The nucleotide sequence of 5 S ribosomal RNA from a sea anemone, *Anthopleura japonica*

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Sea anemone (Anthopleura japonica) *5 S rRNA* *Nucleotide sequence* *Evolution* *Phylogenic tree* *Secondary structure*

1. INTRODUCTION

The phylogenic position of sea anemone has been settled by morphological studies, but there has been no such approach on a molecular basis. Since 5 S rRNA sequences are useful for establishing the evolutionary relationships of organisms [1], we have sequenced the 5 S rRNA from a sea anemone, *Anthopleura japonica* and found that the sequence is almost equally similar to those of vertebrates, Arthropoda, sea urchin and *Lingula* and is less related to those of plants, fungi and protozoa.

2. EXPERIMENTAL

The 5 S rRNA of a sea anemone, *Anthopleura japonica*, was isolated as follows: 100 g of whole organisms were homogenized in a buffer containing 10 mM Tris–HCl (pH 7.7), 1 mM $MgCl_2$ and 1% SDS. The homogenate was shaken with an equal volume of 90% phenol for 30 min, and centrifuged for 60 min at 9000 rev./min. Nucleic acids were precipitated with ethanol from the aqueous phase and treated with 50 μg DNase/ml at 37°C for 20 min. The RNA was recovered by the phenol method. The crude 5 S rRNA was isolated from the RNA preparation by electrophoresis on a 15% polyacrylamide gel containing 7 M urea, 0.1 M Tris–borate (pH 8.3) and 1 mM EDTA. The 5 S rRNA was eluted from the gel pieces with 0.5 M ammonium acetate, 0.1 mM EDTA and 0.1% SDS at 37°C overnight and precipitated with 3 vol. ethanol. This process was repeated again to purify the 5 S rRNA.

The sequence was analyzed by the chemical method [2] using 5 Sr [3′-^{32}P]RNA and by the enzymatic method [3] using [3′- *or* 5′-^{32}P]RNA.

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3. RESULTS

3.1. *5′- and 3′-end analyses*

When the RNase T_2-digest of the 5 Sr[3′-^{32}P]-RNA was developed on a cellulose thin-layer plate [4], only Up* (* = radioactive) was detected. In the case of nuclease P_1-digest of the 5 Sr[5′-^{32}P]RNA, only p*G was observed. These results indicate that the 3′-terminal base was U and the 5′-terminal was G.

3.2. *Sequence*

The sequence of 118 nucleotides from the 3′-terminus except for positions 14–18 and 53–57 was determined by the chemical degradation of [3′-^{32}P]RNA, followed by electrophoresis [2]. These sequences were confirmed by the enzymatic method [3] using [3′-^{32}P]RNA. The first and second residues from the 5′-terminus and the sequences of positions 14–18 and 53–57 were determined by the same method using [5′-^{32}P]RNA with confirmations of the sequences of other regions. Autoradiograms of the sequencing gels obtained by the enzymatic method are shown in fig.1, where nucleotides of position 1–80 are readable. The sea anemone 5 S rRNA is 120 nucleotides long and its sequence is shown in fig.2 with the se-

