Plant Molecular Biology 7: 63–70, 1986

Characterization of the spinach chloroplast genes for the S4 ribosomal protein, $tRNA^{Thr}$ (UGU) and $tRNA^{Ser}$ (GGA)

S. Ben Tahar*, W. Bottomley & P. R. Whitfeld
Division of Plant Industry, CSIRO, GPO Box 1600, Canberra, A.C.T. 2601, Australia
**Permanent address: Laboratoire de Biologie Moleculaire et Cellulaire, Limagrain BP51, 63360 Gerzat, France*

Keywords: chloroplast DNA, ribosomal protein gene, transfer RNA genes, *Spinacia oleracea,* nucleotide sequence

Summary

The map location and nucleotide sequence of the genes for the S4 ribosomal protein (*rps*4) and for $tRNA^{Thr}$ (UGU) (*trn*T) and $tRNA^{Ser}$ (GGA) (*trn*S) on spinach chloroplast DNA have been determined. *rps*4 lies approximately 5 kb 3′ to *atp*BE in the large single copy region and is transcribed in the same direction as *atp*BE. It has a 178 bp leader sequence, a 603 bp coding region and 620 bp 3′ tail. The sequence of the coding region is 83% homologous with that of maize *rps*4 (29) and the deduced amino acid sequences from the two species are 7% homologous. The spinach and *Escherichia coli* S4 proteins are only 36% homologous. As in the case of maize, *trn*T lies upstream from, and on the same strand as *rps*4 whereas *trn*S lies downstream and on the opposite strand. Transcription of *rps*4 apparently proceeds past *trn*S.

Introduction

Approximately one third of the 55 or so chloroplast ribosomal proteins are synthesised in isolated chloroplasts (4, 6, 9, 19, 23) and are presumed to be encoded in the organelle genome. Genes for the S19 protein of tobacco (30) and *Vicia faba* (26), for the S19 and L2 proteins of spinach and *Nicotiana debneyi* (36), for the S4 protein of maize (29), for the S14 protein of *Marchantia polymorpha* (31) and for the S7 and S12 proteins of *Euglena gracilis* chloroplasts (16) have been mapped precisely and their sequences determined. Other genes, e.g. for the L24 and L25 proteins of spinach (3) and for the L28 protein of *Spirodela oligorhiza* (18), have been approximately mapped on the chloroplast genome. Evidence so far indicates that the genes are dispersed around the 150 kb-DNA.

The assembled ribosome contains equimolar amounts of most of the constituent proteins and therefore it is likely that expression of the ribosomal protein genes is coordinated. In the absence of a suitable *in vivo* system for studying the regulation of chloroplast gene expression, the identification of putative regulatory sequences may be approached by comparing the 5′ flanking sequences of a number of these genes. The limited sequence data available has precluded the identification of such conserved regulatory sequences. We are therefore accumulating DNA sequence data on chloroplast ribosomal protein genes from a single species, *Spinacia oleracea.* In this paper we present the sequence of the *rps*4 gene and of the genes for $tRNA^{Ser}$ (GGA) and $tRNA^{Thr}$ (UGU) which, as in the case of maize (29), flank *rps*4.

Materials and methods

The recombinant plasmid pSocB149 (32), containing the 11 kb *Bam*HI fragment 3 of spinach chloroplast DNA, was restricted with *Bam*Hl and *Sal*I and the 2.9 kb *Bam*HI-*Sal*I fragment (Fig. 1A) subcloned into pUC8. Smaller fragments for se-

Address for offprints: Dr P. R. Whitfeld, CSIRO, Division of Plant Industry, GPO Box 1600, Canberra, A.C.T. 2601, Australia.

