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Silkworm 5S RNA and Alanine tRNA Genes Share Highly Conserved 5′ Flanking and Coding Sequences

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A fragment of *Bombyx mori* genomic DNA containing one $tRNA_2^{Ala}$ gene and one 5S RNA gene has been used to compare the structural features of silkworm 5S RNA and tRNA genes. The nucleotide sequences of both genes and of the primary transcripts produced from them in homologous in vitro transcription systems have been determined. Comparison of the sequences of these two genes with that of another previously analyzed *B. mori* $tRNA_2^{Ala}$ gene reveals common oligonucleotides which may be important transcriptional signals. The oligonucleotides TA(C)TAT, AATTTT, and TTC are located approximately (±1 nucleotide) 29, 19, and 3 nucleotides, respectively, before the transcription initiation sites of the two $tRNA_2^{Ala}$ genes and the one 5S RNA gene we have analyzed. The sequence GGGCGTAG(C)TCAG lies within the coding regions of all three genes. The functional significance of these sequences is suggested by their location within regions required for the transcription of silkworm alanine tRNA genes in vitro.

The identification of DNA sequences that control the initiation of transcription is important for understanding the mechanisms governing gene activity. In procaryotes, conserved sequences upstream from genes interact directly with RNA polymerase to promote transcription (34, 40). Eucaryotic genes transcribed by RNA polymerase II are also preceded by conserved sequences whose exact role is not known but whose effect on the activity of these templates is significant (7). In contrast, the sequences upstream from eucaryotic genes transcribed by RNA polymerase III are less conserved, and at least some control regions for these genes lie downstream from transcription initiation sites.

Specifically, DNA sequences controlling transcription of 5S RNA genes (6, 36), tRNA genes (10, 21, 25, 29, 39), an adenovirus VAI RNA gene (18, 23), and human *Alu* family sequences (19) have been shown to lie within the coding regions. For these genes, normal 5′ flanking DNA can be replaced by heterologous DNA sequences without abolishing transcriptional activity in several in vitro systems. Such alterations are not entirely without effect, however. The 5′ flanking DNA sequences can influence both the transcriptional efficiency of certain templates and the selection of particular transcription initiation points (11, 13, 14, 18, 46). In at least one case, dependence on normal 5′ DNA is extreme. *Bombyx mori* $tRNA_2^{Ala}$ genes are completely inactive in homologous transcription extracts when sequences upstream from the transcription start point are replaced by unrelated DNA (44). These results indicate that signals controlling transcription by RNA polymerase III may lie upstream from genes as well as inside them.

Although distinct transcription factors may be involved in controlling the activity of 5S RNA and tRNA genes (16, 46), both classes of genes are transcribed by RNA polymerase III, and both may also require certain common transcription factors (37). We wish to compare transcriptional control regions in silkworm 5S RNA and tRNA genes to learn whether some signals are common to all genes transcribed by RNA polymerase III, whereas others are specific for 5S RNA or tRNA genes. For example, signals recognized by RNA polymerase III itself, rather than by gene-specific transcription factors, may be conserved among both 5S and tRNA genes.

In this paper, we compare two $tRNA_2^{Ala}$ genes and one 5S RNA gene isolated from the *B. mori* genome. To look for common DNA sequences that could be involved in transcriptional control, we have characterized the primary in vitro transcription products of these genes and have determined the DNA sequences corresponding to both coding and flanking regions. We find striking sequence homologies both within the coding regions and in the DNA preceding the transcription initiation sites of all three genes.

MATERIALS AND METHODS

Isolation of a *B. mori* genomic DNA fragment containing $tRNA^{Ala}$ and 5S RNA genes. The pBm13 *Eco*RI fragment of *B. mori* genomic DNA was originally cloned in bacteriophage λgtB as described earlier (24) and was then subcloned into the bacterial plasmid

pBR322 by standard procedures. DNA fragments were joined with T4 polynucleotide ligase (New England Biolabs; 15) and were inserted into *Escherichia coli* RRI cells by transformation (12). Bacterial colonies with $tRNA_2^{Ala}$ and 5S gene-containing plasmids were detected by colony hybridization (2) to ^{32}P-labeled $tRNA_2^{Ala}$ (24) or 5S RNA.

Restriction mapping and DNA sequence determination. The locations of $tRNA^{Ala}$ and 5S RNA genes in *B. mori* DNA fragments were established by restriction mapping of unlabeled and end-labeled DNA, and by hybridization (42) with ^{32}P-labeled $tRNA^{Ala}$ or 5S RNA. Partial restriction endonuclease cleavage (41) of the entire 9.5-kilobase *Eco*RI fragment was used to determine the orientation of each gene. These locations and orientations were confirmed by determination of appropriate DNA sequences.

