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Transcriptional activity and factor binding are stimulated by separate and distinct sequences in the 5' flanking region of a mouse $tRNA^{Asp}$ gene

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ABSTRACT

The transcriptional properties of two cloned mouse $tRNA^{Asp}$ genes were examined *in vitro*. The $tRNA_2^{Asp}$ gene displays a five fold greater transcriptional activity than the $tRNA_1^{Asp}$ gene and a greater ability to form stable complexes with transcription factors. Transcription of a hybrid gene with swapped 5' flanking sequences and of 5' flanking region deletion mutants demonstrates that the differential transcription of the genes results from stimulatory sequences in the 5' flanking region of the $tRNA_2^{Asp}$ gene. Distal sequences including those between positions -53 and -31 stimulate transcription but do not affect factor binding. Proximal sequences between positions -9 and-1 enhance factor binding. Thus, binding of transcription factors and later steps required for transcription can be modulated by separate and distinct 5' flanking sequence motifs in eukaryotic tRNA genes.

INTRODUCTION

At least two distinct sets of reactions are required for transcription of a eukaryotic tRNA gene. First, specific protein factors recognize and interact with sequences in the tRNA coding region and a stable protein-gene complex is formed (1-6). The tRNA gene coding sequences which exert a dominant influence on factor binding are often termed the A and B blocks, and encode the D stem and loop and the T stem and loop of the tRNA molecule, respectively (7-11). Once the stable transcription factor-gene complex has formed, a second set of reactions, including recognition and transcription by RNA polymerase III, occurs (12,13). Thus, modulation of transcription of different tRNA genes can potentially occur either as a consequence of differences in the efficiency of factor-gene complex formation or stability, or alternatively, differences in the steps subsequent to formation of the complex.

A number of studies have shown that the 5' flanking regions of eukaryotic tRNA genes can influence transcription. Several genes contain stimulatory sequences in their 5' flanking regions (14-19) and some contain inhibitory

sequences (15,20,21). A major question in regard to modulatory 5' flanking sequences is which set of reactions required for transcription is affected. In some cases, deletion of stimulatory sequences inhibits both transcription and factor binding (16,17), while in others, only transcription appears to be affected (7,22). These results therefore suggest that a given 5' stimulatory sequence can influence either the interaction of internal control regions with factors, or later steps in transcription initiation.

To gain insight into the mechanisms by which 5' flanking sequences of mammalian tRNA genes influence transcription, we have analyzed the activities of two mouse $tRNA^{Asp}$ genes which contain identical coding regions and different flanking sequences. The transcriptional activities of the two genes differ by five fold *in vitro*. Transcription of deletion mutants of the more active gene reveals the presence of two separate and distinct 5' flanking sequences which influence transcriptional activity and factor binding, respectively.

MATERIALS AND METHODS

Cloned Genes and Gene Fragments. We have previously isolated and sequenced two mouse $tRNA^{Asp}$ genes from a phage Charon 4A-genomic DNA library (23). All clones used in this paper were derived from sequences obtained from the recombinant phage subcloned into M13mp9 or M13mp19 vectors. Clone mAsp1 has a 510 bp Sma I fragment from phage Mt2 containing the $tRNA_1^{Asp}$ gene with 119 bp of 5' flank. Clone mAsp2 has a 309 bp Alu I fragment from phage Mt4 containing the $tRNA_2^{Asp}$ gene with 255 bp of 5' flank. Subclone mA1.5'-35 contains sequences from mAsp1 between the Acc I site at position -35 (relative to the start of the mature tRNA coding sequence) and the 3' end of the insert, including the $tRNA_1^{Asp}$ coding region. Subclone mA2.5'-15 contains sequences from mAsp2 between the Hae III site at position -15 and the 3' end of the insert, including the $tRNA_2^{Asp}$ coding region. Subclone mA2.FL contains sequences from mAsp2 between the 5' end of the insert and the Hae III site at position -15 in the 5' flank. Subclone mA2.D contains sequences from mAsp2 between the Hae III site at position -15 and the Tha I site at position +39 and thus includes A block sequences but not B block sequences. Subclone mA2.A1, containing the 5' flank of mAsp2 and the 3' flank of mAsp1, was constructed using a unique Hinf I site in the $tRNA^{Asp}$ coding region. All M13 subclones were sequenced by the dideoxy chain termination method (24). The plasmid pH3B*R contains the VA I gene from the Adenovirus-2 genome in pBR322 (25) and was used as a reference template in competition experiments.