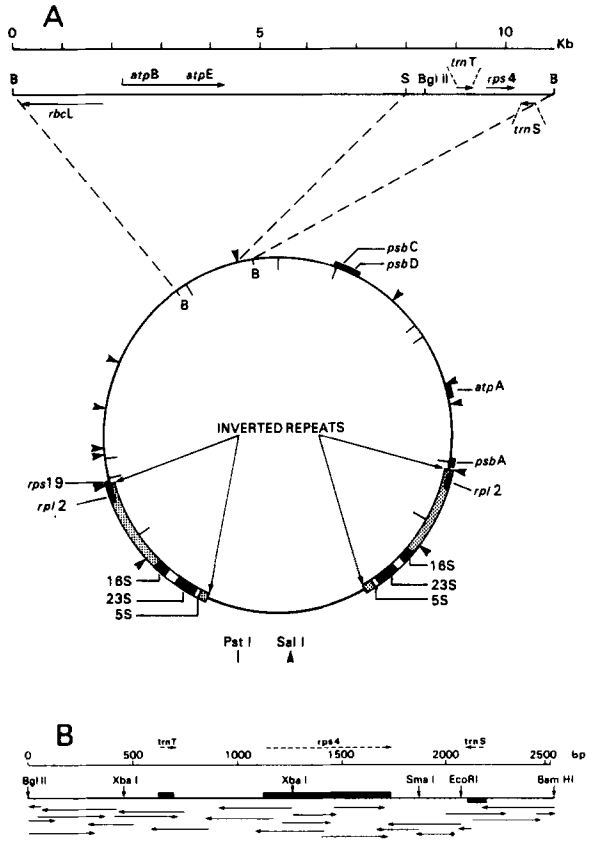

Fig. 1. Location of the genes for the S4 ribosomal protein (*rps*4), and for $tRNA^{Thr}$ (*trn*T) and $tRNA^{Ser}$ (*trn*S) on spinach chloroplast DNA. A. *Sal*I/*Pst*I restriction map of spinach chloroplast DNA (10) showing the position of *Bam*HI fragment 3. The arrows indicate the direction of transcription of the various genes. B. Sequencing strategy for the 2.5 kb *Bgl*II-*Bam*HI fragment from the right-hand end of *Bam*HI fragment 3. Black bars indicate the positions of *trn*T, *rps*4 and *trn*5. The arrow commencing with a filled circle denotes that the fragment was 5′ end labeled with [γ^{32}-P]ATP and sequenced by the Maxam and Gilbert method (15). All other fragments were sequenced as indicated by the arrows by the dideoxynucleotide chain termination method (21). All sequences were determined at least twice except for one region of 100 bp.

quencing were subcloned into M13mp18 and mp19. Plasmid DNA was purified from bacterial lysates by ethidium bromide-CsCl density gradient centrifugation (14). Total spinach leaf RNA was extracted from leaves 2 to 3 cm long and from very young leaves (< 0.5 cm in length) by the SDS-phenol method (34). RNA was separated from DNA by precipitation with 2 M lithium acetate.

Nucleotide sequences were determined by the dideoxynucleotide chain-termination method (21) and by the chemical cleavage method (15). Single-stranded DNA probes for S1 nuclease mapping experiments (1) were prepared as described below (12). Spinach leaf RNA (50 to 200 μg) was ethanol precipitated with 5×10^4 to 2×10^5 Cerenkov cpm of the single-stranded probe and 2 μg sonicated salmon sperm DNA, resuspended in 20 μl 50% deionised formamide, 0.5 M NaCl, 1 mM EDTA, 40 mM PIPES pH 6.4 buffer and incubated at 42° for 15 h. Ice-cold S1 buffer (200 μl of 30 mM sodium acetate, pH 4.5, 250 mM NaCl, 1 mM $ZnSO_4$, 5% glycerol) was added together with S1 nuclease (10 units per μg RNA) and incubated for 30 min at 37 °C. After phenol extraction the ethanol precipitated nucleic acid was resuspended in 4 μl 10 mM Tris-HCl, pH 8.0, 1 mM EDTA and 4 μl formamide containing 0.1% Bromophenol Blue and Xylene Cyanol FF. The samples were placed in a boiling water bath for 3 min and loaded onto a 6% polyacrylamide/8 M urea sequencing gel.

Results and discussion

The relative location of many of the genes on the spinach and maize chloroplast genomes is the same. Thus we anticipated that the gene for the spinach chloroplast ribosomal S4 protein (*rps*4) might lie in the centre of the large single-copy region, approximately 5 kb downstream from the end of *atp*BE (35), as it does in maize (29). This would place it on *Bam*HI fragment 3 at the end opposite to *rbc*L (Fig. 1A). The sequence of a 2.5 kb *Bgl*II-*Bam*HI fragment derived from *Bam*HI fragment 3 was determined as indicated in Fig. 1B. It contained a single, major open reading frame of 603 bp (nucleotides 1120 to 1722, Fig. 2), the orientation of which was the same as that of *atp*BE. Comparison of this nucleotide sequence with that of maize *rps*4 (29) showed the two be very similar (83% homology, Fig. 2) and we concluded that this open reading frame was the spinach equivalent of the maize gene. Most (50%) of the differences that occur between them are in the third position of the codons, the rest are distributed equally between the first and second positions.

Downstream from *rps*4 the homology between the spinach and maize sequences drops sharply (Fig. 2). Upstream for 50 to 60 bp there is signifi-

