Volume 11 Number 23 1983 Nucleic Acids Research

The nucleotide sequences of tRNA^{Ser} (GCU) and tRNA^{Gln} (UUG) genes from tobacco chloroplasts

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Received 30 September 1983; Accepted 1 November 1983

ABSTRACT

The nucleotide sequence of tobacco chloroplast genes for tRNA^{Ser}(GCU) and tRNA^{Gln}(UUG) have been determined. These tRNA genes are encoded on the same DNA strand and separated by 1144 bp. Two open reading frames of 52 codons and 98 codons have been found in this spacer region. The tRNA^{Ser}(GCU) and tRNA^{Gln}(UUG) deduced from the DNA sequences show 67% and 76% sequence homologies with E. coli tRNA^{Ser}(GCU) and tRNA^{Gln}(UUG), respectively.

INTRODUCTION

Chloroplast DNA codes for a set of tRNAs, probably all of the species necessary for the protein synthesis of 70S ribosomes in chloroplasts (1). Chloroplast tRNAs show higher sequence homologies with bacterial tRNAs than eukaryotic cytoplasmic tRNAs (2) and sequences similar to E. coli "Pribnow box" and "-35 regions" are found in the regions upstream from chloroplast tRNA genes (3-10). On the other hand, chloroplast tRNA genes have some eukaryotic features: the 3' terminal CCA sequences of chloroplast tRNAs are not encoded by chloroplast DNA (3-17) and introns are found in some chloroplast tRNA genes (7,10-13).

To analyse the organization and fine structure of tRNA genes of tobacco chloroplasts, we have determined nucleotide sequences of tobacco chloroplast DNA fragments which were hybridized with its chloroplast tRNAs (3,4,7,10,12). In this report, we show the nucleotide sequences of tRNA^{Ser}(GCU) and tRNA^{Gln}(UUG) genes found in the 3.5 kbp BamHI fragment (Ba10) from tobacco chloroplast DNA. The spacer region between these two tRNA genes contained two open reading frames.

MATERIALS AND METHODS

Recombinant plasmid pTB24 containing a 12.4 kbp partial BamHI fragment of Nicotiana tabacum (var. Bright Yellow 4) chloroplast DNA had been constructed (18). The plasmid DNA was digested with BamHI and a 3.5 kbp BamHI fragment was separated from 0.17 and 8.7 kbp BamHI fragments and vector pBR322 by electrophoresis in a 1% agarose gel. DNA sequence was determined by the method of Maxam and Gilbert (19).

RESULTS AND DISCUSSION

We had cloned a 12.4 kbp partial BamHI fragment of tobacco chloroplast DNA which codes for several tRNAs and the α subunit

