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Generation of long read-through transcripts *in vivo* and *in vitro* by deletion of 3' termination and processing sequences in the human $tRNA_i^{met}$ gene

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ABSTRACT

The effects of 3' deletions of the coding and flanking regions of the human $tRNA_i^{met}$ gene on its transcription and subsequent processing have been studied both in vitro and in vivo. We demonstrate that in the absence of the oligo T stop signal, polymerase III will read-through efficiently to the next available downstream stop signal. In mutations preserving the 3' terminal sequence of the coding region these read-through transcripts are efficiently processed, irrespective of their length and sequence by an endonucleolytic cleavage to yield both a mature tRNA and an intact trailer RNA. However, deletions involving the terminal regions up to +62 in the coding sequence produce an unprocessed co-transcript of tRNA and downstream sequences. Deletions further within the B promoter box abolish transcription. The use of these mutants as possible "portable" promoters is discussed.

INTRODUCTION

The eukaryotic tRNA gene appears to contain information within the tRNA structural sequence as well as outside its borders which are essential for efficient transcription and processing. In addition to an intragenic promoter, divided into 5' and 3' components (1-9), the tRNA gene may contain 5' flanking sequences which modulate transcriptional activity (10-14). Termination signals of the transcription unit appear to reside 3' to the tRNA sequence (2,3,5,15-19). In this study we have explored the effects of several 3' deletions in the human $tRNA_i^{met}$ gene on termination of the primary transcripts and their subsequent processing. The constructions have been assayed in both a homologous cell-free system capable of efficient processing as well as within the intact X. laevis oocyte. In addition to extending our understanding of the function of sequences within the tRNA gene discovered recently, this report demonstrates that the tRNA gene can be used to promote the RNA polymerase III-dependent transcription of otherwise transcriptionally inactive DNA sequences. These constructions may serve as the basis of utilizing the tRNA gene as a "portable promoter" in engineered genetic constructions.

METHODS

Plasmids and Construction of 3' Deletion Mutants:

phH2D, containing the human $tRNA_i^{met}$ gene, has been described in detail elsewhere (20). It contains a 3.7kb Hind III fragment of human chromosomal DNA bearing a single $tRNA_i^{met}$ gene cloned into the Hind III site of pBR322. 3' deletion mutants of the $tRNA_i^{met}$ gene were constructed as follows: phH2D was digested with Bam HI, (Bethesda Research Laboratories) and the larger of the two fragments generated purified by agarose gel electrophoresis. The fragment was trimmed on both ends with S1 nuclease (Sigma) as described by Mizuuchi (21). The trimmed fragment was purified by phenol extraction, collected by ethanol precipitation and the ends filled with Klenow polymerase (Bethesda Research Laboratories). Bam HI linkers (CCGGATCCGG, Collaborative Research Laboratories) were ligated to the fragments. Following digestion with Bam HI the fragments were circularized by incubation with T4 ligase (PL Biochemicals). The preparation was transferred into E. coli LE 392 (22). Plasmids were isolated from individual colonies and screened by sizing of fragments generated following Bam HI-Hinf I digestion. Plasmids were selected in which the characteristic 220 bp HinfI-BamHI fragment containing the $tRNA_i^{met}$ gene was shortened. In the original sequence the HinfI site lies 120 bp 5' to the $tRNA_i^{met}$ sequence and the Bam HI site lies 40 bp beyond the 3' end of the $tRNA_i^{met}$ gene (20). Plasmids were sequenced by the chemical methods of Maxam and Gilbert (23). Plasmids were linearized with Bam HI, treated with T4 polynucleotide kinase (PL Biochemical) and digested with HinfI (Bethesda Research Laboratories). The labelled fragments were isolated by electrophoresis in 2% low gelling temperature agarose. In addition, the TaqI fragment from position 53 in the gene to positions 3' within pBR322 was isolated, end labelled with T4 polynucleotide kinase, and redigested with SphI (Bethesda Research Laboratories). The smaller fragment was isolated by electrophoresis in 3% low gelling temperature agarose and sequenced.

Transcription

In vitro transcription reactions were performed as described (18) utilizing a cell-free system from KB cells in which faithful $tRNA_i^{met}$ transcription and processing occur. Plasmids were isolated by the acid-phenol extraction procedure and further purified by chromatography over Sepharose 4B (24). Equivalent amounts of each plasmid were added to each reaction. Each reaction contained (in 50 µl): 30 mM Tris-HCl, pH 8.0, 1.0 mM $MgCl_2$ (added exogenously), 0.6 mM unlabeled ATP, CTP, and UTP, 10-20 µCi (α-^{32}P) GTP (specific radioactivity, 600 Ci/mmol, New England Nuclear), 25 µM

GTP, 0.15 µg plasmid, 1.0 mM dithiothreitol, and 20 µl of the KB fraction. The KB cell fraction utilized was partially purified over DEAE-cellulose as described (18). The reactions were incubated at 30°C for the times stated and the RNA products extracted as described (18). The samples were analyzed by gel electrophoresis in 5% acrylamide gels in 7M urea and 1 x TBE (23), and autoradiographed at -70°C.

