Two thraustochytrid 5S ribosomal RNAs

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ABSTRACT

The complete nucleotide sequences of the 5S ribosomal RNAs (rRNAs) of two thraustochytrids, *Thraustochytrium visurgense* and *Schizochytrium aggregation*, are AUGAGCCCUCAUAUCAUGUGGAGUGCACCGGAUCUCAUCCGAACUCCGUAGUUAAGCCACAUAGAGCGCGUC UAGUACUGCUGGGCGAAGCGAGGGCGUGCUCAUU and ACAGCCGUUCAUACCACGAGAUAACCGGAUCUGGCUACUCCGAACUCCGUAGGCGUGGCGUGCCAGUACUACCACGAGGACUGGGUGGGA AGCGUGCGUAGCGCGUG, respectively. These sequences are discussed in terms of the apparent unity in secondary structure and strong divergence in primary structure exhibited by protist 5S rRNAs.

INTRODUCTION

Thraustochytrids are marine protists of uncertain phylogenetic affinity. Sporangia of these organisms attach to algae and marine angiosperms by rhizoid-like structures which penetrate host tissue [1]. Ultrastructural studies of these systems suggest that they are similar in developmental origin and anatomy to the ectoplasmic nets of labyrinthuloid slime net organisms [2]. Here we present the nucleotide sequences of the cytoplasmic 5S rRNAs of two thraustochytrids, *Thraustochytrium visurgense* and *Schizochytrium aggregation*, and then compare them to each other and to 5S rRNA sequences from other protists.

MATERIALS AND METHODS

*Thraustochytrium visurgense* and *Schizochytrium aggregation* were obtained from the American Type Culture Collection (ATCC 28208 and 28209) and were each cultured for 5 days in Byt medium (1 g Difco yeast extract, 1 g Difco peptone and 5 g glucose in 1 l seawater) at 24 C. RNA extraction, 5S rRNA purification and preparation of RNA labeled at either the 5' or 3' terminus with 32P have been described previously [3]. The partial chemical degradation method of Peattie [4] was used only on 3'-labeled RNA, while the partial ribonuclease digestion method of Donis-Keller et al. [5], supplemented by the use of ribonuclease PhyM [6] and pancreatic ribonuclease was used for both 3'- and 5'-
labeled RNA. Ribonucleases T1 and U2 were obtained from P.L. Biochemicals, pancreatic ribonuclease was from Worthington, and PhyM was a gift from Helen Donis-Keller. Alkaline digests of terminally labeled RNA were obtained by incubation in 20 μl of freshly prepared 0.15 M ammonium hydroxide for 1 min at 90°C, followed by lyophilization and suspension of the digest in the buffer used for ribonuclease digestion [5]. Very nearly all of each 5S rRNA nucleotide sequence could be determined by chemical sequencing of 3'-labeled RNA. However, enzymatic sequencing of 5'-labeled RNA was necessary to confirm sequences at 5' termini, and at places where band compression was observed in sequencing gels of 3'-labeled material.

RESULTS AND DISCUSSION

The data used to derive 5S sequences for T. visurgense and S. aggregatum have been provided to the editor and referees, and are available from the authors. The sequence of T. visurgense 5S rRNA, broken at intervals of ten residues, is AUGAGCCUC AUUAUCUGUG GAGUCUCGGU GAUCAGGCCCCA AGUGAGCAACG GAGAUGGGGA CUAGGUGGGA AGCGGCGUGG GGGUCCUUU. That of S. aggregatum is ACAGCCGGCG AUAACACAGCG GAGAUGGGGA AGCGGCGUGG GGGUCCUUU. In Fig. 1, the sequence of T. visurgense 5S rRNA (large letters) is displayed according to a secondary-structural model proposed by Böhm et al. [7]. The sequence of S. aggregatum 5S rRNA is displayed outside this, with residues which differ from those in homologous positions in T. visurgense 5S rRNA shown in small letters. Nucleotide residues which are identical to those in homologous positions in T. visurgense 5S rRNA are indicated by open or closed circles, the latter indicating positions which are occupied by identical residues in nine diverse eukaryotic 5S rRNAs compared in this way previously [8].

Both 5S rRNA sequences do conform to the expectations of the secondary-structural model of Böhm et al. [7]. Of the 37 differences between them, 26 are confined to regions of presumed base-pairing. Of these 26, 22 represent compensatory base changes in both strands of such regions, and therefore allow retention of proposed base-pairing, while 4 represent G-C/G-U or U-G/U-A transitions which would also preserve such structure [7,8].

When each of these sequences is compared with each of ten diverse protist and/or fungal 5S rRNA sequences (those of the amoeboid Acanthamoeba castellanii [3], the cellular slime mold Dictyostelium discoideum [9], the unicellular alga Chlorella pyrenoidosa [10], the zygomycete fungus Phycomyces blakesleeanus [11], the flagellate protist Crithidia fasciculata [12], the ciliate protist
Fig. 1. The nucleotide sequences of *T. visurgense* and *S. aggregatum* 5S rRNAs displayed as suggested by Böhm et al. [7]. See text for explanation.

*Tetrahymena thermophila* [13], the ascomycete fungus *Saccharomyces cerevisiae* [14], the flagellate unicellular alga *Euglena gracilis* [15], the dinoflagellate alga *Cryptothecodinium cohnii* [16], and the ascomycete fungus *Schizosaccharomyces pombe* [17]), mean differences of 51 ± 5.4 (S.D.) and 52 ± 4.0 (S.D.) are obtained for *T. visurgense* and *S. aggregatum*, respectively. These are considerably greater than the difference of 37 residues (out of 119) observed between the two, and their phylogenetic affinity seems thus confirmed. Nevertheless, the apparent phylogenetic distance between these two species, which are considered members of the same family [1], is greater than that between any two members of the kingdom of multicellular animals yet examined [13,18]. This bespeaks either the great antiquity of the protists or substantially more rapid rates of 5S rRNA sequence divergence within them.

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REFERENCES