Modulation of Transcriptional Activity and Stable Complex Formation by 5'-Flanking Regions of Mouse tRNA^{His} Genes

MARY J. MORRY[†] AND JOHN D. HARDING^{*}

Department of Biological Sciences, Columbia University, New York, New York 10027

Received 7 May 1985/Accepted 10 October 1985

We determined the nucleotide sequences of three mouse tRNA^{His} genes and a tRNA^{Gly} gene present in two different λ clones. One λ clone contained two tRNA^{His} genes 600 base pairs (bp) apart in opposite orientations. The other clone contained a tRNA^{His} and a tRNA^{Gly} gene 569 bp apart in the same orientation. The coding regions of the three tRNA^{His} genes were identical to sequenced mammalian tRNA^{His} if posttranscriptional modifications are not considered. Notably, the three tRNA^{His} genes and a fourth gene previously sequenced by us contained within the flanking regions, various amounts of short, conserved 5' leader sequences and 3' trailer sequences directly abutting the coding regions. Otherwise the flanking regions were not homologous. Deletion mutants of one of the tRNA^{His} genes were constructed which contained 228, 99, 9, and 3 bp of the wild-type 5'-flanking region, respectively. Deletion of 5'-flanking sequences from positions -9 to -4 reduced transcriptional activity substantially (ca. fivefold) in a HeLa cell S-100 lysate. This effect was independent of the vector sequences in the deletion clone, implying that the region from -4 to -9 of the intact gene contains a positive modulatory element for transcription in vitro. The deletion mutant containing 3 bp of wild-type 5'-flanking sequence also had a greatly reduced ability to inhibit the transcription of a second tRNA gene in a competition assay. Thus, the normal 5'-flanking region influences the ability of the gene to form stable complexes with transcription factors. These data further indicate that a mammalian transcription extract is sensitive to 5'-flanking-region effects if a suitable tRNA gene is assayed.

Mammalian tRNA genes are reiterated and dispersed in the genome. Individual members of some mammalian tRNA gene families vary in respect to coding- or flanking-region sequences or both (26, 37, 41, 45). Similar types of variation are also seen in tRNA genes isolated from amphibians (6), insects (e.g., see references 1, 10, 20), and yeasts (reviewed in reference 16). To obtain a detailed understanding of the mechanisms by which tRNA gene expression is regulated, it is necessary to determine the effects of intrafamilial sequence variation on gene activity.

The efficiency of transcription, as measured by in vitro assays, is one level at which sequence variation can modulate tRNA gene activity. Eucaryotic tRNA genes contain highly conserved sequences within the coding regions that are absolutely required for transcription (4, 14, 19, 42). These "internal" control regions appear to be sites of interaction of specific transcription factors with the gene (12, 13, 24, 43, 47). In addition, sequences in the 5'-flanking regions of certain *Xenopus* (18), *Drosophila* (8, 10, 11, 38), *Bombyx mori* (23), and yeast (22, 44) tRNA genes can modulate transcription either positively or negatively. Thus, major questions in regard to any specific tRNA gene family are whether modulatory sequences are present in the flanking regions and the mechanisms by which these sequences affect gene activity.

To address these issues for mammalian tRNA genes, we isolated and sequenced four members of the mouse $tRNA^{His}$ gene family. We show that the flanking regions of the mouse $tRNA^{His}$ genes contain different amounts of short conserved 5' leader and 3' trailer sequences directly abutting the coding regions. Part of the conserved 5' leader sequence is a

positive regulatory element for transcription in vitro and promotes stable interaction of the gene with transcription factors present in cell extracts.

MATERIALS AND METHODS

Isolation of λ clones containing tRNA^{His} genes. A recombinant DNA library containing DBA-2 mouse genomic DNA cloned in phage λ Charon 4A (17) was plated, and the plaques were transferred to cellulose nitrate filters (BA85; Schleicher & Schuell, Inc., Keene, N.H.) by the method of Benton and Davis (2). Filters were hybridized with 5×10^6 cpm of ³²P-labeled tRNA^{His}-specific probe as described in reference 27. After hybridization, the filters were washed for 1 h at 68°C in 4× SET-0.1% sodium dodecyl sulfate-0.1% sodium PP_i and for 1 h at 68°C in 2× SET-0.1% sodium dodecyl sulfate-0.1% sodium PP_i. (1× SET is 0.15 M NaCl, 2 mM EDTA, 30 mM Tris hydrochloride, pH 7.5.) The filters were autoradiographed with Kodak XS-5 film and an intensifying screen. Phage plaques which gave a signal on duplicate filters in the first screening were picked, and the phage were plaque purified.

Hybridization probes. A tRNA^{His}-specific probe was prepared from the tRNA^{His} coding region of phage λ Mt1 (17). This probe contains 51 base pairs (bp) of the tRNA^{His} coding region cloned into the *Eco*RI site of pBR322. It was labeled with ³²P by nick translation and used as a probe to detect tRNA^{His} coding sequences in λ and M13 phages.

Total mouse liver tRNA was labeled with $12^{\frac{5}{5}}$ I as described in reference 17.

DNA sequence analysis. All DNA sequences were determined from M13 subclones derived from λ clones. Phage λ Mt5 and λ Mt6 DNAs were digested with various restriction enzymes (see Fig. 1 legend), and the resulting fragments made blunt ended, if necessary, by incubating 600 ng of DNA in 30 µl of 20 mM Tris hydrochloride (pH 7.5)–10 mM

^{*} Corresponding author.

⁺ Present address: Laboratory of Molecular Parasitology. The Rockefeller University, New York, NY 10021.

MgCl₂–10 mM dithiothreitol containing 33 μ M each deoxynucleoside triphosphate and 0.1 U of DNA polymerase I (large fragment) at 37°C for 30 min. The target fragments were ligated with *Hinc*II-cut M13 mp7 DNA (29) essentially as described in reference 17, transfected into CaCl₂-treated *Escherichia coli* JM103, and plated as described in reference 29. M13 plaques were screened as described above for λ phage except that sonicated *E. coli* DNA carrier was not used in the hybridization and each filter was hybridized with 2.5 × 10⁶ cpm of ³²P-labeled tRNA^{His}-specific probe. White plaques corresponding to autoradiographic signals were picked, and the mouse DNA inserts were sequenced by the dideoxy chain termination method, using 8 or 6% thin sequencing gels (35, 36).

Isolation of M13 subclones for transcription analysis. Clone nomenclature is as follows, e.g., for the clone m5A-228R: m = M13 vector, 5A = the tRNA^{His} gene designated *Mt5A* (see Fig. 1 and 2), -228 = the last 5'-flanking residue of mouse DNA in the clone, R = coding strand of the tRNA gene inserted into the noncoding strand of the vector (as defined in reference 29). Clones m5A-228R (termed Av 1 in Fig. 1), m5A-99R, m5A-9R, and m5A-3R (termed A30 in Fig. 1) were isolated during DNA sequencing from AvaI, SmaI, HpaII, and AluI digests of λ Mt5 DNA, respectively. Clone m5B-34R was isolated during DNA sequencing from an HhaI digest of λMt5 DNA. Clone m5B-1R was isolated from a HpaII-HhaI double digest of λ Mt5 DNA, the 5' flank being anomalously replaced by a foreign DNA sequence. Clone m6-175R was isolated from a Sau 96-DdeI double digest, and clone m6-9R was isolated from an HpaII digest of λ Mt6 DNA.

In vitro transcription. HeLa cell S-100 transcription extract was prepared essentially as described in reference 49. The final step involved dialysis in 20 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (pH 7.9)–100 mM KCl-2 mM MgCl₂–2mM dithiothreitol–20% glycerol. The dialyzed extract was aliquoted and stored at -80° C.

HeLa cell nuclear extract was prepared as follows. Nuclei obtained during the preparation of the S-100 extract were suspended in a volume of hypotonic buffer equal to the original packed volume of HeLa cells. Hypotonic buffer is 10 mM Tris hydrochloride (pH 7.9), 10 mM KCl, 1 mM MgCl₂, and 1 mM dithiothreitol. Saturated ammonium sulfate solution (0.1 volume) was added dropwise to the nuclei, and the solution was incubated on ice for 20 min with occasional gentle shaking. One packed cell volume of hypotonic buffer and glycerol (1:1, vol/vol) was added, and the suspension was centrifuged at 100,000 × g for 2 h. The supernatant was removed, dialyzed as for the S-100 lysate, and stored at -80° C.

A typical 15-µl transcription reaction mixture contained 7.5 µl of HeLa cell S-100, 5 µl of HeLa cell nuclear extract, 500 µM ATP, CTP, and UTP, 25 µM GTP, 10 µCi of $[\alpha^{-32}P]$ GTP (410 Ci/mmol), and 4 mM creatine phosphate. DNA template solution (1 µl) (typically containing 0.2 µg of M13 clone DNA in 10 mM Tris hydrochloride [pH 7.4]–0.1 mM EDTA) was added, and the reaction mixture was incubated for 1 h at 30°C. Stop buffer (1 volume) (8 M urea, 150 mM NaCl, 20 mM Tris hydrochloride [pH 8], 10 mM EDTA, 0.5% sodium dodecyl sulfate, 100 µg of yeast RNA per ml) and gel buffer (1 volume) (7 M urea, 15% glycerol, 1× TBE buffer [35], 5% dimethyl sulfoxide, 0.03% xylene cyanole) were added, and the sample was incubated at 70°C for 5 min. Samples were run on 8% thin polyacrylamide–8 M urea gels (35) which were dried and autoradiographed.

RESULTS

Isolation and sequence analysis of mouse tRNA^{His} genes. From a mouse recombinant DNA library, we plaque purified 12 λ phage which hybridized with a tRNA^{His}-specific probe. The λ clones can be divided into three groups based on *Eco*RI (and other restriction enzyme) digestion patterns. Of the 12 clones, 9 contained a 10-kilobase *Eco*RI mouse DNA insert and, in addition, identical *XbaI* and *Bam*HI fragments. One of these phage, termed λ Mt5, was further characterized. One of the 12 λ clones, termed λ Mt6, contained two *Eco*RI mouse DNA fragments, 2.2 and 4.3 kilobases in size, respectively. The remaining two λ clones were identical to phage λ Mt1, which contains a single tRNA^{His} gene (17).

Two noncontiguous sequences, termed Mt5A and Mt5B, were obtained from phage λ Mt5 (Fig. 1, 2, and 3). The Mt5A and Mt5B sequences comprise 956 and 799 bp, respectively (Fig. 2 and 3). Restriction mapping experiments based on known sites in the sequences established that the Mt5A and Mt5B sequences are separated by ca. 250 bp of unsequenced DNA (Fig. 1). Each sequence contains a single tRNA^{His} gene. The two tRNA^{His} coding regions are present in opposite orientations, ca. 600 bp apart.

We derived a 1,373-bp sequence from phage λ Mt6 which contains a tRNA^{Gly} and a tRNA^{His} gene, 569 bp apart in the same orientation (Fig. 1 and 4).