DNA fragments for sequence analysis were 5'-terminally labeled with [γ-^{32}P]ATP and polynucleotide kinase (P-L Biochemicals; 3, 30) or were 3'-terminally labeled with [α-^{32}P]CTP (ICN Pharmaceuticals, Inc.) and terminal deoxynucleotidyltransferase (Bethesda Research Laboratories; 35) or α-^{32}P-labeled deoxynucleoside triphosphates ([α-^{32}P]dXTPs) (New England Nuclear Corp. Schwarz/Mann) and the Klenow fragment of DNA polymerase (Boehringer Mannheim Corp.; 9). Sequencing was performed by partial chemical cleavage (30). Both strands of DNA were analyzed in all cases. DNA sequences were compared for homologous regions with the SEQ computer program (8) through the Stanford MOLGEN project and the National Institutes of Health SUMEX-AIM facility.

Analysis of in vitro transcription products. *B. mori* transcription extracts were prepared and used as described earlier (44). In vitro transcription with *Xenopus laevis* germinal vesicle lysates was carried out as previously described (4). α-^{32}P-labeled nucleoside triphosphates were obtained from ICN, Schwarz/Mann, or New England Nuclear Corp. Transcription products were fractionated on 10% polyacrylamide gels in the presence of 7 M urea (43). Nucleotide sequence analysis of internally labeled RNAs was carried out by standard procedures (1). Oligonucleotides generated by digestion with T_1 RNase (Sankyo) were fractionated two-dimensionally by electrophoresis at pH 3.5 (first dimension) and by homochromatography on polyethyleneimine thin-layer plates (second dimension) (33). The resulting oligonucleotides were identified by secondary digestion with pancreatic RNase. Further analysis of secondary digestion products was done by cleavage with RNase T_2 and fractionation of nucleotides by chromatography on cellulose thin-layer plates (Analtech, Inc.) in isopropanol-HCl or isobutyric acid-NH_4OH solvents (31). The 5'-terminal nucleotides of transcripts were produced by complete digestion with a mixture of T_2 (125 U/ml), T_1 (0.2 mg/ml), and pancreatic (0.2 mg/ml) RNases for 3 h at 37°C. They were separated from internal mononucleotides by electrophoresis on DEAE paper at pH 3.5. Quantitation was done by scintillation counting of appropriate regions of DEAE paper after localization of mononucleotides by autoradiography. Nucleoside tetraphosphates were cleaved with P_1 nuclease (20) to yield the corresponding nucleoside 5'-triphosphates plus P_i. These products were analyzed by chromatography on polyethyleneimine thin-layer plates in 1 M KH_2PO_4 (pH 3.5) in parallel with marker nucleotides.

RESULTS

Transcription of pBm13 DNA in vitro. To study both tRNA and 5S RNA genes from silkworms, we examined a cloned *B. mori* genomic fragment (pBm13) which contains one $tRNA_2^{Ala}$ gene and one 5S RNA gene. Detailed restriction mapping of this fragment combined with hybridization of DNA fragments to radioactive $tRNA^{Ala}$ or 5S RNA (42) established the structure diagrammed in Fig. 1 (data not shown). The two genes are separated by 4.3 kilobases and are oriented in opposite directions within a 9.5-kilobase restriction fragment. We have determined the sequence of 225 base pairs of DNA containing the tRNA gene and 275 base pairs of DNA containing the 5S RNA gene. This analysis shows that the alanine tRNA gene on pBm13 encodes $tRNA_2^{Ala}$ (43)—an abundant tRNA species in a variety of silkworm cells in vivo. Like the pBm11 $tRNA_2^{Ala}$ gene previously analyzed (24), this tRNA gene does not contain any intervening sequences and does not encode the 3'-terminal CCA of the tRNA.

The 5S RNA gene on pBm13 differs in 5 of 120 positions from the reported sequence of *B. mori* 5S RNA found in vivo (28, 45). However, silkworms, like other organisms (17, 38), probably have multiple forms of 5S RNA genes. At least three different *B. mori* 5S RNAs synthesized in vivo can be separated on highly resolving gels.

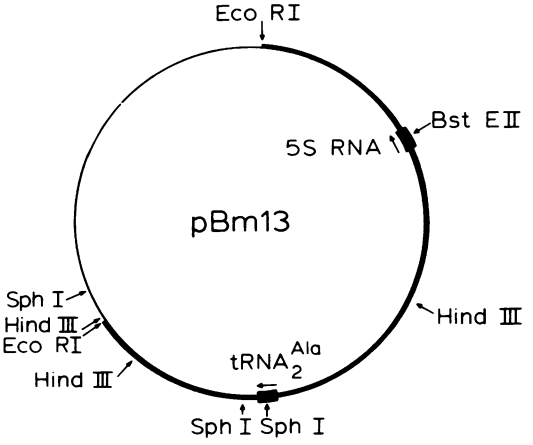

FIG. 1. Restriction map of pBm13 DNA. pBm13 contains a 9.5-kilobase *Eco*RI fragment of *B. mori* genomic DNA (heavy line) inserted into the *Eco*RI site of the plasmid vector, pBR322 (thin line). The map was determined by cleavage of pBm13 DNA with a variety of restriction endonucleases, followed by hybridization of resulting DNA fragments to ^{32}P-labeled $tRNA^{Ala}$ or 5S RNA. Partial restriction endonuclease cleavage of end-labeled DNA (41) was used to determine the orientation of each gene. The arrows next to the 5S RNA and alanine tRNA genes indicate the direction in which each gene is transcribed.