Construction of 5' Deletion Mutants. Clone mAsp2 was restricted with Eco RI and digested with nuclease Bal31 for 0 to 30 minutes. The reaction was terminated, the DNA was repaired by DNA Polymerase I (large fragment) and restricted with Hind III. DNA fragments 150 to 300 bp in size were isolated by gel electrophoresis in 1.5% low melting point agarose, purified and cloned into Hinc II-Hind III cut M13mp9 or Sma I-Hind III cut M13mp19. Clones of interest were identified from the pattern of T residues obtained in a dideoxy sequencing reaction and deletion endpoints confirmed by complete sequencing (24). Deletion mutants are specified by mA2.5', to designate 5' to 3' deletion of mAsp2 sequences, followed by the position of the first remaining bp of mouse DNA relative to the start of the mature tRNA coding region (e.g., mA2.5'-114 contains 114 bp of the original mAsp2 5' flanking sequence). Constructs specified by pA2.5' contain M13 deletion mutant inserts that were excised with Bam HI and Hind III and subcloned into pBR322.

***In Vitro* Transcription of tRNA Genes.** HeLa cell S100 extracts were prepared according to Weil, et al. (26). Transcription reactions were typically performed at 30°C for 60 minutes in a volume of 7.5 µl. Reactions contain 5.0 µl HeLa S100 extract, 10 mM Hepes (pH 7.9), 66 mM KCl, 1 mM MgCl2, 1 mM dithiothreitol, 0.1 mM EDTA, 12% glycerol, 0.5 mM each of ATP, CTP and UTP, 25 µCi [a -32P]GTP (3000 Ci/mmol), 2.5 mM creatine phosphate, and 25 ng of DNA. All DNA was in the form of supercoiled replicative form I M13 DNA or supercoiled plasmid DNA. Optimal concentration of total DNA was established by transcription of a suboptimal amount of template with increasing amounts of vector DNA. Optimal template concentration was then established by varying the amount of template DNA while holding the total DNA concentration constant with the appropriate amount of vector DNA (7,22). Transcripts were resolved on 8% or 12% polyacrylamide gels containing 7 M urea, visualized by autoradiography and quantitated by computerized densitometry. Several sets of bands were also excised and quantitated by liquid scintillation counting for comparison with densitometric measurements to ensure reliable quantitation.

Transcription complex formation was measured by "preincubation" competition assays (4) as follows. The template to be tested (20 ng) was transcribed in the S100 for 15 minutes. Reference template pH3B*R (5 ng), containing the VA I gene, was then added and the reaction continued for 90 minutes. The degree of competition is calculated as the percent reduction of VA I transcription in the presence of the tRNA gene template as compared to VA I transcription with the same amount of vector DNA. Competitive strength is then