On the basis of the sequences shown in Fig. 2, 3, and 4, we analyzed $\lambda Mt5$ and $\lambda Mt6$ DNA by Southern blotting (46), using both mouse tRNA- and tRNA^{His}-specific probes. These experiments indicated that the two λ clones contain no additional unsequenced tRNA genes (data not shown).

The tRNA coding regions. The three tRNA^{His} coding regions present in the Mt5A, Mt5B, and Mt6 sequences can encode a tRNA^{His} identical to sequenced mouse tRNA^{His} (34) if posttranscriptional modifications are not considered. The tRNA^{His} coding region of phage λ Mt1 differs at a single position in the D loop (17).

The tRNA^{His} genes do not encode the 3'-terminal CCA residues of the tRNA, as is usual for eucaryotic tRNA genes. In addition, cytoplasmic tRNA^{His} contains a 5'-terminal G residue (modified to methyl-G in mouse tRNA^{His} [34]) that is not encoded in the DNA of λ Mt1, λ Mt5, or λ Mt6. Cooley et al. (7) have shown that extracts of *Drosophila* Kc cells add a G residue to the 5' end of *Drosophila* tRNA^{His} transcribed in vitro. The mouse gene sequences suggest that the 5'-terminal G residue of mammalian tRNA^{His} is also added post-transcriptionally.

The tDNA^{Gly} encoded by λ Mt6 does not correspond to any sequenced tRNA^{Gly} listed in a recent compilation (15). It is identical to the tRNA^{Gly} coding region of two other sequenced mouse genes (21, 26).

Comparison of tRNA^{HIs} gene flanking regions. Inspection of the sequences shown in Fig. 2, 3, and 4 indicates that the tRNA^{HIs} genes contain short homologous 5' leader and 3' trailer sequences contiguous to the coding regions. No other obvious flanking region homologies are evident.

The proximal 5'-flanking regions of the tRNA^{His} genes are compared in Fig. 5A. The first 13 bp of the Mt5A 5' flank are homologous to the first 14 bp of the Mt5B sequence, the latter containing one additional C residue. Mt5B and Mt6 share a common 13-bp leader sequence containing two mismatches. The proximal 5'-flanking region of the Mt1 gene is less homologous to those of the other three genes. It is most similar to the Mt6 sequence, with five of the first seven bp homologous.

A comparison of the proximal 3'-flanking residues of the



FIG. 1. M13 subclones used to sequence the tRNA genes in clones λ Mt5 and λ Mt6. (A and B) The bar represents the sequenced region of clone λ Mt5. The dotted lines mark ca. 250 bp of unsequenced DNA identified by restriction mapping. The tRNA^{His} coding regions are filled in. The leftward (5') gene is termed Mt5A and the rightward gene is termed Mt5B. (C) The bar represents the sequenced region of clone λ Mt6. The tRNA^{Gly} and tRNA^{His} coding regions are filled in. The labeled arrows below the bars represent the sequenced regions of M13 subclones derived from λ Mt5 and λ Mt6 DNAs, respectively. The beginning of the mouse DNA insert relative to the M13 sequencing primer is indicated by a vertical line; the arrowhead denotes the end of the sequence read from the gel. An arrowhead followed by a vertical line indicates that the entire mouse DNA insert of the M13 subclone was sequenced. Arrows pointing to the right indicate that DNA corresponding to the coding strand (as defined by the tRNA^{His} gene in the particular sequence) was sequenced; arrows pointing to the lambda phage from which it was isolated: A, *Alu*I; Av, *Ava*I; D, *Dde*I; H, *Hae*III; Hn, *Hin*fI; Hh, *Hha*I; Hp, *Hpa*II; S, *Sau*96I; Sa, *Sau*3A; Sm, *Sma*I.

tRNA^{His} genes is shown in Fig. 5B. The Mt5A and Mt5B genes share a homologous 29-bp sequence with one mismatch. The homology ends just before the putative transcription termination site, consisting of a string of four or five consecutive T residues in the two genes. There is no obvious homology among the 3'-flanking regions of the Mt6 and Mt1 genes and any of the other tRNA^{His} genes.

Effects of 5'-flanking sequences on transcription in vitro. To

analyze the effects of 5'-flanking region sequences on tRNA^{His} gene transcription, we prepared several deletion mutants of the tRNA^{His} genes. Each clone was transcribed in a modified HeLa cell S-100 lysate as described in Materials and Methods. Addition of HeLa cell nuclear extract to the basic S-100 lysate substantially increases the transcriptional activity of the lysate (R. Rooney and J. Harding, unpublished data). In preliminary experiments (data not shown), we established that each tRNA gene is transcribed by RNA

MOL. CELL. BIOL.