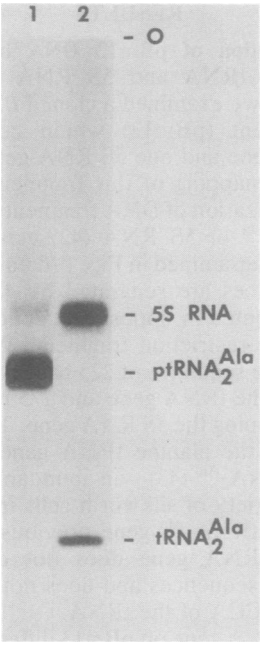

FIG. 2. In vitro transcription of pBm13 DNA. RNA was labeled with [α-^{32}P]GTP during transcription of pBm13 DNA in vitro. Samples were run on a 10% polyacrylamide gel containing 7 M urea. Lane 1, RNA transcripts produced from pBm13 DNA in *B. mori* ovary extracts. Lane 2, RNA transcripts produced from pBm13 DNA in *X. laevis* germinal vesicle lysates. Positions of 5S RNA, precursor tRNA$_2^{Ala}$ (ptRNA$_2^{Ala}$), and mature tRNA$_2^{Ala}$ are indicated. Heterogeneity in size of both ptRNA^{Ala} and 5S RNA transcripts is responsible for the width of the RNA bands on the gel and is due to transcription termination at multiple sites.

Fingerprint analysis of these RNAs indicates that the major species has the sequence reported by Komiya et al. (28), whereas one of the other forms (present in vivo at about one-fourth the level of the major species) is encoded by the pBm13 5S RNA gene (K. Sprague, unpublished data). Thus, the 5S RNA gene we have studied belongs to a class which is active in vivo.

To assay the transcriptional activity of cloned 5S RNA and tRNA genes in vitro, we have used extracts made from *B. mori* pupal ovaries (44) and from *X. laevis* germinal vesicles (4). Figure 2 shows that when pBm13 is used as a template in either kind of extract, RNAs resembling putative alanine tRNA and 5S RNA are synthesized. Most of the tRNA transcripts are converted to mature length in *X. laevis* extracts but accumulate as precursors in *B. mori* extracts. Structural analysis established, however, that both extracts synthesize identical tRNA precursors and process them correctly (data not shown). The 5S RNAs synthesized by these extracts are also indistinguishable from each other. The difference in yield of 5S RNA compared with alanine tRNA transcripts is reproducible, and we are examining the basis for this phenomenon.

Partial sequence determination of each of the transcripts from pBm13 was undertaken with two aims. We wished first to learn whether the RNAs tentatively characterized on the basis of electrophoretic mobility as alanine tRNA and 5S RNA were in fact the products of the pBm13 genes identified by DNA sequence analysis. Second, we wished to determine the sites of transcription initiation and termination for each gene, information which defines the transcription unit and permits alignment of genes with respect to their transcription initiation sites. It thus allows comparison of sequences having a common spatial relationship to these sites. RNA sequence analysis confirmed that the 5S RNA-sized molecule is the primary transcript of the pBm13 5S RNA gene and that the putative precursor tRNA is a 93-nucleotide primary transcription product of the tRNA$_2^{Ala}$ gene. The oligonucleotides resulting from digestion of each transcript of RNase T_1 coincide with those predicted from the gene sequences (Fig. 3 and 4; Table 1).

Transcription initiation sites. For precise identification of the sites at which the transcription of tRNA and 5S RNA genes initiates, the 5′ termini of the corresponding transcripts were examined in detail. Fractionation and quantitation of the products obtained by digesting precursor tRNA and 5S RNA to mononucleotides indicated that both molecules possess triphosphorylated 5′ ends characteristic of primary transcripts. Nearest-neighbor analysis of nucleoside tetraphosphates derived from transcripts labeled with each of the four [α-^{32}P]XTPs identified with the first two nucleotides in each RNA. We conclude that more than 90% of the tRNA and 5S RNA transcripts begin with pppApAp and pppGpCp, respectively. Oligonucleotides containing these 5′-terminal nucleotides have been identified in T_1 RNase (Fig. 3) and pancreatic RNase (not shown) digests of both RNAs. Alignment of RNA sequences with the corresponding DNA sequences shows that transcription of the tRNA$_2^{Ala}$ gene is initiated three nucleotides upstream from the mature coding sequence and that transcription of the 5S RNA gene begins at the nucleotide corresponding to the 5′ end of mature 5S RNA. These initiation sites are typical for genes transcribed by RNA polymerase III in that initiation occurs at a purine preceded by a pyrimidine residue.