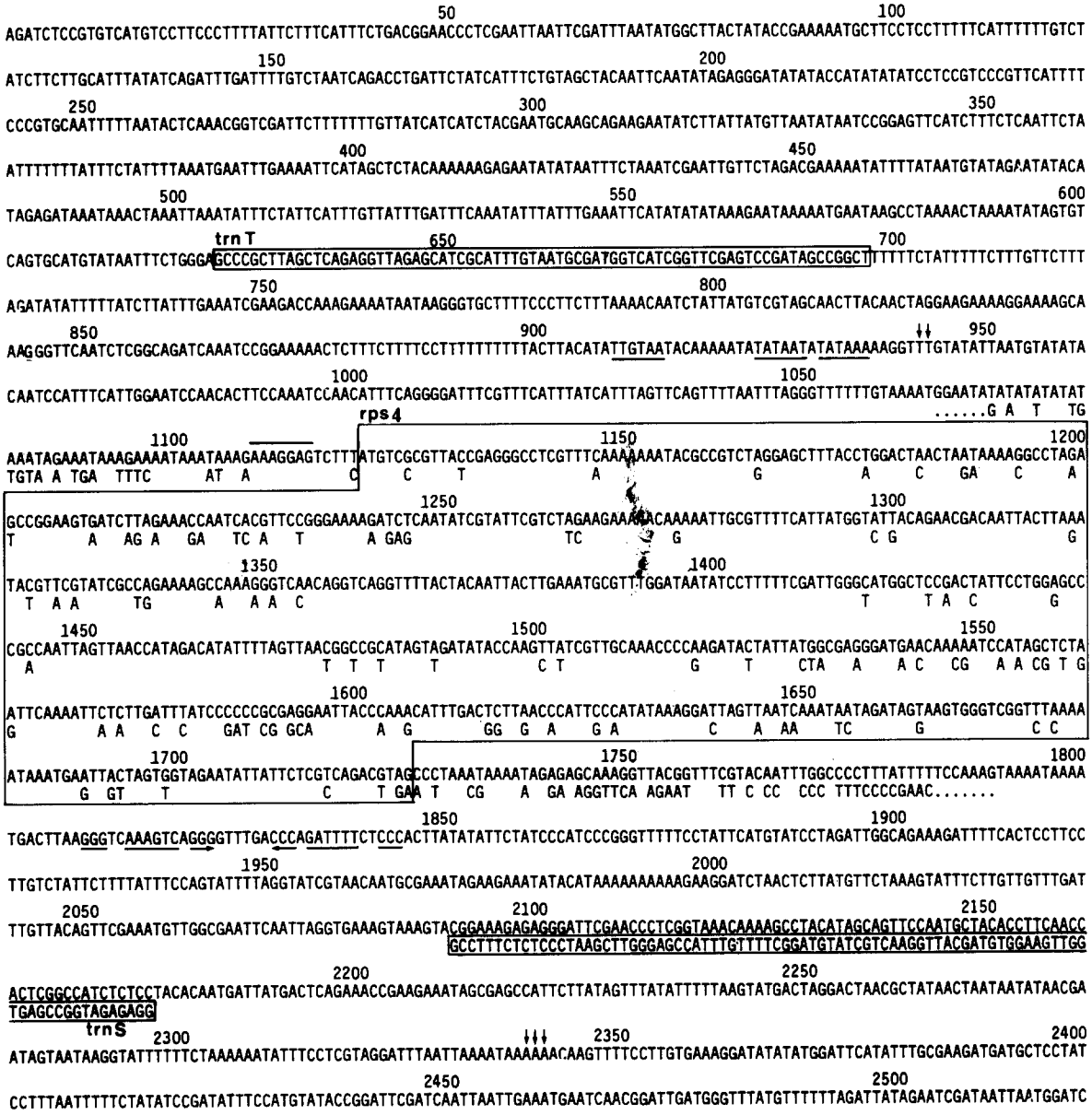

Fig. 2. Nucleotide sequence of the 2.5 kb *Bgl*II-*Bam*HI fragment from *Bam*HI fragment 3 of spinach chloroplast DNA. Only the sequence of the mRNA-like strand is given. Part of the sequence (1070 to 1783) is compared with the published sequence of *rps*4 from maize chloroplast DNA (29), only those nucleotides which are different being shown below the spinach sequence. No attempt is made to compare the spinach and maize sequences outside the 1070 – 1783 coordinates because the homology is so weak. The open reading frame of *rps*4 is enclosed in the large box. A ribosome binding site at -6 to -12 with reference to the ATG codon is overlined. A '-35 region' and two possible '-10 regions' with respect to the 5′ end of the *rps*4 transcript (arrows at positions 942 – 943) are underlined. The 3′ end of the *rps*4 transcript is indicated by arrows (positions 2339 – 2341). A region of dyad symmetry 3′ to *rps*4 is underlined with inverted arrows. *trn*T is boxed, as is also *trn*S which is on the opposite strand.

cant homology (60%) but beyond that point the two sequences diverge. As in maize, the sequence AAAGGAG occurs 5 bp proximal to the initiation codon. This sequence is complementary to the sequence at the 3′ end of maize chloroplast 16S rRNA (24) and, by analogy with the *E. coli* system (25), may be considered to be a strong ribosome binding site.

