The nucleotide sequence of the chloroplast 5S ribosomal RNA from spinach

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ABSTRACT

Spinacia oleracia chloroplast 5S ribosomal RNA was end-labeled with [^{32}P] and the complete nucleotide sequence was determined. The sequence is: pUAUU CUGGUGUCCUAGGCGUAGAGGAACCACACCA AUCCAUCCCGAACUUGGUGGUUAAACUCUACUGCGGUGACGAUACU GUAGGGGAGGUCCUGCGGAAAAAUAGCUCGACGCCAGGAUG$_{OH}$.

This sequence can be fitted to the secondary structural model proposed for prokaryotic 5S ribosomal RNAs by Fox and Woese (1). However, the lengths of several single- and double-stranded regions differ from those common to prokaryotes. The spinach chloroplast 5S ribosomal RNA is homologous to the 5S ribosomal RNA of Lemna chloroplasts with the exception that the spinach RNA is longer by one nucleotide at the 3' end and has a purine base substitution at position 119. The sequence of spinach chloroplast 5S RNA is identical to the chloroplast 5S ribosomal RNA gene of tobacco. Thus the structures of the chloroplast 5S ribosomal RNAs from some of the higher plants appear to be almost totally conserved. This does not appear to be the case for the higher plant cytoplasmic 5S ribosomal RNAs.

INTRODUCTION

This paper reports the nucleotide sequence of the chloroplast 5S ribo-

somal RNA from Spinacia oleracia. The chloroplast 5S RNA can be fitted to the

structural model for prokaryotic 5S RNAs of Fox and Woese (1) but the chloro-

plast RNA differs with respect to the lengths of the single- and double-

stranded regions. The nucleotide sequence of the chloroplast 5S RNA of spin-

ach is identical to that of the 5S ribosomal RNA gene from tobacco chloro-

plasts (2) and is nearly identical to the chloroplast 5S RNA of duckweed (3).

In contrast to the remarkable homology between some of the higher plant chloro-

plast 5S RNAs, the 5S RNAs of the higher plant cytoplasmic ribosomes appear

to be less homologous (4-6).

EXPERIMENTAL PROCEDURE

Isolation and Nucleotide Sequence Analysis of Chloroplast 5S Ribosomal

RNA. Chloroplasts were purified from freshly harvested spinach, total RNA

was extracted from the chloroplasts and purified by DEAE cellulose column
chromatography as described (7). Spinach chloroplast 5S RNA was obtained by
a three step chromatographic procedure involving BD-cellulose column chroma-
tography at pH 7.5, followed by RPC-5 column chromatography at pH 4.5 and
RPC-5 column chromatography at pH 7.5. These procedures produced highly
purified chloroplast 5S RNA which ran as a single band on a 20% polyacryl-
amide gel in 7 M urea and was free of tRNAs and cytoplasmic 5S RNA. The
chloroplast origin of this RNA was verified by hybridization to spinach
chloroplast DNA, performed as described (7).

The nucleotide sequence of the spinach chloroplast 5S ribosomal RNA was
determined by three methods: RNA sequencing gels using 5' and 3' [^{32}P] la-
beled 5S RNA (8), partial formamide hydrolysis (9) and mobility shift analy-
sis (10-12).

RESULTS

The three methods of nucleotide sequence analyses employed provided over-
lapping and confirming data throughout the molecule and allowed us to unam-
biguously determine the complete nucleotide sequence of the spinach chloro-
plast 5S ribosomal RNA. A secondary structural model constructed according
to that proposed for prokaryotic 5S RNAs by Fox and Woese (1) is shown in
Figure 1 along with a model of the 5S RNA of the cyanobacterium Anacystis
nidulans (13). Large stretches of homology in single- and double-stranded
regions between these two molecules are indicated in Figure 1. The structure
of the spinach cytoplasmic 5S ribosomal RNA (5) is also shown in Figure 1.
This RNA has features typical of eukaryotic 5S ribosomal RNAs (14) and has
few homologies in primary or secondary structure to that of the chloroplast
5S RNA.