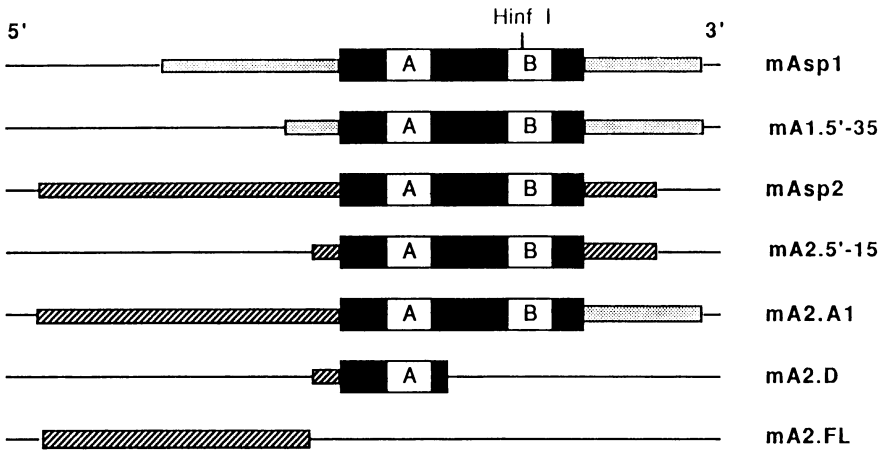

Fig. 1. Schematic representation of the DNA sequences contained in each tRNA^{Asp} gene containing subclone. Black areas represent mature tRNA^{Asp} coding sequences; stippled areas represent sequences flanking the tRNA$_1^{Asp}$ gene; striped areas represent sequences flanking the tRNA$_2^{Asp}$ gene; the thin line denotes vector sequences. The Hinf I site was used to construct the hybrid clone, mA2.A1. See Materials and Methods for a precise description of the sequences in each subclone.

taken as the inverse of that number, with competition by mAsp2 normalized to 100% (see Table 1).

RESULTS

Differential Transcription of Two Mouse tRNA^{Asp} Genes *in vitro*. The tRNA$_1^{Asp}$ and tRNA$_2^{Asp}$ genes, previously isolated and sequenced in our laboratory (23), contain identical tRNA^{Asp} coding sequences but different 5' and 3' flanking sequences. M13 subclones containing these genes are depicted in Fig. 1. Transcription of clone mAsp1 in a HeLa cell S100 extract yields one predominant RNA species (Fig. 2, lane b). Fingerprint analysis indicates that this species is the primary transcript, initiating with pppA at position -11 (relative to the start of the mature tRNA coding sequence) (27). This transcript is eventually processed through an intermediate form to tRNA sized molecules (28). Transcription of clone mAsp2 yields two predominant RNA species (Fig. 2, lane c). Fingerprint analysis reveals both of these species to be primary transcripts, the larger one initiating with pppA at position -10 and the smaller one initiating with pppG at position -7 (data not shown). In longer incubation, these transcripts are also processed through an intermediate form to tRNA sized molecules (see e.g. Fig. 3, lane c).

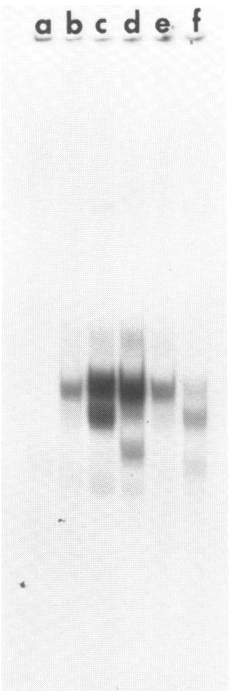

Fig. 2. Transcription of the tRNA^{Asp} gene containing subclones. Each reaction contained 25 ng of template DNA. Templates used: lane a, M13mp9 vector; lane b, mAsp1; lane c, mAsp2; lane d, mA2.A1; lane e, mA1.5'-35; lane f, mA2.5'-15.

Under conditions of optimal transcriptional activity for both genes, clone mAsp1 is about 20% as active as clone mAsp2 (Fig. 2, lanes b and c and Table 1). In order to determine whether this effect is due to sequence differences in the 5' or 3' flanking regions, a hybrid gene, clone mA2.A1, was constructed containing the 5' flanking region of mAsp2, the tRNA^{Asp} coding region and the 3' flanking region of mAsp1 (Fig. 1). The transcriptional activity of mA2.A1 is as high as that of mAsp2 (Fig. 2, lane d and Table 1), indicating that the differential transcription of mAsp1 and mAsp2 is determined by sequences in the 5' flanking regions. Transcription of mA2.A1 results in the synthesis of a sole primary transcript initiating at position -10, which is processed through an intermediate form to tRNA sized molecules. The absence of a second primary transcript initiating at position -7 suggests that the appearance of those transcripts (as occurs with mAsp2) is affected by the 3' flanking sequence, in some way. We have not, as yet, determined the reason for this.