CAGGAAAAGAA CTGTTGGTGT GTCGGTGGTA GTTCATTCGT AAGTTCTCGA TCCACCTTTT788898188118128TTCACTTCCA TTTGGCCTAA TGCGAATAA TAGACCCGAA TTTGTGAGGCTTAAACACCCG AACCCCAAA138148159168178188TTTTTAGAGG CACACAAAAC AACGTGATAA TTTGTTGAGGCTT AAACACCCCC AACTGGAAGAAAAAAATTCTC GTGTGTTTG TTGCACTATT AAACGAAATT ACACTCCATC TGACCCTTCT198288218228238248GGAGAAAAAC ACCAGCACC ACGAGGCTAT GTTAGCACGC GTGTCACTGG TGTTTTTGACACAAAAAACCCACCTCCT198288218228CACAAGTGACC ACAAAAAACCCTCTTTTTG TGGTCGTGG TGCTCCGATA CATAGGGCG GTGTCACTGG TGTTTTTGAGTTGGCAGTAG ATCTTCATTGTAGCACCAA AAAAAAACT258268278288298388AGTCCCTTCA GTGTGGTAGT ATCTTCATTTGTCATATGATCTGAACCAA AAAAGGTTCG258268278288358368GTTGGTAGT ATCTTTCATTTGTCAATGAAAAAAGGTTCGAGACTTGGTT258268278388358368GTTTGTTTCCTACCTAACC CTAATTTTAA TTCTAACCCTAATTTTCGAAGGATA318328338348358368GTTGTTGTG GCCTCCGTGA TGAACTTTA AGAACTATA AGAATTAACAGACTTGAAATTAGACTAAAAT338398488418428AATCGTATG CCTCCGTGA TGAACATTAA AGAATAGAA AGTACCACGAGAGAAAATAA AGAAAAATAGACTAAAATT438448458468478488GTGATTAACC GTTGATATAC CTTTAATGG GAAAATAGAA AGTACCACGGAGAGAAATAA AGAAAAGAA AGTACCACG GAAAATAACAA AGTAACACAG G
768696168168118128TTCACTTCCATTGGCGTAATGCCGAATAATAGACCCGAATTTGTGGAGCTTGAGGGTTTAAACCGGATAAACCGGGTTATAACCGGCTTATTAGACCCGAATTTGGGAGCAAACACCCCCA138148158168178188TTTTTAAGAGCACACAAAACAAACGTGATAATTTGCTTTAATGGAGGTAGACTGGGAAGAAAAAATTCTCGTGTGTTTTGTTGCACTATAAACGAGAAAAACGTGAAAAAACGTGAAAA198288218228238248GGAGAAAAAACCAGCACCCACGAGGCTATGTTAGCAGCCCACAAGAAAACCCTCTTTTGTGGTCGTGGTTGCTCCGATACATCGTGCGCACAAAAAACCCTCTTTTGTGGTGGTAGTATCTTTCATTTGTCATATGATCTGAACCAAAGTCCCTTCAGGTGGTAGTATCTTTCATTTGTCATATGATCTGAACCAACCAGGGAAGTCACACCATCATAGAAAGTAAACAGTATACTAGACTTGGTT258268278288298388AGTCCCTTCAGTGGTGTAGTATCTTCATTTGTCAACAAAAAAGGTTCGTCAGGGAAGTCACACAATAAACAGTTTAATAGACTTGGTTTTTTCCAAGC318328338348358368GTTTGTTTCCTAACGGAATTAAAAATTAAGGAATAAAGACTTGGCAATAAAATCCTATGCCACCGGAATAGTTTTAAAAGACTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
TTCACTTCCATTGGCCTAATGCCGAATAATAGACCCGAATTTGGGCCTTTGAGGGTTTAAGTGAAGGTAAACCGGATTACGGCTTATTATCTGGGCTTAAACACCCGAAAAAACGGGATATTGGAGGTTAACCACCCCAAAA130140150160170180180TTTTAAGAGGCACAAAAACAACGGAATAATTGCTATTAATGGGAGAAAAACCGGGAAGAAAAAAATTCCCGTGTGTTTGTTGCACTATTAAACGAAATTACACTCCATCTGACCCTTCT190200210220230240240GGAGAAAAACACCAGCACCCACGAGGCTATGTTACCAGCCACAAAAAACTCCTCTTTTGTGGCGTGGGTGCTCCGATACAACCACCGCACAAAAAACT250260270280290380AGTCCCTTCAGTGTGGTAGTATCTTTCATTTGTGAACCAAAAAAGGTTCGTCAGGGAAGTACACCACCACCATAGAAAGTAAACAGTATACTAGACTTGGTT170180320330340350360GTTTGTTTCCTACCTAACCCTAATTTAATTCTAACCCCTAATTTCCAACC180320330340350360GTTTGTTTCCTACCTAACCCTAATTTTAAAGACTAAAATAGACTTGGATA190220380390400410420AATTCCTATGCCTCCGGAATCAACAGGAAAAGTGGATAAACAAAAACT190380390400410420AATTCCTATGCCTCCGGAATCAAGAACAACACGGAGAAAAACACTCAAAAAACT10031038039040
138148158168178188TTTTTAAAGAG CACACAAAACC GTGTGTTTTGACGTGATAA TTGCACTATTTGCTTTAA AAACGAAATTTGTGAGGAAG ACACTCCATCACTGGGAAGA TGACCCCTCT198208218228238248GGAGGAAAAAC CCTCTTTTGACCAGCACCC TGGTCGTGGGACGAGGCTAT TGCCCTCGATAGTTAGCACGC CACAGTGGCGACAAAAAACT GTTAGCACGC258268268278288298388AGTCCCTTCA CCAGGGAAGTGTGTGGTAGTA CACACCACACAATCTTCATT TGTCATATGATCTGAACCAA AAAAGGTACAAAAAGGTTCG AGACTTGGT318328338348358368GTTTGTTTTC CAACCAAAAAG GATGGATTGGATTAAAATT AAGATTGGGAGACTTGGTT TTTTCCAAGCAAAAGGTACA AGACTTGGG318328338348358368GTTTGTTTC CAACCAAAAAG GATGGATTG388398488418AATTCCTATG CCCTCCGTGAGAAAATAGAA ACTTGAGAATAGTCTTGTAA TCAGAAATT TCAGAAATT TCAGAAATT428AATTCTATG GGGAGGCACTGAAAATAGAA AGTACCACGCAGAGATATAA AGTCTTGTAA TCAGAAATT TCAGAAATT TCAGAAATT TCAGAAATT488GTGATTAAAC GGTGACTAAAGAAAATAGAA GAAAATAGAAAGTACCACGC AGGAAATAGAAGTACCACG AGTACATAC476438448458468478AGAAAAAGTAA AGTACCACGC538548GTGACTAAA CCAACAAAGCT518528538548GTGACTAAA CCAACAAACCT518528538548GTGAC
TTTTTAAGAG AAAAATTGTCCACACAAAAC GTGTGTTTTGCACGTGATAA TTGCACTATTTTTGCTTTAA AAACGAAATTTGTGAGGTAG ACACACCATCCTGGGGAAGA TGACCTTCT190200210220230240GGAGAAAAAC CCTCTTTTGACCAGCACCC TGGTCGTGGGACGAGGACCC TGCTCCGATACACAGGACCC CACAAGAAACT CAATCGTGCGCACAACAAAACT TGTCATGGG240AGTCCCTTCA CCTCTTTTGTGGTCGTGGG TGGTCGTGGTAGTACTCTTCAAT ACACCACCACA ACACACACACA TCAGGGAAGTCACACCACACA ACACCACACA ACACCACCACCA TAGAAAGTAAG TCAGGGAAGT250260270 ACAAAAAGGTTCG TGTCATGGG280290 ACACAAAAAG AAAAGGTTCG TGTCATGGGT300310320270280350360 AAAAGGTTCG TTTTCCAAGC350360 360GTTTGTTTC CTACCTAACC CTAACTTAGG CAACCAAAAAG GTTTTGTTTC320330340350 350360 350GTTGGTTGGGTAGT CAACCAAAAAG TTGGATTTAA AAACAGGAATAGGA AAACAGAAACT TTAAGGAAAC AAACAGAAAACT TCAGGAGCACT330340350 350360 360370380380390400410 400420 AAAAAGCT AAAAAGCAAAAAT TCTGAATTAG TCAGGAGCACT480 440420 440430440450460470 480480 440480 450GGTGACTAAA CCAACTAAAGC450520530 530540 TGTTTTCATT430500500510520530 530540 TGTTTTTGAG ACAAAAACTC4900500500570580590 5
AAAAATTCTCGTGTGTTTTGTTGCACTATTAAACGAAATTACACTCCATCTGACCTTCT198288218228238248GGAGAAAAACACCAGCACCCACGAGGCTATGTTAGCACGCCACAGTGACCACAAAAAACTCCTCTTTTGTGGTCGTGGGTGCTCCGATACAATCGTGCGGTTAGCACGCACAAAAAAACTCTCTTTTGTGGTCGTGGGTGCTCCGATACAATCGTGCGGTTGTCTTTGATGTTTTTTGAAGTCCCTTCAGTGTGGTGAGTATCTTTCATTTGTCATATGAAGACTTGGTTTTTCCAAGCTGTGAGGGAAGTCAACCACACACATAGAAAAGGTAACTAGACTTGGTTTTTCCAAGCTTTTCCAAGC318328338348358368ATTGTCTATAGACTTGGCATTGTCCATCAACCAAAAAGATGGATTGGGATTAAAATTAAGATTGGCATTAACAGGATAAAGACTAAAAATAGACTAAAAAT378388398488418428AATTCCTATGCCCTCCGTGATGAACTTTAAGTCTTGTAAGGTTTTTAAAAGACTAAAAT378388448458468478AGACTAAAAT438448458468478ACAAAAAGTAAGTGACTAAACGTTGTTCGAGAAAATAGAAAGTACCACGCAGAAAAGTAA438448458528538548GGTGACTAAACCAACAAAGCTGTAACATCAGTGCCTCGTGACATAGAACGA498588518528598688GGTGACTAAAGCAACAAAGCTGAACAACACGGAACAACACGGAACAAAACTC558568578588598<
198288218228238248GGAGAAAAACACCAGCACCCACGAGGCTATGTTAGCACGCCACAGTGACCACAAAAAACTCTCTTTTTGTGGTCGTGGGTGCTCCGATACAATCGTGCGGTGTCACTGGTGTTTTTTGA258268278288298388AGTCCCTTCAGTGTGGTAGTATCTTTCATTTGTCATATGATCTGAACCAAAAAAGGTTCG108328338348358368368GTTTGTTTCCTACCTAACCCTAATTTTAATTCTAACCCCTAATTTTCGCATTGTCCTATAAACAAAAAGGATGGATTGGGATTAAAAATTAAGATTGGGGATTAAAAGCGTAACAGGATA378388398489418428AATTCCTATGCCCTCCGTGATGAACTCTTAAGTCTTGTAAGGTTTTTAA378388398468478468AATTCATAGGGGGAGGCACTACTGGAGAAAAGTACCACGCAGGATATTA438448455468478488GTGATAACCGTTGAATAGGAAATAGAAAGTACCACGCAGGAGATATA438588518528538548GGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAC498588518528538548GGTGACTAAACCAACAAGCTGTAACATCAGTGGCTCGTGACATAGTATAC498568568578588598688AATTAACACGCATTATATAGGGAAGTAGTGGACATAGGAGAATTAGCCAATTAACCACGCATTATATAGCGGAAGT
GGAGAAAAAC CCTCTTTTG TGGTCGTGGGACCAGCGACCC TGCTCCGATAACGAGGCACCC CCTCCGATA CAATCGTCGGCACAACAGGCTAT CCACACCATCAGTTAGCACGC CCACACCATCA TAGAAAGTAA ACAGTATACT ACAGTATACT ACAGTATACT AGACTTGGT280 290 290 290 290 3000 3000 3000 3000 3000 3500 3
CCTCTTTTTGTGGTCGTGGGTGCTCCGATACAATCGTGCGGTGTCACTGGTGTTTTTGA250260270280290300AGTCCCTTCAGTGTGGTAGTATCTTTCATTTGTCATATGAAGACCTAGACAAAAGGTTCGTCAGGGAAGTCACACCATCATAGAAAGTAAACAGTATACTAGACTTGGTTTTTTCCAAGC310320330340350360GTTTGTTTTCCTACCTAACCCTAATTTTAATTCTAACCCCTAATTTTCCAAGCCAAACAAAAGGATGGATTGGGATTAAAATTAAGATTGGGGATTGTCCTATCAAACAAAAGGATGGATTGGGATTAAAAATTAAGACTAAAAGCGATTGTCCTAT370380390400410420AATTCCTATGCCTCCGTGATGAACTCTTAAGTCTTGTAAGGTTTTTAAATTAAGGATACGGGAGGCACTACTTGAGAAATTCAGAACATTCCAAAAAATT430440450460470480GTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGATATTAACAAAAGTAA430440450460470480GTGACTAAACGAACTTAATCCTTTTATCTTTCATGGTGCGAGAGATATTAACAAAAGTAA430500500510520530540GGTGACTAAACCAACAAGCTGTAACATCAGGGCACTGATATCACAAAAACTC490500500570580590600AATTAACCACGCATTATATGCCTTCATCATGAGCAACAGGGACATAGGAGAATTAGCCC550560570580590600 </td
250260270280290300AGTCCCTTCAGTGTGGTAGTATCTTTCATTTGTCATATGATCTGAACCAAAAAAGGTTCGTCAGGGAAGTCACACCATCAAGAAGTAAACAGTATACTAGACTTGGTTTTTTCCAAGC310320330340350360GTTTGTTTCCTACCTAACCCTAATTTAATTCTAACCCATTGTCCATCAAACAAAAGGATGGATTGGCTAATTTAAATTCTAACCCATTGTCCTATAAAAGGGTACGGAGGCACTCTAATTTAAAGATTGGAGGATTGTCATAAAAGC370380390400410420AATTCCTATGCCCTCCGTGATGAACTCTTAAGTCTTGTAAGGTTTTTAAAAAGGATACCGGGAGGCACTTGAACTACAAAGTCCTGGAAAGACTAAAAT430440450460470480GTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGATATTAAAATTAACCGTTGAATAGGAAAATAGAAAGTACCACGCAGAGATATAACACTAATTGGAACTTAACCGTAACATCAGTCGCTCGTGAAGAGATATAAGGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGGGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGGGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGGGTGACTAAACCAACAAGCTGAACATCAGGAGCCACACAGGGAAAAACTCA90500510520530540GGTGACTAAACCAACAAGCTGCCTTCATCATGAGCAACAGGGACATAGGAAATTAACCACGCATTATAAT <t< td=""></t<>