Expression of the plasmids in the intact stage 6 X. laevis oocyte were performed as described (25). Approximately 20 nl of each circular plasmid in 88 mM NaCl, 20 mM Tris-HCl, pH 8.0 at 0.5 mg/ml, were introduced per nucleus. 40 nl of (α-^{32}P) CTP (10 µCi/µl, SA 600 Ci/mmol, New England Nuclear) was injected in a second injection into the vegetal pole. The oocytes were maintained at 21° in OR2 buffer (26). Total RNA was isolated by suspension of 3-4 oocytes into 0.3 ml of a buffer containing 20 mM Tris-HCl, pH 8.0, 20 mM NaCl, 5 mM $MgCl_2$, and 2% SDS. Proteinase K (Boehringer Mannheim) was added to 1 mg/ml, 50 µg of yeast tRNA was added as carrier, and the reactions maintained at 21° for 30 min. The samples were then adjusted to 0.2 M NaCl and extracted once with one volume of phenol together with one volume of choloform/isoamyl alcohol (24/1). One ml of 95% ethanol was added to the aqueous phase and the nucleic acids precipitated at -70° for 15 min, and collected by centrifugation at 15,000 x g for 20 min. The pellets were dried and resuspended into 15 µl of electrophoresis loading solution containing 50% urea, 0.05% bromphenol blue, 0.05% Xylene cyanol FF, 1 x TBE, heated at 68°C for 5 min and electrophoresed on either 5 or 10% acrylamide gels containing 7M urea. Gels were autoradiographed at -70°C.

RESULTS

3' Deletion Mutants

The 3' deletion mutants of the human $tRNA_i^{met}$ gene were constructed as outlined in Figure 1 and detailed in Methods. The plasmid, phH2D, was linearized by digestion with Bam HI which opens the plasmid at a site 40 bp downstream from the $tRNA_i^{met}$ sequence. The large fragment, containing pBR322 joined with a 2 kb segment of human DNA, was trimmed at both ends with S1 nuclease under controlled digestion conditions (21). The trimmed fragment was recircularized following ligation of Bam HI linkers to each end.

The nucleic acid sequences of the six 3' deletion constructions studied are shown in Figure 2. In Δ 3'-1 the wild type sequence is deleted 20 bp from the 3' boundary of the $tRNA_i^{met}$ sequence preserving the block of 11 T residues and 6 of the 8 C residues immediately downstream of the T block. Δ 3'-2 and Δ

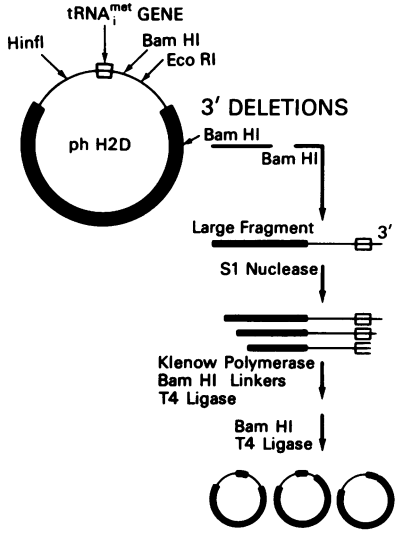

Figure 1. Construction of 3' deletion mutants of the human tRNA$_i^{met}$ gene. Plasmid phH2D contains a 3.7 kb fragment of human chromosomal DNA containing a single tRNA$_i^{met}$ gene inserted into the Hind III site of pBR322 (20). Thick line represents pBR322 sequences, the thin line, insert sequences. Experimental details are presented in Methods.

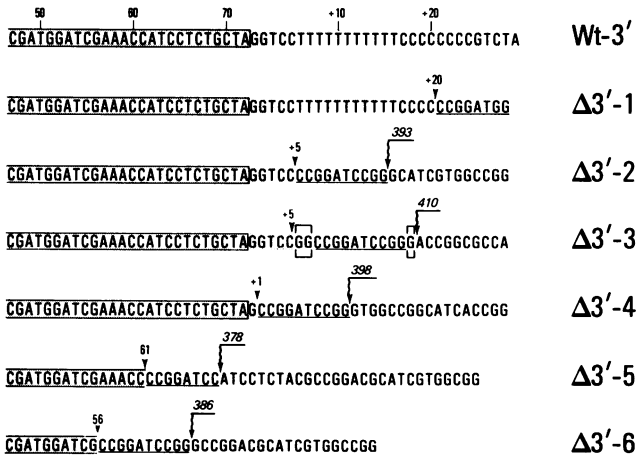

Figure 2. Sequences of the deletion borders of the 3' deletion constructions. Sequences present in the wild type gene are hatched. The Bam HI linker derived sequences are underlined. Boxes enclosing nucleotides on either side of the linker in Δ 3'-3 are presumed to have arisen from heterogenity in the linker preparation. Numbers 3' to the linker sequence denote position in pBR322 (27). Sequences were determined by the chemical methods of Maxam and Gilbert (23) using the strategy outlined in Methods.