Subclones of mAsp1 and mAsp2 in which 5' flanking sequences are deleted were constructed to preliminarily examine the location of modulatory regions (Fig. 1). The transcription of subclone mA1.5'-35 (Fig. 2, lane e) is the same as that of mAsp1, indicating the sequences upstream of position -35 in the 5'

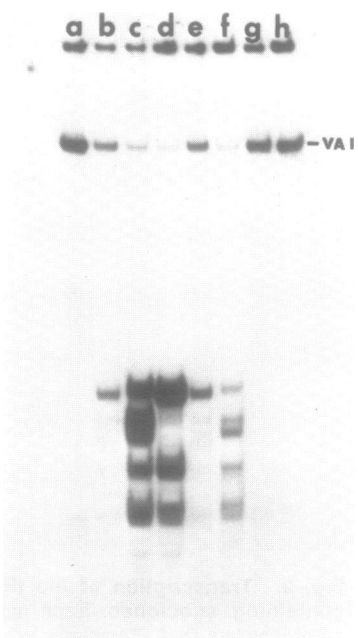

Fig. 3. Stable complex formation by the tRNA^{Asp} gene containing subclones. Competitor DNA (20 ng) was incubated in a transcription reaction for 15 minutes prior to addition of 5 ng of the reference template pH3B*R (containing the VA I gene). Competitors used: lane a, M13mp9 vector; lane b, mAsp1; lane c, mAsp2; lane d, mA2.A1; lane e, mA1.5'-35; lane f, mA2.5'-15; lane g, mA2.FL; lane h, mA2.D.

flanking region of mAsp1 are neutral with respect to transcription. In contrast, the transcription of mA2.5'-15 (Fig. 2, lane f) is diminished relative to that of its parent clone, mAsp2, and is similar to the level of mAsp1. This result has also been observed with this deletion mutant cloned into pBR322 (clone pA2.5'-15, Table 1), eliminating the possibility of transcription inhibition by M13 vector sequences. Thus, sequences upstream of position -15 in the 5' flanking region of mAsp2 stimulate transcription and are responsible for the five fold difference in activity between mAsp1 and mAsp2.

Effect of 5' Flanking Sequences on Formation of Stable Transcription Complexes. The subclones depicted in Fig. 1 were also tested for their ability to compete for transcription factors when forming transcription complexes by means of preincubation competition assays (see Materials and Methods and Ref. 4). The adenovirus VA I gene was used as the reference template in these experiments because its transcript is readily distinguishable from tRNA^{Asp} gene transcripts, and it binds the same transcription factors as tRNA

Table 1.
Transcription and competition by the $tRNA_1^{Asp}$ and $tRNA_2^{Asp}$ genes and recombinant subclones.

Clone	Transcriptional activity,[1] (% ± S.D.)	Competitive strength,[2] (% ± S.D.)
mAsp1	20 ± 5	41 ± 10
mA1.5'-35	20 ± 5	39 ± 9
mAsp2	100	100
mA2.A1	112 ± 12	122 ± 16
mA2.5'-114	91 ± 7	100 ± 17
mA2.5'-77	106 ± 9	137 ± 27
mA2.5'-60	101 ± 8	122 ± 21
mA2.5'-53	100 ± 8	100 ± 19
mA2.5'-31	53 ± 3	122 ± 28
mA2.5'-15	31 ± 6	100 ± 19
mA2.5'-12	20 ± 2	157 ± 45
mA2.5'-9	45 ± 2	137 ± 26
mA2.5'+2	12 ± 4	48 ± 9
mA2.5'+17	<1	23 ± 8
mA2.FL	<1	<1
mA2.D	<1	<1
pA2.5'-53	100	100
pA2.5'-15	32 ± 7	95 ± 14
pA2.5'+2	36 ± 5	51 ± 10

Transcriptional activities and competitive strengths were calculated as described in Materials and Methods. Values for M13 subclones are relative to those for mAsp2; values for pBR322 subclones are relative to those for pA2.5'-53.

[1]Values presented here are the average of six or more independent experiments.

[2]Values for M13 subclones are the average of five or more experiments; values for pBR322 subclones are the average of four experiments.

genes (2,3). In preliminary experiments (data not shown), we established that maximal competition by mAsp1 or mAsp2 in our reaction conditions was obtained by addition of 15 to 20 ng of template as competitor DNA and remained relatively unchanged between 10 and 30 minutes of preincubation.