AGTCCCTTCA TCAGGGAAGTGTGTGGGTAGT ACACCCATCA TAGAAAGTAAATCTTTCATT TGTCATATGA ACAGTATACTTGTGAACCAA AGACTTGGTT AGACTTGGTT TTTTCCAAGC31Ø GTTTGTTTC CAACCAAAAG32Ø GATGGATTGG GATGGATTGG GATGAACTTAAAATT CAAACAAAAG33Ø AGACTTGAACC CTAATTTTAA AAGATTGGGG34Ø TGTAATTTCCAAGC TAATTTCCAAGCA AACAGGATA35Ø ATTGTCCTAT AACAGGATA37Ø AATTCCTATG CCCTCCGTGA CCCTCCGTGA TTAAGGATAC GGGAGGCACT39Ø GGAACTCTTA AGTCTTGAAATT AGTCTTGTAA AGTCTTGTAA AGTCTTGAAAATT CCAAAAATTT CCAAAAATTT CCAAAAATTT CCAAAAATTT CCAAAAATTA CCAACTAAAA CACTTAATC GGTGACTAAA CCACTGATT41Ø A2Ø AGACTTAAAC CCAACTAAAC CCAACTAAAC CCAACTAAAC CCAACTAAAC CCAACTAAAC CCAACAAAGCT45Ø A6Ø AGAAATAGAA AGTACCACGC AGGAGATATTA AGACTTAAAT CCAAAAATTA CCAACAAAGCT AGTGATTTCAATT48Ø AGACATATAA AGAAATAGAA AGTACCACGC AGAGATATTA AGAAATAGAA AGTACCACGC CCATAGTATAC CCATAGTATAC CCAACAAAGCT45Ø AGTGATTAAAA CCAACAAACTC41Ø A2Ø AGAAATAGAA AGTACCACGC AGAAATAGAA AGTACCACGC CCATAGTATAC CCAACAAAACTC45Ø AGTACATCAC AGAAATAGAA AGTACCACGC CCATAGTATAC CCATAGTAAACC CCATGTATCCAAAACTC41Ø ACAAAAACTC49Ø GGTGACTAAA CCAACAAACCC CCACCAAACCC GGAACTACACACG GGACATAGGA GGACATAGGA GGACATAGGA CCACTGATATA CCAACAAAACTC410 ACAAAAACTC49Ø GGTGACTAAAA CCAACAAAACCC CCACCACACACG GGACATAGGA CCACTGACACACACACACACACACACACACACACACACAC
TCAGGGAAGTCACACCATCATAGAAAGTAAACAGTATACTAGACTTGGTTTTTTCCAAGC310320330340350360GTTTGTTTCCTACCTAACCCTAATTTTAATTCTAACCCTAATTTTCGCATTGTCCTATCAAACAAAAGGATGGATTGGGATTAAAATTAAGATTGGGGATTAAAAGCGAATACAGGATA370380390400410420AATTCCTATGCCCTCCGTGATGAACTCTAAAGTCTTGTAAGGTTTTTAAAAGACTAAAATTTAAGGATACGGGAGGCACTACTTGAGAATTCAGAACATTCCCAAAAATTTCTGATTTAA430440450460470480GTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGGATATTAACAAAAGTAACACTAATTGGCAACTTAATCCTTTTATCTTTCATGGTGCGTCTCTATAATTGTTTTCATT490500510520530540GGTGACTAAACCAACAAGCTGTAACATCAGTGGCGCGCACTGTATCATATCACAAAAACTC550560570580590600AATTAACCACGCATTATATAGCGTTCATCATGAGCAACAGGGACATAGGAGAATTAGCCC550560570580590600G00AATTAACCACGCATTATATAGCGTTCATCATGAGCAACAGGGACATAGGAGAATTAGCCC550560570580590600G00AATTAACCACGCATATATATACGGAAGTAGTACTCGTTGTCCCTGATACCCTTAATCGGG
310320330340350360GTTTGTTTTCCTACCTAACCCTAATTTTAATTCTAACCCCTAATTTTCGCATTGTCCTATAAACAAAAAGGATGGATTGGCAATAAAATTAAGATTGGGGATTAAAAAGCGATTGTCCTATAATTCCTATGCCCTCCGTGATGAACTCTTAAGTCTTGTAAGGTTTTTAAAAGACTAAAATTAATTCCTATGCCCTCCGTGATGAACTCTTAAGTCTTGTAAGGTTTTTAAAAGACTAAAAATAATTCCTATGCCCTCCGTGATGAACTCTTAAGTCTTGTAAAGTCTAAAAATTAGACTAAAAATAATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGATATTAAGACAAAAGTAAGGTGACTAAACCAACAAGCTGTAACATCAGAGCTCGTGGAATTTTTCATT490050005100520053005400GGTGACTAAACCAACAAGCTGTAACATCAGAGCGAGCACTGTATCATATC550056005700580059006000AATTAACCACGCATTATAAATGCCTTCATCATGAGCAACAGGGACATAGGAATAATTGGTGCGAATATTACGGAAGTAGTACTCGTTGTCCCTGTATCCT
GTTTGTTTTC CAAACAAAAG GATGGATTGGCTACTTAACC GATGAATTGGCTAATTTTAA GATTAAAAATT AAGATTGGGGTTCTAATTTCCC ATTAAAAGCGATTGTCCTAT TAACAGGATA370380390400410420AATTCCTATG CCCTCCGTGA GGGAGGCACTTGAACTCTTA AGTCTGAGAAT ACTTGAGAATAGTCTTGTAA GGTTTTAAA AGTCTGAAAATT TCAGAACATTAGACTAAAAAT TCTGAATTTA AGACTAAAAAT TCTGATTTAAA430440450460470480GTGATTAACC CACTAATTG CACTAATTG450460470480GTGATTAACC CACTAATTGGTTGAATAGA CAACTTAATCAGTACCACGC GAAAATAGAAAGTACCACGC AGTACCACGCAGAGATATTA ACAAAAGTAA TCTTTTCATT490500510520530540GGTGACTAAA CCAACAAGCT510520530540GGTGACTAAA CCAACAAGCT550560570580590600AATTAACCAC TTAATTGGTG560570580590600AATTAACCAC CGTAATATTAGCCTTCATCA CGGAAGTAGTTGAGCAACAG ACTCGTTGTCGGACATAGGA CCTGATCCT6000
CAAACAAAAGGATGGATTGGGATTAAAATTAAGATTGGGGATTAAAAGCGTAACAGGGATA370380390400410420AATTCCTATGCCCTCCGTGATGAACTCTTAAGTCTTGTAAGGTTTTTAAAAGACTAAAATTTAAGGATACGGGAGGCACTACTTGAGAATTCAGAACATTCCAAAAATTTTCTGATTTAAA430440450460470480GTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGATATTAACAAAAGTAACACTAATTGGCAACTTAATCCTTTTATCTTTCATGGTGCGAGAGATATAAATGTTTTCATT490500510520530540TGTTTTGAGGGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGACAAAAAACTC550560570580590600GAATTAGCCC550560570580590600GAATTAGCCCTTAATGGTGCGTAATATTACGGAAGTAGTACTCGTTGTCCCTTAATCGGG
37Ø38Ø39Ø4ØØ41Ø42ØAATTCCTATGCCCTCCGTGATGAACTCTTAAGTCTTGTAAGGTTTTTAAAAGACTAAAATTTAAGGATACGGGAGGCACTACTTGAGAATAGTCTTGTAACAAAAATTTAGACTAAAATGTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGAAATTAAGACAAAGTAACACTAATTGGCAACTTAATCCTTTTATCTTAGTACCACGCAGAGAATATAAACAAAAGTAAGGTGACTAAAACCAACAAGCTGTAACATCAGTCGCTCGTGAACTTAATCTGTTTTCATTGGTGACTAAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGTGTTTTGAGGCCACTGATTTGGTTGTTCGASTØØSTØØCAAAAACTCGTAACAATCAGGCCATCATATCS5ØS5ØS6ØS7ØTGAGCAACAGGGACATAGGAGAATTAGCCCTTAATTGGTGCGTAATATTACGGAAGTAGTACTCGTTGTCCTGTATCCTCTTAATCGGG
AATTCCTATG TTAAGGATACCCCTCCGTGA GGGAGGCACTTGAACTCTTA ACTTGAGAAT ACTTGAGAATAGTCTTGTAA CCAAAAATTTGGTTTTTAAA AGACTAAAAT AGACTAAAAT AGACTTTA430440450460470480430440450460470480GTGATTAACC CACTAATTG CACTAATTG CACTAATTG CACTAATTG450460470480GTGATTAACC CACTAATTG GTTGATTAG450460470480GTGATTAACC CACTAATTG CACTAATTG450460470480GTGATTAACC GCACTAATCGTAAAATAGAA CTTTTATCTTAGTCCCCCGC TCATGGTGCGAGAGATATTA TCTTTTCATT490500510520530540GGTGACTAAA CCAACAAGCT510520530540GGTGACTAAA CCAACAAGCTGTAACATCAG CATTGTAGTCAGCGAGCACT AGCGAGCACTGTTTTTGAG GTTTTTGAG550560570580590600AATTAACCAC TTAATTGGTG560570580590600GGACATAGGA CCTGTATCTCGGAAGTAGT ACTCGTTGTCCTGTATCCTCTTAATGCCC
TTAAGGATACGGGAGGCACTACTTGAGAATTCAGAACATTCCAAAAATTTTCTGATTTA430440450460470480GTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGATATTAACAAAAGTAACACTAATTGGCAACTTAATCCTTTTATCTTTCATGGTGCGTCTCTATAATTGTTTTCATT490500510520530540GGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGTGTTTTTGAGCCACTGATTTGGTTGTTCGACATTGTAGTCAGCGAGCACTGTATCATATCACAAAAACTC550560570580590600AATTAACCACGCATTATAATGCCTTCATCATGAGCAACAGCCTGTATCCTCTTAATGGCGTTAATTGGTGCGTAATATTACGGAAGTAGTACTCGTTGTCCTGTATCCTCTTAATCGGG
430440450460470480GTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGATATTAACAAAAGTAACACTAATTGGCAACTTAATCCTTTTATCTTTCATGGTGCGAGAGATATTAAGAAAAGTAA490500510520530540GGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGAATAGTATAGAGAAAAACTCCCACTGATTTGGTTGTTCGACATTGTAGTCAGCGAGCACTS00600AATTAACCACS60570580590600GGACATAGTGCGTAATATTACGGAAGTAGTACTCGTTGTCCTTAATGGGG
GTGATTAACCGTTGAATTAGGAAAATAGAAAGTACCACGCAGAGATATTAACAAAAGTAACACTAATTGGCAACTTAATCCTTTTATCTTTCATGGTGCGTCTCTATAATTGTTTTCATT490500510520530540GGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGTGTTTTTGAGCCACTGATTTGGTTGTTCGACATTGTAGTCAGCGAGCACTGTACATAACACCGTTTTTGAG550560570580590600AATTAACCACGCATTATAATGCCTTCATCATGAGCAACAGGGACATAGGATTAATTGGTGCGTAATATTACGGAAGTAGTACTCGTTGTCCTTAATCGGG
CACTAATTGGCAACTTAATCCTITTATCTTTCATGGTGCGTCTCTATAATTGTTTTCATT490500510520530540GGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGTGTTTTTGAGCCACTGATTTGGTTGTTCGACATTGTAGTCAGCGAGCACTGTATCATATCACAAAAACTC550560570580590600AATTAACCACGCATTATAATGCCTTCATCATGAGCAACAGGGACATAGGAGAATTAGCCCTTAATTGGTGCGTAATATTACGGAAGTAGTACTCGTTGTCCCTGTATCCTCTTAATCGGG
49Ø5ØØ51Ø52Ø53Ø54ØGGTGACTAAACCAACAAGCTGTAACATCAGTCGCTCGTGACATAGTATAGTGTTTTTGAGCCACTGATTTGGTTGTTCGACATTGTAGTCAGCGAGCACTGTATCATATCACAAAAACTC55Ø56Ø57Ø58Ø59Ø6ØØAATTAACCACGCATTATAATGCCTTCATCATGAGCAACAGGGACATAGGATTAATTGGTGCGTAATATTACGGAAGTAGTACTCGTTGTCCTGTATCCT
GGTGACTAAA CCAACAAGCT GTAACATCAG TCGCTCGTGA CATAGTATAG TGTTTTTGAG CCACTGATTT GGTTGTTCGA CATTGTAGTC AGCGAGCACT GTATCATATC ACAAAAACTC 550 560 570 580 590 600 AATTAACCAC GCATTATAAT GCCTTCATCA TGAGCAACAG GGACATAGGA GAATTAGCCC TTAATTGGTG CGTAATATTA CGGAAGTAGT ACTCGTTGTC CCTGTATCCT CTTAATCGGG
CCACTGATTT GGTTGTTCGA CATTGTAGTC AGCGAGCACT GTATCATATC ACAAAAACTC 550 560 570 580 590 600 AATTAACCAC GCATTATAAT GCCTTCATCA TGAGCAACAG GGACATAGGA GAATTAGCCC TTAATTGGTG CGTAATATTA CGGAAGTAGT ACTCGTTGTC CCTGTATCCT CTTAATCGGG
550 560 570 580 590 600 AATTAACCAC GCATTATAAT GCCTTCATCA TGAGCAACAG GGACATAGGA GAATTAGCCC TTAATTGGTG CGTAATATTA CGGAAGTAGT ACTCGTTGTC CCTGTATCCT CTTAATCGGG
AATTAACCAC GCATTATAAT GCCTTCATCA TGAGCAACAG GGACATAGGA GAATTAGCCC Ttaattggtg cgtaatatta cggaagtagt actcgttgtc cctgtatcct cttaatcggg
TTAATTGGTG CGTAATATTA CGGAAGTAGT ACTCGTIGIC CCIGIAICCI CIIAAICGGG
61Ø 62Ø 63Ø 64Ø 65Ø – 66Ø
GGGCATTACA ACGCCTTCAC TAAAAAGGCG CCCTTGCCGT GCCATTGTTG CCACAATGCC His
CCCGTAATGT TGCGGAAGTG ATTTTTCCGC GGGAACGGCA CGGTAACAAC GGIGIIACGG
67 <i>Ø</i> 68 <i>Ø</i> 69 <i>Ø</i> 7 <i>ØØ</i> 71 <i>Ø</i> 72 <i>Ø</i>
GTGACTCGGA TTCGAACCGA GGTTGCTGCG GCCACAACGC AGAGTACTAA CCACTATACG
CACTGAGCCT AAGCTTGGCT CCAACGACGC CGGTGTTGCG TCTCATGATT GGTGATATGC
<u> </u>
ATCACGGC <mark>G</mark> A GCTACCGGAA CGCCACAGTG TGCTGCGTAG CCAGGGCGAC TCTTGAGACA
TAGTGCCGCT CGATGGCCTT GCGGTGTCAC ACGACGCATC GGTCCCGCTG AGAACTCTGT
< −79ø 8øø 81ø 82ø 83ø 84ø
GGGACGATCC ATCCCTGCC ATCCCTATGC TGATGTACTG GGGGGGGCCCC AGCGAACCAC
CCCTGCTAGG TAGGGGACGG TAGGGATACG ACTACATGAC CCCCCGGGG TCGCTTGGTG
85Ø 86Ø 87Ø 88Ø 89Ø 9ØØ
AACGATTGAC CACAGTCTTC TTGAGTTTCT ATTTGGCTTG GAAACGCACC ACAAAAAGAC
TTGCTAACTG GTGTCAGAAG AACTCAAAGA TAAACCGAAC CTTTGCGTGG TGTTTTICIG
91Ø 92Ø 93Ø 94Ø 95Ø 956
AGCCATGAAA ATGCTTGCAA GCCCAGCGTG GTGGCGCCTA GGAACCTAAA ACTCGG
TCGGTACITT TACGAACGTT CGGGTCGCAC CACCGCGGAT CCTTGGATTT TGAGCC Sequence of the A region of clone) Mt5. The coding (non-tRNA-like) strand is shown on the ton line with the 5' end to the left