In Figure 3 the deduced 201 amino acid sequences of spinach and maize (29) chloroplast *rps*4 are compared with the sequence of the S4 protein of *E. coli* ribosomes (22). Of the amino acids in the spinach protein, 154 are identical in maize (77% homology). Most of the differences occur in the regions between residues 23 and 42 and between 133 and 179. The homology between the spinach and *E. coli* proteins (36%) is about the same as that between the maize and *E. coli* proteins (39%) (29). The spinach protein has a deduced molecular weight of 22906 and its overall amino acid composition is very similar to that of the maize and the *E. coli* proteins. The excess of basic over acidic amino acids (28 for spinach compared to 33 for maize and 21 for *E. coli*) is a characteristic feature of most ribosomal proteins.

Analysis of the rps4 *transcript*

Northern blot analysis of chloroplast and total leaf RNAs with labeled restriction fragments internal to *rps*4 showed a number of faintly hybridising bands all of which corresponded to one or other of the various ribosomal RNAs or their major degradation products (data not shown). As these bands were detectable with either plus- or minus-strand probes they could only be considered artifacts. A transcript of *rps*4 would remain undetected if it coincided with one of these bands and was of low abundance so we defined the 5′ and 3′ extremities of the mRNA by S1 mapping experiments.

5′ terminus. Preliminary S1 mapping experiments indicated that the 5′ end of *rps*4 was approximately 180 bp upstream from the initiation codon. This site was determined more precisely using a ^{32}P-labeled single-stranded probe (12) prepared by extending a synthetic oligonucleotide complementary to nucleotides 995-1015 (Fig. 2) on the 817 bp *Xba*I fragment (444-1260) cloned into M13mp19. The extended primer was cut with *Aha*III (i.e. at position 787) and the 227-nucleotide, single-stranded fragment (including the 21-residue primer) was isolated by electrophoresis on a denaturing polyacrylamide gel.

When this probe was hybridized to total RNA either from young leaves (2-3 cm) or from very young leaves (< 3 mm) and digested with S1 nuclease, a fragment 73-74 nucleotides long was left intact (Fig. 4A). The predominant *rps*4 transcript therefore has a 5′ end at position 943-944 (Fig. 2) and a 5′ leader sequence of 177-178 nucleotides. As there was also some full-length protected probe and

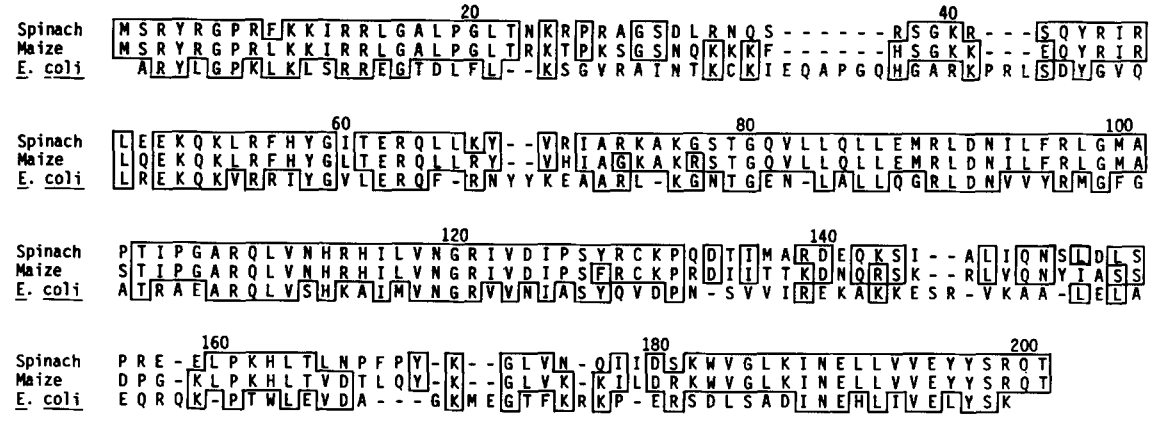

Fig. 3. Comparison of the amino acid sequences of the S4 ribosomal proteins from spinach and maize (29) chloroplasts and *E. coli* (22). Gaps have been introduced into the sequences in order to maximise homology. Identical residues are enclosed in boxes. Numbering refers to the spinach sequence.

the amount was greater with RNA from very young leaves than with RNA from older leaves (Fig. 4A, lanes 1 and 2), it is possible that transcription of *rps*4 initiates further upstream and that the 5′ end corresponding to the 943-944 position is the result of processing. Variable, developmentally-dependent processing of a long initial transcript would also account for the minor fragment about 200 nucleotides long protected by RNA from very young leaves but not by RNA from older leaves (Fig. 4A, lanes 1 and 2). Attemps to identify by S1 mapping another 5′ end further upstream, other than that of the mature $tRNA^{Thr}$, were unsuccessful.