3'-3 share the same 5 nucleotides at the 3' border of the tRNA gene, identical to the wild-type sequence, but differ in the corresponding sequences 3' to the deletion border. Δ 3'-3 appears to have incorporated two G residues 5' and one G residue 3' to the linker sequence (boxed sequences) presumed to have arisen from chemical heterogeneity of the linker preparation. Δ 3'-4 retains one original nucleotide 3' to the tRNA sequence, with 3' distal sequences unrelated to the original downstream region. In Δ 3'-5 the deletion has entered the $tRNA_i^{met}$ sequence, to position 61 in the DNA sequence. With respect to the transcription unit, this deletion should lie just 3' to the B promoter box (1-9); with respect to the $tRNA_i^{met}$ RNA sequence, the deletion enters the stem of the T loop. In Δ 3'-6 the deletion extends to DNA position 56, cutting within the 3' promoter element (2). With respect to the RNA sequence, the deletion replaces the 3' portion of the $tRNA_i^{met}$ up to the middle of the T loop.

Expression of the 3' deletion constructions in vitro

The plasmids were transcribed in a cell-free system from KB cells previously shown to accurately transcribe the human $tRNA_i^{met}$ gene and process the primary transcript to a mature tRNA sequence (18). This processing reaction involves sequential processing of the 5' and 3' terminii, respectively (18), a property of the two enzymes which process the transcript (Castano and Zasloff, Manuscript in preparation). The in vitro system exhibits relatively slow processing kinetics. As we have shown previously, at 1 hour under our conditions only the primary transcript accumulates in the reaction (18). The 5' processed intermediate accumulates within 2 hours after initiation, and is converted almost completely to mature $tRNA_i^{met}$ by about 5 hours. In these extracts, tRNA gene transcription is dependent on RNA polymerase III and ceases by about 2 hours (18). The results of the in vitro transcription of the 3' deletion plasmids are shown in Figure 3. At 1 hour the 89 nucleotide primary transcript of the $tRNA_i^{met}$ gene is observed both from the wild type gene and from the deletion Δ 3'-1, which retains 20 nucleotides 3' from the mature sequence. Removal of the 3' oligo T tract (plasmids Δ 3'-2, Δ 3'-3, Δ 3'-4, Δ 3'-5) leads to the appearance of high molecular weight RNA species ranging in size from 200 to 1200 nucleotides (Figure 3A). Deletion, however, up to DNA position 56 results in an inactive construction (Figure 3A, Δ 3'-6). The absence of high molecular weight RNA species in the presence of Δ 3'-6 is consistent with the argument that these RNA species arise from within the $tRNA_i^{met}$ sequence promoter, and not from some aberrant transcription unit elsewhere on the plasmid.

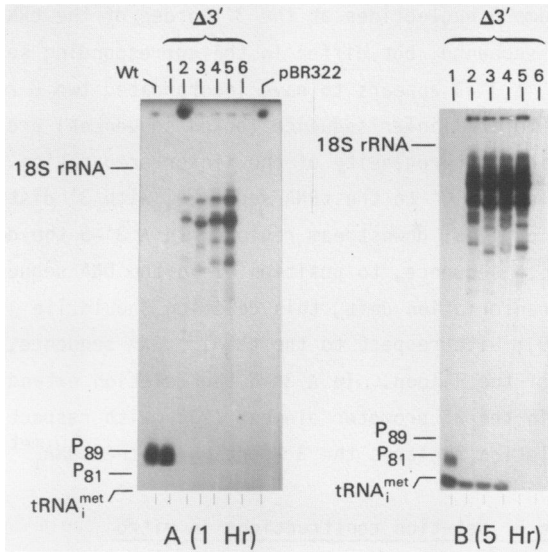

Figure 3. In vitro transcription of 3' deletion mutants. The transcription products obtained in 1 hour and 5 hour reactions are displayed. Processing is slow in this cell-free system, and products accumulate to detectable levels only after 90 to 120 min (18). P_{89} represents the primary transcript of the $tRNA_i^{met}$ gene, and contains an 8 base leader and trailer sequence. P_{81} represents the primary transcript from which the 5' leader has been cleaved (18). The position of 18 S rRNA (1900 nucleotides), visualized by ethidium bromide staining of the gel, is noted. Panel A, reaction products after 1 hour incubation; Panel B, products appearing after a 5 hour incubation.

