Volume 14 Number 19 1986 Nucleic Acids Research

A subpopulation of spinach chloroplast tRNA genes does not require upstream promoter elements for transcription

W.Gruissem*, C.Elsner-Menzel, S.Latshaw, J.O.Narita, M.A.Schaffer and G.Zurawski[1]

Department of Botany, University of California, Berkeley, CA 94720 and [1]DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304, USA

Received 8 August 1986; Accepted 5 September 1986

ABSTRACT

We have identified a class of spinach plastid tRNA genes which do not require 5' upstream promoter elements for their expression in a chloroplast transcription system. The 5' DNA sequences flanking the trnR1 and trnS1 coding regions have little or no homology to previously characterized chloroplast promoter sequences. The deletion of the 5' DNA sequences from these genes to positions close to the start of the coding regions has little effect on their transcription in vitro. In addition, a synthetic DNA fragment homologous to the 5' region of trnS1 does not support the transcription of the promoter (-) trnM2 mutant 51 in a promoter/trnM2-51 fusion assay. In a dicistronic construct the wild type trnS1 gene does not support transcription of the trnM2-51 promoter mutant, indicating that termination of trnS1 transcription occurs immediately following the 3' end of the coding region. Both trnS1 and trnR1 compete with trnM2 for the same chloroplast RNA polymerase and/or common transcription factors.

INTRODUCTION

The development of chloroplast transcription systems has allowed the assessment of DNA sequences which are required for the in vitro transcription of transfer RNA and protein coding genes coded by chloroplast DNA (1,2,3,4). The comparison of DNA sequences 5' upstream of transcription start sites from several genes or polycistronic transcription units in higher plant chloroplast DNA has revealed DNA sequences with homology to prokaryotic promoter elements (5,6). Subsequent analysis of DNA sequences which support accurate transcription of cloned plastid genes in the chloroplast transcription system has confirmed the importance of a -35 promoter element (ctp1) for the transcription of the spinach trnM2 (7) and mustard psbA genes (3). The detailed mutational analysis of the promoter region for the spinach trnM2 gene has provided additional information for the requirement of a -10 analog (ctp2) as well as a specific arrangement of ctp1 and

ctp2 for maximal transcription (7). In analogy to the prokaryotic promoter elements, base substitutions in ctp1 and ctp2 result in reduced transcription rates for the trnM2 gene. A trnM2 promoter-deletion derivative has been used as a tool to identify and characterize the promoter regions for the spinach rbcL, atpB and psbA plastid protein coding genes (8). The fusion constructs have demonstrated that approximately 40 bp regions, including the defined in vivo transcriptional start sites and proximal residues, from rbcL, atpB and psbA, direct the correct transcription of the trnM2 gene. All three regions have DNA sequences homologous to ctp1 and ctp2, and thus the results are consistent with rules that have been established for the spinach plastid trnM2 and prokaryotic promoters.

In addition to the above genes with established promoter regions, we have identified a class of plastid tRNA genes with 5' upstream DNA sequences that have little or no homology to the previously characterized ctp1 and ctp2 regions. Two of these genes, trnR1 and trnS1, have been analyzed in detail for their transcription properties in vitro. We report here that both tRNA genes have no 5' upstream promoter elements that are comparable in their function to ctp1 and ctp2, which are essential for the transcription of trnM2, rbcL, atpB and psbA.

MATERIALS AND METHODS

Plasmid DNA

The trnS1 gene was subcloned from the spinach plastid BamHI fragment 16 (9) as a 380 bp Sau3A fragment and inserted into the BglII site of the vector pMT11. The pMT11 vector is a truncated pBR322 plasmid and has a polylinker insertion as well as additional pUC8 sequences, but lacks the lacZ' promoter region (H. Huang and K. Moore, unpublished). The trnR1 gene was subcloned from the spinach plastid SalI fragment 10 as a SalI-HindIII fragment and inserted into the vector pUC18. Plasmid DNA for enzymatic reactions and in vitro transcription experiments was purified from bacterial lysates by ethidium bromide-CsCl gradient centrifugation.

Construction of 5' Deletion Mutants

The trnS1 gene in pMT11 was digested with HindIII, and 15 µg of the restricted DNA was incubated with 4 units of the 3',5'-

exonuclease Bal31 for 12 min at 37°C. Aliquots were removed from the reaction at various intervals. The aliquots were combined, extracted with phenol-chloroform-isoamylalcohol, and precipitated with ethanol. Blunt ends were generated by incubation of the Bal31-treated DNA with T4 DNA polymerase, and the plasmid DNA was subsequently digested with PstI. The Bal31-treated fragments containing the trnS1 gene were separated on a 1.5% agarose gel, and fractions of different DNA fragment sizes were isolated from the gel. The DNA fragments were ligated into pMT11 which had been digested with SmaI and PstI. The initial Bal31 deletion mutants were selected from a screen of the transformants digested with EcoRI and PstI. The 5' endpoints of the Bal31 deletion mutants were determined by direct sequencing of the double-stranded plasmid DNA using the pBR322 EcoRI primer (5'GTATCAC-GAGGCCCTT) and reverse transcriptase (10). The trnR1 gene in pUC18 was digested with XbaI, and subsequently incubated with Bal31 using the above protocol. After treatment with T4 DNA polymerase, digestion with HindIII and electrophoretic separation, DNA fragments of appropriate sizes were ligated into pUC18 digested with SmaI and HindIII. Selection of Bal31 deletion mutants and DNA sequencing followed the above procedures. All selected Bal31 mutants of the trnRI gene were also cloned as EcoRI-HindIII fragments into pUC19 and pMT11.