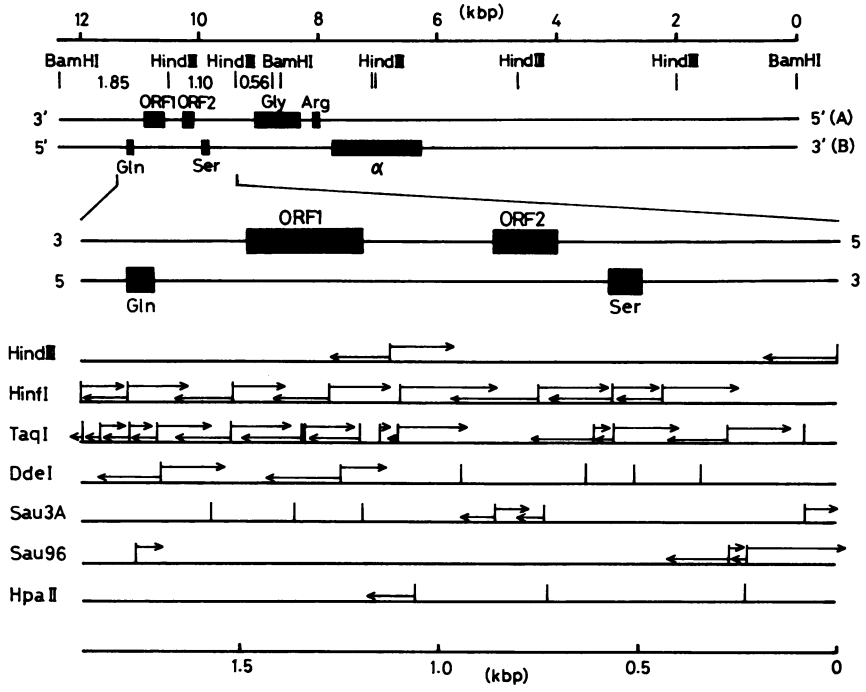
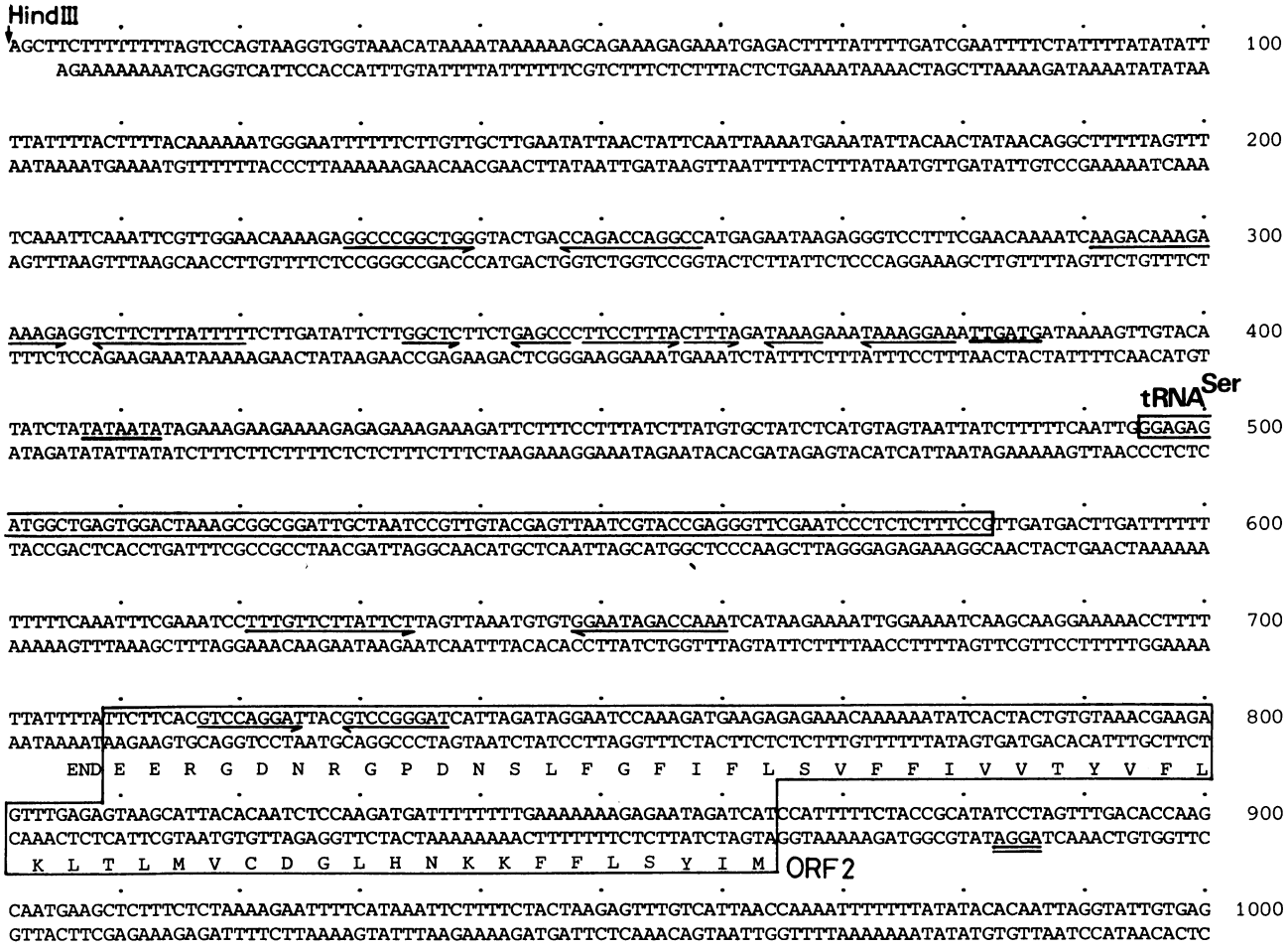