Competition by $tRNA^{Asp}$ genes and gene fragments is shown in Fig. 3 and their competitive strengths are listed in Table 1. The greatest competitive strength is shown by mAsp2 (Fig. 3, lane c) and mA2.A1 (Fig. 3, lane d). The competitive strengths of mAsp1 (Fig. 3, lane b) and mA1.5'-35 (Fig. 3, lane e) are 40% that of mAsp2. The competitive strength of mA2.5'-15 (Fig. 3, lane f), however, is not reduced from that of its parent clone, mAsp2 (see Table 1).

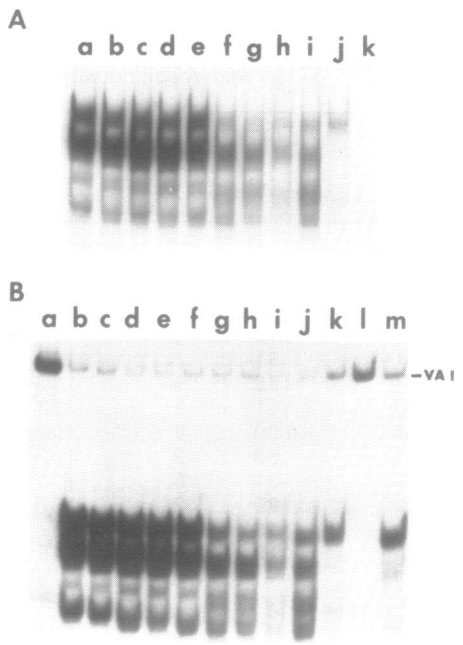

Fig. 4. Transcription of and stable complex formation by 5' flanking sequence deletion mutants of the $tRNA_2^{Asp}$ gene.
(A) Transcription of 25 ng of each 5' deletion mutant. Templates: lane a, mAsp2; lane b, mA2.5'-114; lane c, mA2.5'-77; lane d, mA2.5'-60; lane e, mA2.5'-53; lane f, mA2.5'-31; lane g, mA2.5'-15; lane h, mA2.5'-12; lane i, mA2.5'-9; lane j, mA2.5'+2; lane k, mA2.5'+17. **(B)** Competition by 20 ng of each 5' deletion mutant against 5 ng of reference template, pH3B*R (VA I RNA gene). Competitors: lane a, M13mp9 vector; lane b, mAsp2; lane c, mA2.5'-114; lane d, mA2.5'-77; lane e, mA2.5'-60; lane f, mA2.5'-53; lane g, mA2.5'-31; lane h, mA2.5'-15; lane i, mA2.5'-12; lane j, mA2.5'-9; lane k, mA2.5'+2; lane l, mA2.5'+17; lane m, mAsp1.

Neither subclone mA2.FL, containing mAsp2 5' flanking sequences upstream of position -15, nor subclone mA2.D, containing mAsp2 sequences between positions -15 and +39, display any competitve ability (Fig. 3, lanes g and h) indicating that these sequences individually cannot stably bind transcription factors in these assays. These data indicate that those sequences in the mAsp2 5' flanking region which stimulate transcriptional activity are not responsible for the enhanced ability of mAsp2 to stably bind and sequester transcription factors.

To confirm that the differential competitive strengths of mAsp1 and mAsp2 are not due to some peculiarity of the VA I reference gene, we also competed the two genes against a $tRNA^{Asp}$ "maxigene". The maxigene was constructed by deleting the termination site of clone mAsp2; transcription

