The nucleotide sequence of 5S rRNA from *Scenedesmus obliquus*

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ABSTRACT

The nucleotide sequence of the cytoplasmic 5S ribosomal RNA from *Scenedesmus obliquus* has been determined using post-labelling techniques. The sequence is closely related to the 5S RNA sequence of *Chlorella* and contains all the areas of invariant sequence which appear to be conserved in plant cytoplasmic 5S RNA species.

INTRODUCTION

Comparisons of the nucleotide sequences of 5S RNA from different species have made possible predictions concerning its generalised secondary structure [1, 2, 3, 4], and the importance of conserved sequences [5, 6]. As more primary structures become known, so these predictions can become more reliable.

The nucleotide sequences of more than 40 eukaryotic cytoplasmic 5S rRNA species are now known [7]. Two sequences for *Chlorella* 5S rRNA have been reported [3,8], and in one of these reports [3] mention is made of the sequence of *Scenedesmus quadricauda* 5S rRNA.

Scenedesmus obliquus is a unicellular green alga which is included in the order of the chlorococcales. *Chlorella* is included in the same order, and so these two algae are closely related morphologically.

A knowledge of the nucleotide sequence of *S. obliquus* 5S rRNA has enabled us to compare it with the sequences of 5S rRNA isolated from a different strain of the same organism; a closely related green alga; a more distantly related green alga, *Chlamydomonas reinhardtii* [9]; and from higher plants.

MATERIALS AND METHODS

Chemicals, enzymes, radiochemicals and chromatographic materials were obtained as described previously [10]. Crude low molecular weight RNA (mostly tRNA and 5S rRNA) was isolated from *S. obliquus* by the method of Osterman *et al* [11]. 5S RNA was separated from tRNA by chromatography on Lysine-Sepharose [12], and the 5S RNA finally purified by 10% polyacrylamide gel electrophoresis.

The primary sequence was deduced using a combination of [^{32}P] post-labelling techniques performed as described previously [10]. These included the limited hydrolysis method [13], direct read-off gels [14] and mobility shift analysis [15]. The latter two methods were performed on the whole molecule and a fragment (residues 75-120) which was obtained during the labelling process. The 5'- and 3'- terminal nucleotides were characterised as described previously [16].

RESULTS

The purification and sequential analysis of the 5S rRNA was relatively straightforward. Sequence analysis of the 3'- end of the molecule was facilitated by the isolation of a 5'-[^{32}P]-labelled fragment (75-120) during the labelling reaction of the whole molecule. This showed up on the autoradiogram of the polyacrylamide gel as a sharp major band. Results from the limited hydrolysis method, in general, enabled conclusive deductions to be made. However, for residues 118 and 119 the data was not so clear. No [^{32}P]-labelled nucleotide was detected after digestion of the fragment expected to have residue 118 at its 5'-end and, although pG was present after digestion of the fragment expected to have residue 119 at its 5'-end, background contamination made this deduction less definitive than those for other residues in the molecule.

The figure shows the nucleotide sequence of S. obliquus 5S rRNA which has been deduced, and indicates the individual methods which provided the sequence data for the different parts of the molecule. It can be seen that, for most of the molecule, information was gained from more than one set of results.

DISCUSSION

The nucleotide sequence for S. obliquus 5S rRNA, shown in the figure, is compared with that of Chlorella which has been published independently by two groups of workers [3, 8].

It can be seen that the sequence deduced here for S. obliquus 5S rRNA resembles more closely the Chlorella sequence deduced by Luehrsen and Fox [3]. These workers also report that the sequence of 5S rRNA from another strain of Scenedesmus (S. quadricauda) is identical to the Chlorella sequence except that it has a C at position 93. S. obliquus 5S rRNA also has C93 but differs from S. quadricauda and Chlorella in having U residues in place of C residues at positions 70 and 73. These algal 5S rRNAs have A rather than G as their 5'-terminal residue, as do both species of Chlamydomonas reinhardtii 5S rRNA [9]. Although this feature is common to these algal 5S rRNAs, neither species of Chlamydomonas cytoplasmic 5S rRNA has close sequence homology to S. obliquus and Chlorella 5S rRNA. Indeed, the homology between S. obliquus and Chlamydomonas (68%) is less than between S. obliquus and higher plant 5S rRNAs (e.g.

Primary sequence of *S. obliquus* 5S rRNA.

- (i) —————, Sequences deduced using the 'Limited hydrolysis' method [13]
 (ii) - - - - -, Sequences deduced using 'Direct read-off' gels [14]
 (iii) , Sequences deduced using 'Mobility shift' analysis [15]
 (a) Differences in the *Chlorella* 5S rRNA sequence deduced by Leuhrsen and Fox [3]
 (b) Differences in the *Chlorella* 5S rRNA sequence deduced by Jordan et al. [8].

spinach (73%) [6] and wheat embryo (74%) [17].

The replacement of C73 in *Chlorella* by U in *S. obliquus* 5S rRNA gives rise to the palindromic sequence U70GAUUAGU. The position of this palindrome is, however, in the proposed prokaryotic palindrome location (70-80) rather than the plant palindrome location (80-85) [6].

The 5S rRNA sequence deduced for *S. obliquus* can be arranged in the five helix structure proposed by Luehrsen and Fox [3], and Garrett et al [4] as a generalised secondary structure for eukaryotic 5S rRNA. The changes from the *Chlorella* to the *S. obliquus* sequence can be accommodated in this secondary structure. The C73 U73 and U93 C93 changes occur in single stranded regions. The C70 U70 change occurs in a helical region and requires a G107-U70 base pair in *S. obliquus*, rather than a G-C base pair in *Chlorella*.

S. obliquus 5S rRNA therefore has typical eukaryotic characteristics. In addition, it contains all the invariant regions of primary sequence which have been proposed to be present in plant cytoplasmic 5S rRNA [6].

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