It is, of course, also possible that the transcripts

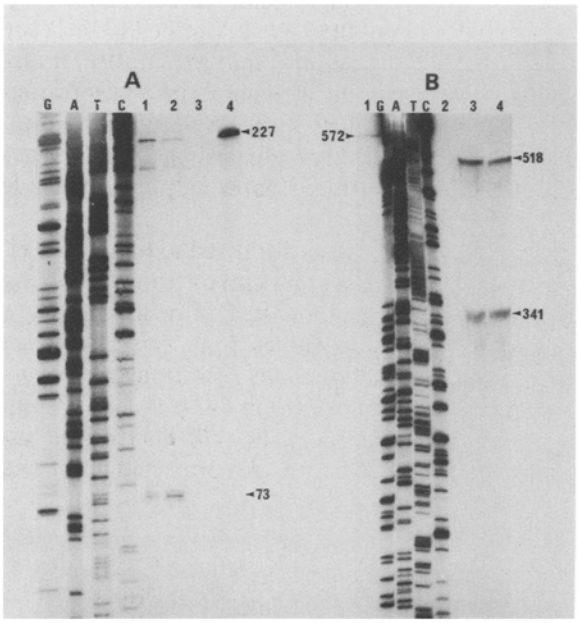

Fig. 4. S1 mapping of the *rps*4 transcript. A. Analysis of the 5′ end. Spinach total RNA (50 μg) from very young (lane 1) or young (lane 2) leaves was hybridized to 5×10^4 Cerenkov cpm of a ^{32}P-labeled 227-nucleotide single-stranded probe (788–1015, see Text and Fig. 2) and then digested with S1 nuclease. Digestion of the probe without hybridization to RNA is shown in lane 3 and undigested probe is run in lane 4. The sequencing ladder (tracks GATC) was derived from a different fragment of spinach chloroplast DNA. B. Analysis of the 3′ end. Total RNA (200 μg) from young leaves (lanes 3 and 4) was hybridized to 2×10^5 Cerenkov cpm of a ^{32}P-labeled 572-nucleotide single-stranded probe (2001 to end of *Bam*HI fragment 3, see Text and Fig. 2) and then digested with S1 nuclease. Digestion of the probe without hybridization to RNA is shown in lane 2 and undigested probe is in lane 1. Tracks GATC are as for 4A. Numbers give the size of the protected band as determined from the sequencing ladder.

responsible for the fully protected probe are the result of chloroplast RNA polymerase occasionally reading through the transcription termination signal of the upstream *trn*T gene and that the 943-944 position is a valid transcription initiation site. In support of this view it can be noted that the sequences TTGTAA and TATAAA occur 35 bp and 10 bp respectively upstream from that site (Fig. 2). These sequences are similar to the *E. coli* -35 and -10 consensus promoter sequences (20) and there is now ample evidence from *in vitro* chloroplast systems that such sequences function as promoters in chloroplast DNA transcription (7, 8, 17). A sequence identical to the *E. coli* -10 consensus sequence (TATAAT) (20) lies 17 bp upstream from the 5′ end at position 943-944 but as it is only 9 bp from the putative -35 sequences it is unlikely to be functional.

3′ terminus. Transcription termination sites in prokaryote genes are frequently preceded by regions of dyad symmetry (20) and similar association has been observed in a number of chloroplast genes (33). A sequence capable of forming a stem-loop structure with a ΔG (25 °C) of -16.6 kcal occurs approximately 100 bp beyond the *rps*4 stop codon (Fig. 2). S1 mapping with probes extending more than 250 bp downstream from the stop codon, however, showed only full-length protection after hybridization to leaf RNA. Further mapping was therefore carried out with a single-stranded probe prepared from a *Sau*3A fragment (position 2517-2000, Fig. 2) cloned into M13mp19. After synthesis of a labeled second strand the DNA was digested with *Sal*I which cuts in the polylinker cloning site of mp19 and a 572 nucleotide single-stranded fragment, including primer and part of the polylinker, was recovered from a preparative denaturing acrylamide gel. A 340-342 nucleotide fragment of this probe was protected from S1 nuclease digestion by hybridization to total leaf RNA (Fig. 4B). This corresponded to a transcript with a 3′ end at position 2339-2341 (Fig. 2). Some fully protected probe (Fig. 4B, lanes 3, 4) was also observed, indicating the possible existence of transcripts extending beyond the end of *Bam*HI fragment 3 or the presence of a small amount of contaminating chloroplast DNA in the total leaf RNA preparation.

Transcription termination or processing at posi-

tion 2340 would give a 3′ untranslated sequence of 619 nucleotides, an unusually long 3′ tail for a chloroplast gene, and a total messenger RNA length of 1.4 kb. This 3′ end is not preceded by a sequence of dyad symmetry and for transcription of *rps*4 to reach this point it must pass the gene for $tRNA^{Ser}$ which is on the opposite strand (see below and Fig. 2). Whether the latter arrangement would cause significant cross-interference between the transcription of *rps*4 and *trn*S is not known. In connection with our failure to detect a specific *rps*4 transcript by Northern analysis, it is worth noting that a 1.4 kb mRNA would coincide with the major artifact band associated with the chloroplast 16S ribosomal RNA.