Promoter/trnM2 and Polycistronic trnS1/trnM2 Fusion Constructs

The trnS1 5' region from -1 to -34, which supports transcription of trnS1 in the deletion mutant 2-9, was constructed from synthetic oligonucleotides as a DNA fragment with EcoRI and BamHI restriction sites and ligated to the trnM2 promoter deletion mutant 51 (7) in pdX11 as described (8) to yield the trnM2-51/S2-9 fusion construct. A polycistronic trnS1-trnM2 locus was constructed using the trnS1 gene in pMT11 and the trnM2 promoter deletion mutant 51. The trnS1 template was cut with XmnI, which cleaves 20 bp 3' of the coding region, and subsequently with XbaI. The isolated fragment was ligated to the trnM2-51 fragment which was cut with SmaI and XbaI. The ligated fragments were cloned into the XbaI cut pIBI vector DNA.

Chloroplast Transcription Extract

Intact chloroplasts were isolated from hydroponically grown

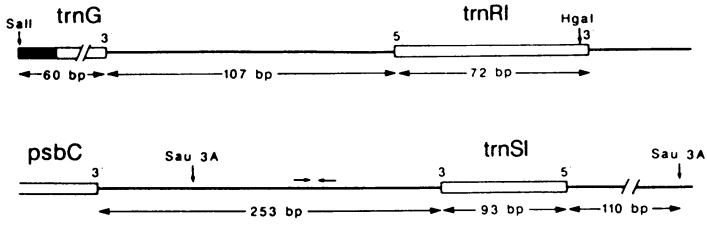

Figure 1. Location and organization of the trnR1 and trnM2 spinach chloroplast genes. The trnR1 gene, located 107 bp downstream from the trnG gene, was subcloned as a SalI/HindIII fragment, which was used for the Bal31 deletion analysis. The trnS1 gene was subcloned as a Sau3A as shown on the map. Open boxes indicate the positions of the trnR1, trnG, trnS1 and psbC coding regions, black bars represent non-coding regions, and the black box indicates part of the intron in trnG.

Spinacea oleracea (cv. Marathon hybrid) and used for the preparation of the transcription extract as previously described (1,11).

In Vitro Transcription and Analysis of Transcripts

Plasmid DNAs (predominately form I DNA; 60 µg/ml) were incubated under standard conditions in 25 µl reactions according to a published protocol (11). After termination of the in vitro transcription reaction and isolation of RNA, the labeled tRNA products were seperated on 10% polyacrylamide-50% urea gels. After localization of the tRNA products on X-ray film they were excised from the gel and incorporation of [α-^{32}P]-UMP into the mature tRNA transcripts was determined by scintillation counting.

RESULTS

The Spinach Chloroplast trnR1 and trnS1 Transcription Units

The trnR1 gene is located downstream from the atpA transcription unit and 107 bp upstream from the 5' end of the trnG1 gene. The trnR1 and trnG1 genes are transcribed in the same orientation, with the trnR1 gene being transcribed towards the 3' end of atpA (Figure 1A). The spinach chloroplast SalI fragment 10 was initially used as a template for the detailed characterization of the $tRNA^{Arg}$ transcript. RNAase T1 fingerprint analysis unequivocally established the correct transcription and processing of the $tRNA^{Arg}$ in the chloroplast in vitro transcription system (12). No transcript has been detected for the atpA gene in vitro, which

is consistent with the observation that this gene is cotranscribed with atpF and atpH in vivo (13). The trnS1 gene is located 253 bp downstream from the 3' end of the psbD coding region, and is transcribed towards the 3' end of this gene (9; Figure 2B). An 11 bp inverted repeat occurs 80 bp downstream from trnS1, which has been proposed to serve as a transcription terminator for trnS1 and/or psbD (9). It has been noted that a 270 bp open reading frame is located 273 bp upstream from the 5' end of trnS1, which does not appear to be expressed in chloroplast (9). In addition, since the Sau3A fragment in pMT11, which excludes this region (Figure 1B), is transcribed in the chloroplast extract, we conclude that the sequence is not important for the expression of trnS1.

