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Nucleotide sequences of 5S ribosomal RNA from four oomycete and chytrid water molds

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ABSTRACT

The nucleotide sequences of the 5S rRNAs of the oomycete water molds Saprolegnia ferax and Pythium hydnosporum and of the chytrid water molds Blastocladiella simplex and Phlyctochytrium irregulare were determined by chemical and enzymatic partial degradation of 3' and 5' end-labelled molecules, followed by gel sequence analysis. The two oomycete sequences differed in 24 positions and the two chytrid sequences differed in 27 positions. These pairs differed in a mean of 44 positions. The chytrid sequences clearly most resemble the sequence from the zygomycete Phycomyces, while the oomycete sequences appear to be allied with those from protozoa and slime molds.

INTRODUCTION

5S rRNA provides a favorable molecule for investigating phylogenetic relationships among fungi. Nearly all macromolecular sequence data from fungi have been obtained from ascomycetes (1,2), although recently a 5S rRNA sequence from the zygomycete Phycomyces blakesleeanus was reported (3). It has been widely thought that chytrid water molds represent morphological 'relics', resembling the earliest eumycota (e.g. Ref. 4). In contrast, both morphological and biochemical criteria have suggested (5-7) that the oomycete water molds represent a lineage distinct from other fungi, perhaps derived from algae (4,8). Here we report the 5S rRNA nucleotide sequences from 2 oomycetes and 2 chytrid water molds, each representing a different order within each class of water molds.

MATERIALS AND METHODS

Preparation of 5S

Cultures of Blastocladiella simplex (ATCC 24579), Phlyctochytrium irregulare (ATCC 32066), Saprolegnia ferax (ATCC 26116) and Pythium hydnosporum (ATCC 26929) were grown in shake cultures containing 0.3% glucose and 0.15% each of peptone and yeast extract. Mycelia or cell aggregates were filtered, washed

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and resuspended in 2 volumes of cold 10 mM Tris, pH 7.4, 1 mM Na_2EDTA, 100 mM NaCl and 100 ppm heparin. 30 sec exposure to a Polytron at full speed produced partial lysates, providing a good yield of 5S rRNA with minimal release of larger RNAs, except in the case of P. irregulare. After 2 extractions with buffer-saturated phenol, the ethanol precipitate was fractionated on a 10% polyacrylamide gel and 5S rRNA extracted from the gel (9). In the case of P. irregulare, it was necessary to remove starch from the salt soluble RNA fraction. This was accomplished by electroelution of the RNA after the salt soluble fraction was dissolved in elution buffer (10) to which 5M urea was added. This solution was placed in the cathode chamber and elution buffer without urea was layered on top and in the anode chamber.

Sequence Analysis

The 3' and 5' termini were labelled with ^{32}P as previously described (10). The chemical sequencing method of Peattie (11) was used with 3' end labelled RNA and the enzymatic method of Donis-Keller (12) was used with 5' end labelled material. T_1 ribonuclease partial digestions were in some cases run with chemical digests to confirm the presence of guanosine where chemical cleavage produced only a weak or blurred band. Partial alkaline digests of labelled RNA were obtained by incubation of RNA in 20 ul of 0.15 N NH_4OH for 90 sec at 90°C, followed by lyophilization (D. Spencer, personal communication). Terminal nucleotide analyses were performed as previously described (10). Sequencing gels were 40 x 33 x .04 cm, 10% polyacrylamide and 60 x 33 x .04 cm, 6 or 8% polyacrylamide.

RESULTS

The sequences are aligned with each other (Fig. 1), and the helical regions, according to the models of Bohm et al. (13) and Diels et al. (14), are indicated. Watson-Crick pairs or G:U pairs are present in all positions within these proposed helical regions. In addition, a possible extension of helix B (II according to Refs. 15 and 16) to include positions 12 and 26 as a G:C pair is suggested in the case of the sequence from P. hydnosporum. Nearly all other 5S rRNA sequences from eukaryotes have an adenosine in position 12 (1), although the sequence from chickens also has a cytosine in this position (17). The suggested deletion in position 114 of the S. ferax sequence is based upon comparisons with all eukaryotic sequences as well as that of P. hydnosporon. Alignment of this sequence near the 3' terminus may possibly be subject to revision with additional sequences from oomycetes. A matrix of nucleotide differences between these sequences and in addition