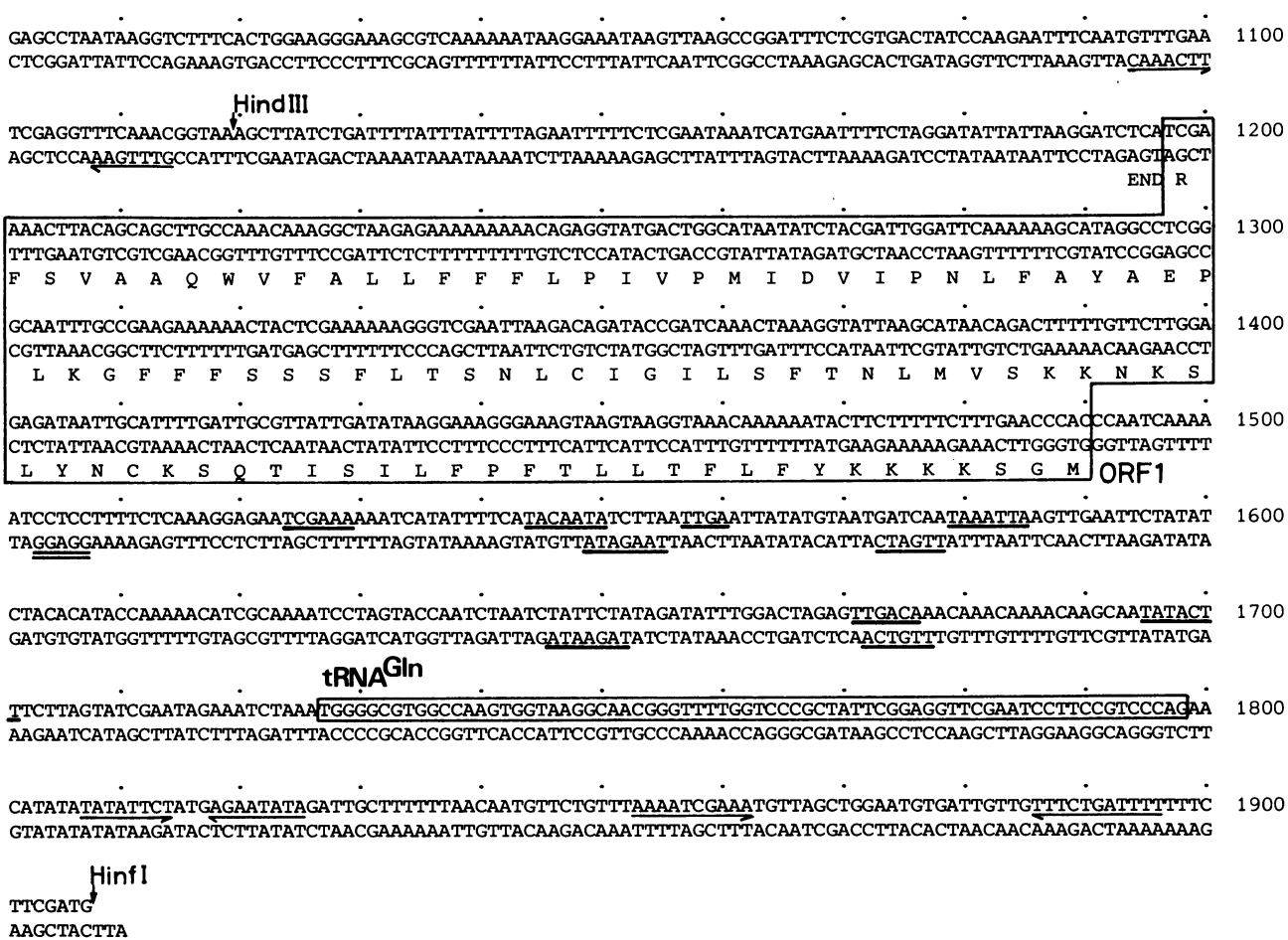