Transcription termination sites. Sequence analysis of RNA oligonucleotides and comparison with the corresponding gene sequences established the site of transcription termination of

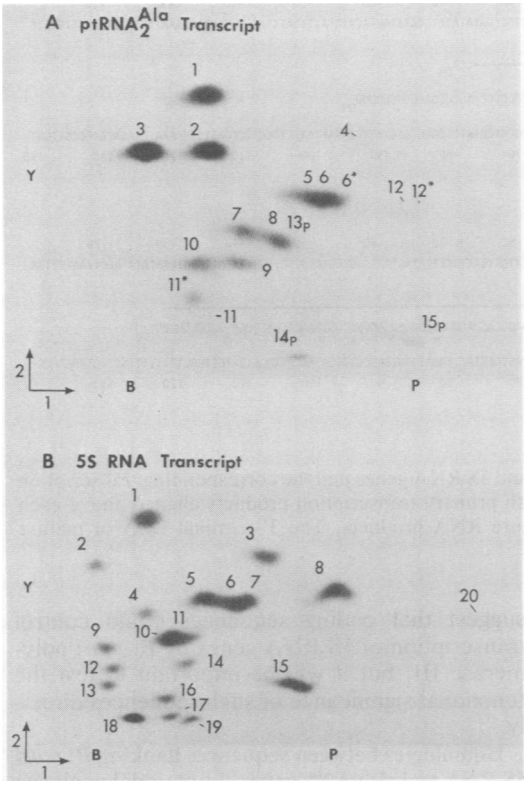

FIG. 3. T_1 RNase fingerprints of the primary $tRNA_2^{Ala}$ transcript (A) and primary 5S RNA transcript (B) synthesized from pBm13 DNA in *B. mori* ovary extracts. T_1 oligonucleotides labeled by [α-^{32}P]GTP were fractionated two-dimensionally (see text). B, P, and Y mark the positions of blue, pink, and yellow tracking dyes. The identities of numbered oligonucleotides are given in Table 1. P indicates an oligonucleotide derived from the extra sequences unique to the primary transcript of the tRNA gene. Oligonucleotide 15p is from the 5′ end; 13p and 14p are from the 3′ end. Asterisks (*) denote modified oligonucleotides which also appear in unmodified form.

each gene. When these genes are transcribed with low ($\leq$25 μM) concentrations of UTP, termination at different positions generates oligonucleotides with variable numbers of 3′-terminal U residues, each in less than full molar yield (data not shown). It is unlikely that this length heterogeneity arises posttranscriptionally, since the number of U residues is influenced by the concentration of UTP (and not other XTPs) in the transcription reaction (24) but not by reincubation of isolated transcripts in *B. mori* transcription extracts (data not shown). Thus, termination of both transcripts occurs in clusters of T residues on the noncoding DNA strand. The $tRNA_2^{Ala}$ termination site is a sequence of six consecutive T's lying 13 base pairs downstream from the mature coding sequence whereas the 5S RNA site is a stretch of eight T's immediately following the 5S RNA gene (Fig. 4).

Having determined the points at which transcription of $tRNA_2^{Ala}$ and 5S RNA genes starts and stops, we can now compare equivalent regions of the two kinds of genes. Below we discuss the possible significance of sequence homologies revealed by such a comparison.

DISCUSSION

Homologies between *B. mori* 5S RNA and $tRNA^{Ala}$ coding sequences. If common sequence signals are recognized by a component of the transcriptional apparatus that participates in the synthesis of both tRNA and 5S RNA, these sequences should appear as homologies when the structures of the corresponding genes are compared. One such signal is the oligothymidylate sequence which terminates transcription in the genes we have analyzed, as well as in others that are transcribed by RNA polymerase III (5).

Our search for additional homologies between *B. mori* 5S RNA and tRNA genes has revealed the common sequence GGGCGTAG(C)TCAG within the coding regions of both kinds of genes. This sequence occurs at nucleotide positions 6 to 18 (relative to the transcription initiation site) in the alanine tRNA genes and at nucleotide positions 64 to 75 in the pBm13 5S RNA gene (Fig. 5). The sequence is similar to parts of internal control regions that have been functionally defined for tRNA genes (10, 21, 25, 39) and a VAI RNA gene (18, 23) in other organisms. It is partially homologous (~75%) to sequences within *B. mori* glycine (22, 26, 47) and phenylalanine (27) tRNAs—the only other silkworm tRNAs for which complete structural information is available. Moreover, there is direct evidence that this region of *B. mori* alanine tRNA genes is functionally important. Deletion analysis of the pBm11 $tRNA_2^{Ala}$ gene shows that although altering much of the coding sequence has little effect, replacing this particular region by nonhomologous sequences drastically reduces transcriptional efficiency (D. Larson, J. S. Wilcox, L. S. Young, and K. U. Sprague, manuscript in preparation).