Analysis of the RNA products accumulating in the in vitro transcription reaction at 5 hours shows that tRNA is processed from the primary transcripts. The wild type gene yields, in addition to the tRNA, the expected 81 nucleotide intermediate from which the 5' terminus has been removed (18). Intermediate species of similar size are not observed in the plasmids generating run-through transcripts, as expected, since the corresponding 5' processed species would still be of high molecular weight. One should note that the absence of intermediates of this size is consistent with the obligatory order of the processing pathway (18), in that a primary 3' nucleolytic event would be expected to generate an 80 nucleotide species consisting of the an intact 5' terminus joined to the 72 nucleotide tRNA sequence. Mature tRNA appears on transcription of the deletion series Δ 3'-2, Δ 3'-3, and Δ 3'-4. Mature tRNA is generated when α-amanitin is added at 1 hr to the system at a concentration of 100 μg/ml, sufficient to inhibit RNA

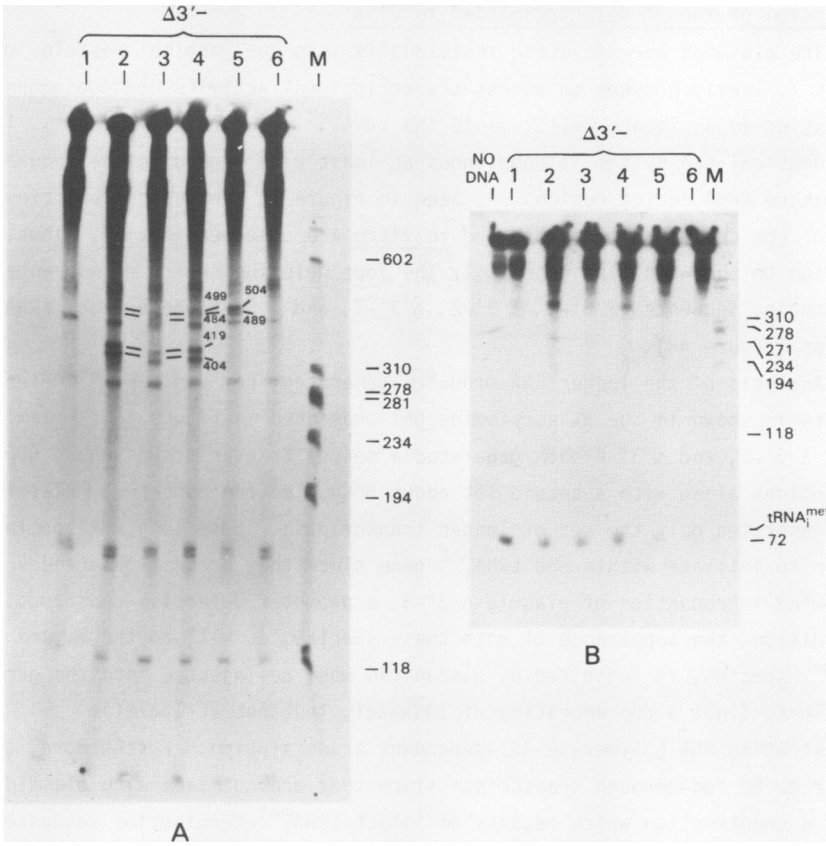

Figure 4. Transcription of the 3' deletion mutants following introduction into the germinal vesicle of intact X. laevis oocytes. Injections, RNA extraction, and electrophoretic analysis of the transcription products are described in Methods. Each reaction was divided into two portions, one displayed on 5% acrylamide (Panel A), the other on 10% (Panel B), in order to resolve high and low molecular weight RNA products. Numbers within Panel B denote size of specific RNA species. Small amount of labeling of tRNA species in control, Δ3'-5, and Δ3'-6, arises from incorporation of (α-^{32}P) CTP in terminal -C-C-A of endogenous tRNA. These "background" species are not visualized if (α^{32}P) GTP is injected instead (Adeniyi-Jones, unpublished). M, ^{32}P-labelled Hae III digest of ΦX-174.

polymerase III-dependent transcription, confirming that processing activity rather than continued transcription accounts for the appearance of the mature tRNA species (data not shown). The products of Δ 3'-5, in which 11 nucleotides at the 3' end of the mature tRNA$_i^{met}$ sequence have been replaced by a plasmid sequence are not processed to a tRNA (Fig. 3B).

Expression of the 3' deletion series in vivo

The plasmids were injected individually into the germinal vesicles of intact X. laevis oocytes to assess transcriptional activity and subsequent processing in an intact cell. Since the $tRNA_i^{met}$ sequence of man and X. laevis are identical the system is homologous at least with regard to the sequence of the mature tRNA coding region. As seen in Figure 4, the basic properties of each of the constructions exhibited in vitro are observed in vivo. Thus, in addition to the wild type gene, only the four deletions which do not enter the tRNA coding sequence (Δ 3'-1, Δ 3'-2, Δ 3'-3, and Δ 3'-4) generate a $tRNA_i^{met}$ species (Figure 4B).

