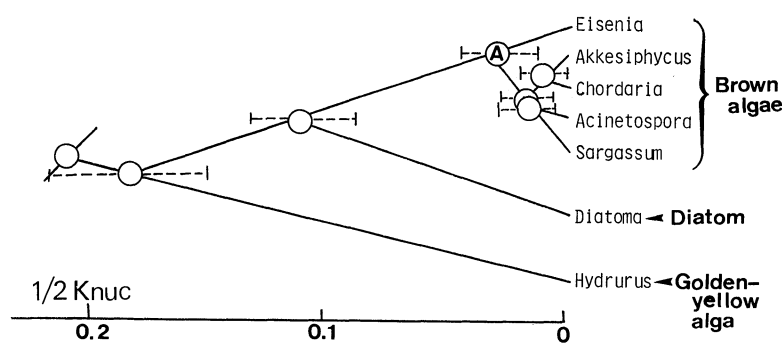
Fig. 3. Phylogenic tree of seven 5S rRNAs from Chromophyta species. 1/2 *Knuc*: relative evolutionary distance; |---○---| : σ_k (range of standard error of 1/2 *Knuc*; see Kimura 1980).

In classical taxonomy, the diatoms are sometimes included in the golden-yellow algal group, Chrysophyta (Alexopoulos and Bold, 1967), because of both having chrysolaminarin as photosynthetic food reserve, although the life cycle, cell structure and division of the diatoms differ considerably from those of golden-yellow algae. The similarity matrix (Table 3) indicates that the diatom 5S rRNA sequence is more related to those from seven species of brown algae (80-81% identity; 81% on average) than to that of golden-yellow alga *Hydrurus* (68% identity). The 5S rRNA from *Hydrurus* is less similar to those from the brown algae and the diatom (68-75% identity; 73% on average). The Chromophyta branch of the 5S rRNA phylogenic tree clearly shows that the golden-yellow alga (*Hydrurus*) separated first, and thereafter, the diatom (*Diatoma*) and brown algae separated from each other (Fig. 3).

Table 3. *Similarity matrix of 7 5S rRNA sequences*
from Chromophyta (%)

	a	b	c	d	e	f	g
a. *Hydrurus*	—						
b. *Diatoma*	68	—					
c. *Sargassum*	75	81	—				
d. *Acinetospora*	72	81	98	—			
e. *Chordaria*	74	81	97	97	—		
f. *Akkesiphycus*	74	81	98	98	99	—	
g. *Eisenia*	74	80	97	97	96	97	—

Thus, this picture suggests that the diatom and the golden-yellow alga are phylogenically rather remote, supporting the view that these two organisms should be placed in the independent groups taxonomically.

The brown algal group is one of the most diversified ones from morphological point of view. We have determined the sequences of 5S rRNA from typical five brown algal species, *Eisenia bicyclis* (Order Laminariales), *Sargassum fulvellum* (Order Fucales), *Acinetospora crinita* (Order Ectocarpales), *Chordaria flagelliformis f. chordaeformis* (Order Chordariales) and *Akkesiphycus lubricum* (Order Dictyosiphonales) to cover the representative major orders of this phylum. Although these 5 species are very different in morphology and life history, the 5S rRNA data clearly indicate that the similarity percent among these brown algal 5S rRNA sequences is very high (96–99%). For example, 5S rRNA sequences from *Eisenia* and *Akkesiphycus* which are taxonomically placed in the separate orders reveal 97% identity. Thus, all the brown algae examined here separated to each other within a very short time (point A in Fig. 3), long after the separation from the ancestor common to the diatom. This diversification point corresponds to about 0.2 billion years ago, when the time of divergence between yeasts and animals is assumed to have occurred 1.2 billion years ago (Kimura and Ohta 1973). The *Knuc* values among brown algae are so close to each other that it is difficult to estimate their precise phylogenic relationships.

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