Do coding sequences also influence the transcription of 5S RNA genes by *B. mori* RNA polymerase III? We do not yet know the answer to this question, but the activity of *B. mori* 5S RNA genes in *X. laevis* extracts (Fig. 2) indicates that these genes are recognized by components of the *X. laevis* RNA polymerase III transcription complex. One of these transcription factors is known to bind to a control site within the coding region of *Xenopus borealis* 5S RNA genes (16). Comparison of the *B. mori* 5S

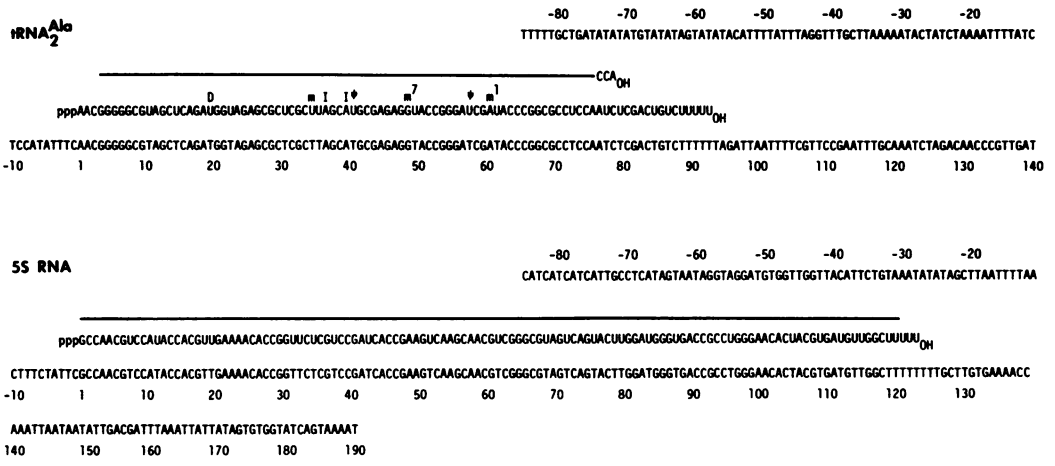

FIG. 4. Nucleotide sequences of the pBm13 $tRNA_2^{Ala}$ and 5S RNA genes and the corresponding transcription products. The noncoding strand of the DNA is shown with primary transcription products aligned above each gene. The solid lines represent the sequence of the mature RNA products. The 3'-terminal CCA of mature $tRNA_2^{Ala}$ is added posttranscriptionally.

RNA gene sequence with the *X. borealis* Xbs1 5S RNA gene (32) shows that 28 of the 34 nucleotides (82%) in the *X. borealis* transcriptional control region (6, 36) are also present in the corresponding part of the *B. mori* 5S RNA gene. The GGGCGTAGTCAG sequence that is shared by *B. mori* 5S RNA and $tRNA_2^{Ala}$ genes is included in this region. These homologies suggest that coding sequences could control transcription of 5S RNA genes by *B. mori* polymerase III, but it will be important to test the functional significance of such sequences directly.

Homologies between sequences flanking *B. mori* 5S RNA and $tRNA^{Ala'}$ genes. Functional analyses indicate that transcriptional signals also lie up-

TABLE 1. Oligonucleotides produced by T_1 RNase digestion of G-labeled primary transcripts from the pBm13 $tRNA_2^{Ala}$ and 5S RNA genes[a]

Precursor $tRNA_2^{Ala}$		5S RNA	
Fingerprint spot	Sequences of oligonucleotides	Fingerprint spot	Sequences of oligonucleotides
1	Gp	1	Gp
2	AGp	2	CGp
3	CGp	3	UGp
4	UGp	4	AAGp
5	UAGp	5	UCGp
6	AUGp	6	UAGp
6*	ADGp	7	AUGp
7	CUUGp	8	UUGp
8	$A^{\psi}_{U}CGp + CA^{\psi}_{U}$	9	ACCGp
9	CUCAGp	10	CCUGp + UCCGp
10	m^7GUACCGp	11	UCAGp
11	AUACCCGp	12	CAACGp
11*	m^1AUACCCGp	13	CCAACGp
12	CUUIp	14	UCAAGp
12*	CUmUIp	15	UACUUGp + UUCUCGp
13p	ACUGp	16	AUCACCGp
14p	CCUCCAAUCUCGp	17	AACACUACGp
15p	pppAACGp	18	AAAACACCGp
		19	UCCAUACCACGp
		20	pppGp

[a] Fingerprint spot numbers are keyed to Fig. 3. Oligonucleotide sequences were verified by secondary digestion of T_1 oligonucleotides with pancreatic RNase as previously described (24). Further analysis of secondary digestion products was done by digestion with T_2 RNase as described in the text. Asterisks (*) indicate modified oligonucleotides which also appear in unmodified form in the $tRNA_2^{Ala}$ transcript.

