Nucleotide sequences of 5S rRNAs from sponge *Halichondria japonica* and tunicate *Halocynthia roretzi* and their phylogenetic positions

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ABSTRACT

The nucleotide sequences of 5S rRNAs from sponge *Halichondria japonica* and tunicate *Halocynthia roretzi* were determined by chemical and enzymatic gel methods. Their phylogenetic positions among metazoans were derived from the 5S rRNA sequences by a computer analysis based on the maximum parsimony principle. It was suggested that the sponge is closely related to several invertebrates and the tunicate has affinity to vertebrates rather than invertebrates.

INTRODUCTION

Because information on 5S rRNA sequences is being accumulated at an accelerated speed, it is quite interesting to analyze phylogenetic relationships of organisms using these data. Sponges are considered to be the simplest form of multicellular animals, whereas tunicates to be closely related to vertebrates (1). It is expected that their 5S rRNA sequences may give useful insight into animal evolution at the early stages. Thus we have determined the nucleotide sequences of 5S rRNAs from sponge *Halichondria japonica* and tunicate *Halocynthia roretzi*.

For construction of a phylogenetic tree of 5S rRNAs, the difference matrix method has been mainly employed (2-5). The sequence method based on the principle of maximum parsimony utilizes the information contained in the sequence data better than the matrix method (6). So we applied this method to the phylogenetic analysis of 5S rRNAs from 30 metazoans so far sequenced.

MATERIALS AND METHODS

Low molecular weight RNA fractions were obtained from the total tissues of *H. japonica* and the branchial pouches of *H. roretzi* (500 g each) by the procedures described previously (7). About 15 A₂₆₀ units of 5S rRNAs were purified by electrophoresis of the fractions on polyacrylamide-7M urea

gel (0.3x20x20 cm). The RNAs were labeled with [$5^{\prime}$ - 32 P]pCp and [γ - 32 P]ATP at the 3' and 5' ends, respectively, and then purified again electrophoretically on a long gel (0.1x25x40 cm) to separate RNA species of slightly different chain lengths. The 3' and 5' end-nucleotides were identified by thin layer chromatography after complete digestions of the labeled RNAs with RNase T₂ and nuclease P1. For these methods, see references (7,8). Sequence analyses were performed by chemical and enzymatic gel methods of Peattie and Donis-Keller *et al.*, respectively (9,10).

Table 1 lists the sources of cytoplasmic 5S rRNAs from metazoan somatic cells, whose sequences were used for phylogenetic analysis (11-23). In addition, 46 protozoan, fungal and vegetable 5S rRNAs were also used in order to determine the phylogenetic positions of metazoans in eukaryotes. 5S rRNAs from oocytes, embryos, organelles and bacteria were excluded from the analysis. The phylogenetic tree was constructed by a computer program system based on the maximum parsimony method, which is similar to that of Moore *et al.* for the analysis of protein phylogeny (24) and was described briefly by Hasegawa (25). Full detail of this method and the phylogenetic tree of whole eukaryotic 5S rRNAs will be published elsewhere. This method conjectures the ancestral sequences for the nodes of tree and searches for the most plausible tree which requires minimum evolutionary change (*i.e.* the minimum number of base-replacements) from a great number of conceivable trees.

Table 1. Sources and references of cytoplasmic 5S rRNAs from metazoan somatic cells whose sequences were used for phylogenetic analysis.

<i>Homo sapiens</i> (human)	(11)	<i>Iguana iguana</i> (lizard)	(11)
<i>Terrapene carolina</i> (turtle)	(11)	<i>Xenopus laevis</i> (toad)	(11)
<i>Xenopus mulleri</i> (toad)	(11)	<i>Tinca tinca</i> (tench)	(12)
<i>Salmo gairdnerii</i> (trout)	(13)	<i>Misgurnus fossilis</i> (loach)	(11)
<i>Scylliorhinus caniculus</i> (dogfish)	(14)	<i>Drosophila melanogaster</i> (fly)	(11)
<i>Acyrtosiphon magnoliae</i> (aphid)	(15)	<i>Bombyx mori</i> (silkworm)	(16)
<i>Philosamia cynthia</i> (silkworm)	(17)	<i>Lingula anatina</i> (brachiopod)	(7)
<i>Mytilus edulis</i> (mussel)	(18)	<i>Helix pomatia</i> (snail)	(18)
<i>Arion rufus</i> (snail)	(18)	<i>Caenorhabditis elegans</i> (nematode)	(19)
<i>Rhabditis tokai</i> (nematode)	(20)	<i>Halocynthia roretzi</i> (tunicate)	*
<i>Halichondria japonica</i> (sponge)	*	<i>Halichondria panicea</i> (sponge)	(21)
<i>Hymeniacidon sanguinea</i> (sponge)	(21)	<i>Haloclona oculata</i> (sponge)	(21)
<i>Anthopleura japonica</i> (sea anemone)	(22)	<i>Brachionus plicatilis</i> (rotifer)	(20)
<i>Spirocodon saltatrix</i> (jellyfish)	(23)	<i>Nemopsis dofleini</i> (jellyfish)	(23)
<i>Aurelia aurita</i> (jellyfish)	(23)	<i>Chrysaora quinquecirrha</i> (jellyfish)	(23)

*, this paper.