Analysis of the longer RNA products generated from the $tRNA_i^{met}$ deletion mutants is shown in the 5% acrylamide gel presented in Figure 4A. Plasmids Δ 3'-2, Δ 3'-3, and Δ 3'-4 each generated a set of transcripts of about 500 nucleotides along with a second set about 80 nucleotides shorter. Plasmid Δ 3'-5 generated only the set of longer transcripts. These long RNA species appear to initiate within the $tRNA_i^{met}$ gene since they are not observed following introduction of plasmid Δ 3'-6, a promoter defective construction. In addition, the appearance of both these species, as well as the mature $tRNA_i^{met}$ species, is inhibited by α-amanitin when co-injected into the germinal vesicle to final a concentration of 100μg/ml, (but not at 1μg/ml) demonstrating RNA polymerase III-dependent transcription. Furthermore, they appear to be run-through transcripts since they are not seen with plasmid Δ 3'-1, a construction which retains an intact $tRNA_i^{met}$ termination sequence. These run-through transcripts represent the predominant RNA products synthesized in the oocyte dependent on introduction of the plasmids, a feature better visualized on gel electrophoretic analysis in which the products have been run further from the origin than shown in the gel presented in Figure 4A (data not shown).

The set of transcripts of 484 and 499 nucleotides observed in the oocytes injected with plasmid Δ 3'-4, as well as similar sized products seen with plasmids Δ 3'-2 and Δ 3'-3, are the sizes expected of transcripts which initiate within the normal 5' start of the human $tRNA_i^{met}$ gene (18) and extend into the pBR322 sequence at the 3' border, terminating within the oligo T tract at pBR322 sequence positions 786-793 (GATTTTCG) and 803-809 (GCTTTCG) (27). The first stop sequence recognized represents the first run of 4T residues in the 3' pBR322 sequence downstream from the $tRNA_i^{met}$ gene and corresponds to a predicted strong RNA polymerase III termination sequence (28). The second stop recognized, also a strong stop in this system,

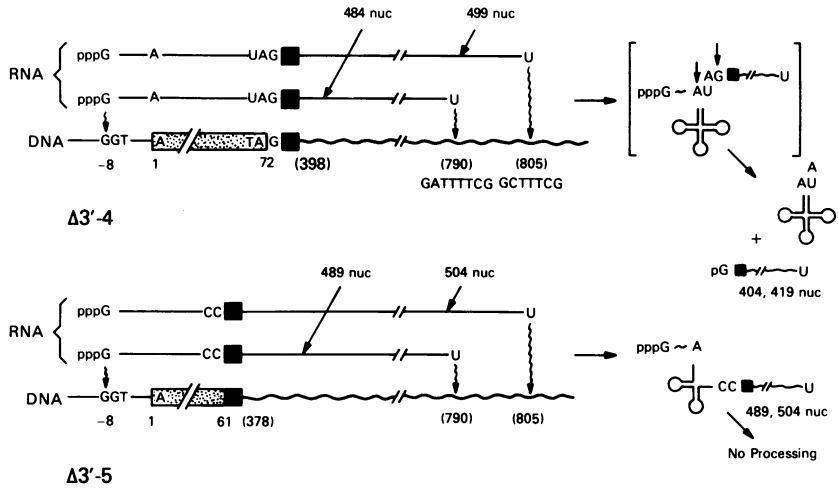

Figure 5. Schematic of processing pathway observed in vivo for transcripts of Δ 3'-4 and Δ 3'-5. Initiation site with respect to the $tRNA_i^{met}$ gene has been determined elsewhere and is presumed to be identical in these constructions. $tRNA_i^{met}$ structural sequence is noted by thick stippled line. Bam HI linker is denoted by the solid box and is 10 nucleotides long in Δ 3'-4 and 8 long in Δ 3'-5. Fused pBR322 sequences are noted by wavy lines with specific plasmid sequences noted in parentheses. The 404 and 419 processed trailers in Δ 3-4 are assumed but not proven to contain a 5' pG.

consisting of 3T residues bracketed by a GC-rich border, has been classified previously as a "weak" stop in vitro (28). Δ 3'-5 generated a set of similiar sized transcripts of 489 and 504 nucleotides, as expected for transcripts initiating and terminating at sequences identical to those of plasmid Δ 3'-4. The additional smaller set of species observed with plasmid Δ 3'-4, 404 and 419 nucleotides in length, correspond precisely in size to the long 3' trailer of the primary transcript, excised as an intact species following processing of the 5' tRNA leader sequence. As we would expect for processed products, RNA species of equivalent size are not found in oocytes injected with plasmid Δ 3'-5, a construction which generates a run-through transcript from which the $tRNA_i^{met}$ leader can not be excised in the oocyte.

A schematic of these reactions is shown in Figure 5.