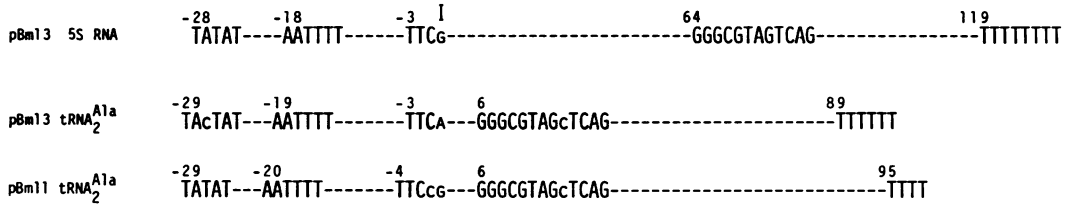

FIG. 5. DNA sequences common to *B. mori* 5S RNA and $tRNA_2^{Ala}$ genes. Common oligonucleotide sequences on the noncoding DNA strands of a *B. mori* 5S RNA gene and two *B. mori* $tRNA_2^{Ala}$ genes are shown. Nucleotide positions are numbered relative to the transcription initiation site (I) for each gene.

stream from *B. mori* tRNA genes. One *B. mori* alanine tRNA gene (pBm11) has been shown to require normal 5′ flanking sequences for activity in homologous in vitro transcription systems (44). The transcriptional properties of partially deleted derivations of this gene have placed the upstream limit of the crucial region between nucleotides 34 and 11 preceding the transcription initiation site (Larson et al., manuscript in preparation). Comparisons between the pBm11 and the pBm13 tRNA and 5S RNA genes suggest that specific sequences within this region are likely to be particularly important. Examination of extensive regions flanking both sides of these genes revealed significant homologies only in the 5′ flanking regions (excluding the oligothymidylate terminators). Strikingly, these homologies occur within the 34 nucleotides upstream from each transcription initiation site. As shown in Fig. 5, the sequences TA(C)TAT, AATTTT, and TTC are common to all three genes and are located at positions -29 ± 1, -19 ± 1, and -3 ± 1 nucleotides, respectively.

The TTC sequence cannot itself be the entire control signal, since this sequence is retained in a transcriptionally silent $tRNA_2^{Ala}$ gene truncated at position −11 (44). Attention is therefore focused on the conserved oligonucleotides TA(C)TAT and AATTTT as possible parts of an essential control region. These two oligonucleotides are not common to the 5′ flanking DNA or tRNA and 5S RNA genes in other organisms. Thus, if these sequences are involved in controlling gene transcription, they are likely to be needed specifically for recognition by *B. mori* RNA polymerase III (or associated factors). Since a 5′-truncated *B. mori* $tRNA_2^{Ala}$ gene which is inactive in *B. mori* transcription extracts can still serve as a template for efficient transcription in *X. laevis* extracts (44), it appears that the *B. mori* RNA polymerase III transcription apparatus is more highly dependent upon specific 5′ flanking sequence signals than is the *X. laevis* RNA polymerase III complex. Genes from a variety of organisms can be transcribed by extracts from *X. laevis* germinal vesicles. We have tested several of these genes, including *Drosophila melanogaster* $tRNA^{Lys}$ genes (13) and a VAI RNA gene (18, 23), and find that they are either completely inactive or very poorly transcribed in *B. mori* extracts (D. G. Morton, unpublished data). The inability of these genes to serve as templates for transcription in *B. mori* extracts may reflect the absence of appropriate 5′ flanking sequence signals. In fact, all of them lack at least two of the three conserved oligonucleotides that precede *B. mori* genes.

Although 5′ flanking sequences have been found to modulate the efficiency and position of transcription initiation by *Xenopus* spp. and *D. melanogaster* RNA polymerase III (13, 14, 46), the effect of 5′ flanking DNA in governing tRNA gene transcription by *B. mori* RNA polymerase III is much more pronounced (44). The first 34 flanking nucleotides includes sequences required for activity of an alanine tRNA gene in vitro (Larson et al., manuscript in preparation). Since the conserved oligonucleotides that we have identified lie within this region, they are likely to contribute to the control of tRNA gene transcription. To determine whether sequences in this region also have a more general function in controlling transcription of other kinds of polymerase III templates, we are constructing mutant derivatives of *B. mori* 5S RNA genes lacking all or part of them.