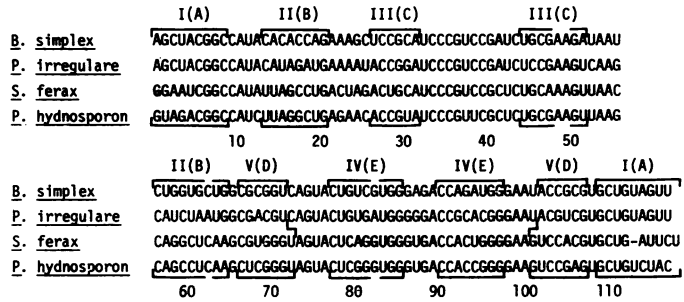

Figure 1. The 5S rRNA sequences for Blastocladiella simplex, Phlyctochytrium irregulare, Saprolegnia ferax and Pythium hydnosporon aligned with presumed homologous positions. The proposed helical regions according to the model of Böhm et al. (13) and Diels et al. (14) are indicated by brackets. Both the lettering nomenclature used by these authors and the numbering nomenclature used by Luehrsen and Fox (15) and Garrett et al. (16) are indicated.

those of the fungi Phycomyces blakesleeanus (3) and Saccharomyces cerevisiae (18) and the cellular slime mold Dictyostelium discoideum (19) is given in Table 1.

DISCUSSION

On the basis of total nucleotide differences, the sequence from P. irregulare clearly is the most similar known 5S sequence to those from the zygomycete Phycomyces blakesleeanus (3) and the chytrid B. simplex (Table 1), being nearly equally divergent from each. Comparisons with other sequences consistently yield values for P. blakesleeanus much higher than for B. simplex (Table 1). This suggests that the lineage leading to the P.

Table 1. A matrix of total number of positions with a different nucleotide in 5S rRNA sequences from various fungi and slime molds.

	S.f.	P.h.	B.s.	P.i.	P.b.	S.c.	D.d.
Saprolegnia ferax	-	24	41	46	56	46	39
Pythium hydnosporon	24	-	42	44	52	49	35
Blastocladiella simplex	41	42	-	27	37	40	44
Phlyctochytrium irregulare	46	44	27	-	28	45	46
Phycomyces blakesleeanus	56	52	37	28	-	56	52
Saccharomyces cerevisiae	46	49	40	45	56	-	53
Dictyostelium discoideum	39	35	44	46	52	53	-

blakesleeanus 5S sequence has been substituting faster than the lineage leading to the B. simplex sequence, thus implying a more recent divergence of the P. irregulare and P. blakesleeanus sequences. The results of several qualitative enzyme surveys as well as molecular weight measurements of the large rRNA in various fungi and slime molds (5-7) are most consistent with the proposal that zygomycetes are derived from chytrids of the order Chytridiales, exemplified by P. irregulare.

The two oomycete sequences show indications of being most recently diverged from sequences from protozoa and slime molds, but are not clearly most similar to any given known sequence within this group. They have a uridine in position 15 and an adenosine in position 64 (Fig. 1) as do sequences from the protozoa or slime molds Acanthamoeba castellanii (20), Physarum polycephalum (21), Crithidia fasciculata (22), Euglena gracilis (23), Tetrahymena thermophila (24), and Dictyostelium discoideum (19). No other known eukaryotic 5S sequence has these nucleotides in these positions (1). Also, the oomycete sequences share with all known protozoan, slime mold and fungal sequences except that of P. irregulare (Fig. 1) a guanosine in position 83, whereas all known sequences from metazoa and the cytosol of plants have an adenosine or, rarely, a uracil in this position (1). Although it apparently has escaped notice, surveys among fungi and slime molds of the qualitative properties of several enzymes or metabolic pathways as well as the molecular weights of the large rRNA consistently indicated that oomycete and dictyostelid cellular slime mold sources were the most similar to each other (5-7).

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