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A SEQUENCE ANALYSIS OF 5 S rRNA FROM THE BLUE-GREEN ALGA *OSCILLATORIA TENUIS* AND A COMPARISON OF BLUE-GREEN ALGA 5 S rRNA WITH THOSE OF BACTERIAL AND EUKARYOTIC ORIGIN

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1. Introduction

Blue-green algae appear to have formed a distinct group very early in the evolution of living organisms [1]. They were first classified as lower plants but now, on account of their lack of organelles, they are thought to be prokaryotes [2] and possibly the progenitors of chloroplasts [3].

In the previous paper we reported the complete nucleotide sequence of 5 S rRNA from *Anacystis nidulans.* Here we describe a sequence analysis of 5 S rRNA from another blue-green alga, *Oscillatoria tenuis,* and discuss sequence homologies between the two. We also compare the sequence of blue-green algal 5 S rRNA with those of two eubacteria and two eukaryotes.

It is suggested that the blue-green algae are a widely divergent group of organisms that have closest affinities with the bacteria and are better described as blue-green bacteria.

2. Materials and methods

A culture of *O. tenuis,* strain 1459/4 Manten, was obtained from the Culture Centre for Algae and Protozoa, Cambridge, UK. Cells were incubated with $[^{32}P]$-orthophosphate under the same conditions as *A. nidulans* [4] except that the culture was maintained at 20°C and was not aerated. The 5 S rRNA was extracted and digested as previously described [4]. The digestion products were fractionated and characterised using standard methods [5].

3. Results and discussion

Table 1 lists the partial or complete sequences of all the oligonucleotides derived from the complete T_1 or pancreatic A ribonuclease digestion of 5 S rRNA from *O. tenuis.* Of the 18 products larger than trinucleotides only six have the same sequence as those from the 5 S rRNA of *A. nidulans.* Although a few others are clearly similar (pU(C_2U_2)Gp and pU–C–C–U–Gp, C–C–A–C–Gp and C–A–A–C–Gp, A–A–C(C,U)Gp and A–A–C–U–C–A–Gp, and G–A–C–A–A–U–A–Gp and C–U–A–A–A–A–U–A–Gp) the two 5 S rRNA molecules are not sufficiently alike for us to deduce a sequence of *O. tenuis* 5 S rRNA by alignment of its oligonucleotides with sequences in the *A. nidulans* molecule. However, this method has been used to estimate the maximum degree of homology which could possibly exist between the two 5 S rRNAs. It is only about 75%; i.e. a quarter of the nucleotides in equivalent positions in the two molecules are not the same. Therefore, like the bacteria, the blue-green algae are probably a widely divergent group of organisms and this, no doubt, is a reflection of their great antiquity [1]. In contrast, the sequences of 5 S rRNAs from the eukaryotic groups studied are much less varied; they are probably identical in the mammalia [6] and only vary by about 10% in the flowering

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[++]
Gp	8.6	Up	–
C–Gp*	1.9	Cp	–
A–C–Gp*	0.9	A–Cp*	5.7
C–C–A–Gp*	0.7	G–Cp*	5.6
C–C–A–C–Gp	0.7		
U–Gp*	0.6	A–Up*	1.4
C–U–Gp	2.2	A–G–Cp*	3.2
A–U–Gp	0.9	G–A–Cp*	
(C$_3$,U)Gp	0.7	A–A–Up	1.1
A–A–C(C,U)Gp	0.7	G–A–A–Cp*	1.0
C–A–C(C$_2$,U)A–C–C–Gp	0.6	G–A–A–A–Cp*	1.0
A–C–C–C–C–A–U–C–C–C–Gp*	0.7	G–Up*	2.6
A–C–A–A–U–A–Gp	0.8	G–G–Cp*	2.8
(C,U)A–C–U–Gp	0.8	G–A–Up*	1.8
C–A–U–U–Gp	0.9	G–G–Up*	3.0
(U$_2$,C)Gp	1.0	A–G–G–Up*	1.2
A–A–A–C–U–U–U–Gp	0.8	G–G–G–A–Cp	0.7
A–U–A–C–U–U–Gp	0.9	G–G–G–Up*	0.6
pU(U$_2$,C$_2$)Gp	0.8	pUp*	0.8
U–C$_{OH}$	–		

* Oligonucleotides common to *O. tenuis* and *A. nidulans* 5 S rRNAs.

[+] Expressed relative to the mean radioactivities in A–C–Gp, $\frac{\text{C–U–Gp}}{2}$, and A–U–Gp.

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

plants [7].

The 5 S rRNAs of *O. tenuis* and *A. nidulans* have features which are also common to all the known prokaryotic 5 S rRNA sequences but not all the known eukaryotic ones. They both contain the sequence –pyr–G–A–A–C–, which may interact with –G–T–Ψ–C–purin tRNA [8] and they both have a monophosphate at their 5′ termini which is consistent with their being derived from larger precursor molecules [9]. In addition the 5 S rRNA of *A. nidulans* has a sequence remarkably like that of *Escherichia coli* (63% homologous) and *Pseudomonas fluorescens* (59% homologous) (fig. 1). In particular there is a long sequence between nucleotides 20 and 62 which has been highly conserved. It is probably of significance that the *O. tenuis* oligonucleotides A–C–C–C–C–A–U–C–C–C–Gp, A–A–C(C,U)Gp and G–A–A–A–Cp closely resemble sequences in this region. In contrast, the sequence of 5 S rRNA from *A. nidulans* is less similar to eukaryotic 5 S rRNAs (fig. 2) both in terms of overall homology (human 48% and *Chlorella pyrenoidosa* 53%) and the lengths of identical sequences. However, the degree of homology is much greater than the 25% that would be expected if two molecules with random sequences but same overall composition were compared. There can be no doubt therefore that the prokaryotic and eukaryotic 5 S rRNAs had a common origin.

