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Nucleotide sequence of *Physarum polycephalum* 5.8S rRNA gene and its flanking regions

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ABSTRACT

The nucleotide sequence of Physarum polycephalum 5.8S rRNA gene and its flanking regions has been determined. The homologies of the 5.8S rRNA sequence with those of Saccharomyces, Chlamydomonas and Xenopus were 56%, 50% and 52%, respectively. In spite of these relatively low homologies, its possible secondary structure was very similar to those of other species.

INTRODUCTION

5.8S rRNA is present only in eukaryotic ribosomes, not in prokaryotic ones. The RNAs (or their genes) from more than 10 species of lower and higher eukaryotes have been sequenced (See ref.1) and the sequences have been found to be highly or moderately conserved. It was recently shown that these 5.8S rRNAs have some sequence homology with the 5' terminal region of E.coli 23S rRNA (2,3). Accumulation of data on the 5.8S rRNA gene should make it possible to discuss the evolutionary origin and change of this gene.

In this work we determine the nucleotide sequence of the 5.8S rRNA gene and its flanking region of a true slime mold, Physarum polycephalum. The structural characteristics are discussed in terms of evolution of the gene.

MATERIALS AND METHODS

Preparation of DNA fragment

The preparation of recombinant plasmid pRD4 carrying the 5.8S rRNA gene and its purification were described previously (4). The purified plasmid was digested with XhoI and the 3.3 kb

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fragment carrying the 5.8S rRNA gene was isolated by 1% agarose gel electrophoresis.

Isolation of 5.8S rRNA

26S rRNA was isolated from the ribosomal pellet of Physarum polycephalum as described by Hall and Brown (5). The sample as a solution in 0.1M LiCl, 10mM EDTA, 10mM Tris-HCl (pH 7.8) and 0.2% SDS was heated at 60°C for 5 min, cooled to room temperature and sujected to 10-30% sucrose density gradient centrifugation (Dupont-Sorval AH627 rotor, 24000 r.p.m. for 20 hrs at 4°C). 4S-7S RNA were precipitated with ethanol and further fractionated on 10% polyacrylamide-7M urea gel. The gel was stained with ethidium bromide, the region of 5.8S RNA was cut out and the RNA was eluted with a solution of 0.5M ammonium acetate, 0.01M magnesium acetate, 0.1% SDS and 0.1mM EDTA at room temperature. The RNA was collected by ethanol precipitation.

Sequencing of terminal regions of 5.8S rRNA

The 5' end of RNA was dephosphorylated with bacterial alkaline phosphatase and labelled with [γ-^{32}P]-ATP and T4 polynucleotide kinase. Then the 5' RNA sequence was determined by wandering spot analysis (6). The 5' terminal nucleotide was determined by complete digestion of the labelled RNA with Nuclease P1 and then two-dimensional thin layer cellulose chromatography.

The 3' end of the RNA was labelled by the method of Bruce and Uhlenbeck (7). The labelled RNA was partially digested with RNase T1, and treated with alkali (pH 9.0),and subjected to 20% polyacrylamide-7M urea gel electrophoresis as described by Donis-Keller et al.(8). The 3' terminal nucleotide was determined by digestion of 3' end labelled 5.8S rRNA with RNase T2, and then analysis by PEI cellulose thin layer chromatography.

Other methods

Southern hybridization was carried out as described previously (4,9). DNA sequencing was done by the method of Maxam and Gilbert (10).

Materials

Restriction enzymes were purchased from Takara Shuzo

Co.,Inc, Japan (Kyoto), Bethesda Research Lab. and New England Biolab. Bacterial alkaline phosphatase was obtained from Worthington and purified further by the method of Weiss et al.(11). T4 RNA ligase was a gift from K. Hirai, Mitsubishi Kasei Institute of Life Science.

RESULTS AND DISCUSSION

First, the location of the 5.8S rRNA gene was determined by Southern blotting with ^{32}P-labelled 5.8S rRNA as a probe (See Fig.1). Plasmid pRD4 was digested with XhoI and the 3.3 kb (kilobase) fragment of the digest was found to hybridize with 5.8S rRNA. Digestion of the 3.3 kb fragment with AvaI produced five fragments, of which the 0.94 kb fragment was specifically hybridized with 5.8S rRNA. A restriction map of the 0.94 kb AvaI fragment was constructed (Fig.1) and 5.8S rRNA was found to hybridize with 0.1 kb TaqI, 0.15 kb TaqI/AvaI, 0.72 kb HinfI and