DISCUSSION

The spinach chloroplast 5S ribosomal RNA can be folded to fit the second-
ary structural model proposed for prokaryotes by Fox and Woese (1) (Fig. 1)
but there are deviations from the prokaryotic structures in the lengths of
the single- and double-stranded regions. For example, in the common arm
single-stranded region, the prokaryotic RNAs have a chain length of 13 nucleo-
tides whereas the chloroplast RNAs have 12. The prokaryotic common arm stem
is almost universally 4 base pairs in length in contrast to the 6 base pairs
that can be formed in the chloroplast 5S RNAs. Also, the stem of the chloro-
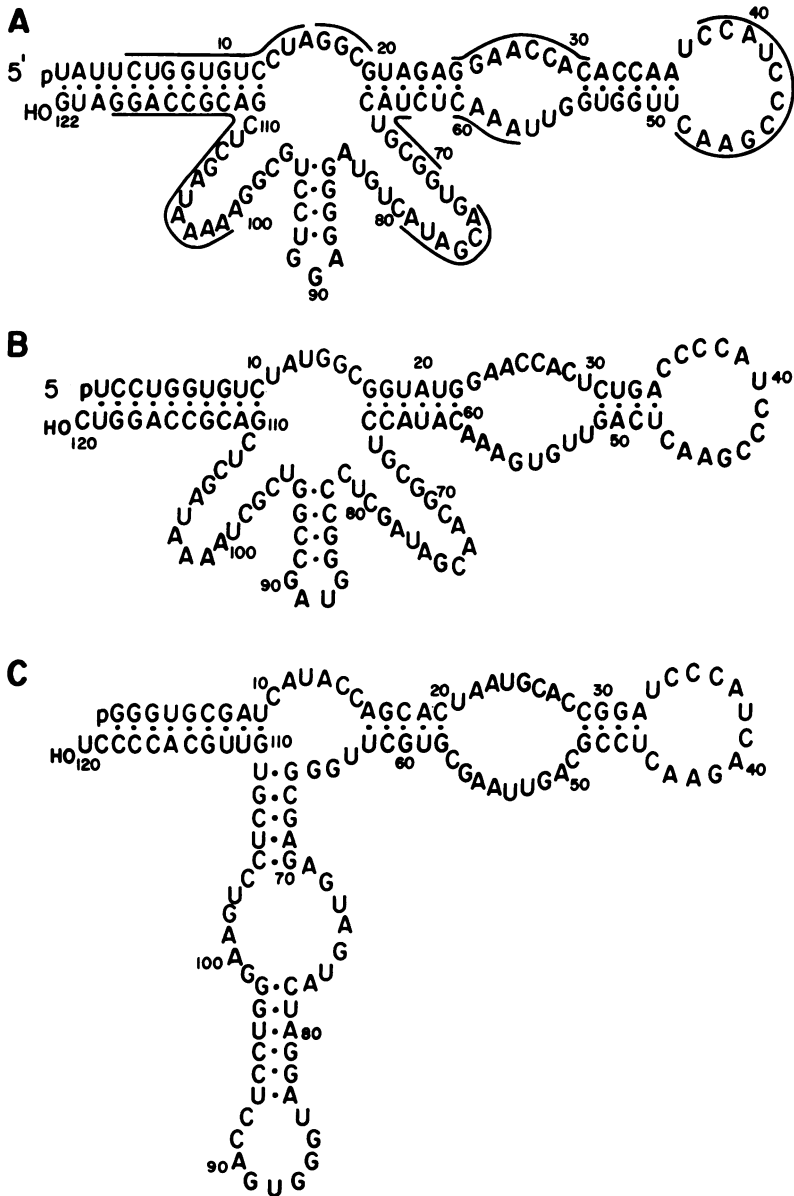plast 5S ribosomal RNA from spinach is 12 base pairs in length whereas the

Figure 1. Secondary structural models of 5S ribosomal RNAs.

A. Spinach chloroplast 5S ribosomal RNA. B. 5S ribosomal RNA from Anacystis nidulans. C. Spinach cytoplasmic 5S ribosomal RNA. The lines drawn around the sequence in A. denote large stretches of homology between spinach chloroplast and Anacystis nidulans 5S RNAs.

stem length is 9-10 base pairs in prokaryotes. Nevertheless, close homolo-
gies in nucleotide sequences can be seen when comparing the primary structures
of the 5S RNAs from Anacystis nidulans and chloroplasts (13). A comparison
of the structures of the 5S RNA from spinach chloroplasts and the 5S RNA from
Anacystis nidulans shows large stretches of nucleotide sequence in both
single- and double-stranded regions that are homologous (Fig. 1). The uni-
versal prokaryotic sequence of the common arm CCGAAC is also present in chloro-
plast 5S RNAs. In contrast to the spinach chloroplast 5S ribosomal RNA, the
cytoplasmic 5S ribosomal RNA from spinach has the primary and secondary
structural features typical of eukaryotic 5S RNAs (5).

