THE NUCLEOTIDE SEQUENCE OF 5 S rRNA FROM THE BLUE-GREEN ALGA *ANACYSTIS NIDULANS*

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1. Introduction

The 5 S rRNA of ribosomes is a particularly suitable molecule for sequence studies as it is small and easily purified. Sequences of the molecule from three eubacteria [1–3], two fungi [4,5], a green alga [6], an amphibian [7], and human KB cells [8] have been determined.

In this paper we present evidence for the complete nucleotide sequence of 5 S rRNA from the blue-green alga *Anacystis nidulans* and point out structural features that it has in common with 5 S rRNAs from other organisms.

2. Materials and methods

A culture of *Anacystis nidulans,* strain 1405/1 Kratz/Allen, was obtained from the Culture Centre for Algae and Protozoa, Cambridge, UK.

Cells, at an initial density of about 4.0×10^4/ml, were cultured in Medium C [9], modified to contain 0.1–0.2 μg K_2HPO_4/ml and supplemented with 35–55 μCi [^{32}P]orthophosphate/ml. Cultures were incubated at 40°C and 4000 lux (Philips 500 W M.B.T.L. bulb) for 24 hr with continuous aeration. Aqueous K_2HPO_4 was then added to a final concentration of 0.25 mg/ml and incubation was continued for a further 24 hr. The final cell density was about 1.0×10^6/ml. Cells were harvested by centrifugation and freeze dried. RNA was extracted as previously described [10] then fractionated by electrophoresis on a slab of 12.5% polyacrylamide gel. The 5 S rRNA was located by autoradiography then eluted, concentrated and freed of soluble acrylamide [11].

Standard procedures were used for the enzymic digestion of 5 S rRNA, the fractionation of the products by two dimensional electrophoresis, and the analysis of the products by further enzymic digestions [12].

Partial digestion of the 5 S rRNA with T_1 or pancreatic A ribonucleases were carried out with enzyme to substrate ratios of 1:500 and 1:1000 (w/w) at 0°C for 30 min. The products were fractionated by high-voltage electrophoresis in cellulose acetate followed either by thin layer chromatography on PEI-cellulose with 7 M urea, 4 M formic acid, 7.5% (v/v) pyridine [13], or by homochromatography on DEAE-cellulose thin layers with homomix A or B [14]. Oligonucleotides derived from the partial digestion products by complete digestion with either T_1 or pancreatic A ribonucleases were identified in most cases by further enzymic digestion as well as by their R_B values.

3. Results and discussion

The sequences of the oligonucleotides from a complete digestion of 5 S rRNA with T_1 ribonuclease are given in table 1. Most were determined by further digestion with pancreatic A and U_2 ribonucleases. Others required modification with *N*-cyclohexyl-*N'*-[2-(4-morpholinyl)ethylcarbodiimide] toluene-p-sulphonate before digestion with pancreatic A ribonuclease. Oligonucleotides T_{14} and T_{15} were sequenced by partial digestions with snake venom phosphodiesterase. Table 1 also lists the sequences of the oligonucleotides from a complete digestion with pancreatic A ribonuclease. All but one were determined after digestion with T_1 ribonuclease. Oligonucleotide P_{20} required a