Fig. 1. Physical map of the cloned 12.4 kbp partial BamHI fragment from tobacco chloroplast DNA and the strategy for sequencing part of it. Strand B codes for the $tRNA^{Gln}$, $tRNA^{Ser}$ and α genes. Coding regions are shown by thick lines. The lower part shows the sequencing strategy. Horizontal arrows indicate the direction and extent of DNA segments sequenced. Parentheses show kbp of HindIII sub-fragments derived from the 3.5 kbp BamHI fragment.


HindIII
tRNA Ser
ORF2
END E E R G D N R G P D N S L F G F I F L S V F F I V V T Y V F L
K L T L M V C D G L H N K K F F L S Y I M


HindIII
END R
ORF1
tRNAGln
HinfI

of proton-translocating ATPase (20). The 12.4 kbp fragment contains 3.5 kbp, 0.17 kbp and 8.7 kbp BamHI fragments in this order. On digestion with HindIII, the 3.5 kbp BamHI fragment isolated from recombinant plasmid pTB24 yields 1.85, 1.10 and 0.56 kbp sub-fragments (Fig. 1). Total tobacco chloroplast tRNA hybridized to the 1.85 kbp BamHI-HindIII and 1.10 kbp HindIII-HindIII sub-fragments (data not shown). We then sequenced the 1.10 kbp sub-fragment and a portion of the 1.85 kbp sub-fragment by the sequence strategy shown in Fig. 1. Fig. 2 shows the nucleotide sequence of a 1907 bp portion (HindIII-HinfI) of the 3.5 kbp BamHI fragment.

A tRNA gene was found between positions 495 to 582 in the 1.10 kbp sub-fragment (Fig. 2). Judging by the anticodon sequence (GCT), it is a $tRNA^{Ser}$(GCU) gene (Fig. 3a). The tobacco chloroplast $tRNA^{Ser}$(GCU) deduced from the DNA sequence shows 67%, 70% and 77% sequence homologies with E. coli and Euglena gracilis $tRNA^{Ser}$(GCU)s and maize chloroplast $tRNA^{Ser}$(GGA), respectively (2,8,9). Another tRNA gene was found between positions 1727 to 1798 in the 1.85 kbp sub-fragment (Fig. 2). Judging by the anticodon sequence (TTG), it is a $tRNA^{Gln}$(UUG) gene (Fig. 3b). The tobacco chloroplast $tRNA^{Gln}$(UUG) deduced from the DNA sequence shows 76% and 79% sequence homologies with E. coli $tRNA^{Gln}$(UUG) and $tRNA^{Gln}$(CUG) (2). The 3'-CCA sequences are not coded for by the chloroplast DNA and no intron was found in these two tRNA genes. The $tRNA^{Ser}$ and $tRNA^{Gln}$ genes are separated by a 1144 bp spacer (Fig. 2). Based on the physical map of tobacco chloroplast DNA (21) and sequence analysis of the tRNA and α genes, the two tRNA genes were found to be oriented in the same direction as the α gene (strand B, ref. 20) and to be located between the α gene and the P32 gene (22) in the large single-copy region on the chloroplast DNA map.

A "Pribnow box"-like sequence TATAATA (407 to 413) and a

Fig. 2. Nucleotide sequence of a 1907 bp portion of the 3.5 kbp BamHI fragment encoding the $tRNA^{Ser}$(GCU), $tRNA^{Gln}$(UUG) and ORFs. Coding regions are boxed. The deduced amino acid sequences are indicated below the nucleotide sequence. "Pribnow box"-like sequences and "-35 region"-like sequences are underlined. Double-underlines show putative ribosome binding sites. Horizontal arrows indicate inverted repeat sequences.