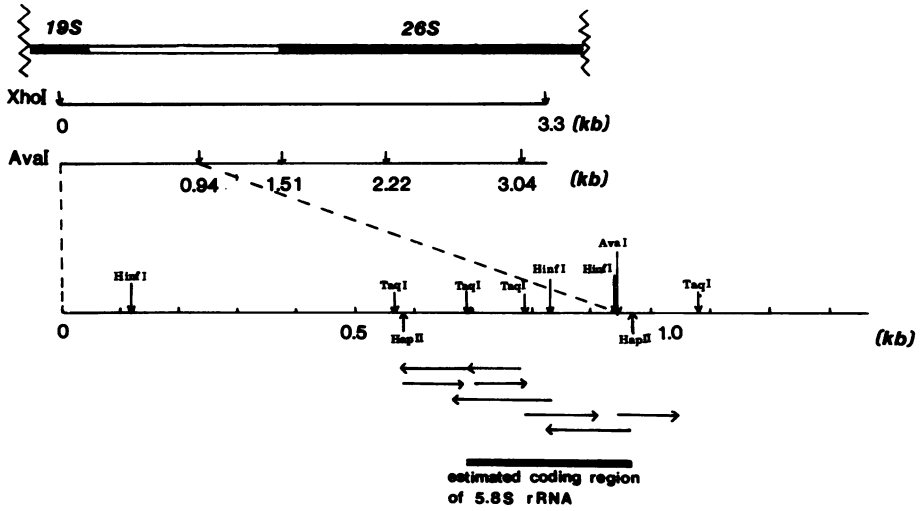

Figure 1. Restriction map of the 5.8S rRNA coding region and strategy for DNA sequencing. The physical position of the 3.3 kb XhoI fragment on ribosomal DNA was determined previously (4). The restriction map of the fragment was constructed by the conventional method. The coding region of the 5.8S rRNA gene, determined by Southern blotting, is shown by a bar. Horizontal arrows show the region and the direction of DNA sequencing.

0.12 kb HinfI fragments. These results localized the 5.8S rRNA coding region within ~250 bases, as shown by the bar in Fig.1.

Then the region containing the 5.8S rRNA gene was sequenced by the method of Maxam and Gilbert (10) by the strategy shown in Fig.1 and the results are presented in Fig.2. To determine the exact position of the 5.8S rRNA gene, we sequenced both terminal regions of the 5.8S rRNA as described in the Materials and Methods. The sequence of the 5' terminal region was determined by wandering spot analysis (6) to be "5' ACCGUUGG---- 3'" and the 3' terminal region was determined by the method of Donis-Keller et al. (8) to be "5' ----GGGNNGNNNNGNNNNU 3'". From these results together with the DNA sequence data in Fig.2, we concluded that the 5.8S rRNA gene corresponds to the region underlined in Fig.2.

5.8S rRNA sequences are known to be considerably conserved: sequence homologies are more than 96% among vertebrates (12), 92% between Neurospora and Saccharomyces (13), 79% between Chlamydomonas and Saccharomyces (14) and 75% between rat and Saccharomyces (15). We examined the sequence homologies between Physarum 5.8S rRNA and 14 published 5.8S rRNA sequences (1, 14, 18) and found relatively low sequence homology (less than 60%). For example, Physarum 5.8S rRNA has 56%, 50% and 52% sequence homologies with those of Saccharomyces (16), Chlamydomonas (14) and Xenopus (12), respectively. Since sequence homology is

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5' GACTGCTTTCCCCTTACCCGTTTCCCCCGCTTAGCAAACTTTAACCGTTA 50
   TaqI
   AATTAAACAACGGAACGTACACCGTTGGGCGATGGATTGCTTGGTGCCTG 100

   CTTCGACGAAGAGCGCAGTGAAACGCGATAACTTTTGTGACTCGCACTCT 150
      TaqI                                  HinfI
   CTGTGATCAACGTCTCCTTGAACATTAGTGCGGCCTTGCCTTCGGGCACT 200

   GGCCCCCTTGGGATGCCTTGTCCTTTTACAGTGCTTACGACCGACTCGGG 250
                                          HinfI AvaI
   GGGATTAACCTTTTCTCGCGTTATACCACCTTTAACC  3'
                                   HaeII
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Figure 2. Base sequence of the 5.8S rRNA gene and its flanking regions. The region shown by a bar in Fig.1 was sequenced by the method of Maxam and Gilbert (10). The underlined part is the 5.8S rRNA coding region.

generally correlated with evolutionary distance, the results suggest that Physarum was separated evolutionally from these species. Our findings were compatible with those of Komiya and Takemura (17), who found that Physarum 5S RNA has relatively low sequence homology with 5S RNAs of species of fungi, metazoa, plants and algae.