Most prokaryotic 5S ribosomal RNAs have hexanucleotide palindromic
sequences in the 3' half of the molecule. In E. coli and other Gram-negative
bacteria (species of Bacillus, Clostridium pasteurianum and Mycobacterium
smegmatis) the hexanucleotide palindromic sequence AUGGUA is present between
positions 70-80 (15). In other organisms, the palindromic sequences are as
follows: Anacystis nidulans, G_{70}CAACG, Thermus aquaticus, G_{91}UCCUG and in
Halobacterium cutirubrum, C_{111}GCCGC. The spinach and duckweed chloroplast 5S
ribosomal RNAs and the tobacco chloroplast 5S RNA gene also have a palindromic
sequence: U_{79}AGGAU. The significance of these palindromic sequences is not
known, but their common occurrence in prokaryotic and chloroplast 5S RNAs may
denote an important functional capacity.

The nucleotide sequence of the spinach chloroplast 5S ribosomal RNA is
identical to that of the 5S ribosomal RNA gene from tobacco chloroplasts (2).
The spinach chloroplast 5S RNA is homologous to the 5S RNA from duckweed
chloroplasts except that the spinach RNA has a guanine residue at position
119 where the duckweed RNA has adenine. Also, the spinach 5S RNA is 122
nucleotides in length whereas the duckweed is 121. No divergences in single-
stranded regions of the chloroplast 5S ribosomal RNAs sequenced to date have
been found.

The high degree of homology between some of the higher plant chloroplast
5S ribosomal RNAs contrasts with a lower degree of homology between the cyto-
plasmic 5S ribosomal RNAs. Wheat embryo and spinach cytoplasmic 5S RNAs vary
by six base changes (4,5). More base substitutions appear to be evident in
duckweed, rye and other higher plant cytoplasmic 5S RNAs (3,6), however, these
sequences need to be reevaluated (4,5). It is possible that there are more
constraints on changes of the 5S ribosomal RNA genes of chloroplasts relative
to that of the nuclear genes.

Although the higher plant chloroplast 5S ribosomal RNAs appear to be

identical or nearly identical in structure, this may not be the case with
the lower eukaryotic chloroplast 5S RNAs. Little homology was found between
the Euglena chloroplast 5S ribosomal RNA gene and spinach chloroplast 5S
ribosomal RNA in DNA hybridization tests (16). The nucleotide sequences of
the lower eukaryotic 5S ribosomal RNAs, both cytoplasmic and chloroplast RNAs,
should prove to be interesting when compared with those of the higher plants.

REFERENCES
 1. Fox, G.E. and Woese, C.R. (1975) Nature 256, 505-507.
 2. Takaiwa, F. and Sugiura, M. (1980) Molec. gen. Genet. 180, 1-4.
 3. Dyer, T.A. and Bowman, C.M. (1979) Biochem. J. 183, 595-604.
 4. Mackay, R.M., Spencer, D.F., Doolittle, W.F. and Gray, M.W. (1980)
     Eur. J. Biochem. 112, 561-576.
 5. Delihas, N., Andersen, J., Sprouse, H.M., Kashdan, M. and Dudock, B.
     in press, J. Biol. Chem.
 6. Payne, P.I. and Dyer, T.A. (1976) Eur. J. Biochem. 71, 33-38.
 7. Kashdan, M.A., Pirtle, R.M., Pirtle, I.L., Calagan, J.L., Vreman, H.J.
     and Dudock, B.S. (1980) J. Biol. Chem. 255, 8831-8835.
 8. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Nucleic Acids Res.
     4, 2527-2538.
 9. Stanley, J. and Vassilenko, S. (1978) Nature 274, 87-89.
10. Pirtle, R., Kashdan, M., Pirtle, I. and Dudock, B. (1980) Nucleic Acids
     Res. 8, 805-815.
11. Sanger, F., Donelson, J.E., Coulson, A.R., Kossel, H. and Fischer, D.
     (1973) Proc. Natl. Acad. Sci. USA 70. 1209-1213.
12. Silberklang, M., Prochiantz, A., Haenni, A.-L. and RajBhandary, U.L.
     (1977) Eur. J. Biochem. 72, 465-478.
13. Corry, M.J., Payne, P.I. and Dyer, T.A. (1974) FEBS Lett. 46, 63-66.
14. Luehrsen, K.R. and Fox, G.E. (1981) in press, Proc. Natl. Acad. Sci. USA.
15. Erdmann, V.A. (1981) Nucleic Acids Res. 9, r25-r42.
16. Bohnert, H.J. and Gordon, K.H.J. (1980) Molec. gen. Genet. 179, 539-545.