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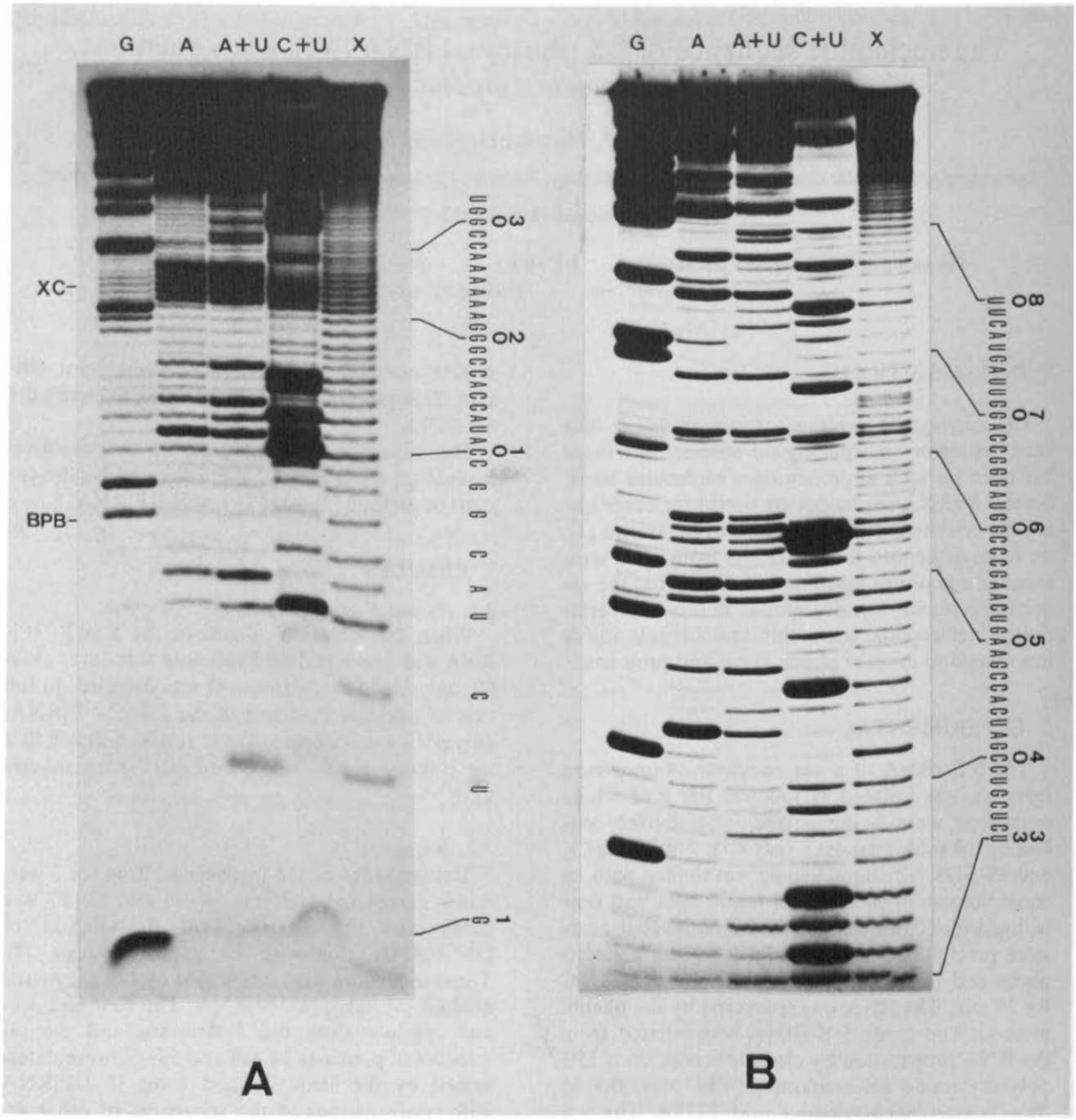

Fig.1. Autoradiograms of 5 S r[5′-^{32}P]RNA of *Anthopleura japonica*. The partial digests obtained by incubating 5 S r[5′-^{32}P]RNA with RNase T_1 (G), RNase U_2 (A), RNase phy M (A + U), *Bacillus cereus* RNase (C + U) and alkali (X) were subjected to electrophoresis on 20% polyacrylamide gels in 7 M urea, 0.1 M Tris–borate (pH 8.3) and 1 mM EDTA at 1500 V for 4 h (A) and 19.5 h (B). The residue number corresponds to that in the alignment in fig.2.
Abbreviations: XC and BPB, marker dyes xylene cyanol and bromophenol blue, respectively.