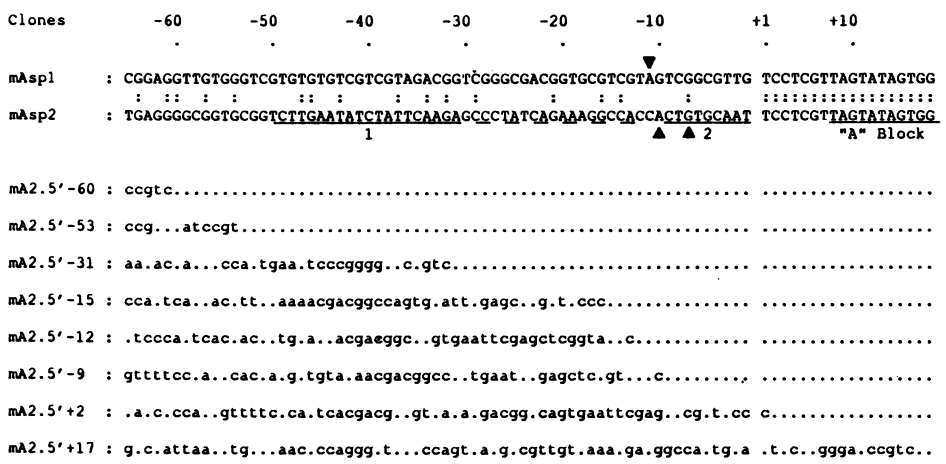

Fig. 5. 5' Flanking sequence deletion mutants of the $tRNA_2^{Asp}$ gene. The first two lines show the noncoding strand of the 5' flanking sequences of the $tRNA_1^{Asp}$ gene (mAsp1) and the $tRNA_2^{Asp}$ gene (mAsp2), respectively. Colons between these lines indicate homologous nucleotides. Triangles point to initiating nucleotides. The last eight lines show the sequences of mA2.5' deletion mutants. Nucleotides homologous to the mAsp2 sequence are denoted by a period; nonhomologous M13 vector sequence is in lowercase. Underlined regions 1 and 2 (in the mAsp2 sequence) are those that influence transcriptional behavior and are explained in the text (the broken line appended to region 1 indicates that the 3' boundary of the distal region has not been defined by deletion mapping).

termination occurs in M13mp9 vector sequences and results in a longer transcript readily distinguishable from mAsp1 or mAsp2 transcripts. Competitions performed with the maxigene as the reference template produced the same results as those shown in Fig. 3 (data not shown).

Transcriptional Activities of 5' Deletion Mutants of the $tRNA_2^{Asp}$ Gene. To perform a more detailed analysis of the sequences responsible for stimulating transcription and enhancing factor binding, a series of deletions in the 5' flanking region of mAsp2 were constructed. Each deletion mutant was tested with regard to transcriptional activity (Fig. 4A and Table 1). Clones with deletions extending to positions -114, -77, -60, and -53 (Fig. 4A, lanes b-e) are transcribed at about the same level as mAsp2 (Fig. 4A, lane a). In contrast, a deletion extending to position -31 results in a 50% decrease in activity (Fig. 4A, lane f) and deletions extending to positions -15 and -12 reduce activity to 31% and 20%, respectively (Fig. 4A, lanes g and h), essentially the same activity as mAsp1 (Table1). Some transcriptional activity is regained in a -9 deletion mutant but then deletion to position +2 reduces transcription to 12% of the wild type value (Fig. 4A, lanes i and j). Further deletion of sequences to position +17 in the

coding region, including most of the A block, eliminates transcription (Fig. 4A, lane k). Similar results were also obtained with deletion mutants subcloned into pBR322 (Table 1). Using pA2.5'-53 as the wild type standard, both pA2.5'-15 and pA2.5'+2 had reduced transcriptional activity (32% and 36%, respectively). This analysis localizes the upstream boundary of the sequences that stimulate the transcription of mAsp2 (relative to mAsp1) to residues between positions -53 and -31.

Competitive Strengths of 5' Deletion Mutants of the $tRNA_2^{Asp}$ Gene. The ability of each mutant to compete for and stably sequester transcription factors is shown in Fig. 4B. The competitive strengths of clones with deletions extending to positions -114, -77, -60, -53, -31, -15, -12, and -9 (Fig. 4B, lanes c-j) are all greater than or equal to that of the parent clone, mAsp2 (Fig. 4B, lane b). Deletion to position +2 (Fig. 4B, lane k) reduces competitive strength to 48% , which is similar to that of mAsp1 (41%). This result occurs in both M13 and pBR322 vectors (see Table 1) and indicates that the 5' boundary of the sequences enhancing transcription factor binding is between positions - 9 and +2. Further deletion to position +17 (Fig. 4B, lane l), removing most of the A block, decreases competitive strength to 23%, half that of mAsp1. Thus, the sequences in mAsp2 which enhance transcription factor binding relative to mAsp1 are located between position -9 and, since the coding regions of both genes are identical, position -1.