FIG. 2. Sequence of the A region of clone λ Mt5. The coding (non-tRNA-like) strand is show The tRNA^{His} coding region is boxed. The arrow denotes the direction of transcription.

polymerase III and characterized the optimal range of DNA concentrations that yielded maximal transcription. Optima are nearly the same for all M13 clones (400 ng of DNA per $25-\mu l$ reaction).

Figure 6 shows the transcripts obtained from each M13

clone. (See Materials and Methods for an explanation of clone nomenclature.) Based on parallel electrophoresis of a DNA sequencing ladder, all of the transcripts in the figure are larger than mature tRNA. Thus, during a 60-min incubation in this extract, little or no processing occurs. Compar-

10	2Ø	3Ø	40	5Ø	6Ø	
GGCCGTTGAT	AGGCAGAAAC	AAATGCCAGC	AACCCGATGA	CGTCAAAGGG	GGCGGGGCAT	
CCGGCAACTA	TCCGTCTTTG	TTTACGGTCG	TTGGGCTACT	GCAGTTTCCC	CCGCCCCGTA	
					->	
7Ø	8Ø	9Ø	1ØØ	11Ø	120	
CCAACACAGA	AGAGACCAAG	GAAGGAGTCC	TGACATCAAG	TTCCCGGTAG	CTOCCGTGA	His
GGTTGTGTCT	TCTCTGGTTC	CTTCCTCAGG	ACTGTAGTTC	AAGGGCCATC	GAGCGGCACT	
					••••••••	
1 3 <i>Ø</i> -	1 4 <i>Ø</i> -		1 6 <i>Ø</i> -		1 8Ø	
TCGTATAGTG	GTTAGTACTC	TGCGTTGTGG	CCGCAGCAAC	CTCGGTTCGA	ATCCGAGTCA	
AGCATATCAC	CAATCATGAG	ACGCAACACC	GGCGTCGTTG	GAGCCAAGCT	TAGGCTCAGT	
					~ ~	
190	200	210	220	230	240	
CGGCAIIGIG	GCAACAAIGG	CACGGCAAGG	GAGCIGIAII	TIATICTICT	GIICGAIIII	
GCCGTAACAC	CGTTGTTACC	GTGCCGTTCC	CTCGACATAA	AATAAGAAGA	CAAGCTAAAA	
250	260	2/10	280	290	300	
AATACTTAGC	TAACATICIA	GAAAAIGAAI	CICAAACIGC	CIGIIGIGAA	IIAGGGIIII	
TTATGAATCG	ATTGTAAGAT	CTTTTACTTA	GAGTTTGACG	GACAACACTT	AATCCCAAAA	
310	32Ø	33Ø	34Ø	35Ø	36Ø	
ATACCAGCGC	GGGCTAAATT	AAACTGCAGG	CGCACAAAAA	GCTTCCAGTA	TGTTAGAGGG	
TATGGTCGCG	CCCGATTTAA	TTTGACGTCC	GCGTGTTTTT	CGAAGGTCAT	ACAATCTCCC	
37Ø	38Ø	39Ø	400	4 1Ø	42Ø	
ATGGTGTTTG	CACCTAAGTA	CCAGACAATT	TGTCAGTCAG	AAAAATGCAA	TGTATATATG	
TACCACAAAC	GTGGATTCAT	GGTCTGTTAA	ACAGTCAGTC	TTTTTACGTT	ACATATATAC	
43Ø	44Ø	45Ø	46Ø	47Ø	48Ø	
TAACTGTTTT	CAATATATGT	TGTAAACATA	CGTTGGTTTT	TCTTAGTCTC	CTATACATAC	
ATTGACAAAA	GTTATATACA	ACATTTGTAT	GCAACCAAAA	AGAATCAGAG	GATATGTATG	
49Ø	500	51Ø	52Ø	53Ø	54Ø	
TTTTTACAAT	TTCCATCCAA	GCAGTAACGT	AGACGCCGAA	GTGATTTAAA	AAAGAGGAAG	
AAAAATGTTA	AAGGTAGGTT	CGTCATTGCA	TCTGCGGCTT	CACTAAATTT	TTTCTCCTTC	
55Ø	56Ø	57Ø	58Ø	59Ø	600	
AGAAGAAGAA	GAAGAAGAAG	AAGAAGAAGA	AGAAGCAACA	AGCAGCAGCA	GCAGCAGCAG	
TCTTCTTCTT	CTTCTTCTTC	TTCTTCTTCT	TCTTCGTTGT	TCGTCGTCGT	CGTCGTCGTC	
61Ø	62Ø	63Ø	64Ø	65Ø	66Ø	
CAGCAGCAGC	TGCACCGCAG	AGGGTGTAAC	ATTTGAGATA	GCTTTTACAA	GGAGAATGGG	
GTCGTCGTCG	ACGTGGCGTC	TCCCACATTG	TAAACTCTAT	CGAAAATGTT	CCTCTTACCC	
67Ø	68Ø	69Ø	7ØØ	71Ø	72Ø	
AATCTGTCAA	ACAGCAATTT	AATGTACAAA	GGGTTGAACA	CAGTCATGAA	AAGGCTCTGT	
TTAGACAGTT	TGTCGTTAAA	TTACATGTTT	CCCAACTTGT	GTCAGTACTT	TTCCGAGACA	
73Ø	74Ø	75Ø	76Ø	77Ø	78Ø	
CTGTCGTGAT	TCCTCTCCTG	AAGTGTTCTC	CCTGGGTCAT	TTTTAGGACC	GTGCGCCGCT	
GACAGCACTA	AGGAGAGGAC	TTCACAAGAG	GGACCCAGTA	AAAATCCTGG	CACGCGGCGA	
79Ø	799					
CCCACATCGC	TCTAGGGCC					
GGGTGTAGCG	AGATCCCGG					

FIG. 3. Sequence of the B region of clone λ Mt5. The noncoding (tRNA-like) strand is shown on the top line with the 5' end to the left. The tRNA^{His} coding region is boxed. The arrow denotes the direction of transcription.

ison of the intensities of the autoradiographic bands in lanes b, c, f, h, j, and k indicates that all four tRNA^{His} genes (Mt5A, Mt5B, Mt6, and Mt1) are transcribed with qualitatively similar efficiencies when they contain 28 to 228 residues of their original 5'-flanking-region DNA. The M13 vector does not yield transcripts in the size range of the unprocessed tRNA^{His} gene transcripts (lane a). Variable and minor amounts of larger transcripts are sometimes copied from the vector.

Comparison of lanes d and e with lanes b and c of Fig. 6 indicates that the conserved 5' leader sequence of the Mt5A gene contains sequences that markedly affect transcription in vitro. When the original 5'-flanking residues between positions -99 and -10 are replaced by M13 vector sequences, a modest reduction in transcription occurs (compare lanes c and d). A similar result is seen for the Mt6 gene (lanes h and i). Further deletion of 5'-flanking residues of the Mt5A gene to position -3 (lane e) causes a much more severe reduction in transcription and synthesis of a more heterogeneous set of transcripts. Scans of autoradiographs obtained from six independent experiments of the type shown in Fig. 6 indicate that the average relative transcriptional efficiencies of clones -228R, -9R, and -3R are 1, 0.85, and 0.23, respectively. The slightly greater activity of