The trn*T (UGU) gene lies 5′ to, and on the same strand as* rps*4*

Examination of the sequence of the 2.5 kb *Bgl*II-*Bam*HI fragment (Fig. 2) showed that the gene for $tRNA^{Thr}$ (UGU) lies upstream from *rps*4. It was identified by the conserved sequence (GTTCNA) common to the Tψ loop of most tRNAs, by the sequence of the anticodon triplet, and by comparison with the maize chloroplast gene (28). As in the case of maize, *trn*T is on the same strand as *rps*4 but the intergenic distance in spinach (424 bp) is greater than it is in maize (319 bp, (29)). The spinach and maize sequences differ in 5 positions (93% homology; (Fig. 5A)). Both species are characterized by having a mis-matching base-pair (C-C) in the middle of the amino-acyl acceptor stem. This does not occur in the equivalent tRNA from *Euglena gracilis* chloroplasts which has an overall level of homology of 82% when compared to spinach (13). The $tRNA^{Thr}$ from *E. coli* (27) is 75% and that from *Bacillus subtilis* (27) is 69% homologous with spinach $tRNA^{Thr}$. There is no significant homology between the two species in the regions 3′ and 5′ to the $tRNA^{Thr}$ coding sequence.

Driesel *et al.* (5) mapped a $tRNA^{Thr}$ gene to the region of the spinach chloroplast genome where we have located *trn*T. This observation together with our Northern blotting analysis (data not shown) confirms that this *trn*T gene is expressed in chloroplasts *in vivo*.

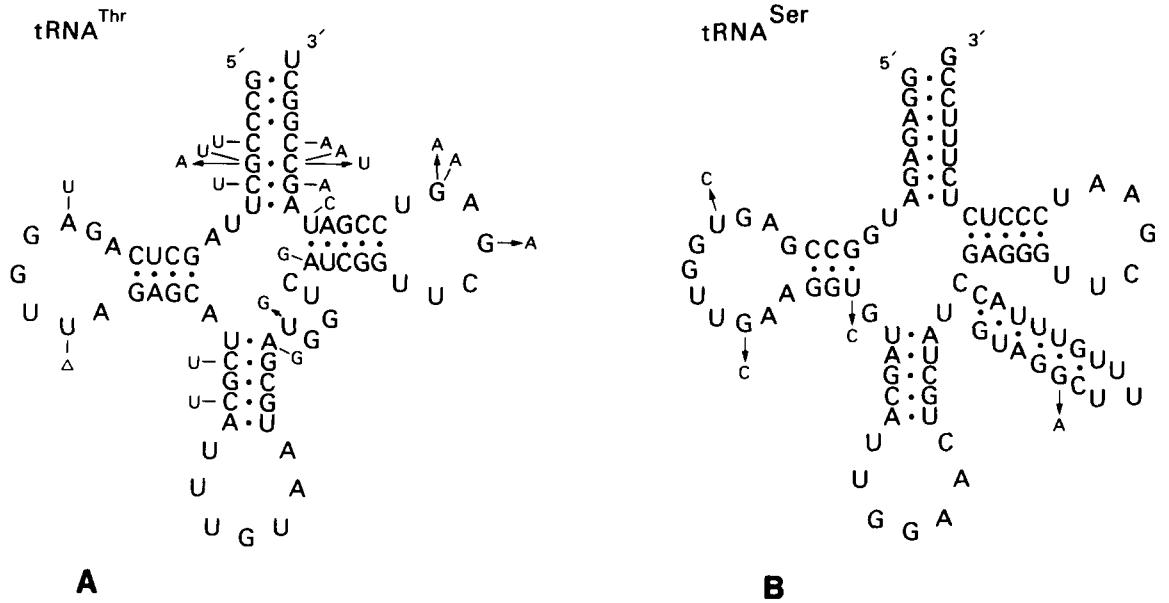

Fig. 5. Clover-leaf representations of spinach chloroplast $tRNA^{Thr}$ (A) and $tRNA^{Ser}$ (B) as deduced from the DNA sequence. In both figures the arrows indicate nucleotide differences in the analogous maize isoacceptor tRNAs (28). In A, the lines not arrowed show the differences in *Euglena* $tRNA^{Thr}$ (UGU) (13), and Δ at position 20 denotes the absence of that nucleotide in the *Euglena* tRNA (13).

The trn*S (GGA) gene lies 3′ to, and on the opposite strand to* rps*4*

The gene for $tRNA^{Ser}$ (GGA) was identified 366 bp downstream from the *rps*4 stop codon (Fig. 2) by applying the same criteria as were used above to identify *trn*T. It is on the opposite strand to *rps*4 and occupies the same relative position with respect to *rps*4 as *trn*S (GGA) does in maize (29) although the intergenic distance is slightly less in maize (281 bp). There are only 3 differences between the spinach and maize sequences (95% homology, Fig. 5B). Outside the coding region of the mature $tRNA^{Ser}$, however, there is little similarity between the two sequences.

