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The nucleotide sequence of the cytoplasmic 5S rRNA from the horsetail, *Equisetum arvense*

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ABSTRACT

Using 3'- and 5'-end labelling sequencing techniques, the following sequence for the cytoplasmic 5S rRNA of the horsetail Equisetum arvense could be determined: pGUGGUGCGGUCAUACCAGCGCUAAUGCACCGGAUCCCAUCAGAACUCCGCAGUUA AGCGCGCUUGGGCCAGAACAGUACUGGGAUGGGUGACCUCCCGGGAAGUCCUGGUGCCGCACCCC$_{OH}$. This sequence exhibits all features expected for higher plant cytoplasmic 5S rRNAs, and can be fitted to the secondary structure model for 5S rRNA proposed by De Wachter et al. (15).

INTRODUCTION

Cytoplasmic ribosomes from eukaryotes contain two low molecular weight RNAs, 5S and 5.8S rRNA; the 5.8S rRNA is hydrogen bonded to the largest ribosomal RNA component (1-3). Chloroplast ribosomes of flowering plants have also been shown to contain two low molecular weight RNAs (4-6), an analogous 5S rRNA and a 4.5S rRNA molecule. The 4.5S rRNA appears not to be hydrogen bonded to the larger ribosomal RNA, and is not analogous to the cytoplasmic 5.8S rRNA. Sequence comparison suggests that this molecule is represented by the 3'-end region of the eubacterial 23S rRNA in those organisms (7).

The Equisetinae appeared and developed very strongly in the Palaeozoic, but only one family, the Equisetacae which appeared in the Carbon aera, is still represented today by the single genus Equisetum, in which there are 32 species (8). The collection of 5S rRNA sequences from plants is increasing steadily, and we can now add one further sequence, that of the ancient horsetail Equisetum arvense.

MATERIALS AND METHODS

Wild grown Equisetum arvense was harvested in the summer in Berlin-Frohnau, frozen with liquid nitrogen and stored at -20°C. Total RNA from Equisetum arvense was extracted from the whole plant tissue and purified by Sephadex gelfiltration. The cytoplasmic 5S rRNA containing fractions were rechromato-

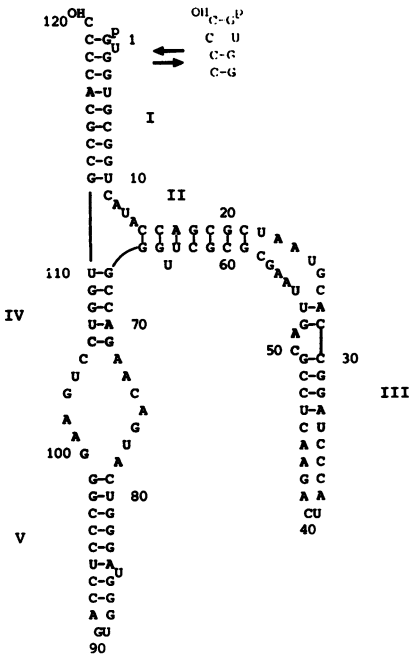

Figure 1: The sequence of Equisetum arvense 5S rRNA arranged in the secondary structure model of De Wachter (15).

graphed and then labelled at the 3'- and 5'-ends as reported (9,10). Labelled 5S rRNA was purified by electrophoresis and then sequenced using the chemical degradation method of Peattie (11) and the enzymatic digestion method of Donis-Keller (12). The terminal nucleotides were determined by thin-layer chromatography of total T2 and P1 digests (13).

RESULTS

RNase T2 digestion of 3'-end labelled material gave cytidine as the 3'-terminal nucleotide. The chemical and enzymatic sequencing methods enabled us to analyse 70 nucleotides starting from the 3'-end. P1 digests of 5'-end labelled 5S rRNA yielded only guanosine spots on TLC thus identifying this as the 5'end nucleotide. Using the enzymatic sequencing method, 70 nucleotides starting from the 5'-end could be identified, thus overlapping the sequence data from the 3'-end labelled material by 20 bases.