DISCUSSION

The deletions at the 3' border of the human $tRNA_i^{met}$ gene described here were constructed to define the sequence requirements in this portion of the

human $tRNA_i^{met}$ gene for transcription, termination, and transcript processing as assayed both in vitro and in an intact cell. Several observations have been made in this study with regard to $tRNA_i^{met}$ gene expression:

1. The human $tRNA_i^{met}$ gene, like the corresponding X. laevis gene (2), contains a 3' internal promoter element essential for transcription, demonstrated both in a homologous cell-free system and in the intact X. laevis oocyte. These two genes, along with the X. laevis tRNA ^{leu} (3) and the C. elegans tRNA ^{pro} (5) genes appear to contain sequences within the T-loop coding sequence of the tRNA gene (referred to as the T or B box) required for efficient transcription. Deletion into a region lying within nucleotides 50 to 70 in these genes results in almost total transcriptional inactivation. In contrast, a second class of tRNA genes appears to retain transcriptional activity, albeit reduced below that of the wild type sequence, when the corresponding sequences are deleted (4,6,7,9,12). The reason for these differences in sequence requirements is not clear at the present time, but may reflect basic differences in the fuctional organization of the promoter sequences in these two classes of tRNA genes, or differences in the capacity of the 5' elements within certain genes to function as independent promoters (9,12). The 3' boundary of the 3' promoter in the X. laevis $tRNA_i^{met}$ gene, previously localized to position 72 (2), can be placed somewhere between position 56 and 61 in the human gene, similar in sequence location to the corresponding regions in X. laevis tRNA ^{leu} (3) and C. elegans tRNA ^{pro} (5).

2. Sequences present within the 3' border of the $tRNA_i^{met}$ gene do not appear to affect transcriptional activity in vitro or in the X. laevis oocyte. Within the 3' sequences flanking the two non-allelic human $tRNA_i^{met}$ genes we have cloned (20), at least two nucleotide blocks of significant length have been conserved, despite the absence of significant homology throughout the 3' flanking region in general. The sequence PyPyPyPyCTGAGGAT is located about 40 bp 3' from the end of each gene, and the sequence PyPyACAGTT, is located about 60 bp from the end of each gene (20). Since replacement of the entire 3' border of the human $tRNA_i^{met}$ gene by pBR322 sequences neither reduces nor increases overall transcription in the systems utilized here, we conclude that these conserved sequence blocks do not appear to be of functional importance in transcription, at least as assayed on templates exogeneous to the genome. The absence of enhancement or inhibition on removal of 3' flanking sequences is in contrast to the effects observed of short sequences at the 5' border of several eukaryotic genes such as B. mori tRNA ^{ala} (7,10), D. melanogaster tRNA ^{lys} (11), X. laevis $tRNA_i^{met}$ (14), and D. melanogaster $tRNA^{arg}$ (12,13).

3. The oligo T tract at the 3' boundary of the human $tRNA_i^{met}$ is essential for transcription termination. Removal of this tract results in run-through transcription into adjoining pBR322 sequences. These termination sequences have been remarkably conserved in the two non-allelic human $tRNA_i^{met}$ we have cloned. The gene studied in this report contains the sequence $GGCC(T)_{11}$ $(C)_8$, while the other contains the sequence, $GGTGC(T)_{15}$ $(C)_{10}$ (20). Each T tract is bracketed by a GC-rich sequence at its 5' boundary and by a run of C residues at the 3' border. Conservation of this particular sequence suggests functional importance. In this report we show that RNA polymerase III, initiated within the $tRNA_i^{met}$ sequence, will terminate within pBR322 sequences, demonstrating that despite the structure of the $tRNA_i^{met}$ stop sequence prokaryotic sequences can function in termination. The first sequence recognized as a termination sequence in pBR322 appears to correspond to the first run of T residues located 3' from the tRNA gene within the adjoining plasmid sequence. Sequences not recognized between these points include CTTGTTTCG and CTTCTTTATC (present at pBR322 positions 500 and 738, respectively) and at least 15 sequences containing 2 T residues flanked by GC-rich borders (27). This result suggests that the RNA polymerase III complex which transcribes $tRNA_i^{met}$ recognizes at least 4T residues as a termination sequence. Termination is not absolute at this site, however, since a major fraction of the transcripts generated *in vivo* appear to run through this sequence terminating in the sequence GCTTTCG located 15 bases downstream. The relative inefficiency of the prokaryotic sequences recognized as termination signals by RNA polymerase III is more dramatically demonstrated *in vitro* where transcription proceeds through the first stop sequence recognized *in vivo* terminating some 1000 nucleotides downstream from the $tRNA_i^{met}$ sequence in pBR322. In contrast, no significant transcription of sequences beyond the normal termination sequence is observed from the wild type $tRNA_i^{met}$ *in vitro* or in the X. laevis oocyte (18). It would appear that the long T tract conserved at the 3' boundary of the human $tRNA_i^{met}$ genes has been conserved to ensure efficient transcription termination, limiting the possibility of read-through transcription. Why the human $tRNA_i^{met}$ gene appears to require a termination sequence of this structure (and apparent high efficiency) is not clear.