Apart from the spinach and maize genes, *trn*S (GGA) has not been sequenced from any other organism. Comparison with *trn*S (UGA) from spinach chloroplasts (11) shows that the sequence of the D-loop, the Tψ stem and loop, and the amino-acyl acceptor stem is highly conserved but that the rest of the sequence diverges considerably. Likewise, comparison of spinach *trn*S (GGA) with *trn*S (GCU) of *Euglena gracilis* (13) and tobacco (2) chloroplasts and with *trn*S (UGA) of *E. coli* (27) shows good conservation of the D-loop and Tψ stem and loop but far less similarity elsewhere.

The gene for $tRNA^{Ser}$ has been mapped previously (5) to the region of the spinach chloroplast genome corresponding to the end of *Bam*HI fragment 3. Hybridization of a probe containing the *trn*S sequence to chloroplast RNA (data not shown) confirmed that this *trn*S gene is transcribed *in vivo*.

General conclusion

The sequence of a third spinach chloroplast ribosomal protein gene has now been determined and the 5′ end of the transcript identified. It is not possible, however, to draw any conclusions with respect to likely regulatory sequences because the transcription initiation site for the only other sequenced spinach ribosomal protein genes, *rpl*2 and *rps*19 (which are co-transcribed) (36), has not yet been determined. The low abundance of mRNAs for these proteins makes it difficult to detect specific transcripts by Northern blotting and hinders the precise mapping of 5′ and 3′ termini by S1 nuclease protection experiments. Nevertheless we are confident that as more chloroplast ribosomal protein genes are identified and sequenced and a concerted effort is made to determine their transcription initiation sites it will become possible to recognize regulatory regions for these genes by sequence comparison.

Acknowledgements

The authors wish to thank John Mason for his help with cloning and sequencing techniques, and Graham Hudson for his advice on the use of 'prime-cut' probes in S1 mapping. SBT gratefully acknowledges the support of a French Government Fellowship (CNRS).

References

1. Bird CR, Koller B, Auffret AD, Huttly AK, Howe CJ, Dyer TA, Gray JC: The wheat chloroplast gene for CF_o subunit I of ATP synthase contains a large intron. EMBO J 4:1381–1388, 1985.
2. Deno H, Sugiura M: The nucleotide sequences of the $tRNA^{Ser}$ (GCU) and $tRNA^{Gln}$ (UUG) genes from tobacco chloroplasts. Nucleic Acids Res 11:8407–8414, 1983.
3. Dorne AM, Heizmann P, Alt J, Mache R: Spinach chloroplast genes coding for three ribosomal proteins. Mol Gen Genet 198:521–524, 1985.
4. Dorne AM, Lescure AM, Mache R: Site of synthesis of spinach chloroplast ribosomal proteins and formation of incomplete ribosomal particles in isolated chloroplasts. Plant Mol Biol 3:83–90, 1984.
5. Driesel AJ, Crouse EJ, Gordon K, Bohnert H-J, Herrmann RG, Steinmetz A, Mubumbila M, Keller G, Burkard G, Weil JH: Fractionation and identification of spinach chloroplast transfer RNAs and mapping of their genes on the restriction map of chloroplast DNA. Gene 6:285–306, 1979.
6. Eneas-Filho J, Hartley MR, Mache R: Pea chloroplast ribosomal proteins: characterization and site of synthesis. Mol Gen Genet 184:484–488, 1981.
7. Gruissem W, Zurawski G: Identification and mutational analysis of the promoter for a spinach chloroplast transfer RNA gene. EMBO J 4:1637–1644, 1985.
8. Gruissem W, Zurawski G: Analysis of promoter regions for the spinach chloroplast *rbc*L, *atp*B and *psb*A genes. EMBO J 4:3375–3383, 1985.
9. Hachtel W: Biosynthesis and assembly of chloroplast ribosomal proteins in isolated chloroplasts from *Vicia faba* L. Biochem Physiol Pflanzen 180:115–124, 1985.
10. Herrmann RG, Whitfeld PR, Bottomley W: Construction of a *Sal*I/*Pst*I restriction map of spinach chloroplast DNA using low-gelling-temperature agarose electrophoresis. Gene 8:179–191, 1980.

