The nucleotide sequences of 5S rRNAs from two red algae, *Gracilaria compressa* and *Porphyra tenera*

Byung-Lak Lim, Hiroshi Hori and Syozo Osawa

Laboratory of Molecular Genetics, Department of Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan

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ABSTRACT
The nucleotide sequences of 5S rRNA from two red algae, *Gracilaria compressa* and *Porphyra tenera* have been determined. The two 5S rRNAs are fairly dissimilar to each other in their sequences (65% identity), although they are both composed of 121 nucleotides. Their secondary structures are generally of the eukaryotic with a prokaryotic characteristic. Judged from the 5S rRNA sequence data, the red algae are phylogenically distinct from green and brown algae, and they, *Porphyra* in particular, are evolutionally most ancient among the eukaryotes of which 5S rRNA sequence has been determined.

INTRODUCTION
In classical biology, the red algae are grouped as the phylum Rhodophyta and classified into two classes, the Protoflorideae containing primitive red algae and the Florideae containing more advanced red algae. To estimate the phylogenetic position of the red algae at the molecular level, we have determined the nucleotide sequences of 5S rRNA from two red algae, *Gracilaria compressa* in the class Florideae and *Porphyra tenera* in the class Protoflorideae, and compared them with those of *Porphyra yezoensis* (1) and other eukaryotes.

MATERIALS AND METHODS
The cytoplasmic 5S rRNAs from *Gracilaria compressa* and *Porphyra tenera* (a close relative of *P. yezoensis*) were directly isolated by phenol method and purified by DE52 chromatography and electrophoresis on a 12% polyacrylamide gel as previously described (2).

The sequences were analyzed by the chemical method of Peattie (3) or the enzymatic method of Donis-Keller (4) using 3'- or 5'-end labelled 5S rRNAs. The 3'- and 5'-terminal nucleotides were confirmed by the TLC plate analysis (5).
Fig. 1 Comparison of the 5S rRNA sequences of *Gracilaria* compressa, *Porphyra tenera*, *P. yezoensis* (1), *Sargassum fulvum* (brown alga; 6) and *Ulva pertusa* (multicellular green alga; 6). Squared-off sequences (A,A',B,B',C,C', etc. in the lowest line) are possibly involved in base pairing in the secondary structural model. The aLb, blc, etc. are symbols for loop regions. (for symbols, see ref. 7). *: *Porphyra*.

Fig. 2 Secondary structure models of 5S rRNAs from *Gracilaria compressa* and *Porphyra tenera*. Dotted lines indicate potential base pairs (8).
RESULTS AND DISCUSSION

The nucleotide sequences were determined mostly by the method involving chemical digestion of 3'-P-labelled RNAs followed by the electrophoresis (3). The sequencing from the 5'-end was also done by the enzymatic method using 5'-32P-labelled RNAs (4). In the case of Porphyra tenera 5S rRNA, autoradiograms obtained with the chemical method alone could not tell us whether the nucleotides of position 80, 89 and 90 from the 5'-end were G or A. Those with the enzymatic method using 3'-P-labelled RNAs clearly showed that they were all G. The 5'- and 3'-terminal nucleotides confirmed by TLC chromatography were A and U in Gracilaria compressa, and A and C in Porphyra tenera, respectively. The 5S rRNA sequences from two red algae were both 121 nucleotides long. These sequences are compared with those of other algae (Fig. 1).

The secondary structure models from these red algae agree with those from other eukaryotes including green and brown algae in having five base-paired regions (Fig. 2) (6). However, the total number of nucleotides in the C-C' helix region plus the cLc' region is 21 in all the red algae examined as has been found in all the prokayotic 5S rRNAs, but in contrast to 20 in all the cytoplasmic 5S rRNAs from eukaryotes (see position 30-50 in fig. 1). Thus, the 5S rRNA from red algae seems to have characteristics of partly eukaryotic and partly prokayrotic. In addition to the above, the red algal 5S rRNAs have several unique characteristics. The Porphyra 5S rRNA has a bulged A in the B-B' helix, and, like in the 5S rRNAs from fungi and protozoa a bulged G can be recognized in the D-D' helix. On the other hands, the B-B' helix from the Gracilaria 5S rRNA has an U/C mismatch. More interesting feature of this 5S rRNA is found in the D-D1 helix where two mismatches of C/A and G/A can be recognized. Such a structure of this region has never

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

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* Mean similarity values calculated from 5S rRNA sequences of 2 brown algae, 5 green algae, 16 higher plants, 17 fungi, 15 protozoa, and 67 multicellular animals.
been found in all the 5S rRNA species so far examined. The sequence of
position 43-46 from the 5'-end of Porphyra is GAAU which is unique, while
that of Gracilaria is GAAC like those of green algae and plants.

Table 1 is a similarity matrix of the 5S rRNA sequences from eukaryotes
with special reference to those of Gracilaria, Porphyra and other algae. It
shows that (i) the sequence of two Porphyra species are very similar to each
other differing only in position 80, 89 and 90 (all G in P. tenera as already
mentioned vs. all A in P. yezoensis), (ii) the sequence of Gracilaria is
quite dissimilar to those of the two Porphyra species (64-65% identity), and
(iii) the similarity of either Gracilaria or Porphyra to green and brown
algae is as low (58-62% identity) as to other eukaryotes (55-64% identity).

The facts mentioned above suggest that Gracilaria and Porphyra separated
from each other at a quite ancient time, though they are classified in the
same phylum. Red algae, especially Porphyra, seem to be the most anciently
emerged group among eukaryotes of which 5S rRNA sequence has been determined.

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REFERENCES

Res. 10, 6037-6040.
Acids Res. 6, 3459-3469.
1909-1912.