1Ø	2Ø	3Ø	4Ø	5Ø	6Ø	
Cgtggagata	ACATTCAGAC	CAAAAACTAA	TCACTTAAAA	TATGAAAATG	TAAAGTGCAA	
Gcacctctat	TGTAAGTCTG	GTTTTTGÅTT	AGTGAATTTT	ATACTTTTAC	ATTTCACGTT	
7Ø	8Ø	9Ø	1ØØ	11Ø	12Ø	
TCTTTTCTAC	ATTTGTACTA	AAGAACGTCA	CACATTAAAA	AACATCCACA	GCAGAGCCTT	
AGAAAAGATG	TAAACATGAT	TTCTTGCAGT	GTGTAATTTT	TTGTAGGTGT	CGTCTCGGAA	
13Ø	14Ø	15Ø	16Ø	17Ø	18Ø	
TTACAGGATC	ACAGGATTCC	Agaagctccc	TTCAAACGAT	AAAAAGAATT	AATTTTCTTT	
AATGTCCTAG	TGTCCTAAGG	Tcttcgaggg	AAGTTTGCTA	TTTTTCTTAA	TTAAAAGAAA	
19Ø	2ØØ	21Ø	22Ø	23Ø	24Ø	
AAACTTGGCT	ATCCTAGAGT	GTTTGTCTGT	CATCTCTCTC	CGTCTGTCTT	ACAGGTCCCC	
TTTGAACCGA	TAGGATCTCA	CAAACAGACA	GTAGAGAGAG	GCAGACAGAA	TGTCCAGGGG	
25Ø	26Ø	27Ø	28Ø	29Ø	3ØØ	
CCCGCCCCCG	TCTTAATTTT	TCTTCAATCA	AAACAAATTT	GCAAGAAAAG	AAACAAAAAA	
GGGCGGGGGC	AGAATTAAAA	AGAAGTTAGT	TTTGTTTAAA	CGTTCTTTTC	TTTGTTTTTT	
31Ø	32Ø	33Ø	34Ø	35Ø	36Ø	GIy.
CTTTCTGTCA	TAGCTTTCAT	TAAGATATCC	AAACAGAACA	Atcgctaacc	TGCGTTGGTG	
GAAAGACAGT	ATCGAAAGTA	ATTCTATAGG	TTTGTCTTGT	Tagcgattgg	ACGCAACCAC	
37Ø GTATAGTGGT CATATCACCA	GAGCATAGCT CTCGTATCGA	GCCTTCCAAG CGGAAGGTTC	4ØØ CAGTTGACCC GTCAACTGGG	41Ø GGGTTCGATT CCCAAGCTAA	42Ø CCCGGCCAAC GGGCCGGTTG	
43Ø	44Ø	45Ø	46Ø	47Ø	48Ø	
GCATAAGGTT	GTCTTTTAC	GTTTAGAAAT	CCGAAGATCT	CTGATCCCTG	GATCTAACTC	
CGTATTCCAA	CAGAAAAATG	CAAATCTTTA	GGCTTCTAGA	GACTAGGGAC	CTAGATTGAG	
49Ø	5ØØ	51Ø	52Ø	53Ø	54Ø	
CACCGAGTTT	CTTCTCAAGT	TTATGTTATT	CCTATCTGTT	AGTACAGCGA	ATGGCGTCAC	
GTGGCTCAAA	GAAGAGTTCA	AATACAATAA	GGATAGACAA	TCATGTCGCT	TACCGCAGTG	
55Ø	56Ø	57Ø	58Ø	59Ø	6ØØ	
Agacttgtag	GATTATCCTT	TTGGGATTGT	TCAGGAAAGT	ATCTAAAATG	GAGTCTGGCT	
Tctgaacatc	CTAATAGGAA	AACCCTAACA	AGTCCTTTCA	TAGATTTTAC	CTCAGACCGA	
61Ø	62Ø	63Ø	64Ø	65Ø	66Ø	
CTTTGAGACT	TTAGAAGCAG	CCCCGCCTTC	GGTTCGGGAG	GTTTTGTGAG	TGGAACCAGG	
GAAACTCTGA	AATCTTCGTC	GGGGCGGAAG	CCAAGCCCTC	CAAAACACTC	ACCTTGGTCC	
67Ø	68Ø	69Ø	7ØØ	71Ø	72Ø	
AAACAGGCCC	CTGGAGAAGG	ACTCCTTCAC	Agctctgctc	CTTTTCTGGT	TGCACCTTCT	
TTTGTCCGGG	GACCTCTTCC	TGAGGAAGTG	Tcgagacgag	GAAAAGACCA	ACGTGGAAGA	
730	74Ø	75Ø	76Ø	77Ø	78Ø	
GGTAACAAAA	CGTTGATTCC	GTGGCTTGTT	TCTTTCCAGA	GAAACAACAT	AGGTCTGCTG	
CCATTGTTTT	GCAACTAAGG	CACCGAACAA	Agaaaggtct	CTTTGTTGTA	TCCAGACGAC	
79Ø	8ØØ	810	82Ø	83Ø	84Ø	
GTGCGGCGGT	TTCTTATCCC	AGGTCCATGA	AATCCCAGAA	GCAAGGAGCT	GGTGCTCGTG	
CACGCCGCCA	AAGAATAGGG	TCCAGGTACT	TTAGGGTCTT	CGTTCCTCGA	CCACGAGCAC	
850	860	87Ø	880	890	9ØØ	
TTAGTATTGC	GGAAGCACCA	GGGTTTCAAA	GCCAGACTTT	TGATATTGCG	GCCATATAGA	
AATCATAACG	CCTTCGTGGT	CCCAAAGTTT	CGGTCTGAAA	ACTATAACGC	CGGTATATCT	
910	920	930	940	950	96Ø	ft The t
CTATACTAAA	TAAATCAGGC	AACTCTGGAA	GGCTACTCTC	ATTCAAAGAC	CATTAAATTG	
GATATGATTI	ATTTAGTCCG	TTGAGACCTT	CCGATGAGAG	TAAGTTTCTG	GTAATTTAAC	

FIG. 4. Sequence of clone λ Mt6. The noncoding (tRNA-like) strand is shown on the top line with the 5' end to the left. The tRNA^{GIy} and tRNA^{His} coding regions are boxed. Arrows denote the direction of transcription.

the -99 clone relative to the -228 clone in Fig. 6 does not occur reproducibly.

Comparison of lanes f and g and lanes k and l in Fig. 6 suggests that the 5'-flanking regions of the Mt5B and Mt1 genes also influence transcription. Clone m5B-1R (lane g) is transcribed to a lesser extent than clone m5B-34R (lane f). Likewise, the loss of the 5'-flanking region plus 5 bp of the coding region greatly reduces transcription of the Mt1 gene (compare lanes k and l).

While the results of Fig. 6 suggest that the conserved 5'

			_>			
97Ø	98Ø	99Ø	1ØØØ-	<u> </u>	<u> </u>	
AGGCCGCAAC	CCGCTGCTTG	CCCGGTGGCT	CCCGTGATC	GTATAGTGGT	TAGTACTCTG	
TCCGGCGTTG	GGCGACGAAC	GGGCCACCGA	GCGGCACTAG	CATATCACCA	ATCATGAGAC	HIS.
1Ø3Ø-	1040-	<u> </u>	<u> </u>	1Ø7Ø	1Ø8Ø	
CGTTGTGGCC	GCAGCAACCT	CGGTTCGAAT	CCGAGTCACG	GCATTTAGTA	GTACCGTCCC	
GCAACACCGG	CGTCGTTGGA	GCCAAGCTTA	GGCTCAGTGC	CGTAAATCAT	CATGGCAGGG	
1 0 0 0	1100	1110	1120	1130	1149	
TTTCCACCTT	TTTACTTACT	ΑΛΤΤΛΛΓΛΛΛ	ATTCTCATT	TTCCCTTTCC	CCTAAAACTC	
AAACGTCCAA	AAATCAATCA	TTAATTCTTT	TAACACTAAA		CCATTTCAC	
1150	1160	117Ø	118Ø	119Ø	1200	
AGTCACCGAA	ATAATTCCAA	GCGGCTTATC	CAATACGAGC	ATCCTAAACT	CTTGGGAAGA	
TCAGTGGCTT	TATTAAGGTT	CGCCGAATAG	GTTATGCTCG	TAGGATTTGA	GAACCCTTCT	
121Ø	122Ø	123Ø	124Ø	125Ø	126Ø	
GCCAGGTCTG	TAAAGGAGGC	TCCTTCCCCA	TCGCAAGAGA	GAAGTAGAGC	GGTGATACCG	
CGGTCCAGAC	ATTTCCTCCG	AGGAAGGGGT	AGCGTTCTCT	CTTCATCTCG	CCACTATGGC	
127Ø	128Ø	129Ø	1300	131Ø	132Ø	
AAACTTGGCT	CCTCCACTGT	CGGTCGGAGA	ACCAAACACT	TCGGGAGCAG	AGCGCCTGTC	
TTTGAACCGA	GGAGGTGACA	GCCAGCCTCT	TGGTTTGTGA	AGCCCTCGTC	TCGCGGACAG	
133Ø	134Ø	135Ø	136Ø	137Ø		
TCTGCAGCAA	ATGTTCTACG	AGTGTGTCTC	TGTCGATTCT	ACACCAGATG	GCC	
AGACGTCGTT	TACAAGATGC	TCACACAGAG	ACAGCTAAGA	TGTGGTCTAC	CGG	

FIG. 4. Continued.

leader sequence of the Mt5A gene influences transcription, it is also possible that the M13 vector contains a sequence which, when moved a critical distance from the coding region, inhibits transcription. To test for possible vector effects on transcription, we constructed two additional sets of clones which contain the same mouse DNA inserts as those transcribed in Fig. 6, but with different vector sequences flanking the mouse DNA inserts. One set of clones contains the inserts of clones m5A-228R, m5A-99R, m5A-9R, and m5A-3R in the opposite (L) orientation in M13. The second set of clones contains the mouse DNA sequences inserted into the *Bam*HI site of pBR322. When transcribed as in Fig. 6, these clones yielded the same result. Replacement of normal mouse DNA sequences between positions -9 and -4 with vector sequences greatly reduced transcription (data not shown).

Effect of 5'-flanking region on formation of stable transcription complexes. To determine whether the 5'-flanking resi-

A. 5' FLANKING SEQUENCES

Mt5.A	(-19)	TGTGGCGTTCC-GGTAGCTC (-1)
Mt5.B	(-2Ø)	CATCAAGTTCCCGGTAGCTC (-1)
Mt6	(-2Ø)	CGCTGCTTGCCCGGTGGCTC (-1)
Mt1	(-2Ø)	TGCTATCTAGTGTGTGGTCC (-1)

B. 3' FLANKING SEQUENCES

Mt1	(+1)	TTATCCTCTGGTCACT	TTTTTGCTCCACT	CTCTCTCTGAT	(+4Ø)
Mt5.A	(+1)	TTGTGGCAACAATGGC	ACGGCAAGGGCGC	CTTTTTAGTGA	(+4Ø)
Mt5.B	(+1)	TTGTGGCAACAATGGC	ACGGCAAGGGAGC	TGTATTTTATT	(+4Ø)
Mt6	(+1)	TTTAGTAGTACCGTCC	CTTTGCAGCTTTT	TACTTACTAAT	(+4Ø)

Mt6 (+1) TTTAGTAGTACCGTCCCTTTGCAGCTTTTACTAAT (+4%) FIG. 5. Comparison of proximal 5'- and 3'-flanking regions of tRNA^{His} genes. In panel A, (-1) indicates the first residue 5' to the coding region. In panel B, (+1) indicates the first residue 3' to the coding region. The noncoding DNA strand is shown.