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LITERATURE CITED

1. **Barrell, B. G.** 1971. Fractionation and sequence analysis of radioactive nucleotides. Proc. Nucleic Acids Res. 2:751–779.

2. **Beckman, J. S., P. F. Johnson, J. Abelson, and S. A. Fuhrman.** 1977. Isolation and characterization of *Escherichia coli* clones containing genes for the stable yeast RNA species. ICN-UCLA Symp. Mol. Cell. Biol. **8**:213–226.
3. **Berkner, K. L., and W. R. Folk.** 1977. Polynucleotide kinase exchange reaction: quantitative assay for restriction endonuclease-generated 5′-phosphate termini in DNAs. J. Biol. Chem. **252**:3176–3184.
4. **Birkenmeier, E. H., D. D. Brown, and E. Jordan.** 1978. A nuclear extract of *Xenopus laevis* oocytes that accurately transcribes 5S RNA genes. Cell **15**:1077–1086.
5. **Bogenhagen, D. F., and D. D. Brown.** 1981. Nucleotide sequences in *Xenopus* 5S DNA required for transcription termination. Cell **24**:261–270.
6. **Bogenhagen, D. F., S. Sakonju, and D. D. Brown.** 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription. II. The 3′ border of the region. Cell **19**:27–35.
7. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eukaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
8. **Brutlag, D. L., V. Clayton, P. Friedland, and L. H. Kedes.** 1982. SEQ: a nucleotide sequence analysis and recombination system. Nucleic Acids Res. **10**:279–294.
9. **Challberg, M. D., and P. T. Englund.** 1980. Specific labeling of 3′ termini with T4 DNA polymerase. Methods Enzymol. **65**:39–42.
10. **Ciliberto, G., L. Castagnoli, D. A. Melton, and R. Cortese.** 1982. Promoter of a eukaryotic $tRNA^{Pro}$ gene is composed of three noncontiguous regions. Proc. Natl. Acad. Sci. U.S.A. **79**:1195–1199.
11. **Clarkson, S. G., R. A. Koski, V. Corlet, and R. A. Hipskind.** 1981. Influence of 5′ flanking sequences on tRNA transcription *in vitro*. ICN-UCLA Symp. Mol. Cell. Biol. **23**:463–472.
12. **Dagert, M., and S. D. Ehrlich.** 1979. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene (Amsterdam) **6**:23–28.
13. **DeFranco, D., O. Schmidt, and D. Söll.** 1980. Two control regions for eukaryotic tRNA gene transcription. Proc. Natl. Acad. Sci. U.S.A. **77**:3365–3368.
14. **DeFranco, D., S. Sharp, and D. Söll.** 1981. Identification of regulatory sequences contained in the 5′ flanking region of *Drosophila* lysine $tRNA_2$ genes. J. Biol. Chem. **256**:12424–12429.
15. **Dugaiczyk, A., H. W. Boyer, and H. M. Goodman.** 1975. Ligation of *Eco*R1 endonuclease-generated DNA fragments into linear and circular structures. J. Mol. Biol. **96**:171–184.
16. **Engelke, D. R., S.-Y. Ng, B. S. Shastry, and R. G. Roeder.** 1980. Specific interaction of a purified transcription factor with an internal control region of 5S RNA genes. Cell **19**:717–728.
17. **Ford, P. J., and R. D. Brown.** 1976. Sequences of 5S ribosomal RNA from *Xenopus mulleri*, and the evolution of 5S gene-coding sequences. Cell **8**:485–493.
18. **Fowlkes, D. M., and T. Shenk.** 1980. Transcriptional control regions of the adenovirus VAI RNA gene. Cell **22**:405–413.
19. **Fuhrman, S. A., P. L. Deininger, P. LaPorte, T. Friedmann, and E. P. Geiduschek.** 1981. Analysis of transcription of the human Alu family ubiquitous repeating element by eukaryotic RNA polymerase III. Nucleic Acids Res. **9**:6439–6456.
20. **Fujimoto, M., A. Kuninaka, and H. Yoshino.** 1974. Substrate specificity of nuclease P_1. Agric. Biol. Chem. **38**:1555–1561.
21. **Galli, F., H. Hofstetter, and M. L. Birnstiel.** 1981. Two conserved sequence blocks within eukaryotic tRNA genes are major promoter elements. Nature (London) **294**:626–631.
22. **Garel, J. P., and G. Keith.** 1977. Nucleotide sequence of *Bombyx mori* L. $tRNA_1^{Gly}$. Nature (London) **269**:350–352.
23. **Guilfoyle, R., and R. Weinmann.** 1981. Control region for adenovirus VA RNA transcription. Proc. Natl. Acad. Sci. U.S.A. **78**:3378–3382.