DISCUSSION

In this paper we have shown that the five fold difference in transcriptional activity between two mouse $tRNA^{Asp}$ genes is due to the presence of a stimulatory sequence in the 5' flanking region of one of the genes ($tRNA_2^{Asp}$). Notably, analysis of deletion mutants of the $tRNA_2^{Asp}$ gene indicates the presence of at least two of at least two distinct modulatory elements in the 5' flanking sequence. The presence of a distal element, whose 5' boundary is located between positions -53 and -31, stimulates transcription but deletion of those sequences has little or no effect upon the stable binding of transcription factors, as assayed by preincubation competition experiments. In contrast, the presence of the proximal element between positions -9 and -1 enhances stable transcripton factor binding but by itself does not stimulate transcriptional activity. Other studies have shown that eukaryotic tRNA genes contain stimulatory 5' flanking sequence motifs (14-19). In the case of mouse and *Drosophila* $tRNA^{His}$ genes, the same sequences that affect transcriptional activity also influence stable complex formation (16,17). To our knowledge, however, the mouse $tRNA^{Asp}$ genes analyzed

here provide the first published example of a tRNA gene having two separate and distinct 5' flanking sequences that independently affect transcriptional activity and factor binding, respectively.

Major issues raised by our data are precisely which residues in the regions demarcated by the deletion mutants are responsible for these effects, and the mechanisms by which these sequences operate. Although these questions cannot be answered directly from the data presented here, inspection of the sequences in the 5' flanking regions of the two $tRNA^{Asp}$ genes suggests some potentially important residues. DNA sequences from those regions and from the 5' flanking regions of relevent mA2.5' deletion mutants are shown in Fig. 5. Sequences of potential functional importance in mAsp2 are underlined and labelled. Region 1 is comprised of the sequence between positions -50 and -31, the deletion of which results in a 50% decrease in transcriptional activity. This region is 78% A+T and also has the potential to form a perfect hairpin with up to a 9 bp stem. The corresponding region in mAsp1 is 45% A+T and cannot form any stable secondary structure. Two *Xenopus* $tRNA^{Tyr}$ genes analyzed by Gouilloud and Clarkson (18) provide a parallel example. The Xenopus gene with a 79% A+T sequence between positions -46 and -28 has a transcriptional activity approximately five to six fold higher than that of the second gene which has no cognate A+T rich region. Replacement of the 5' flanking region of the first *Xenopus* gene to position -12 with vector DNA, such that the region upstream of position -28 is only 39% A+T, decreases transcriptional activity by 50%. A-T rich sequences of unknown function (10-30 bp in length) occur within 20 to 30 bp of the start of approximately 40% of sequenced eukaryotic tRNA genes, most of which, however, were isolated from invertebrates.

The fact that the -50 to -31 region of the $tRNA_2^{Asp}$ gene stimulates transcription without affecting factor binding suggests that it influences some aspect of the interaction of RNA polymerase III with the transcription factor-gene complex. One obvious possibility is that the A-T rich region melts more readily than the surrounding DNA and thus provides a recognition or entry site for the RNA polymerase.

The sequences that affect stable complex formation (positions -9 to -1) are underlined and designated region 2 in Fig. 5. Inspection of the sequence reveals no obvious candidates for critical residues in this area. We have previously shown that residues -9 to -4 of a mouse $tRNA^{His}$ gene are also necessary for formation of stable complexes (17). There is , however, no homology between the $tRNA_2^{Asp}$ and $tRNA^{His}$ genes in this region. Thus it appears that genes containing different coding regions employ various 5' flanking

sequence motifs to effect and/or enhance stable complex formation. DNAase I footprinting experiments indicate that transcription factors from HeLa cells (3,13) and yeast (6,30) strongly protect sequences in and around the B block of the coding region and, in addition, afford nuclease protection over the A block and 5' flanking region residues proximal to the coding region. Thus, 5' flanking sequences abutting the coding region may directly interact with transcription factors. Possibly, certain 5' flanking sequences compensate for coding region variation to help form binding sites for transcription factors or maintain a particular local DNA conformation conducive to factor binding. Alternatively, there may be multiple isomeric forms of transcription factors that bind to specific combinations of flanking and coding region sequences.