FIG. 6. In vitro transcription analysis of M13 clones containing tRNA^{His} coding sequences. Each DNA (400 ng) was transcribed in 25 μ l of a standard transcription reaction mixture for 60 min at 30°C. An autoradiograph of the gel is shown. Lanes: a, M13 mp7; b, m5A-228R; c, m5A-99R; d, m5A-9R; e, m5A-3R; f, m5B-34R; g, m5B-1R; h, m6-73R; i, m6-9R; j, m1-167L; k, m1-28R; l, m1+6R. Lanes a through e and f through l are from the same gel. Irrelevant lanes between lanes e and f were deleted from the figure.

dues critical for efficient transcription also influence formation of stable transcription complexes in the HeLa cell extract, we performed a competition experiment in which two genes are added sequentially to the same transcription reaction (39). As a reference template, we used a mouse tRNA^{Gly} gene (26), the transcripts of which are readily distinguishable from the tRNA^{His} gene transcripts on a polyacrylamide gel (compare Fig. 7, lanes b and f). Neither

FIG. 7. Competition between tRNA^{His} genes and a tRNA^{Gly} gene in vitro. The tRNA^{Gly} gene is contained in the M13 clone Nar8 (see reference 26). Each reaction mixture contained 300 ng of total DNA added to 10 µl of standard transcription mix. M13 mp7 DNA was added as necessary to yield this total DNA concentration. Lanes a through d, Transcription of the tRNA^{Gly} gene alone: a, 38 ng; b, 75 ng; c, 150 ng; d, 300 ng of Nar8 DNA, respectively. Lanes e through j, Competition experiments. A given amount of tRNA^{His} gene DNA and M13 mp7 DNA (total DNA concentration, 225 ng) were incubated for 15 min at 30°C in 10 µl of a standard transcription reaction. tRNA^{Gly} gene DNA (75 ng) was then added, and incuba-tion was continued for 60 min at 30°C. Lanes: e, no tRNA^{His} gene DNA; f, 150 ng of m5A-228R; g, 225 ng of m5A-228R; h, no tRNA^{His} gene DNA; i, 150 ng of m5A-3R DNA; j, 225 ng of m5A-3R DNA. An autoradiograph of the gel is shown. On the left of the figure, his marks the position of tRNA^{His} gene transcripts, gly marks tRNA^{Gly} gene transcripts, and e marks an endogenous band labeled in the lysate. Neither tRNA^{His} nor tRNA^{Gly} transcripts were processed in these reactions.

Vol. 6, 1986

the tRNA^{Gly} nor the tRNA^{His} gene transcripts are processed appreciably in these reactions.

Before performing the actual competition experiments, we determined the lowest amount of tRNA^{Gly} gene DNA that would yield a strong signal on the autoradiograph. In the presence of M13 DNA, 75 ng of tRNA^{Gly} gene DNA yielded a maximal amount of transcript which was not increased appreciably by additional tRNA^{Gly} gene template (Fig. 7, lanes a through d). The rather low and sharp concentration optimum in the presence of inert vector DNA (compare lanes a and b) is characteristic of S-100 extracts and has also been noted by others (43).

For the competition experiments in lanes e through j of Fig. 7, a given tRNA^{His} gene DNA and M13 DNA were first incubated with the transcription lysate, followed by tRNA^{Gly} gene DNA. Preincubation of 225 ng of clone m5A-228R DNA significantly reduced the transcription of the tRNA^{Gly} gene (compare lanes e and g), indicating that stable transcription complexes had been formed on the tRNA^{His} gene before addition of the tRNA^{Gly} gene. In contrast, preincubation of clone m5A-3R DNA in the lysate affects the transcription of the tRNA^{Gly} gene to a much lesser extent (compare lanes h through j). Thus, a tRNA^{His} gene which contains only three residues of the 5'-flanking region has a greatly reduced ability to form stable complexes with transcription factors present in the S-100 lysate.

DISCUSSION

Effects of 5'-flanking sequences on transcription. Several invertebrate tRNA genes contain 5'-flanking sequences that stimulate transcription in homologous extracts (8, 11, 22, 23, 38, 44). However, in some cases, the 5'-flanking dependence of transcription appears to be reduced in mammalian cell extracts (3, 11, 32, 38, 43). These observations have therefore raised questions regarding the sensitivity of mammalian cell extracts to 5'-flanking-region effects and the existence of modulatory sequences in the 5' flanks of mammalian tRNA genes. We show here that a mouse tRNA^{His} gene contains sequences located between positions -4 and -9 in the 5'-flanking region that have a marked positive influence on transcription in a HeLa cell extract. Thus, at least some mammalian tRNA genes are similar to invertebrate genes in regard to 5'-flanking-sequence requirements for efficient transcription in vitro. Furthermore, the HeLa cell extract clearly is sensitive to 5'-flanking effects if a suitable tRNA gene is assaved.

From the experiments reported in this paper we cannot infer whether the entire -4 to -9 region of the Mt5A tRNA^{His} gene is necessary for modulation of transcription. Inspection of the sequences of the M13 and pBR322 clones we constructed suggests the potential importance of residues at two particular positions. All actively transcribed mouse tRNA^{His} gene clones (representing all four sequenced genes) contain a G residue on the noncoding strand at position -4. None of the poorly transcribed clones (such as m5A-3R) contains a G residue at this position. Another important position may be at -6, which is always a pyrimidine in transcribed clones and, with one exception, a purine in poorly transcribed clones. The importance of these two consensus positions may also explain why a Drosophila tRNA^{His} gene is transcribed with much lower efficiency in a HeLa cell extract than in a Drosophila cell extract (3, 8). Although the Drosophila gene contains a proximal 5'flanking region which is similar in some respects to that of the mouse $tRNA^{His}$ genes, it lacks a G at position -4 and a pyrimidine at -6. Alternatively, other sequences as well as differences in human and *Drosophila* transcription factors (3) may influence transcription of the *Drosophila* gene in the HeLa cell extract.

A major question raised by our data is the mechanism by which the proximal 5'-flanking region of the tRNA^{His} gene influences transcription. There is currently no evidence for factors that bind solely to the 5' flanks of eucarvotic tRNA genes, although this possibility has not been ruled out definitively. Rather, it is more likely that the 5'-flanking region influences the interaction of the gene either with factors that bind to the internal control regions or with RNA polymerase III. The competition experiment shown in Fig. 7 demonstrates that the 5'-flanking sequence influences stable complex formation with transcription factors. Drosophila and HeLa cell transcription extracts contain at least two components essential for formation of stable complexes in vitro (3, 24). Transcription factor C probably interacts with the 3' internal control element (or B block) of a tRNA gene. Its association with the gene is stabilized by factor B(3, 24). The resulting complex can then be transcribed by RNA polymerase III. Burke and Soll (3) have suggested that the 5'-flanking region of a *Drosophila* tRNA^{His} gene, which is required for efficient transcription in a Drosophila extract, probably influences at least the interaction of factor B with the factor C-gene complex. By analogy, the -4 to -9 region of the mouse tRNA^{His} gene may also be involved in the interaction of the B factor with the gene, perhaps serving as part of the binding site, as is suggested by the apparent sequence requirements at specific positions, as noted above. The -4 to -9 region may also influence some aspect of interaction of RNA polymerase III with the gene, as is suggested by the heterogeneous array of transcripts obtained from the mutants containing only 3 bp of wild-type flankingregion sequence. These hypotheses are directly testable by construction of specific point mutations in the wild-type 5'-flanking sequence and transcription analyses with fractionated extracts.

The members of several tRNA gene families present in yeasts (22, 30, 31, 40, 48), Drosophila melanogaster (1, 9, 20, 33, 50), and rats (41) also contain variable amounts of short, conserved 5' leader and 3' trailer sequences contiguous with the coding region. In most cases the influence of these sequences on transcription has not been analyzed. However, the transcriptional properties of yeast tRNA^{Leu} genes are strikingly similar to those of the mouse tRNA^{His} genes. The yeast genes contain a conserved 5' leader that is a positive modulator of in vitro transcription (22, 32). Components present in yeast transcription extracts protect the 5' leader from DNase digestion (47). The 5' leaders of the yeast and mouse genes are, however, completely different in sequence, showing that different flanking-sequence motifs can influence transcription. These results suggest that the combination of a particular 5'-flanking sequence with a particular tRNA coding sequence to produce a more active transcription unit may be a common (although not universal) feature of eucarvotic tRNA gene organization.

Sequence organization and evolution of the mouse tRNA^{His} gene family. It is not known whether all of the members of a mammalian tRNA gene family are expressed equally in vivo. To answer this question it will be necessary to isolate and characterize an entire gene family. In this paper and in reference 17, we report the sequences of four mouse tRNA^{His} genes. A number of observations suggest that these genes are representative, and perhaps make up a majority, of the genes encoding tRNA^{His} in mouse cells.

Histidine is specified by the CAU and CAC codons, both of which can be read by the GUG anticodon present in tRNA encoded by the four sequenced genes. Mouse and human tRNA^{His} isolated from tissue culture cell lysates by immunoprecipitation with anti-Jo-1 antibody is identical in sequence to the tDNA sequences of the Mt5 and Mt6 genes if posttranscriptional modifications are not considered (34). Anti-Jo-1 antibody is isolated from the sera of autoimmune patients having polymyositis and probably recognizes histidyl-tRNA synthetase. The antibody immunoprecipitates all of the tRNA^{His} charging activity from a HeLa cell lysate (28). Thus, if there is a mammalian tRNA^{His} not immunoprecipitated by the antibody (and therefore not sequenced), it must either be present in very small amounts in tissue culture cells or not be recognized by the antigenic tRNA^{His} synthetase. The available evidence therefore suggests that all mouse tRNA^{His} molecules have essentially the same primary structure, as encoded by the genes we sequenced. This structure can probably be modified in various ways, as evidenced by the identification of multiple tRNA^{His} isoacceptors by reverse-phase chromatography (see, e.g., reference 25).

From the Clarke-Carbon equation (5), we calculate a 78% probability that our genomic DNA library contains any given unique sequence. We screened the library rigorously for tRNA^{His} genes. Eighty signals from the initial screening with tRNA^{His}-specific probe were characterized. Most of these were false-positives that did not yield hybridizing phage upon a secondary screening. A total of 12 phage, 9 of which are very similar, if not identical, to λ Mt5, were finally plaque purified. The tRNA^{His}-specific probe obtained from phage λ Mt1 contained one mismatched residue (near its 5' end) relative to the tRNA^{His} coding regions of λ Mt5 and λ Mt6. Therefore, it is likely that other minor sequence variants would have been detected in the screening since the probe identified the Mt5 and Mt6 sequences. The probe might not have hybridized with highly mutated pseudogenes or gene fragments.

An issue that remains unresolved is the precise number of tRNA^{His} genes in the mouse genome. In genomic Southern blots, a tRNA^{His}-specific probe hybridizes only to *Eco*RI fragments of genomic DNA having the same size as those present in phages λ Mt1, λ Mt5, and λ Mt6 (J. Harding, unpublished data). We have not sequenced the multiple copies of the phage λ Mt5 sequence obtained from the DNA library and thus have not yet determined whether this sequence is reiterated in the genome.

Notably, the tRNA^{His} gene sequences exhibit a hierarchy in respect to sequence homology. The Mt6 sequence lacks the 3' trailer and part of the 5' leader relative to the Mt5A and Mt5B sequences. The Mt1 gene has the least homologous leader, no homologous trailer, and a single variant residue in the coding region. The evolution of this pattern of sequence variation can perhaps be explained by two hypotheses. First, the sequence differences may reflect the relative times during evolution at which the various genes duplicated and diverged if it is assumed that there is little or no selective pressure for maintaining many of the flanking-region residues. Thus, the duplication event that produced the Mt5A and Mt5B sequences may have occurred more recently than the duplication that produced the Mt5 and Mt1 (or Mt6) sequences. An alternative explanation is that similarities in flanking-region homologies are related to the relative efficiency of gene conversion, which can homogenize related sequences and has been shown to occur in the tRNA^{ser} genes of Schizosaccharomyces pombe (30).