Analysis of trnR1 5' Deletion Mutants

The SalI-HindIII fragment containing the trnR1 gene in pUC18 directs the transcription of $tRNA^{Arg}$ with approximately the same efficiency at comparable template concentrations in vitro (not shown). The SalI site at -164 of trnR1 provides a convenient start point for resection of the 5' upstream region with Bal31 exonuclease (Figure 1A). Deletion of 5' DNA sequences from the SalI site to -75 does not alter the transcription efficiency of the trnR1 deletion mutants significantly (2-1; Figure 2B). This deletion site for trnR1 is located 32 bp downstream from the 3' end of the trnG1 coding region. Deletions 2-1 and 2-5 also remove one and two T at -76 and -75, respectively, from a sequence 5'TTGTCT, which has partial homology to ctp1 and was identified as a promoter element for trnM2. Removal of these nucleotides results in a 60% reduction of transcription efficiency for trnM2 (7), but has no effect on transcription of trnR1. Mutation 2-4 removes all 5' upstream sequences to -58, including the sequence 5' TTGTCT, but does not decrease the transcription efficency of the resulting deletion mutant template in the in vitro system (Figures 2A and 2B). Deletion mutants 3-2 and 3-3 have 5' upstream sequences deleted to -29 and -21 of the trnR1 coding region, respectively, including a sequence 5' TTGTTT (-52) with significant homology to ctp1. Again, we find that the deletions have only a marginal effect on in vitro trnR1 transcription. Deletion of the 5' upstream region to -8 decreases

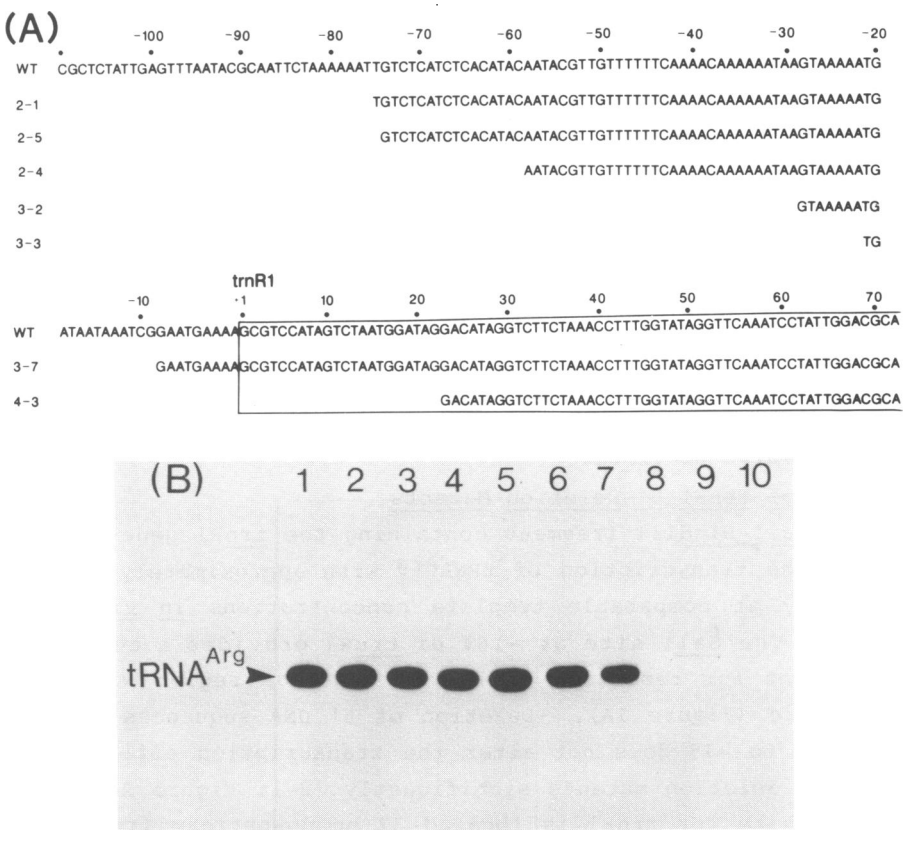

Figure 2. Deletion analysis of the trnR1 5' region. (A) DNA sequence of the trnR1 region and deletions obtained after resection with Bal31. The nucleotides of the trnR1 coding region are enclosed by a box. Blank areas indicate deleted DNA sequences which were replaced by pUC18, pUC19 or pMT11 DNA sequences upstream from the SmaI site. (B) In vitro transcription of trnR1 deletion mutants in the spinach chloroplast extract. The DNA concentrations in the transcription reactions were 60 µg/ml (form I) for wild type and mutant templates. Lanes 1 - 8 correspond to wild type and mutant templates 2-1, 2-5, 2-4, 3-2, 3-3, 3-7, 4-3, respectively. Lanes 9 and 10 are additional mutants with deletions in the coding region (not shown in A). The mature $tRNA^{Arg}$ transcript is 75 nt long and has been previously characterized (12).

transcription of the mutant template to 65%, which could in part be a consequence of alterations at the 5' initiation site. Since the chloroplast transcription extract is active in tRNA processing (14), we have been unable to confirm the transcription start

site for this gene. No mature $tRNA^{Arg}$ or truncated transcript can be detected when part of the coding region of trnR1 is deleted (mutant 4-3).