11. Holschuh K, Bottomley W, Whitfeld PR: Structure of the spinach chloroplast genes for the D2 and 44 kd reaction-centre proteins of photosystem II and for $tRNA^{Ser}$ (UGA). Nucleic Acids Res 12:8819 – 8834, 1984.
12. Hudson GS, Farrell PJ, Barrell BG: Two related but differentially expressed potential membrane proteins encoded by the EcoRI Dhet region of Epstein-Barr virus B95 – 8. J Virol 53:528 – 535, 1985.
13. Karabin GD, Hallick RB: *Euglena gracilis* chloroplast transfer RNA transcription units. Nucleotide sequence analysis of a $tRNA^{Thr}$-$tRNA^{Met}$-$tRNA^{Ser}$-$tRNA^{Gln}$ gene cluster. J Biol Chem 258:5512 – 5518, 1983.
14. Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning; A Laboratory Manual. Cold Spring Harbor Laboratory 1982.
15. Maxam AM, Gilbert W: Sequencing end-labeled DNA with base-specific chemical cleavages. Methods in Enzymology 65:499 – 560, 1980.
16. Montandon P-E, Stutz E: The genes for the ribosomal proteins S12 and S7 are clustered with the gene for the EF-Tu protein on the chloroplast genome of *Euglena gracilis*. Nucleic Acids Res 12:2851 – 2859, 1984.
17. Mullet JE, Orozco EM Jr, Chua N-H: Multiple transcripts for higher plant *rbc*L and *atp*B genes and localization of the transcription initiation site of the *rbc*L gene. Plant Mol Biol 4:39 – 54, 1985.
18. Posno M, Torenvliet DJ, Lustig H, van Noort M, Groot GSP: Localization of three chloroplast ribosomal protein genes at the left junction of the large single copy region and the inverted repeat of *Spirodela oligorhiza* chloroplast DNA. Current Genet 9:211 – 219, 1985.
19. Posno M, van Noort M, Debise R, Groot GSP: Site of synthesis of spinach chloroplast ribosomal proteins. Current Genet 8:147 – 154, 1984.
20. Rosenberg M, Court D: Regulatory sequences involved in the promotion and termination of RNA transcription. Ann Rev Genet 13:319 – 353, 1979.
21. Sanger F, Nicklin S, Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463 – 5467, 1977.
22. Schiltz E, Reinbolt J: Determination of the complete amino acid the sequence of protein S4 from *Escherichia coli* ribosomes. Eur J Biochem 56:467 – 481, 1975.
23. Schmidt RJ, Richardson CB, Gillham NW, Boynton JE: Sites of synthesis of chloroplast ribosomal proteins in *Chlamydomonas*. J Cell Biol 96:1451 – 1463, 1983.
24. Schwarz Z, Kössel H: Sequencing of the 3′ terminal region of a 16S rRNA gene from *Zea mays* chloroplast reveals homology with *E. coli* 16S rRNA. Nature 279:520 – 522, 1979.
25. Shine J, Dalgarno L: Determination of cistron specificity in bacterial ribosomes. Nature 254:34 – 38, 1975.
26. Shinozaki K, Sun C-R, Sugiura M: Gene organization of chloroplast DNA from the broad bean *Vicia faba*. Mol Gen Genet 197:363 – 367, 1984.
27. Sprinzl M, Gauss DH: Compilation of tRNA sequences. Nucleic Acids Res 10:r1 – r55, 1982.
28. Steinmetz AA, Krebbers ET, Schwarz Z, Gubbins EJ, Bogorad L: Nucleotide sequences of five maize chloroplast transfer RNA genes and their flanking regions. J Biol Chem 258:5503 – 5511, 1983.
29. Subramanian AR, Steinmetz A, Bogorad L: Maize chloroplast DNA encodes a protein sequence homologous to the bacterial ribosome assembly protein S4. Nucleic Acids Res 11:5277 – 5286, 1983.
30. Sugita M, Sugiura M: A putative gene of tobacco chloroplast coding for ribosomal protein similar to the *E. coli* ribosomal protein S19. Nucleic Acids Res 11:1913 – 1918, 1983.
31. Umesono K, Inokuchi H, Ohyama K, Ozeki H: Nucleotide sequence of *Marchantia polymorpha* chloroplast DNA: a region possibly encoding three tRNAs and three proteins including a homologue of *E. coli* ribosomal protein S14. Nucleic Acids Res 12:9551 – 9565, 1984.
32. Whitfeld PR, Bottomley W: Mapping of the gene for the large subunit of ribulose bisphosphate carboxylase on spinach chloroplast DNA. Biochem Internat 1:172 – 178, 1980.
33. Whitfeld PR, Bottomley W: Organization and structure of chloroplast genes. Ann Rev Plant Physiol 34:279 – 310, 1983.
34. Whitfeld PR, Herrmann RG, Bottomley W: Mapping of the ribosomal RNA genes on spinach chloroplast DNA. Nucleic Acids Res 5:1741 – 1751, 1978.
35. Zurawski G, Bottomley W, Whitfeld PR: Structures of the genes for the β and ϵ subunits of spinach chloroplast ATPase indicate a dicistronic mRNA and an overlapping translation stop/start signal. Proc Natl Acad Sci USA 79:6260 – 6264, 1982.
36. Zurawski G, Bottomley W, Whitfeld PR: Junctions of the large single copy region and the inverted repeats in *Spinacia oleracea* and *Nicotiana debneyi* chloroplast DNA: sequence of the genes for $tRNA^{His}$ and the ribosomal proteins S19 and L2. Nucleic Acids Res 12:6547 – 6558, 1984.

Received 31 December 1985; in revised form 10 April 1986; accepted 23 April 1986.