24. **Hagenbüchle, O., D. Larson, G. I. Hall, and K. U. Sprague.** 1979. The primary transcription product of a silkworm alanine tRNA gene: identification of *in vitro* sites of initiation, termination and processing. Cell **18**:1217–1229.
25. **Hofstetter, H., A. Kressman, and M. L. Birnstiel.** 1981. A split promoter for a eukaryotic tRNA gene. Cell **24**:573–585.
26. **Kawakami, M., K. Nishio, and S. Takemura.** 1978. Nucleotide sequence of $tRNA_2^{Gly}$ from the posterior silk glands of *Bombyx mori*. FEBS Lett. **87**:288–290.
27. **Keith, G., and G. Dirheimer.** 1980. Primary structure of *Bombyx mori* posterior silkgland $tRNA^{Phe}$. Biochem. Biophys. Res. Commun. **92**:109–115.
28. **Komiya, H., M. Kawakami, and S. Takemura.** 1981. Nucleotide sequence of 5S ribosomal RNA from the posterior silk glands of *Bombyx mori*. J. Biochem. (Tokyo) **89**:717–722.
29. **Koski, R. A., S. G. Clarkson, J. Kurjan, B. D. Hall, and M. Smith.** 1980. Mutations at the yeast *sup*4 $tRNA^{Tyr}$ locus: transcription of the mutant genes *in vitro*. Cell **22**:415–425.
30. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
31. **Nishimura, S.** 1972. Minor components in transfer RNA: their characterization, location, and function. Prog. Nucleic Acid Res. Mol. Biol. **12**:49–85.
32. **Peterson, R. C., J. L. Doering, and D. D. Brown.** 1980. Characterization of two *Xenopus* somatic 5S DNAs and one minor oocyte-specific 5S DNA. Cell **20**:131–141.
33. **Platt, T., and C. Yanofsky.** 1975. An intercistronic region and ribosome-binding site in bacterial messenger RNA. Proc. Natl. Acad. Sci. U.S.A. **72**:2399–2403.
34. **Rosenberg, M., and D. Court.** 1980. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
35. **Roychoudhury, R., and R. Wu.** 1980. Terminal transferase-catalyzed addition of nucleotides to the 3′ termini of DNA. Methods Enzymol. **65**:43–62.
36. **Sakonju, S., D. F. Bogenhagen, and D. D. Brown.** 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription. I. The 5′ border of the region. Cell **19**:13–25.
37. **Segall, J., T. Matsui, and R. G. Roeder.** 1980. Multiple factors are required for the accurate transcription of purified genes by RNA polymerase III. J. Biol. Chem. **255**:11986–11991.
38. **Selker, E. U., C. Yanofsky, K. Driftmier, R. L. Metzenberg, B. Alzner-DeWeerd, and U. L. RajBandary.** 1981. Dispersed 5S RNA genes in *N. crassa*: structure, expression and evolution. Cell **24**:819–828.
39. **Sharp, S., D. DeFranco, T. Dingermann, P. Farrell, and D. Söll.** 1981. Internal control regions for transcription of eukaryotic tRNA genes. Proc. Natl. Acad. Sci. U.S.A. **78**:6657–6661.
40. **Siebenlist, U., R. B. Simpson, and W. Gilbert.** 1980. *E. coli* RNA polymerase interacts homologously with two different promoters. Cell **20**:269–281.
41. **Smith, H. O., and M. L. Birnstiel.** 1976. A simple method for DNA restriction site mapping. Nucleic Acids Res. **3**:2387–2399.
42. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by electrophoresis. J. Mol. Biol. **98**:503–517.
43. **Sprague, K. U., O. Hagenbüchle, and M. C. Zuniga.** 1977. The nucleotide sequence of two silk gland alanine tRNAs: implications for fibroin synthesis and initiator tRNA structure. Cell **11**:561–570.
44. **Sprague, K. U., D. Larson, and D. Morton.** 1980. 5′ flanking sequence signals are requried for activity of silkworm alanine tRNA genes in homologous *in vitro*

transcription systems. Cell 22:171–178.
45. **Troutt, A., T. J. Savin, W. C. Curtiss, J. Celentano, and J. N. Vournakis.** 1982. Secondary structure of *Bombyx mori* and *Dictyostelium discoideum* 5S rRNA from S1 nuclease and cobra venom ribonuclease susceptibility, and computer assisted analysis. Nucleic Acids Res. 10:653–664.
46. **Wormington, W. M., D. F. Bogenhagen, E. Jordan, and D. D. Brown.** 1981. A quantitative assay for *Xenopus* 5S RNA gene transcription *in vitro*. Cell 24:809–817.
47. **Zúñiga, M. C., and J. A. Steitz.** 1977. The nucleotide sequence of a major glycine transfer RNA from the posterior silk gland of *Bombyx mori* L. Nucleic Acids Res. 4:4175–4196.