To exclude that DNA sequences in the pUC18 vector can function as a promoter for the transcription of trnR1 in vitro, and thus cause the observed levels of transcripts from the 5' deletion mutants, we have subcloned all deletion mutants as EcoRI-HindIII fragments into pUC19 and finally into the lacZ' promoter (-) vector pMT11 (see Materials and Methods). All trnR1 deletion templates in pUC19 or pMT11 direct the transcription of $tRNA^{Arg}$ in the chloroplast extract with the same efficiency as described for the pUC18 constructs (results not shown).

Analysis of trnS1 5' Deletion Mutants

The 380 bp Sau3A fragment in pMT11 directs the transcription of the 96 nt $tRNA^{Ser}$ in the chloroplast extract at a high efficiency, indicating that the trnS1 locus is actively expressed in vitro. The analysis of the 5' upstream region does not reveal DNA sequences with good homology to the ctp1 and ctp2 promoter elements of trnM2 (7), indicating that transcription of trnS1 may be similar to trnR1. This notion is supported by transcription experiments with 5' deletion mutants of the trnS1 gene in the chloroplast extract (Figure 3A and 3B). The trnS1 deletion mutants 1-3, 2-4, 2-1, 2-9 and 2-6, in which 5' DNA sequences were removed upstream from positions -79, -44, -41, -35 and -34, respectively, all direct the transcription of $tRNA^{Ser}$ at nearly wild type levels. Further deletion of upstream sequences to -10 results in a decrease of trnS1 transcription. As discussed for the trnR1 gene, we can not exclude that this is a consequence of alterations around the transcription start site. No transcripts can be detected from templates in which part of the trnS1 coding region has been deleted.

Fusion of trnS1 5' DNA sequences to the trnM2 coding region

The analysis of the trnS1 5' deletion mutants lacks the resolution to exclude that DNA sequences between -10 and -35 may have promoter activity in vitro. To evaluate the function of this region, we constructed a synthetic DNA fragment of the 5' region which remains in the trnS1 deletion mutant 2-9, and fused it to the trnM2 promoter deletion mutant 51 (Figure 4A). This

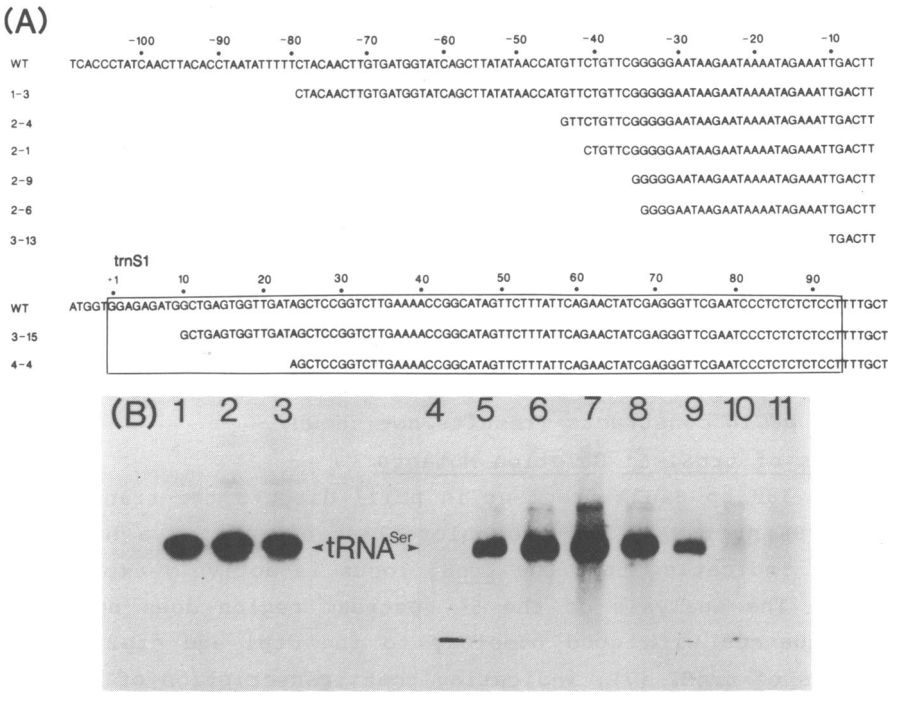

Figure 3. Deletion analysis of the trnS1 5' region. (A) DNA sequence of the trnS1 region and deletions obtained after resection with Bal31. The trnS1 sequence has been previously published (9). The nucleotides of the trnS1 coding region are enclosed by a box. Blank areas indicate deleted DNA sequences which were replaced by pMT11 DNA sequences upstream from the SmaI site. (B) In vitro transcription of trnS1 deletion mutants in the spinach chloroplast extract. The concentrations of DNA templates were 60 µg/ml, except in lane 1, where the concentration was 20 µg/ml. Lane 1: wild type; lane 2: 2-4; lane 3: 2-1; lane 4: no DNA; lanes 5 and 6: wild type; lanes 7 - 11 correspond to mutant templates 2-9, 2-6, 3-13, 3-15 and 4-4, respectively. The mature $tRNA^{Ser}$ transcript is 96 nucleotides long.