4. When we replace all but one nucleotide 3' to the mature tRNA sequence, generating a run through transcript which retains the complete $tRNA_i^{met}$ sequence at the 5' end of the primary transcript, the trailer 3' to the tRNA sequence is subsequently efficiently cleaved. Replacement of the 3' terminal 11 nucleotides of the $tRNA_i^{met}$ gene generates a run-through transcript which

can not be efficiently processed at the 3' end, either in vitro or in vivo. Since this deletion into the 3' portion of the tRNA gene replaces sequences of the amino acid acceptor stem, the T-loop stem, and the T loop, but also clearly disrupts normal tRNA tertiary structure, we can not distingish whether the failure of processing reflects the requirement of the processing endonuclease for a native tRNA structure or for the presence of specific sequences within the deleted region. Both effects may be important in the 3' processing reaction. As we have shown elsewhere, substitution of G by U at tRNA position 57 in the T loop results in a 20-fold reduction in 3' processing of the primary transcript of the gene (18; Castano and Zasloff, Manuscript in preparation), despite evidence that this base substitution may have little effect on tRNA tertiary structure (29). Alternatively, disruption of the D-loop stem in yeast tRNA ^{leu} (30), as well as the introduction of several base substitutions in the yeast tRNA ^{tyr} gene which likely disrupt tertiary interactions (31), also impair processing of the terminii of the respective primary transcripts of these genes.

5. No specific sequence requirement for trailer processing can be demonstrated at the 3' border of the tRNA trailer cleavage site. Replacement of the 3' sequences immediately downstream of the terminal nucleotide of the mature $tRNA_i^{met}$ sequence by several different sequences of pBR322 origin has little demonstrable effect on cleavage of the trailer either in vitro or in vivo. Furthermore, the very long trailer generated in these constructions, amounting to at least 400 nucleotides in vivo, is cleaved efficiently in vivo and in vitro. This demonstrates that processing of the trailer occurs despite its length, which is considerably longer than the 3-20 nucleotide terminii which characterize most eukaryotic tRNA gene transcripts (11,13,16-19,32,33). The processing properties of these run-through transcripts suggest that the processing endonuclease responsible recognizes principally the structure (or specific sequences) of the tRNA portion of the primary transcript, with the precise site of cleavage defined by interactions with the tRNA portion rather than by sequence recognition at the specific cleavage site. This seems an appropriate adaptation for the eukaryotic tRNA processing system since, as a class, tRNA genes are generally dispersed in most eukaryotic genomes, with no general consensus sequence evident within the immediate 3' trailer (based on review of sequences listed in (34)). The apparent lack of specificity of the eukaryotic tRNA processing enzymes which remove the 3' trailer of the $tRNA_i^{met}$ run-through transcripts described here is strikingly reminiscent of the processing nucleases in prokaryotes which also

appear to show little sequence specificity with regard to the terminal sequences cleaved from tRNA precursor species (35).

6. The $tRNA_i^{met}$ run-through transcripts are processed at the 3' end by an endonuclease, and the 3' trailer accumulates *in vivo* as a discrete species. These long transcripts appear to be processed in a fashion similar to the B. mori $tRNA^{tyr}$ primary transcript, in which the 22 nucleotide trailer is cleaved through the action of an endonuclease (16,33). This report demonstrates that *in vivo* a long 3' trailer can accumulate as a major species in the cell, and is not necessarily rapidly degraded.

The properties of the $tRNA_i^{met}$ 3' deletion plasmids described in this study suggest their potential use in certain engineered genetic constructions. The tRNA gene could be used to promote transcription of theoretically any DNA sequence fused to the 3' border of the gene, generating a fusion gene which would utilize the efficient polymerase III promoter of the human $tRNA_i^{met}$ gene (18,25,36). By fusion of the DNA sequence to a $tRNA_i^{met}$ deletion mutant such as Δ 3'-4, a long read-through transcript would be generated *in vivo* (dependent, of course, on the absence of effective RNA polymerase III termination sequences). Fusion of the DNA sequence to a $tRNA_i^{met}$ deletion mutant such as Δ 3'-5 would lead to the generation of a cotranscript from which subsequent processing of the tRNA leader at the 5' portion of the fused transcript would be blocked. Control over processing may be of some biological use in engineered constructions, as suggested by the properties of mRNA species bearing tRNA sequences as 5' leaders in prokaryotes (37,38). Such "dual transcripts" code for several predominant bacterial proteins such as EF-Tu (37) and may use the tRNA leaders as a means of stabilizing the transcript from degradation *in vivo* (39). The potential use of the $tRNA_i^{met}$ gene as a "promoter-leader" in eukaryotic systems has been realized recently in our laboratory. Fusion genes consisting of the deleted $tRNA_i^{met}$ sequences contained on plasmids Δ 3'-4 and Δ 3'-5 in front of a promoter-less Herpes simplex type I thymidine kinase gene yield viral-specific enzyme resulting from RNA polymerase III dependent transcription in both X. laevis oocytes and somatic cells (Adeniyi-Jones and Zasloff, Manuscript in preparation).

