Nucleotide sequence of 5 S rRNA from gonads of a Japanese ascidian, *Holocynthia roretzi*

Tsutomu Kumazaki*, Hiroshi Hori and Syozo Osawa

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

Received 15 February 1983

The nucleotide sequence of 5 S rRNA from gonads of an ascidian *Holocynthia roretzi* has been determined. The sequence is almost equally related to those of vertebrates and most of the multicellular animal groups. The secondary structure of this 5 S rRNA fits in with the general structural model for multicellular 5 S rRNA.

Ascidian Holocynthia roretzi *5 S rRNA* *Nucleotide sequence* *Secondary structure* *Phylogeny*

1. INTRODUCTION

Ascidians have been believed to occupy a unique position in the phylogeny of the Chordata. A series of anatomical evidence suggests that they separated from the main line in an early stage of the Chordata evolution. Here, we have determined the nucleotide sequence of 5 S rRNA from gonads of a Japanese ascidian *Holocynthia roretzi* to deduce its phylogenic relationships with other multicellular animals. The sequence obtained is almost equally related to those of vertebrates and most of the multicellular animals.

2. EXPERIMENTAL

Gonads were taken out from three individuals of a Japanese ascidian *Holocynthia roretzi* (Phylum Protochordata; Class Ascidiacea) kindly provided by Dr T. Numakumai (Asamushi Marine Biological Laboratory, Tohoku University).

The ribosomal RNAs, that had been extracted by the phenol method from the 80 S ribosomes, were fractionated with a 12% polyacrylamide −7 M urea gel electrophoresis [1]. The 5 S rRNA preparation so obtained was purified by electrophoresis and subjected to the sequence analyses by the chemical method [2] and the enzymatic method [3]. The terminal base analyses were also done by the method of [4].

* On leave from: Department of Zoology, Faculty of Science, Hiroshima University, Japan

3. RESULTS

3.1. *3′- and 5′-terminal base analyses*

The 5 S [3′-^{32}P]rRNA was digested completely with RNase T_2 followed by the analysis of radioactive base by thin-layer chromatography [4]. Only Ap* (* = radioactive) was detected. The 5 S [5′-^{32}P]rRNA was digested with nuclease P_1 and only p*A was observed. These results indicated that the 3′- and 5′-terminal bases were both A.

3.2. *Sequence analyses*

The sequence of 118 nucleotides from the 3′-terminus was determined by the chemical method [2] and confirmed by the enzymatic method [3] using [3′-^{32}P]RNA. The sequence of the 5′-terminal region was also confirmed by the same enzymatic method using [5′-^{32}P]RNA. An autoradiogram of the sequencing gel obtained by the chemical method is shown in fig.1, where