technique has been successfully used for the analysis of promoter regions from trnM2, rbcL, atpB and psbA (7,8). As is shown in Figure 4B, trnS1 wild type and deletion mutant 2-9 templates direct the transcription of $tRNA^{Arg}$ at nearly comparable levels. The trnM2-51/S2-9 fusion construct, however, shows almost no increase in $tRNA^{Met}$ transcript levels over the background level detected for the trnM2-51 deletion mutant. As a control, the psbA-promoter/trnM2-51 fusion construct directs the transcription of $tRNA^{Met}$ approaching wild type levels, which is consistent with

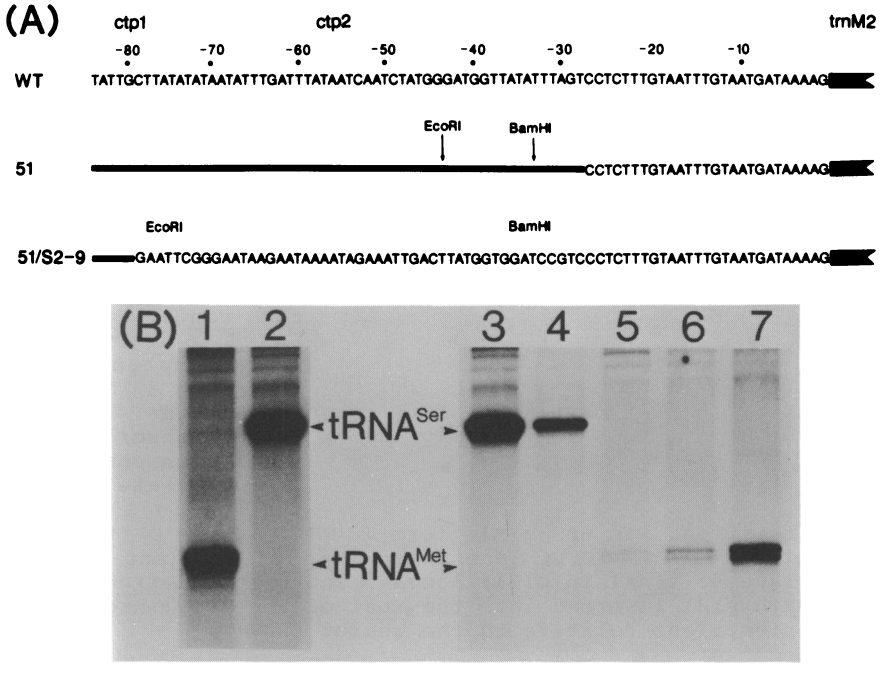

Figure 4. Construction and expression of a trnM2/trnS1 5' fusion template. (A) The trnS1 5' region from -1 to -34 (see Figure 3A) was synthesized and fused to the trnM2 deletion mutant 51 as described in Materials and Methods. The filled boxes and the bar represent the trnM2 coding region and pdX11 sequences, respectively. (B) In vitro transcription of trnM2/promoter fusion templates. Form I plasmid DNAs (60 µg/ml) were transcribed as described in Materials and Methods. Lane 1: trnM2 in pdX11; lane 2: trnS1 in pMT11; lanes 3 and 4: trnS1 5' deletion mutants 2-9 and 3-13, respectively; lane 5: trnM2 deletion mutant 51 (7); lane 6; trnM2-51/S2-9; lane 7: trnM2-psbA promoter fusion control (8).

previously published results (8). We therefore conclude that the DNA sequence present in the trnS1 deletion mutant 2-9 alone is not sufficient to support the transcription of the trnS1 gene in vitro.

Can trnS1 Support The Transcription Of trnM2 Deletion Mutant 51?

To address the question if the trnS1 gene itself can serve as a promoter for the expression of the trnM2 deletion mutant 51, we constructed the polycistronic transcription unit shown in Figure 5A. It was demonstrated in previous experiments that chloroplast polycistronic tRNA transcripts occur in vivo (15,16) and can be