Although the sequence homology is low, Physarum 5.8S rRNA has several sequences common to almost all 5.8S rRNAs of known sequence (Fig.3). Since these sequences are conserved in a variety of species, they must be important for the function of 5.8S rRNA.

Finally, we studied the secondary structure of Physarum 5.8S rRNA by the method of Nazar et al. (15). As shown in Fig.4, it is possible to construct a secondary structure that shows considerable common features for 5.8S rRNAs of various species. It should be noted that conserved sequences are concentrated on right-side branches. We infer that both the secondary structure and the sequences of this region are critical for the biological function of 5.8S rRNA and have been conserved during evolution of the gene.

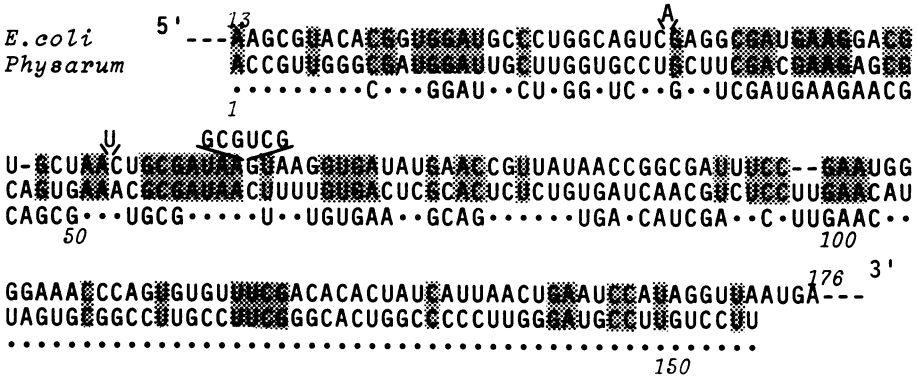

Figure 3. Comparison of sequences of 5.8S rRNA from various species. The Physarum 5.8S rRNA sequence was compared with 14 reported 5.8S rRNA sequences (1, 14, 18). Sequences common to 14 of 15 species are shown by letters in the third line. The Physarum sequence was also compared with the 5' terminal region of E.coli 23S rRNA (19) and homologous sequences are shown by half-toned screening. Numbers show nucleotide numbers from the 5' end.

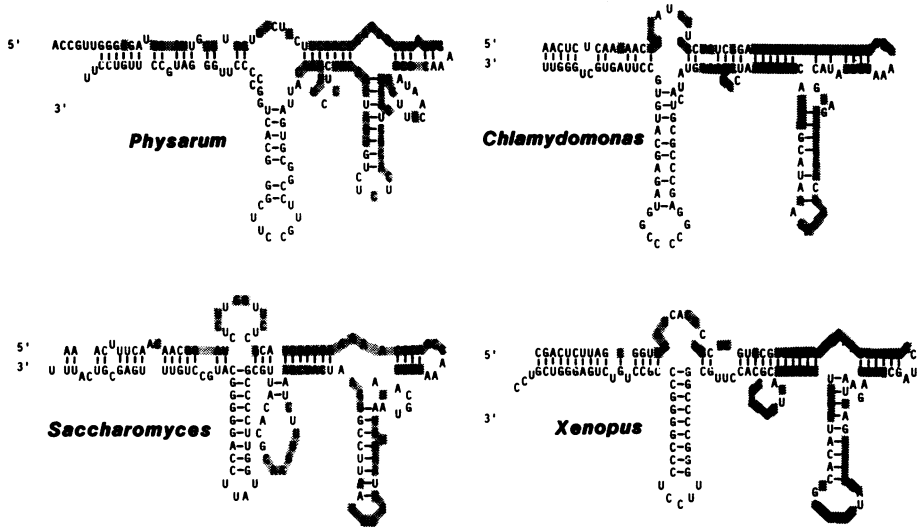

Figure 4. Possible secondary structures of 5.8S rRNA from various species. 5.8S rRNAs from Physarum, Saccharomyces, Chlamydomonas and Xenopus were compared. Data on Saccharomyces and Xenopus and on Chlamydomonas were those of Nazar et al. (15), and Darlix and Rochaix (14),respectively. Conserved sequences described in Fig.3 are shown by half-toned screening.

Physarum 5.8S rRNA also has some sequence homology (40%) with the 5' terminal region of E.coli 23S rRNA (Fig.3), supporting the hypothesis that the 5.8S rRNA gene was derived from the 5' terminal region of the prokaryote large rRNA gene (2,3).

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