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

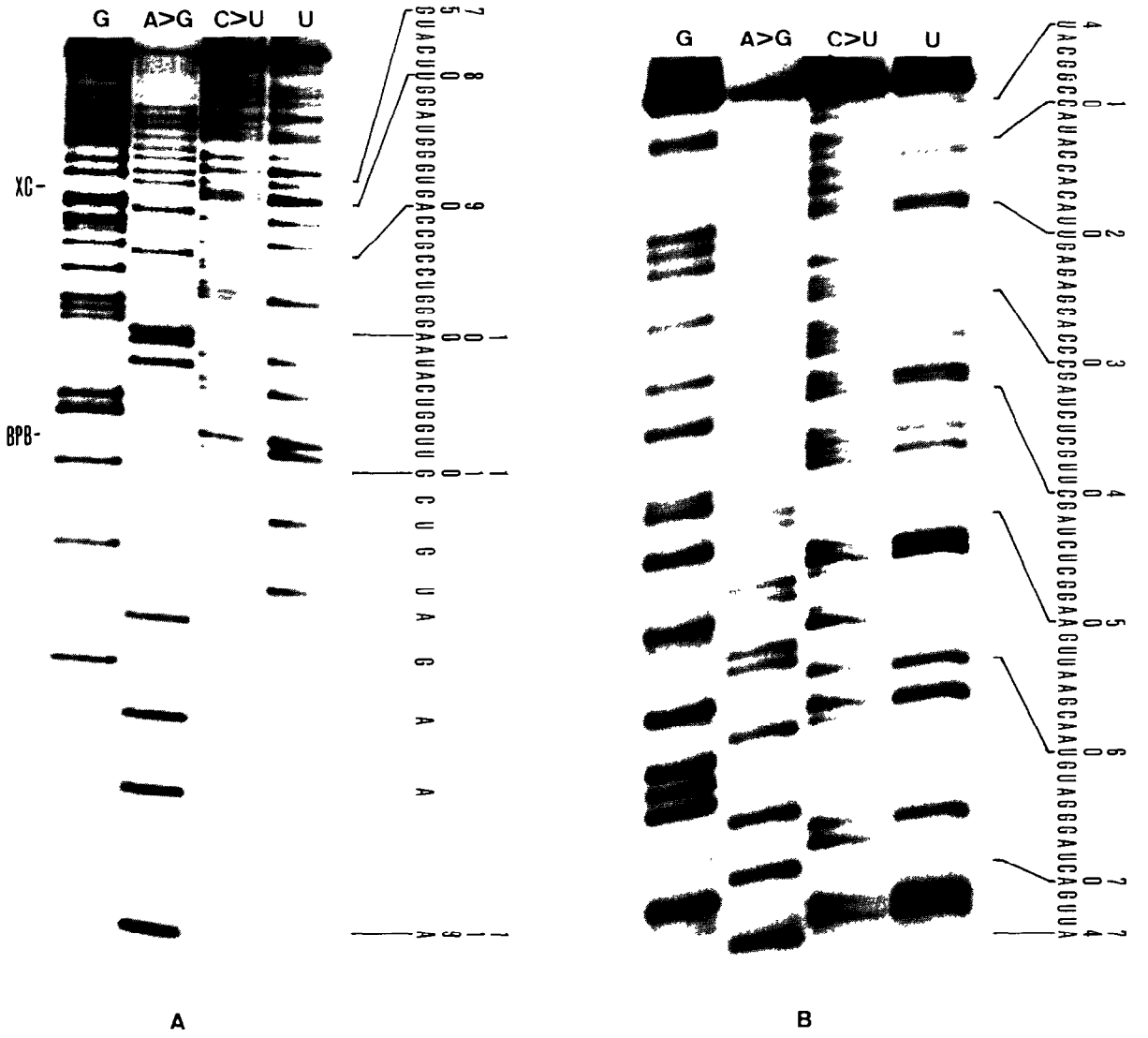

Fig.1. Autoradiograms of 5 S [3′-^{32}P]rRNA of *Holocynthia roretzi*. The partial chemical digests were fractionated on 12% polyacrylamide gel in 7 M urea, 0.1 M Tris–borate (pH 8.3) and 1 mM EDTA at 1500 V for 1.5 h (A) or 7 h (B). The residue number corresponds to that in the secondary structure in fig.2. *Abbreviations:* XC and BPB, marker dyes xylene cyanol and bromophenol blue, respectively.

nucleotides in positions 4–119 are readable. The *Holocynthia* gonad 5 S rRNA was 119 nucleotides long and its sequence is shown in fig.2.

4. DISCUSSION

The secondary structure model of the ascidian gonad 5 S rRNA (fig.2) has 5 base-paired regions which are common to all the eukaryotic 5 S rRNA [1]. In the D–D′ base-paired region (see fig.2), an A/C mismatch can be recognized. As shown in [1], this A/C mismatch can be observed in practically all the multicellular animal 5 S rRNAs so far studied and is absent in 5 S rRNAs from organisms belonging to other kingdoms. Thus the present data of the ascidian gonad 5 S rRNAs supports the view that the A/C mismatch is one of the characteristics of the multicellular animal 5 S

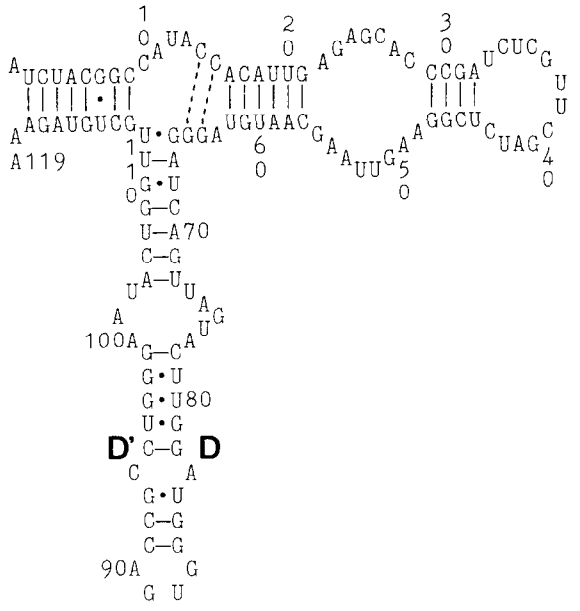

Fig.2. Secondary structure model of *Holocynthia roretzi* 5 S rRNA.

rRNAs. Another characteristic feature of the ascidian gonad 5 S rRNA is the existence of a non-base paired A at the 5′-end and a cluster of 3 A residues at the 3′-end. In the eukaryotic 5 S rRNAs, the 5′- and 3′-ends are mostly G and U, respectively, while in the prokaryotes the 5′-end is usually U and the 3′-end is U or C (see pp.20–21 of [5]). There are only a few examples known for 5 S rRNA sequences having A either at the 3′-end or at the 5′-end. The *Lingula* 5 S rRNA has A at its 3′-end, the *Chlamyodomonas* and *Chlorella* 5 S rRNAs have A at their 5′-end, and the plant mitochondrial 5 S rRNA has two A residues at both 3′- and 5′-ends (for sequence, see pp.20–21 of [5]). The G–C content of the ascidian gonad 5 S rRNA is 48%, which is one of the lowest in the 5 S rRNAs so far known.