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REFERENCES

1. Klemenz, R., Stillman, D. and Geiduschek, E. (1982) Proc. Natl. Acad. Sci. (USA) 79, 6191-6195.
2. Lassar, A., Martin, P. and Roeder, R. (1983) Science 222, 740-748.
3. Fuhrman, S., Engelke, D. and Geiduschek, E. (1984) J. Biol. Chem. 259, 1934-1943.
4. Schaak, J., Sharp, S., Dingermann, T. and Soll, D. (1983) J. Biol. Chem 258, 2447-2453.
5. Dingermann, T., Burke, D., Sharp, S., Schaak, J. and Soll, D. (1983) J. Biol. Chem. 258, 10395-10402.
6. Stillman, D. and Geiduschek, E. (1984) EMBO J. 3, 847-853.
7. Sharp, S., Dingermann, T., Schaak, J., DeFranco, D. and Soll, D. (1983) J. Biol. Chem. 258, 2440-2446.
8. Hofstetter, H., Kressmann, A. and Birnstiel, M. (1981) Cell 24, 573-585.
9. Galli, G., Hofstetter, H. and Birnstiel, M. (1981) Nature (London) 194, 626-631.
10. Ciliberto, G., Castagnoli, L. and Melton, D. (1982) Proc. Natl. Acad. Sci. (USA) 79, 1195-1199.

11. Sharp, S., DeFranco, D., Dingermann, T., Farrell, P. and Soll, D. (1981) Proc. Natl. Acad. Sci. (USA) 78, 6657-6661.
12. Bieker, J., Martin, P. and Roeder, R. (1985) Cell 40, 119-127.
13. Carey, M., Gerrard, S. and Cozzarelli, N. (1986) J. Biol. Chem. 261, 4309-4317.
14. Larson, D., Bradford-Wilson, J., Young, L. and Sprague, K. (1983) Proc. Natl. Acad. Sci. (USA) 80, 3416-3420.
15. Dingermann, T., Burke, D., Sharp, S., Schaak, J. and Soll, D. (1982) J. Biol. Chem. 257, 14738-14744.
16. Cooley, L., Schaak, J., Burke, D., Thomas, B. and Soll, D. (1984) Mol. Cell. Biol. 4, 2714-2722.
17. Morry, M. and Harding, J. (1986) Mol. Cell. Biol. 6, 105-115.
18. Gouilloud, E. and Clarkson, S. (1986) J. Biol. Chem. 261, 486-494.
19. Johnson, J. and Raymond, G. (1984) J. Biol. Chem. 259, 5990-5994.
20. Hipskind, R. and Clarkson, S. (1983) Cell 34, 881-890.
21. DeFranco, D., Sharp, S. and Soll, D. (1981) J. Biol. Chem. 256, 12424-12429.
22. Wilson, E., Larson, D., Young, L. and Sprague, K. (1985) J. Mol. Biol. 183, 153-163.
23. Looney, J. and Harding, J. (1983) Nucleic Acids Res. 11, 8761-8775.
24. Sanger, F., Coulson, A., Barrell, F., Smith, A. and Roe, B. (1980) J. Mol. Biol. 143, 161-178.
25. Jove, R. and Manley, J. (1982) Proc. Natl. Acad. Sci. (USA) 79, 5842-5846.
26. Weil, P., Segall, J., Harris, B., Ng, S-Y. and Roeder, R. (1979) J. Biol. Chem. 254, 6163-6173.
27. Rooney, R. (1986) Ph.D. Thesis, Columbia University, New York, NY.
28. Rooney, R. and Harding, J. (1986) Nucleic Acids Res. 14, 4849-4864.
29. Korn, L. and Brown, D. (1978) Cell 15, 1145-1156.
30. Stillman, D., Caspers, P. and Geiduschek, E. (1985) Cell 40, 311-317.