Table 1
Oligonucleotides produced by enzymic digestion of 5 S rRNA

T_1 ribonuclease digestion products				Pancreatic A ribonuclease digestion products			
Oligonucleotide		Molar yield		Oligonucleotide		Molar yield	
		Experiment+	Theoretical*			Experiment++	Theoretical*
T1	Gp	13	9	P1	Up	12	10
T2	C–Gp	3.6	2	P2	Cp	20	20
T3	C–C–Gp	1.1	1	P3	A–Cp	2.3	2
T5	A–C–Gp	1.0	1	P4	A–A–Cp	1.1	1
T7	C–C–A–Gp	0.8	1	P5	G–Cp	3.0	3
T9	C–A–A–C–Gp	0.9	1	P6	A-Up	4.5	4
T11	U–Gp	3.2	2	P7	G–A–Cp	4.3	2
T12	U–C–Gp	1.0	1	P8	A–G–Cp		3
T14	C–U–C–Gp	1.0	1	P9	G–A–A–Cp	1.0	1
T15	C–U–C–C–C–Gp	0.6	1	P11	G–A–A–A–Cp	0.8	1
T16	U–A–Gp	0.9	1	P12	G–Up	3.6	2
T18	A–U–A–Gp	0.8	1	P13	G–G–Cp	2.6	2
T20	A–A–C–U–C–A–Gp	0.4	1	P14	A–A–A–A–Up	0.9	1
T21	A–C–C–C–C–A–U–C–C–C–Gp	0.5	1	P15	G–A–Up	1.3	1
T22	U–U–Gp	1.1	1	P16	A–G–Up	1.3	1
T24	U–A–U–Gp	0.8	1	P18	G–G–A–A–Cp	0.6	1
T25	pU–C–C–U–Gp	0.4	1	P19	G–G–Up	3.2	3
T26	U–C–U–A–U–Gp	0.7	1	P20	A–G–G–Up	1.2	2
T28	C–U–A–A–A–A–U–A–Gp	0.5	1	P21	G–G–G–Up	0.8	1
T29	A–A–C–C–A–C–U–C–U–Gp	0.6	1	P23	pUp	1.5	1
T30	A–A–A–C–A–U–A–C–C–U–Gp	0.4	1				
3′	$U–C_{OH}$	–	1				

* Calculated from the sequence (fig. 1).

\+ The mean of three experiments and expressed relative to the mean radioactivities in A–C–Gp, U–C–Gp, U–U–Gp and U–A–Gp.

++ Expressed relative to the mean radioactivities in A–A–Cp, G–A–Up, A–G–Up, $\frac{\text{G–A–Cp}}{2}$, $\frac{\text{A–G–Cp}}{2}$, $\frac{\text{G–G–Cp}}{2}$, and $\frac{\text{G–G–Up}}{3}$.

further, partial digestion with U_2 ribonuclease before its sequence could be deduced. The five experiments in which 5 S rRNA was partially digested with T_1 or pancreatic A ribonucleases gave enough sequence overlaps for us to determine unambiguously the complete sequence of the molecule (fig. 1).

The 5 S rRNA of *A. nidulans* contains 120 nucleotides with 26.7, 30.8, 22.5 and 20.0 moles per cent each of G, C, A, and U respectively. No methylated or minor bases were detected and the molecule appears to be homogeneous. In common with all other 5 S rRNAs that have been sequenced, the 5′ and 3′ ends of the molecules are complementary:

pU–C–C–U–G–G–U–G–U - - ->
| | | | | · | | |
HOC–U–G–G–A–C–C–G–C–A - - ->

and there is little doubt that the two ends of the molecules are hydrogen bonded in their native state. There are no other long sequences of nucleotides that are obviously complementary to each other.