The results presented here provide further evidence that sequence of 5 S rRNA has changed slowly during

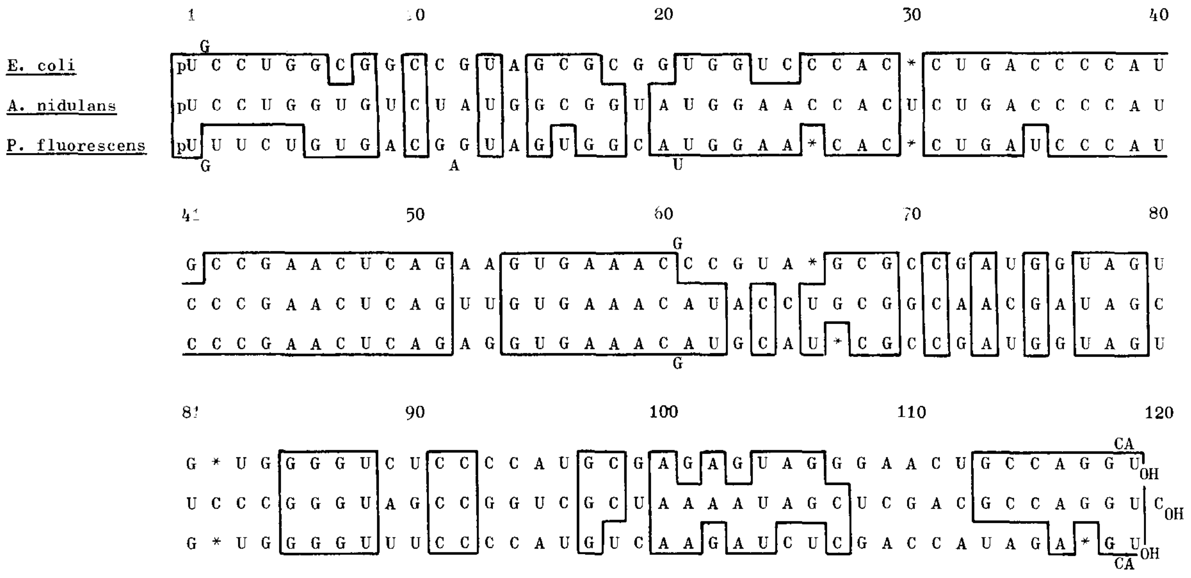

Fig. 1. Comparison of the nucleotide sequence of 5 S RNA from *Anacystis nidulans* with 5 S rRNAs from *Escherichia coli* and *Pseudomonas fluorescens*. Asterisks indicate gaps introduced to obtain maximum sequence homology.

the evolution of living organisms and it seems an excellent molecule to use in comparative studies to establish phylogenic relationships of major groups of organisms.

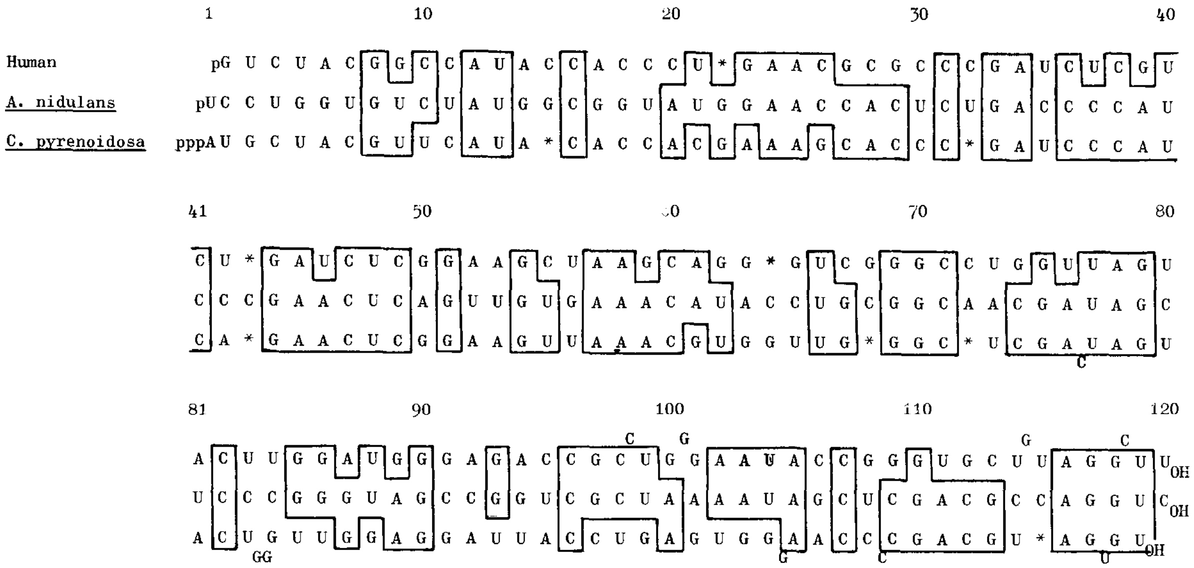

Fig. 2. Comparison of the nucleotide sequence of 5 S rRNA from *Anacystis nidulans* with 5 S rRNAs from Human KB cells and *Chlorella pyrenoidosa*.

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