DISCUSSION

Equisetum arvense is a survivor of the ancient horsetails. It is a primitive plant in which the alternating haploid and diploid generations are independent: it requires a water environment for sexual reproduction. The horse-

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E.a.  GUGGUGCGGUCAUACCAGCGCUAAUGCACCGGAUCCCAUCAGAACUCCGCAGUUAAGCGCGCUUGGGC
S.o.  G-GGUGCGAUCAUACCAGCACUAAUGCACCGGAUCCCAUCAGAACUCCGCAGUUAAGCGUGCUUGGGC
U.p.  GUGAUACGGUCAUACCACCAGGAAAACAGGCGAUCCCAUCAGAACUCGCAACUUAAGCCUGGUUGGGC
P.t.  G-GAUGCGGUCAUACCAAGGCUACUACACCAGAUCCCAUCAGAACUCUGAAGUUAAGCGCCUUUGGGC
                10        20        30        40        50        60

E.a.  CAGAACAGUACUGGGAUGGGUGACCUCCCGGGAAGUCCUGGUGCCGCACCCC-
S.o.  GAGAGUAGUACUAGGAUGGGUGACCUCCUGGGAAGUCCUCGUGUUGCACCCCU
U.p.  AGGAUUAGUACUGGGCUGAGUGAUCUCCUGGGAAUCCCCUGUGCUGUAUCGC-
P.t.  CGGAAUAGUACUGGGAUGGGUGACCUCCCGGGAAGUCCCGGUGCUGCAUCCA-
      70        80        90        100       110       120
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Figure 2: The nucleotide sequence of Equisetum arvense 5S rRNA (E.a.), in comparison to that of Spinacea oleracea (18), Ulva pertusa (19) and Plagiomnium trichomanes (14).

tails are classified with the ferns in the Pteridophyta, a class from which, via intermediates, the higher plants eventually evolved. Most available plant 5S rRNA sequences are from the higher plants although the sequences from several bryophyte 5S rRNAs have been recently publised (14). Most algal 5S rRNA sequences are from simple single celled forms.

The sequence we have determined consists of 120 nucleotides and can be fitted into the secondary structure for 5S rRNA proposed by De Wachter et al. (15) as shown in Fig. 1. The sequence has most of those characteristics expected for a higher plant - the group specific signatures described by Delihas and Andersen (16). Typical for higher plants is the uridine at position 22 which enforces a shorter Helix II than found in all other phylogenetic groups: 7 base-pairs in plants, 8 in all other groups including the algae. The looped out uridine in position 64 is present in both higher plant and green algal 5S rRNAs.

The length of Helix IV is unusual in being of five base pairs, four is typical for higher plants and algae. The looped-out uridine at position 85 is typical for plants and algae. The structure of Helix I is interesting since it again involves a looped-out uridine (at position 2) or a mismatched pair. The overall stability of this helix, according to the empirical model of Ninio (17), is $\Delta G = -20.0$kcal. Diversity of structure in Helix I appears to be prevalent in the eukaryotes, Delihas and Andersen (16) found no group specific signatures in this helix. Higher plant sequences appear to be free of bulge-loops in this helix whilst bulge-loops and even internal loops of two nucleotides may be found in the sequences of 5S rRNA from less sophisticated orga-

nisms, e.g. Chlamydemonas reinhardii, Paramecium tretraurelia and Philosamia cynthia ricini.

Figure 2 shows the alignment of the Equisetum 5S rRNA sequence with that of the cultivated plant Spinacea oleracea, the thalloid green alga Ulva pertusa and the moss Plagiomnium trichomanes. The %-homology between these sequences is:

	E.a.	S.o.	U.p.	P.t.
E.a.	-	89	72	86
S.o.		-	74	80
U.p.			-	74
P.t.				-

Clearly, the Equisetum sequence shows very strong homology to the sequence from spinach and also to the moss Plagiomnium trichomanes. The homology with the relatively well differentiated alga Ulva pertusa is, on the other hand lower, on the same level as that between the alga and spinach. This is, of course, in line with the classical grouping of these organisms. A low homology is observed between the alga and the moss 5S rRNA sequences, suggesting an early divergence of these two groups with the moss liniage more closely related to the horsetail and higher plant lines.

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