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**The nucleotide sequence of 5S rRNA from a cellular slime mold *Dictyostelium discoideum***

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**ABSTRACT**

The nucleotide sequence of ribosomal 5S rRNA from a cellular slime mold *Dictyostelium discoideum* is GUUAACGGCCAUACUAGGUUGGAAACACAUCAUCCCGUUCGAUCUGAUAAGUAAAUCGACCUCAGGCCUCCAAGUACUCUGGUUGGAGACAACAGGGGAACAUAGGGUGCUGUAUACU. A model for the secondary structure of this 5S rRNA is proposed. The sequence is more similar to those of animals (62% similarity on the average) rather than those of yeasts (56%).

**INTRODUCTION**

There have been little data to show the phylogenic position of a cellular slime mold *Dictyostelium discoideum* on the quantitative basis. As we repeatedly mentioned (1, 2), the 5S rRNA sequences can be used for establishing phylogenic relationships of widely separated organisms. We have therefore sequenced the 5S rRNA of *D. discoideum*, and the phylogenic position of this slime mold among eukaryotes has been deduced from a quantitative comparison of the sequence to the known 5S rRNA sequences.

**MATERIALS AND METHODS**

The 80S ribosomes of *D. discoideum* (strain NC-4) were purified as described previously (3). The 5S rRNA, that had been extracted by the phenol method from the 80S ribosomes, was fractionated with a 10% polyacrylamide-7M urea gel electrophoresis (4). Materials and methods for the sequence analyses of 5S rRNA have already been described (4, 5).

**RESULTS****(1) 5'- and 3'-terminal analyses**

When the RNase T<sub>2</sub>-digest of the [3'-<sup>32</sup>P] 5S rRNA was developed on an Avicel TLC plate, the main radioactive nucleotide was Up\* (\* radioactive) with small amounts of other nucleotides (Gp\*:Ap\*:Cp\*:Up\* = 0.01:0.05:0.04:

0.91). In the case of [5'-<sup>32</sup>P] 5S rRNA digested with nuclease P<sub>1</sub>, p\*G was the main, indicating that the 3'-terminal base is U and the 5'-terminal is G.

(2) Sequence analyses by post labelling procedures

The 117 nucleotide sequences from the 3'-terminus of the 5S rRNA were determined by the chemical degradation of [3'-<sup>32</sup>P] RNA followed by the electrophoresis. Fig. 1 shows a representative autoradiograph of the electrophoresis where the sequence of positions 109 to 53 from the 3'-terminus can be read off. Positions 1 to 15 from the 5'-terminus were also determined in this way by using [5'-<sup>32</sup>P] 5S rRNA. The primary sequence of *D. discoideum* 5S rRNA so obtained was shown in Fig. 2, together with the sequences of human KB cell 5S rRNA and yeast 5S rRNA for comparison.

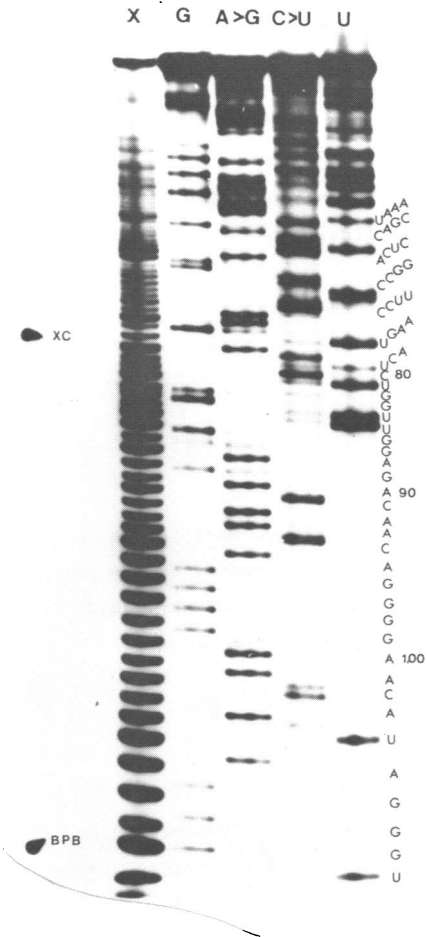


Fig. 1. Autoradiogram of 3'-end labelled *Dictyostelium discoideum* 5S rRNA. The RNA was submitted to the partial chemical cleavage reactions (4, 5). The cleavage products were fractionated on 12% polyacrylamide gel in 7M urea, 0.1 M Tris borate (pH 8.3), and 1 mM EDTA at 1,000 volts for 4 hr. The residue number corresponds to that in the alignment in Fig. 2. Abbreviations: XC and BPB, positions of marker dyes, xylene cyanol and bromophenol blue, respectively. X, lane of alkali-digestion.