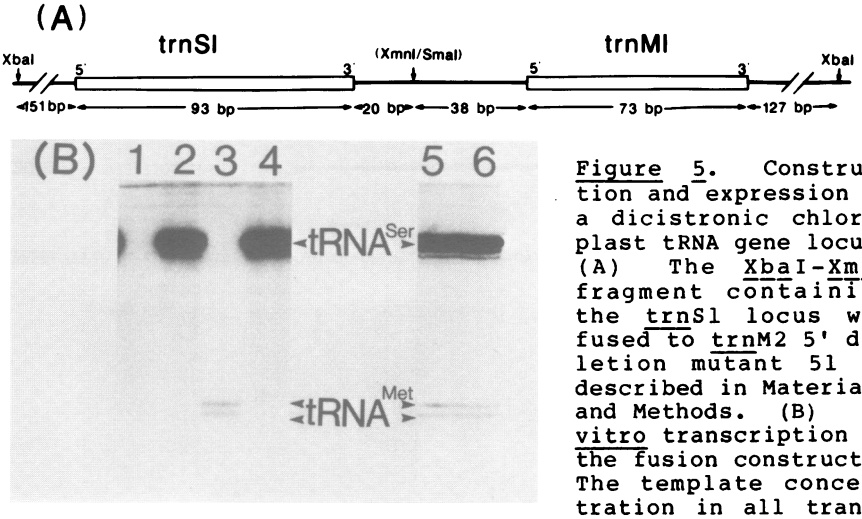

Figure 5. Construction and expression of a dicistronic chloroplast tRNA gene locus. (A) The XbaI-XmnI fragment containing the trnS1 locus was fused to trnM2 5' deletion mutant 51 as described in Materials and Methods. (B) In vitro transcription of the fusion constructs. The template concentration in all transcription reactions was 60 µg/ml (form I DNA). Lane 1: trnM2 deletion mutant 51 in pdX11; lane 2:trnS1 in pMT11; lane 3: trnM2 in pIBI; lane 4: trnS1 in pIBI; lanes 5 and 6: trnS1-trnM2 fusion construct in pIBI inserted into the XbaI of the polylinker region in both orientaions relative to the vector DNA.

transcribed and properly processed in vitro (1,2,14,15). In the dicistronic construct trnS1 and trnM2 are seperated by 58 bp, with 20 bp and 27 bp resulting from the trnS1 3' and trnM2 5' regions, respectively. The 11 bp separating these DNA sequences are derived from the polylinker region of pdX11 (Figure 5A). The single trnS1 gene in pIBI is transcribed efficiently, and we also note that the background transcription of the single trnM2 deletion mutant gene 51 in pIBI has increased as compared to the pdX11 construct (Figure 5B). This is most likely due to non-specific initiation events of the chloroplast RNA polymerase on vector DNA sequences. The transcription of the dicistronic trnS1-trnM2 construct in the chloroplast extract shows that trnS1 is transcribed with an efficiency similar to the monocistronic transcription unit. However, we do not observe a significant increase of $tRNA^{Met}$ transcripts. Since we can not detect increased levels of transcripts of higher molecular weight, it is unlikely that this result is a consequence of inefficient processing of a primary transcript. We therefore conclude that in the dicistronic construct the trnM2 gene is not cotranscribed.

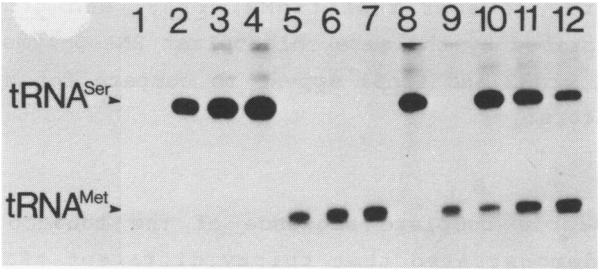

Figure 6. Competition of trnM2 and trnS1 for common transcription factors in the spinach chloroplast extract. All incubations were for 60 min at 25°C with various DNA template (form I) concentrations. Lane 1: no DNA; lane 1 - 4: trnS1 at 20, 60 and 120 µg/ml, respectively; lanes 5 - 7: trnM2 at 20, 40 and 60 µg/ml, respectively; lane 8: trnS1 (60 µg/ml); lane 9: trnM2 at 20 µg/ml; lanes 10 - 12: trnS1 at 60 µg/ml and trnM2 at 20, 40 and 60 µg/ml, respectively.

trnM2 and trnS1 Compete For The Same Transcription Factors

It is possible that tRNA genes in the chloroplast genome which differ in their promoter organization require different transcription factors or are transcribed by different RNA polymerase activities. At least two chloroplast RNA polymerase activities have recently been reported for higher plants and Euglena (17). We have therefore initiated competition experiments with trnM2 and trnS1 in the chloroplast transcription extract (Figure 6). Increasing template concentrations in the transcription reactions result in an approximately 1.5- and 2-fold increase of transcript levels for trnM2 and trnS1, respectively. The addition of vector DNA alone does not result in a significant decrease of transcript levels, indicating little non-specific binding of chloroplast RNA polymerase. Increasing concentrations of trnM2 (20-60 µg/ml) in the presence of a fixed trnS1 concentration (60 µg/ml) in the transcription reaction results in a decrease of $tRNA^{Ser}$ transcripts to levels lower than observed with trnS1 alone. Preincubation experiments with trnS1 or trnM2 suggest that stable transcription complexes can be formed with both genes, which results in decreased transcription levels of the gene added following the preincubation (results not shown). Although these experiments do not clearly distinguish between RNA polymerase and/or additional transcription factor requirements,

they indicate, however that trnM2, trnS1 and trnR1 are most likely transcribed by the same chloroplast RNA polymerase activity, and that trnM2 and trnS1 appear to compete for common transcription factors.