Table 1 is a similarity matrix of the eukaryotic 5 S rRNAs. The table indicates that the sequences of most of the multicellular animal groups are similarly related to one another (~80% identity) (for exceptions, see [6,8]). This would suggest that their ancestors, even those for sponges and the Coelenterata, would have evolved within a relatively short time after the emergence of protozoa, plants and fungi [1,6–8]. This is consistent to the fossil records where most of the multicellular animal phyla begin almost simultaneously in the Cambrian Era (about 0.6 billion years before) and there is little logical order (i.e., structurally primitive to complicated) in time of appearance, although the increasing complexity can be seen among groups within single phyla (pp.24–39 of [9]). The sequence of the ascidian gonad 5 S rRNA is almost equally similar to

Table 1

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

	Ver	Aci	Sea	Mol	Art	Rot	Mar	Coe	Spo	Pro	Pla	Asc
Vertebrata[a]		78	82	79	77	79	77	80	79	67	63	59
Ascidian	78		76	80	75	78	76	75	76	66	60	56
Sea urchin	82	76		85	83	88	81	84	83	69	64	63
Mollusca[a]	79	80	85		85	87	79	84	85	69	64	63
Arthropoda[a]	77	75	83	85		84	80	80	82	68	61	59
Rotifera	79	78	88	87	84		80	83	85	67	65	63
Marine planaria	77	76	81	79	80	80		79	78	67	64	61
Coelenterata[a]	80	75	84	84	80	83	79		84	67	67	61
Sponges[a]	79	76	83	85	82	85	78	84		68	66	65
Protozoa[a]	67	66	69	69	68	67	67	67	68		63	59
Plants[a]	63	60	64	64	61	65	64	67	66	63		56
Ascomycetes[a]	59	56	63	63	59	63	61	61	65	59	56	

[a] The mean similarity values calculated from the sequences of 27 vertebrates, 3 Mollusca species, 5 Arthropoda species, 5 Coelenterata species, 3 sponges, 8 protozoa, 10 plants and 8 Ascomycetes species. For the sources of the sequences, see [1,6–8,11–15]

those of vertebrates (78% identity on average) and other multicellular animals (75–80%). The ascidian gonad sequence is, however, less similar to the sequences of protozoa, plants and fungi (56–66%) as expected. An almost equal similarity of the ascidian sequence to those of vertebrates and other animals supports the classical view that the ascidians branched at an early period of the Chordata evolution (see section 1).

Komiya et al. (unpublished) have independently sequenced the 5 S rRNA from somatic cells of *Holocynthia roretzi*, indicating that the gonad and somatic 5 S rRNAs have exactly the same sequence. This is in sharp contrast to the existence in *Xenopus* of 2 types of 5 S rRNA, each specific to ovary and to somatic cells [10].

ACKNOWLEDGEMENTS

We thank Dr T. Numakumai of Tohoku University, and Professors E. Nakano and M. Hoshi of Nagoya University for their advice. This work was supported by grants 56480377, 56570178 and 57121003 (Special Project Research) from the Ministry of Education of Japan.

REFERENCES

[1] Kumazaki, T., Hori, H. and Osawa, S. (1982) FEBS Lett. 146, 307–310.
[2] Peattie, D.A. (1979) Proc. Natl. Acad. Sci. USA 76, 1760–1764.
[3] Donis-Keller, H. (1980) Nucleic Acids Res. 8, 3133–3142.
[4] Nishimura, S. (1972) Prog. Nucleic Acid Res. Mol. Biol. 12, 49–85.
[5] Hori, H. and Osawa, S. (1982) Zbl. Bact. Hyg. I Abt. Orig. C3, 18–30.
[6] Kumazaki, T., Hori, H., Osawa, S., Ishii, N. and Suzuki, K. (1982) Nucleic Acids Res. 10, 7001–7004.
[7] Hori, H., Ohama, T., Kumazaki, T. and Osawa, S. (1982) Nucleic Acids Res. 10, 7405–7408.
[8] Ohama, T., Kumazaki, T., Hori, H., Osawa, S. and Takai, M. (1983) Nucleic Acids Res. in press.
[9] Simpson, G.C. (1966) The Meaning of Evolution (rev. edn) Yale University Press, London, New Haven.
[10] Ford, P.J. and Southern, E.M. (1973) Nature New Biol. 241, 7.
[11] Goldsbrough, P.B., Ellis, T.H.N. and Lomonossoff, G.P. (1982) Nucleic Acids Res. 10, 4501–4514.
[12] Xian-Rong, G., Nicoghosian, K. and Cedegren, R.J. (1982) Nucleic Acids Res. 10, 5711–5716.
[13] Wildeman, A.G. and Nazar, R.N. (1982) J. Biol. Chem. 257, 11395–11404.
[14] Rafalski, J.A., Wiewiorowski, M. and Söll, D. (1982) Nucleic Acids Res. 10, 7635–7642.
[15] Kumazaki, T., Hori, H. and Osawa, S. (1982) FEBS Lett. 149, 281–284.