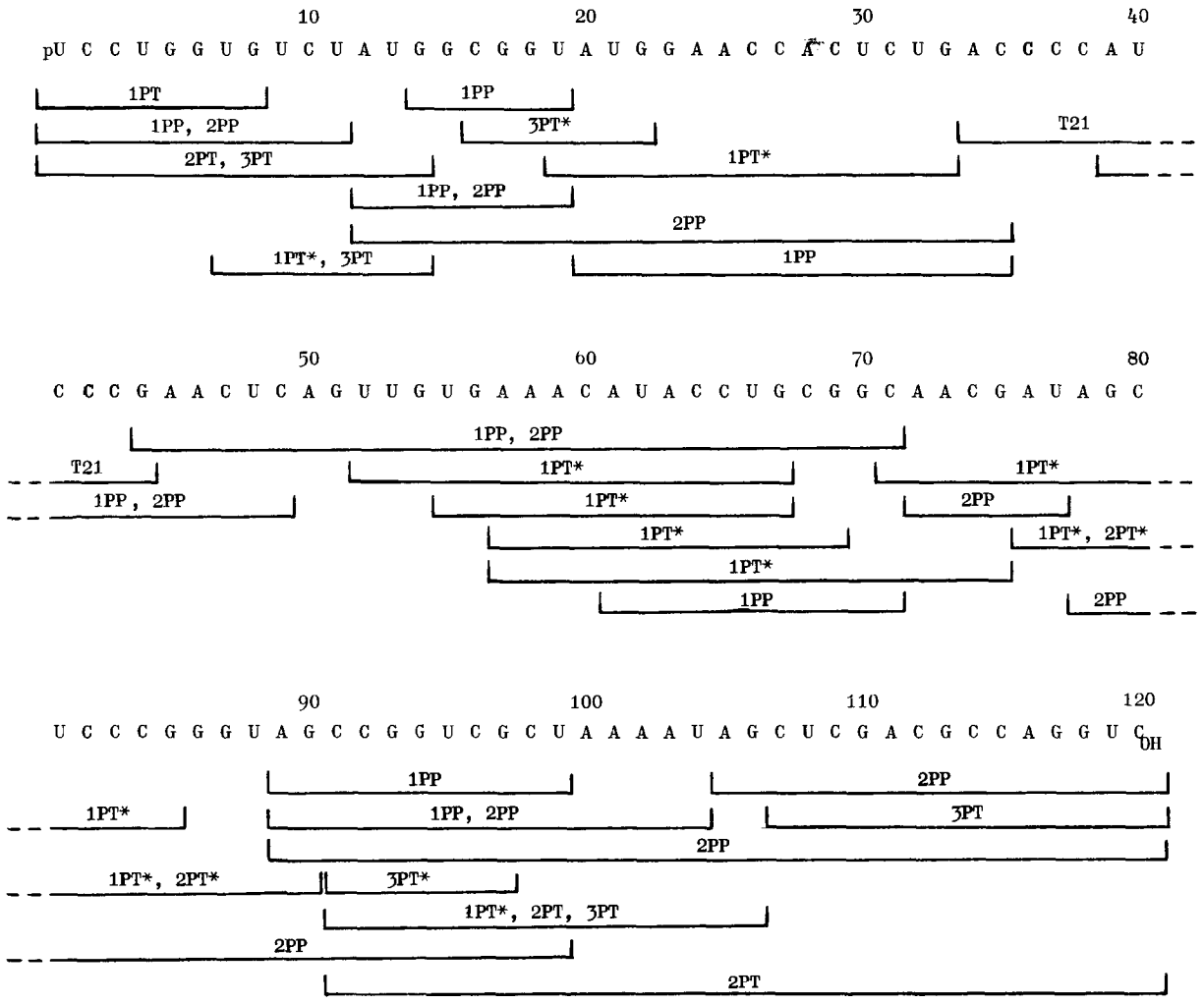

Fig. 1. The nucleotide sequence of 5 S rRNA from *A. nidulans*. The brackets indicate the products of partial T_1 or pancreatic A ribonuclease digestions; 1PT, 2PT and 3PT: three experiments with T_1 ribonuclease; 1PP and 2PP: two experiments with pacreatic A ribonuclease. The oligonucleotides whose partial digestion products were identified by R_B values only (R_B = mobility relative to the blue dye, xylene cyanol F.F.) are marked with asterisks.

Dobson et al. [15] have reported the presence in *A. Nidulans* of a precursor to 5 S rRNA which is slightly larger than the mature molecule. They compared T_1 ribonuclease fingerprints of the precursor and mature molecules and found two spots unique to each. They suggested that these were the 5′ and 3′ terminal oligonucleotides and that the difference in their mobility was due to additional nucleotides in the precursor. Our fingerprints of the mature molecule were very similar to theirs and our sequence analysis of the products confirms their identification of the 5′ terminal oligonucleotide. A difference between it and the corresponding oligonucleotide from the precursor seems indisputable. The spot which they suggested was the 3′ terminal oligonucleotide of the mature molecule corresponds to our T_{21} which comprises nucleotides 34 to 44 of the intact molecule. Variable mobility of this fragment occurred in several of our experiments and is possibly due to its large size and high C content. Dobson et al. [15] did not detect the 3′

oligonucleotide; it has the sequence U–C_{OH}, migrates to the position of Cp and is very faint on autoradiographs and easily missed. As no spot was distinguishable on the fingerprint of the precursor which could represent a 3′ terminal fragment containing additional nucleotides to U–C_{OH}, it seems most likely that the 3′ terminal sequences of both the mature and precursor molecules are the same. Therefore, maturation of the precursor of 5 S rRNA from *A. nidulans* probably occurs by cleavage of nucleotides from the 5′ end of the molecule only, as it does in *E. coli* [16].

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