DISCUSSION

The available complete sequence of the tobacco chloroplast genome has demonstrated that thirty different tRNA genes are encoded by the chloroplast DNA (18). Of these trnL-CAA is co-transcribed with the 16S and 23S rrn transcription units, and trnE-UUC - trnY-GUA - trnD-GUC form the only known polycistronic tRNA transcription unit in higher plant chloroplasts (16). The spinach chloroplast trnM2 (CAU), trnR1 (UCU) and trnS1 (UGA) genes which we have analysed in this and previous reports (9,12) are located in positions which are similar to those reported for the respective tobacco genes. Only the promoter region of the spinach chloroplast trnM2 has been functionally defined (7), although chloroplast promoter regions for tRNA, ribosomal RNAs and protein-coding genes have been compiled based on their structural homologies to the prokaryotic consensus promoter sequence and their location relative to experimentally defined transcript start sites (19). It has been generally assumed that the chloroplast RNA polymerase requires such prokaryote-type 5' upstream regions for binding and transcription initiation. Only recently have promoter regions been functionally defined for psbA (3,8), rbcL and atpB (8,20) by their ability to support transcription in homologous chloroplast extracts, and the results have confirmed the requirement for prokaryote-type promoter elements.

It was therefore unexpected that a subpopulation of chloroplast tRNA genes does not require 5' upstream promoter regions for their transcription in vitro. The absence of functional promoter elements in the 5' regions of the trnR1 and trnS1 coding regions is supported by several criteria. First, comparison of the upstream DNA sequences for these genes with the defined chloroplast promoter regions does not reveal regions of significant homology with ctp1-/ctp2-type sequences (7,8). Second, deletion of the trnS1 and trnR1 5' DNA sequences to or beyond the limit of minimal DNA sequence required for promoter function has

little or no effect on the transcription of these genes in vitro. This is in contrast to results obtained for the trnM2 locus, where deletion of a "-35"-like promoter element around -80 (ctpl) results in a complete loss of transcription from this gene (7). The possibility that the promoter regions for trnS1 and trnR1 are located upstream from the restriction sites used to subclone these genes can be excluded, since longer restriction fragments show no increase in $tRNA^{Ser}$ or $tRNA^{Arg}$ transcript levels. Third, DNA sequences from the 5' region of trnS1 do not support transcription of the trnM2 promoter deletion mutant 51, in contrast to promoter regions from psbA, rbcL and atpB, which can effectively replace the trnM2 wild type promoter.

At present we can only speculate about the location of the promoter regions for trnR1 and trnS1. It is interesting to note that for transcription of eukaryotic nuclear tRNA genes by RNA polymerase III two intragenic sequence blocks are required which are highly conserved in all eukaryotic tRNAs (21). Consequently, bacterial and chloroplast tRNA genes which share this homology are also recognized and transcribed by the eukaryotic RNA polymerase III (22). Comparison of the respective sequences in the spinach chloroplast tRNA genes shows that trnS1 has complete homology with the eukaryotic consensus A- and B-block promoter sequences, while in trnR1 six out of nine (A-block) and 8 out of nine (B-block) base pairs are conserved. However, a similar level of homology is observed for the spinach chloroplast trnM2 gene, which requires upstream promoter elements for transcription in vitro. To demonstrate the presence of internal control regions for transcription of eukaryotic tRNA genes, deletions were constructed extending from the 5' and 3' flanking regions into the tRNA coding region (21,23). Although similar trnR1 and trnM2 deletion mutants were tested, we would not have detected truncated tRNA molecules since they are rapidly degraded in the chloroplast extract. We can not exclude, however that the deletion mutants are no longer transcribed. Experiments are currently underway to introduce base substitutions that change the sequence of the trnR1 coding region, but do not alter the secondary structure of the tRNA molecule.

In the dicistronic trnS1-trnM2-51 locus trnS1 does not sup-

port the transcription of trnM2. It is most likely, therefore that termination of trnS1 transcription occurs within the remaining 20 bp of the 3' DNA sequences. Again, this is consistent with results reported for eukaryotic tRNA genes, in which T-rich 3' DNA regions can serve as termination signals for RNA polymerase III (21). Both trnR1 and trnS1 coding regions are immediately followed by T-rich DNA sequences, whereas such region can not be detected for trnM2. Transcription of trnM2 proceeds through an inverted repeat sequence, which may serve as processing signal and which can be detected in vitro (1). Moreover, analysis of run-on transcripts from the spinach atpB/E-trnM2 region indicates that transcription from the trnM2 gene may even continue into the non-coding strand of the atpB/E region (X.W. Deng and W. Gruissem, manuscript in preparation). Since little is known about transcription termination in higher plant chloroplasts, we can at best speculate about the requirements for the chloroplast RNA polymerase to terminate transcription of the three different tRNA genes.