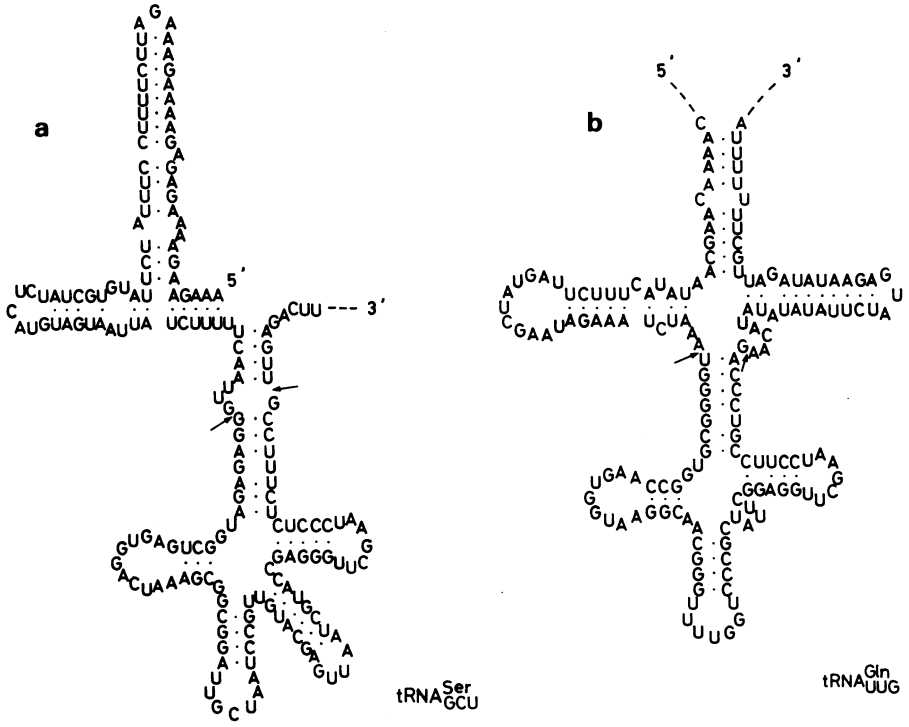


Fig. 3. Possible secondary structures of putative transcripts containing the $tRNA^{Ser}$(GCU) sequence (a) and the $tRNA^{Gln}$(UUG) sequence (b). Arrows indicate possible processing sites.

"-35 region"-like sequence TTGATG (381 to 386) were found in the region upstream from the $tRNA^{Ser}$ gene (Fig. 2). Likewise, three "Pribnow box"-like sequences TACAATA (1544 to 1550), TAAATTA (1579 to 1585) and TATACTT (1695 to 1701) and three "-35 region"-like sequences TCGAAA (1524 to 1529), TTGA (1557 to 1560) and TTGACA (1671 to 1676) were found in the region upstream from the $tRNA^{Gln}$ gene. Two inverted repeat sequences (621 to 660 and 717 to 737) were found in the region downstream from the $tRNA^{Ser}$ gene, and two inverted repeat sequences (1807 to 1825 and 1853 to 1896) followed by T-rich clusters were found in the region downstream from the $tRNA^{Gln}$ gene. These sequences may be transcriptional initiation and termination signals for the $tRNA^{Ser}$ and $tRNA^{Gln}$ genes. These observations suggest that the $tRNA^{Ser}$ and $tRNA^{Gln}$ genes are transcribed independently. Possible secondary

structures of putative transcripts containing the $tRNA^{Ser}$ and the $tRNA^{Gln}$ sequences can be constructed as shown in Fig. 3.

Two open reading frames (ORFs) were found in the spacer between the $tRNA^{Ser}$ and $tRNA^{Gln}$ genes and to be oriented in the opposite direction (strand A) with the tRNA genes (Fig. 2). The ORF1 of 98 codons (1490 to 1197) was found 236 bp apart from the $tRNA^{Gln}$ gene. A putative polypeptide derived from the ORF1 is expected to be hydrophobic and its molecular weight is calculated to be 11,229 daltons. A sequence GGAGG (1507 to 1503) complementary to the 3' end of tobacco chloroplast 16S rRNA (23) was found 17 to 13 bp upstream from the initiation codon (GTG) and should be a putative ribosome binding site. Two "Pribnow box"-like sequences TAGAATA (1652 to 1646) and TAAGATA (1555 to 1549) and two "-35 region"-like sequences TTGTCA (1677 to 1672) and TTGATC (1578 to 1573) were found in the region upstream from the ORF1. An inverted repeat sequence (1114 to 1094) was found in the region downstream from the termination codon (TGA). Several "Pribnow box"-like sequences and "-35 region"-like sequences are present in the 236 bp region between the ORF1 and the $tRNA^{Gln}$ gene (Fig. 2). Therefore, there is a possibility that transcription of the ORF1, if any, and of the $tRNA^{Gln}$ gene overlaps head to head.