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REFERENCES

1. Koski, R.A., Clarkson, S.G., Kurjan, J., Hall, B.D., and Smith, M. (1980) Cell 22, 415-425.
2. Hofstetter, H., Kressmann, A., and Birnstiel, M.L. (1981) Cell 24, 573-585.
3. Galli, G., Hofstetter, H., and Birnstiel, M.L. (1981) Nature 294, 626-631.
4. Sharp, S., DeFranco, D., Dingermann, T., Farrell, P., and Soll, D. (1981) Proc. Natl. Acad. Sci. (USA) 78, 6657-6661.
5. Ciliberto, G., Castagnoli, L., Melton, D.A., and Cortese, R. (1982) Proc. Natl. Acad. Sci. (USA) 79, 1195-1199.
6. Carrara, G., DiSegni, G., Otsuka, A., and Tochini-Valentini, G.. (1981) Cell 27 371-379.
7. Larson, D., Bradford Wilcox, J., Young, L.S., and Sprague, K.U. (1983) Proc. Natl. Acad. Sci (USA) 80, 3416-3420.
8. Klemenz, R., Stillman, D.J., and Geiduschek, E.P. (1982) Proc. Natl. Acad. Sci. (USA) 79 6191-6195.
9. Sharp, S., Dingermann, Schaack, J., DeFranco, D., and Soll, D. (1983) J. Biol. Chem. 258, 2440-2446.
10. Sprague, K.U., Larson, D., and Morton, D. (1980) Cell 22, 171-178.
11. DeFranco, D., Schmidt, O., and Soll, D. (1980) Proc. Natl. Acad.Sci. (USA)77 3365-3368.
12. Sharp, S., Dingermann, T., and Soll, D. (1982) Nucleic Acids Res., 10 5393-5406.
13. Dingermann, T., Burke, D.J., Sharp, S., Schaak, J., and Soll, D. (1982) J. Biol. Chem. 257, 14738-14744.
14. Clarkson, S.G. (1982) in Eukaryotic Genes: Their Structure, activity, and Regulations, eds. Maclean, N., Gregory, S.P., and Flavell, R.A. (Butterworth, London)
15. Ciliberto, G., Castagnoli, L., and Cortese, R. (1983) Current Topics in Develop. Biol. 18, 59-88.
16. Hagenbuchle, O., Larson, D., Hall, G.I., and Sprague, K.U. (1979) Cell, 18, 1217-1229.
17. Koski, R. A., Clarkson, S.G. (1982) J. Biol. Chem. 257, 4514-4521
18. Zasloff, M., Santos, T., Romeo, P. and Rosenberg, M. (1982) J. Biol. Chem 257, 7857-7863.
19. Silverman, S., Schmidt, O., Soll, D., and Hovemann, B. (1979) J. Biol. Chem. 257, 10290-10294.
20. Santos, T., and Zasloff, M. (1981) Cell 23, 699-709.
21. Mizuuchi, M., and Mizuuchi, K. (1980) Proc. Natl. Acad. Sci. (USA), 77, 3220-3224.
22. Dagert, H. and Ehrlich, S.D. (1979), Gene 6, 23-28.
23. Maxam, A.M., and Gilbert, W. (1977) Proc. Natl. Acad. Sci (USA) 74, 560-564.
24. Zasloff, M., Ginder, G., and Felsenfeld, G. (1978) Nucleic Acids Res. 5, 1139-1151.
25. Zasloff, M., Rosenberg, M., and Santos, T. (1982) Nature 300, 81-84.
26. Wallace, R.A., Jared, D.W., Dumont, J.N., and Sega, M.W. (1973) J. of Exp. Zoology 184, 321-334.
27. Sutcliffe, J.G. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 77-90.
28. Bogenhagen, D.F., and Brown, D.D. (1981) Cell 24, 261-270.
29. Kim, S.H. (1979) In, Transfer RNA: Structure, Properties, and Recognition, eds. Schimmel, P., Soll, D., and Abelson, J.N., 83-100 (Cold Spring Harbor, NY).
30. Mattoccia, E., Baldi, M.I., Pande, G., Ogden, R., and Tocchini-Valentini, G.P. (1983) Cell 32, 67-76.

31. Nishikura, K., Kurjan, J., Hall, B.D., and De Robertis, E.D. (1982) EMBO Journal 1, 263-268.
32. De Robertis, E.M., and Olson, M.V. (1979) Nature 278, 137-143.
33. Garber, R.L., and Orge, L.P. (1979) Cell, 18, 817-828.
34. Sprinzl, M., and Gauss, D.M. (1982) Nucleic Acids Res. 10, r57-83.
35. Altman, S. (1981) Cell 23, 3-4.
36. Zasloff, M., Santos, J., and Hamer, D.H. (1982) Nature 295, 533-535.
37. Hudson, L., Rossi, J., and Landy, A. (1981) Nature 294 422-427.
38. Altman, S., Model, P., Dixon, G., and Wosnik, M.A. Cell, 26, 299-304.
39. Lim, L. and Kennel, D.J. (1979) J. Mol. Virol. 135, 369-390.