		1		2		3		4		5
	123456789	012345	678901	2345678	9012	345678901234	5678	90123456		
Human	GUCUACGGG	CAUACC	ACCCUG	AACGCGC	CCGA	UCUCGUCUGAUC	UCGG	AAGCUAAG		
<i>D. disco.</i>	GUAUACGGC	CAUACU	AGGUUG	GAAACAC	AUCA	UCCCGUUCGAUC	UGAU	AAGUAAAU		
<i>S. cerevi.</i>	GGUUGCGGC	CAUAUC	UACCAG	AAAGCAC	CGUU	UCCCGUCCGAUC	AACUG	UAGUUAAG		
	A	aLb	B	bLc	C	cLc'	C'	c'Lb'		
					1		1		1	
	6	7	8	9	0	1	1	2		
	789012	345	6789012	34567	89012	34567890123	456789	012	3456789	012345678901
	CAGGGU	CGG	GCCUGGU	UAGUA	CUUGG	AUGGGAGACCG	CCUGG	AAU	ACCGGGU	GCUGUAGGCUUU
	CGACCU	CAG	GCCUUC	AAGUA	CUCUG	GUUGGAGACAA	CAGGGG	AAC	AUAGGGU	GCUGUAUACU
	CUGGUA	AGA	GCCUGACCGGUA	GUGUA	GUGGGUGACCA	UACGGG	AAA	CUAGGU	GCUGCAUUCU	
	B'	b'Le'	E	eLd	D	dLd'	D'	d'Le'	E'	A'

Fig. 2. Comparison of the 5S rRNA sequences of *Dictyostelium discoideum*, human KB cell and *Saccharomyces cerevisiae*. The squared off-sequences correspond to the base-paired regions in the secondary structures (A,A', B,B', C,C' and D,D' in the lowest line). aLb, bLc, etc. are symbols for loop regions (e.g., aLb is the loop region between A and B; for these symbols, see ref. 1).

**DISCUSSION**

The primary sequence of *D. discoideum* 5S rRNA is more similar to those of animals (62% similarity on the average) than to those of yeasts (56%) or plants (49%; see Table 1). Therefore, this cellular slime mold could be more related to animals rather than either fungi or plants. Since the slime molds are now often considered to be relatives of animalic amoeba (6), it will be interesting to see if 5S rRNAs of both slime molds and amoeba are alike and

Table 1. Sequence similarities of *Dictyostelium discoideum* 5S rRNA to those of animals, yeasts and plants 5S rRNAs.

animals	% <sup>†</sup>	Yeasts	% <sup>†</sup>	Plants	% <sup>†</sup>
Human KB cell	63	<i>S. cerevisiae</i>	57	Wheat	53
Birds	61	<i>T. utilis</i>	57	Sunflower	47
Reptiles	64	<i>P. membranae-</i>	55	Beans	48
Toads	60	<i>fachiens</i>	55	<i>Chlorella</i>	49
Fishes	63	<i>Kl. lactis</i>	57		
<i>Lingula</i> *	65				
Sea urchin*	62				
Fly	56				
Silk worm*	62				

<sup>†</sup> Similarity percent of the 5S rRNA sequence of *D. discoideum* to those of the organisms listed. \* The sequences of organisms with no asterisk were cited from ref. 7. For those of *Lingula*, sea urchin and silk worm, see ref. 8, 9, and 10, respectively.

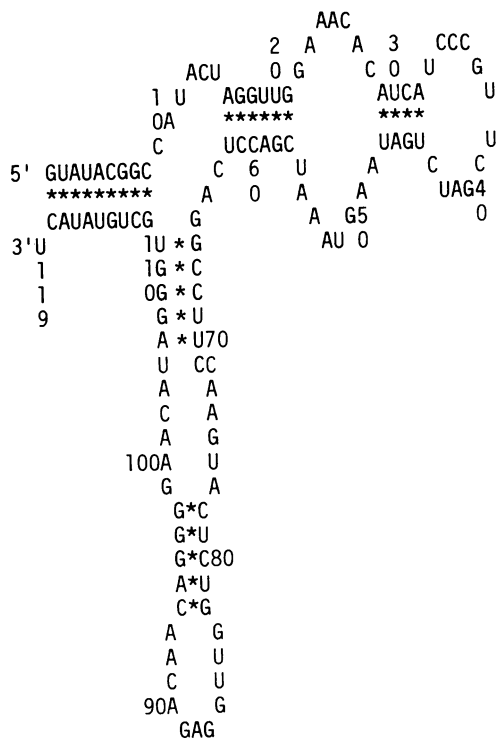


Fig. 3. Secondary structure model of *Dictyostelium discoideum* 5S rRNA.

the 5S rRNA of amoeba is also related to the animal 5S rRNAs.

The secondary structure model of *D. discoideum* 5S rRNA constructed by the method previously described (1, 2) is shown in Fig. 3. As we already pointed out (1, 2), all eukaryotic 5S rRNAs known to date, including the slime mold 5S rRNA, show a common secondary structure, having at least four base-pairing regions (A-A', B-B', C-C' and D-D' in Fig. 2). It seems now reasonable, in addition to the four, to have one more base-paired region E-E' for all the eukaryotic 5S rRNAs. Both the 116-N and 120-N type prokaryotic 5S RNAs (1) can not form the base-pairings at this region.

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