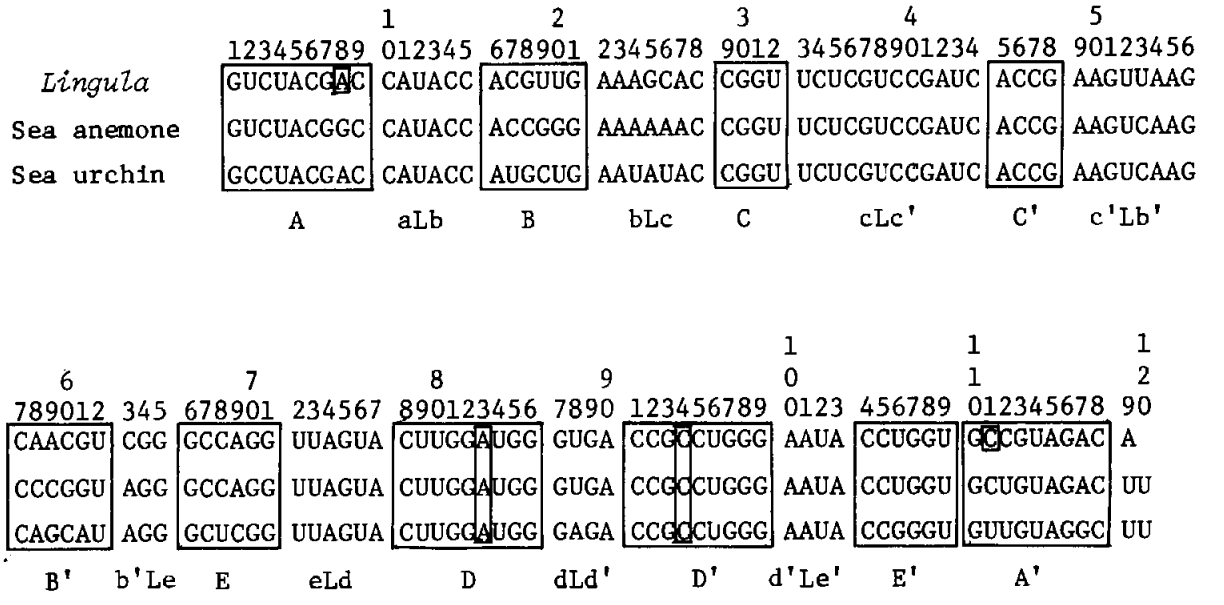

Fig.2. Comparison of the 5 S rRNA sequences of sea anemone, *Lingula anatina* and sea urchin. The squared-off sequences correspond to the base-paired regions in the secondary structures (A,A', B,B', in the lowest line). aLb, bLc, are symbols for loop regions (for these symbols, see [1]).

quences of *Lingula anatina* [5] and sea urchin [6] for comparison.

4. DISCUSSION

Table 1 is a similarity matrix of the eukaryotic 5 S rRNAs. The sequence of the sea anemone 5 S rRNA is almost equally similar to those of vertebrates (76–83% identity, 80% on average), Arthropoda (76–80%, 77% on average), sea urchin (84%) and *Lingula anatina* (87%), and is related less to those of protozoa (48–74%, 65% on average), plants (62–68%, 65% on average) and Ascomycetes (59–74%, 64% on average). This would suggest that the ancestor of sea anemone and those of vertebrates, Arthropoda, Echinoidea and Brachiopoda have evolved to different directions at about the same time after the emergences of protozoa, fungi and plants.

The secondary structure model of the sea anemone 5 S rRNA is shown in fig.3. The model is essentially the same as that for slime mold 5 S rRNA in [7], however, an extended base-paired region

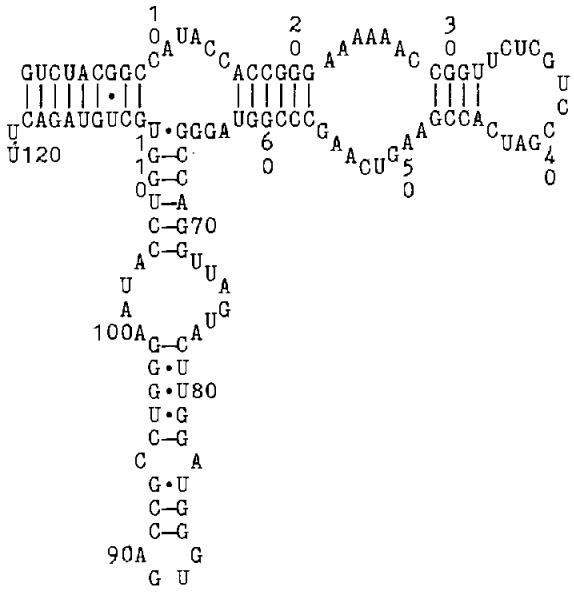

Fig.3. Secondary structure model of *Anthopleura japonica* 5 S rRNA.

Table 1

Similarity of 5 S rRNA sequences of eukaryotes (%)

	VER[a]	ART[a]	SUR	LAN	AJA	PRO[a]	PLA[a]	ASC[a]
VER		76	81	80	80	64	64	60
ART	76		83	83	77	64	61	59
SUR	81	83		84	84	66	64	63
LAN	80	83	84		87	66	67	65
AJA	80	77	84	87		65	65	64
PRO	64	64	66	66	65		61	58
PLA	64	61	64	67	65	61		56
ASC	60	59	63	65	64	58	56	

[a] The mean similarity values calculated from the sequences of 28 vertebrates (VER), 4 Arthropoda species (ART), 11 protozoa (PRO), 9 plants (PLA) and 9 Ascomycetes species (ASC)

SUR, sea urchin; LAN, *Lingula anatina*; AJA, *Anthopleura japonica*. For the sources of the sequences, see [8] and [9–19]

containing an A/C mismatch in the stem D–D′ (see fig.2). This A/C mismatch may be a common characteristic for multicellular animal 5 S rRNAs, because it is observed in 5 S rRNAs from all the animals so far examined and is absent in 5 S rRNAs from plants, fungi and protozoa. Other characteristic features of the sea anemone 5 S rRNA reside:

(i) In the B–B′ stem which is extremely rich in G–C pairs;

(ii) In the 22–27 position which is composed of 6 adenine residues.

On the other hand, the sequence of position 41–44 is GAUC, which is common in 5 S rRNAs from animals and fungi (GAAC in plants and bacteria).

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