FIGURE 1. The nucleotide sequences of 5S rRNAs from sponge *Halichondria japonica* and tunicate *Halocynthia roretzi* arranged into the secondary structural model of Nishikawa and Takemura (26).

RESULTS AND DISCUSSION

Except for the 5' terminal nucleotides, the total sequences of *H. japonica* and *H. roretzi* 5S rRNAs were determined by the chemical method with 3' end-labeled RNAs using various types of polyacrylamide-7M urea gels (20 %, 0.05x25x40 cm; 7 or 12 %, 0.05x25x60 cm). The sequences from positions 1-110 were derived by the enzymatic method with 5' end-labeled

Table 2. Number of base substitutions between metazoan 5S rRNAs in Table 1. The vertebrate, snail, insect, nematode, and jellyfish 5S rRNAs are grouped with each other, respectively, and average values between them and other animal 5S rRNAs are shown. The sequences of *H. japonica* and *H. sanguinea* 5S rRNAs are identical.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1) Vertebrates												
(2) <i>H. roretzi</i>	26											
(3) <i>H. japonica</i>	25	26										
(4) <i>H. panicea</i>	22	28	4									
(5) <i>H. oculata</i>	27	32	20	16								
(6) <i>A. japonica</i>	22	25	17	19	23							
(7) <i>L. anatina</i>	22	20	12	16	16	13						
(8) <i>M. edulis</i>	24	21	11	15	21	16	7					
(9) Snails	26	26	14	16	26	18	14	13				
(10) Insects	27	31	19	18	26	25	18	18	16			
(11) <i>B. plicatilis</i>	24	26	17	17	19	22	12	7	20	19		
(12) Jellyfishes	24	30	17	19	23	18	16	17	20	21	19	
(13) Nematodes	30	31	31	31	32	36	28	24	35	33	23	34

FIGURE 2. A phylogenetic tree of animal 5S rRNAs constructed by the maximum parsimony method. The branch lengths in horizontal direction are proportional to the numbers of base replacements between contemporary and ancestral sequences. The numbers attached to the arrows show the increases of the minimum number of base replacements required for the phylogenetic tree when the branches were grafted into other places.

RNAs and were well consistent with the sequences determined chemically. Figure 1 shows these two sequences thus established, which are arranged into the secondary structural model of Nishikawa and Takemura (26).

Quite recently, Dames *et al.* reported sequences of 5S rRNAs from three species of sponge, *Halichondria panicea*, *Hymeniacidon sanguinea*, and *Haliclona oculata* (21). It is interesting to note that 4 base substitutions are present between the 5S rRNA sequences of sponges belonging to the same genus, *H. japonica* and *H. panicea*, whereas the sequences are identical between different genera, *H. japonica* and *H. sanguinea*. The 5S rRNA from *H. oculata*, which belongs to the different order from that of the above three species, is largely remote from these three 5S rRNAs (Table 2).

Conventional taxonomy teaches us that tunicates are closely related to

vertebrates, classifying them into chordate and that sponges are the simplest form of multicellular animals which diverged at the earliest stage of animal evolution (1). However, the number of nucleotide substitutions between tunicate (*H. roretzi*) and vertebrate 5S rRNAs is as great as those between invertebrate and vertebrate 5S rRNAs and is even comparative to that between sponge and vertebrate 5S rRNAs (Table 2). The values among sponge, brachiopod, mollusk, insect, and coelenterate are relatively small, indicating their close relationship, whereas nematode is largely remote from other animals. Thus, the phylogenetic tree obtained by the maximum parsimony analysis (Fig. 2) is somewhat different from the conventional taxonomy; *i.e.*, 1) nematode separates from other animals at the earliest stage of their evolution, and 2) other invertebrates including sponges form an assemblage, showing their close relationship. Similar phylogenetic tree was also obtained by the difference matrix method of Fitch and Margoliash (27) (data not shown).

Tunicate *H. roretzi* and vertebrates form a branch of chordate (Fig. 2), which is consistent with the conventional animal taxonomy. If the branch of tunicate is grafted into the root of invertebrates, the minimum number of base replacements required for the tree increases by one (Fig. 2). This altered topology is also possible, because the increase is very small. This suggests that the tunicate and vertebrates diverged near the time of divergence of vertebrates and invertebrates. Several other possible topologies are also shown in Fig. 2.

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