The ORF2 of 52 codons (864 to 709) was found 332 bp apart from the ORF1. A sequence AGGA (886 to 883) complementary to the 3' end of tobacco chloroplast 16S rRNA (23) was found 22 to 19 bp upstream from the initiation codon (ATG). However, no "Pribnow box"-like and "-35 region"-like sequences were found in the region upstream from the ORF2. Further studies are necessary to determine whether these ORFs are expressed or not.

ACKNOWLEDGEMENTS

We thank Dr. K. Shinozaki for valuable discussion. This work was supported in part by Grants-In-Aid from the Ministry of Education, Science and Culture of Japan and from the Torey Science Foundation.

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REFERENCES

1. Driesel, A.J., Crouse, E.J., Gordon, K., Bornert, H.J., Herrmann, R.G., Steinmetz,A., Mubumbila, M., Keller, M., Burkard, G. and Weil, J.H. (1979) Gene 6, 285-306
2. Sprinzl, M. and Gauss, D.H. (1983) Nucleic Acids Res. 11, r1-r53
3. Tohdoh, N., Shinozaki, K. and Sugiura, M. (1981) Nucleic Acids Res. 9, 5399-5406
4. Kato, A., Shimada, H., Kusuda, M. and Sugiura, M. (1981) Nucleic Acids Res. 9, 5601-5607
5. Schwarz, Z., Kössel, H., Schwarz, E. and Bogorad, L. (1981) Proc. Natl. Acad. Sci. USA 78, 4748-4752
6. Orozco, Jr., E.M. and Hallick, R.B. (1982) J. Biol. Chem. 257, 3265-3275
7. Deno, H., Kato, A., Shinozaki, K. and Sugiura, M. (1982) Nucleic Acids Res. 10, 7511-7520
8. Steinmetz, A.A., Krebbers, E.T., Schwarz, Z., Gubbins, E.J. and Bogorad, L. (1983) J. Biol. Chem. 258, 5503-5511
9. Karabin, G.D. and Hallick, R.B. (1983) J. Biol. Chem. 258, 5512-5518
10. Deno, H. and Sugiura, M. (1983) submitted.
11. Koch, W., Edwards, K. and Kössel, H. (1981) Cell 25, 203-213
12. Takaiwa, F. and Sugiura, M. (1982) Nucleic Acids Res. 10, 2665-2676
13. Steinmetz, A., Gubbins, E.J. and Bogorad, L. (1982) Nucleic Acids Res. 10, 3027-3037
14. Graf, L., Kössel, H. and Stutz, E. (1980) Nature 286, 908-910
15. Orozco, Jr., E.M., Rushlow, K.E., Dodd, J.R. and Hallick, R. B. (1980) J. Biol. Chem. 255, 10997-11003
16. Schwarz, Z., Jolly, S.O., Steinmetz, A.A. and Bogorad, L. (1981) Proc. Natl. Acad. Sci. USA 78, 3423-3427
17. Kashdan, M.A. and Dudock, B.S. (1982) J. Biol. Chem. 257, 1114-1116
18. Sugiura, M. and Kusuda, J. (1979) Mol. Gen. Genet. 172, 137-141
19. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564
20. Deno, H., Shinozaki, K. and Sugiura, M. (1983) Nucleic Acids Res. 11, 2185-2191
21. Seyer, P., Kowallik, K.V. and Herrmann, R.G. (1981) Current Genetics 3, 189-204
22. Sugita, M. and Sugiura, M. manuscript in preparation.
23. Tohdoh, N. and Sugiura, M. (1982) Gene 17, 213-218