In tobacco, only one trnR1 (ACG) has been found that most likely can read all four Arg codons (18). Although the total number of trnR genes in the spinach chloroplast genome is unknown, the similarity of the two genomes and the efficient transcription of this gene in the chloroplast extract would exclude the formal possibility that trnR1 is a pseudogene. Similarly, the codon recognized by $tRNA^{Ser}$ (UGA) is frequently used for the translation of chloroplast proteins. Thus, both trnR1 and trnS1 appear to be actively expressed genes in spinach chloroplast. This notion is supported by our results that both genes compete with trnM2 for common transcription factors and/or RNA polymerase in the in vitro transcription reaction.

Based on the present evidence we therefore conclude that trnS1 and trnR1 do not require 5' promoter regions for their transcription in vitro. Although the promoter regions for these genes remains to be elucidated, it appears that a prokaryote-type promoter structure can not be generalized for chloroplast genes.

ACKNOWLEDGEMENTS

We thank Drs. Warwick Bottomley and Paul Whitfeld for the DNA sequence information of the trnR1 gene, and Dr. Masahiro Sugiura

for providing the sequence of the tobacco chloroplast genome. This work was supported by a grant from the U.S. Department of Energy (85ER13375).

*To whom correspondence should be addressed

REFERENCES

1. Gruissem, W., Greenberg, B.M., Zurawski, G., Prescott, D.M. and Hallick, R.B. (1983) Cell **35**, 815-828.
2. Gruissem, W., Narita, J.O., Greenberg, B.M., Prescott, D.M. and Hallick, R.B. (1983) J. Cell. Biochem. **22**, 31-46.
3. Link, G. (1984) EMBO J. **3**, 1697-1704.
4. Orozco, E.M., Mullet, J.E. and Chua, N.H. (1985) Nucleic Acids Res. **13**, 1283-1302.
5. Bohnert, H.F., Crouse, E.J. and Schmitt, J.M. (1982) In Parthier, B. and Boulter, D. (eds.), Encyclopedia of Plant Physiology, Springer Verlag, Berlin, Vol. **14B**, pp. 475-530.
6. Whitfeld, P.R. and Bottomley, W. (1983) Annu. Rev. Plant Physiol. **34**, 279-310.
7. Gruissem, W. and Zurawski, G. (1985) EMBO J. **4**, 1637-1644.
8. Gruissem, W. and Zurawski, G. (1985) EMBO J. **4**, 3375-3383.
9. Hohlschuh, K., Bottomley, W. and Whitfeld, P.R. (1984) Nucleic Acids Res. **12**, 8819-8834.
10. Zagursky, R.J., Baumeister, K., Lomax, N. and Berman, M.L. (1985) Gene Anal. Techn. **2**, 89-94.
11. Gruissem, W., Greenberg, B.M., Zurawski, G. and Hallick, R.B. (1986) Methods Enzymol. **118**, 815-828.
12. Gruissem, W. and Zurawski, G. (1985) In van Vloten-Doting, L. Groot, G.S.P. and Hall, T.C. (eds.), Molecular Form and Function of the Plant Genome, Plenum Publishing Company, New York pp. 199-210.
13. Cozens, A.L., Walker, J.E., Phillips, A.L., Huttly, A.K. and Gray, J.C. (1986) EMBO J. **5**, 217-222.
14. Greenberg, B.M., Gruissem, W. and Hallick, R.B. (1984) Plant Mol. Biol. **3**, 97-109.
15. Greenberg, B.M. and Hallick, R.B. (1986) Plant Mol. Biol. **6**, 89-100.
16. Ohme, M., Kamogashira, T., Shinozaki, K. and Sugiura, M. (1985) Nucleic Acids Res. **13**, 1045-1056.
17. Greenberg, B.M., Narita, J.O., DeLuca-Flaherty, C., Gruissem, W., Rushlow, K.A. and Hallick, R.B. (1984) J. Biol. Chem. **259** 14880-14887.
18. Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J. Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. and Sugiura, M (1986) EMBO J., in press.
19. Kung, S.D. and Lin, C.M. (1985) Nucleic Acids Res. **13**, 7543-7549.
20. Hanley-Bowdin, L., Orozco, E.M. and Chua, N.H. (1985) Mol. Cell. Biol. **5**, 2733-2745.

21. Galli, G., Hofstetter, H. and Birnstiel, M.L. (1981) Nature **294**, 626-631.
22. Gruissem, W., Prescott, D.M., Greenberg, B.M. and Hallick, R. B. (1982) Cell **30**, 81-92.
23. Sharp, S., DeFranco, D., Dingermann, T., Farrell, P. and Soll D. (1981) Proc. Natl. Acad. Sci. USA **78**, 6657-6661.