ACKNOWLEDGMENTS

This work was supported by grants 1-802 from the March of Dimes Birth Defects Foundation and Public Health Service grant GM26884 from the National Institutes of Health.

We wish to thank Jang Han for preparing the tRNA^{His}-specific probe and Robert Rooney for providing transcription extracts.

LITERATURE CITED

- Addison, W., C. Astell, A. Delaney, I. Gillam, S. Hayashi, R. Miller, B. Rajput, M. Smith, D. Taylor, and G. Tener. 1982. The structures of genes hybridizing with tRNA^{Val} from *Drosophila melanogaster*. J. Biol. Chem. 257:670–673.
- Benton, W., and R. Davis. 1977. Screening λ recombinant clones by hybridization to single plaques in situ. Science 196:180-182.
- Burke, D., and D. Soll. 1985. Functional analysis of fractionated Drosophila Kc cell tRNA gene transcription components. J. Biol. Chem. 260:816-823.
- Ciliberto, G., L. Castagnoli, D. Melton, and R. Cortese. 1982. Promoter of a eucaryotic tRNA^{Pro} gene is composed of three noncontiguous regions. Proc. Natl. Acad. Sci. USA 79:1195– 1199.
- 5. Clarke, L., and J. Carbon. 1976. A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *E. coli* genome. Cell 9:91–99.
- Clarkson, S., R. Koski, J. Corlet, and R. Hipskind. 1981. Influence of 5' flanking sequences on tRNA transcription in vitro, p. 463–472. *In D. D. Brown* (ed.), Developmental biology using purified genes. Academic Press, Inc., New York.
- Cooley, L., B. Appel, and D. Soll. 1982. Post-transcriptional nucleotide addition is responsible for the formation of the 5' terminus of histidine tRNA. Proc. Natl. Acad. Sci. USA 79:6475-6479.
- Cooley, L., J. Schaack, D. Burke, B. Thomas, and D. Soll. 1984. Transcription factor binding is limited by the 5'-flanking regions of a *Drosophila* tRNA^{His} gene and a tRNA^{His} pseudogene. Mol. Cell. Biol. 4:2714–2722.
- 9. DeFranco, D., O. Schmidt, and D. Soll. 1980. Two control regions for tRNA gene transcription. Proc. Natl. Acad. Sci. USA 77:3365-3368.
- DeFranco, D., S. Sharp, and D. Soll. 1981. Identification of regulatory sequences contained in the 5' flanking region of Drosophila lysine tRNA genes. J. Biol. Chem. 256:12424-12429.
- Dingermann, T., D. Burke, S. Sharp, J. Schaack, and D. Soll. 1982. The 5' flanking sequences of *Drosophila* tRNA^{Arg} genes control their in vitro transcription in a *Drosophila* cell extract. J. Biol. Chem. 257:14738–14744.
- 12. Dingermann, T., S. Sharp, J. Schaack, and D. Soll. 1983. Stable transcription complex formation of eukaryotic tRNA genes is dependent on a limited separation of the two intragenic control regions. J. Biol. Chem. 258:10395–10402.
- Fuhrman, S., D. Engelke, and E. Geiduschek. 1984. HeLa cell RNA polymerase III transcription factors. Functional characterization of a fraction identified by its activity in a second template rescue assay. J. Biol. Chem. 259:1934–1943.
- Galli, G., H. Hofstetter, and M. Birnstiel. 1981. Two conserved sequence blocks within eukaryotic tRNA genes are major promoter elements. Nature (London) 294:626-631.
- 15. Gauss, D., and M. Sprinzl. 1984. Compilation of tRNA sequences. Nucleic Acids Res. 12:r1-r58.
- Guthrie, C., and J. Abelson. 1982. Organization and expression of tRNA genes in *Saccharomyces cerevisiae*, p. 487–528. *In J.* Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 17. Han, J., and J. Harding. 1982. Isolation and nucleotide sequence of a mouse histidine tRNA gene. Nucleic Acids Res. 10:4891-4900.
- Hipskind, R., and S. Clarkson. 1983. 5'-flanking sequences that inhibit in vitro transcription of a *Xenopus laevis* tRNA gene. Cell 34:881-890.
- 19. Hofstetter, H., A. Kressmann, and M. Birnstiel. 1981. A split promoter for a eucaryotic tRNA gene. Cell 24:573-585.

- Hosbach, H., M. Silberklang, and B. McCarthy. 1980. Evolution of a D. melanogaster glutamate tRNA gene cluster. Cell 21:169–178.
- Hu, J., B. Cote, E. Lund, and J. Dahlberg. 1983. Isolation and characterization of genomic mouse DNA clones containing sequences homologous to tRNAs and 5S rRNA. Nucleic Acids Res. 11:4809–4821.
- Johnson, J., and G. Raymond. 1984. Three regions of a yeast tRNA^{1eu} gene promote RNA polymerase III transcription. J. Biol. Chem. 259:5990-5994.
- Larson, D., J. Bradford-Wilcox, L. Young, and K. Sprague. 1983. A short 5' flanking region containing conserved sequences is required for silkworm alanine tRNA gene activity. Proc. Natl. Acad. Sci. USA 80:3416-3420.
- Lassar, A., P. Martin, and R. Roeder. 1983. Transcription of class III genes: formation of preinitiation complexes. Science 222:740-748.
- Lin, V., W. Farkas, and P. Agris. 1980. Specific changes in Q-ribonucleoside containing transfer RNA species during Friend leukemia cell erythroid differentiation. Nucleic Acids Res. 8:3481-3489.
- Looney, J., and J. Harding. 1983. Structure and evolution of a mouse tRNA gene cluster encoding tRNA^{Asp}, tRNA^{Gly} and tRNA^{Glu} and an unlinked, solitary gene encoding tRNA^{Asp}. Nucleic Acids Res. 11:8761–8775.
- Maniatis, T., R. Hardison, E. Lacy, J. Lauer, C. O'Connell, D. Quon, G. Sim, and A. Efstradiatis. The isolation of structural genes from libraries of eucaryotic DNA. Cell 15:687-701.
- Mathews, M., and R. Bernstein. 1983. Myositis auto-antibody inhibits histidyl-tRNA synthetase: a model for autoimmunity. Nature (London) 304:177-179.
- 29. Messing, J., R. Crea, and P. Seeburg. 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. 9:309-321.
- Munz, P., H. Amstutz, J. Kohli, and U. Leupold. 1982. Recombination between dispersed serine tRNA genes in *Schizosac-charomyces pombe*. Nature (London) 300:225-231.
- Page, G., and B. Hall. 1981. Characterization of the yeast tRNA^{Ser} gene family: genomic organization and DNA sequence. Nucleic Acids Res. 9:921-934.
- Raymond, G. J., and J. D. Johnson. 1983. The role of noncoding DNA sequences in transcription and processing of a yeast tRNA gene. Nucleic Acids Res. 11:5969-5988.
- Robinson, R., and N. Davidson. 1981. Analysis of a Drosophila tRNA gene cluster: two tRNA^{Leu} genes contain intervening sequences. Cell 23:251-259.
- Rosa, M., J. Hendrick, Jr., M. Lerner, J. Steitz, and M. Reichlin. 1983. A mammalian tRNA^{His}-containing antigen recognized by the polymyositis-specific antibody anti-Jo-1. Nucleic Acids Res. 11:853–870.
- 35. Sanger, F., and A. Coulson. 1978. The use of thin acrylamide gels for DNA sequencing. FEBS Lett. 87:107-110.
- 36. Sanger, F., A. Coulson, F. Barrell, A. Smith, and B. Roe. 1980.

Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143:**161–178.

- Santos, T., and M. Zasloff. 1981. Comparative analysis of human chromosomal segments bearing nonallelic dispersed tRNA^{Met} genes. Cell 23:699–710.
- Schaack, J., S. Sharp, T. Dingermann, D. Burke, L. Cooley, and D. Soll. 1984. The extent of a eucaryotic tRNA gene. 5' and 3' flanking sequence dependence for transcription and stable complex formation. J. Biol. Chem. 259:1461–1467.
- Schaack, J., S. Sharp, T. Dingermann, and D. Soll. 1983. Transcription of eukaryotic tRNA genes in vitro. II. Formation of stable complexes. J. Biol. Chem. 258:2447-2453.
- Schmidt, O., J. Mao, R. Ogden, J. Beckmann, H. Sakano, J. Abelson, and D. Soll. 1980. Dimeric tRNA precursors in yeast. Nature (London) 287:750-752.
- Sekiya, T., R. Nishizawa, K. Matsuda, Y. Taya, and S. Nishimura. 1982. A rat tRNA gene cluster containing the genes for tRNA^{Pro} and tRNA^{Lys}. Analysis of nucleotide sequences of the genes and the surrounding regions. Nucleic Acids Res. 10:6411-6419.
- 42. Sharp, S., D. DeFranco, T. Dingermann, P. Farrell, and D. Soll. 1981. Internal control regions for transcription of eucaryotic tRNA genes. Proc. Natl. Acad. Sci. USA 78:6657–6661.
- Sharp, S., T. Dingermann, J. Schaack, D. DeFranco, and D. Soll. 1983. Transcription of eukaryotic tRNA genes in vitro. I. Analysis of control regions using a competition assay. J. Biol. Chem. 258:2440-2446.
- 44. Shaw, K., and M. Olson. 1984. Effects of altered 5'-flanking sequences on the in vivo expression of a Saccharomyces cerevisiae tRNA^{Tyr} gene. Mol. Cell. Biol. 4:657–665.
- 45. Shibuya, K., S. Noguchi, S. Nishimura, and T. Sekiya. 1982. Characterization of a rat tRNA gene cluster containing the genes for tRNA^{Asp}, tRNA^{Gly} and tRNA^{Glu} and pseudogenes. Nucleic Acids Res. 10:4441–4448.
- Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.
- Stillman, D. J., and E. P. Geiduschek. 1984. Differential binding of a S. cerevisiae RNA polymerase III transcription factor to two promoter segments of a tRNA gene. EMBO J. 3:847–853.
- 48. Valenzuela, P., A. Venegas, F. Weinberg, R. Bishop, and W. Rutter. 1978. Structure of yeast phenylalanine-tRNA genes: an intervening DNA segment within the region coding for the tRNA. Proc. Natl. Acad. Sci. USA 75:190–194.
- 49. Weil, P., J. Segall, B. Harris, S.-I. Ng, and R. Roeder. 1979. Faithful transcription of eucaryotic genes by RNA polymerase III in systems reconstituted with purified DNA templates. J. Biol. Chem. 254:6163-6173.
- Yen, P., and N. Davidson. 1980. The gross anatomy of a tRNA gene cluster at region 42A of the *D. melanogaster* chromosome. Cell 22:137-148.