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Using iodinated single-stranded M13 probes to facilitate rapid DNA sequence analysis – nucleotide sequence of a mouse lysine tRNA gene

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ABSTRACT

From a recombinant lambda phage, we have determined a 387 bp sequence containing a mouse lysine tRNA gene. The putative lys tRNA (anticodon UUU) differs from rabbit liver lys tRNA at five positions. The flanking regions of the mouse gene are not generally homologous to published human and Drosophila lys tRNA genes. However, the mouse gene contains a 14 bp region comprising 13 A-T base pairs, 30-44 bp from the 5' end of the coding region. Cognate A-T rich regions are present in human and Drosophila genes. The coding region is flanked by two 11 bp direct repeats, similar to those associated with alu family sequences.

The sequence was determined by a "walking" protocol that employs, as a novel feature, iodinated single-stranded M13 probes to identify M13 subclones which contain sequences partially overlapping and contiguous to an initially determined sequence. The probes can also be used to screen lambda phage and in Southern and dot blot experiments.

INTRODUCTION

We have recently isolated several clones which contain tRNA genes from a mouse DNA recombinant library. In this paper we present a general protocol for sequencing the tRNA genes and their flanking regions, which comprise a relatively small amount of the total mouse DNA inserted into a lambda clone. This protocol is generally useful for sequencing other genes.

The sequencing strategy employed here involves the following: First, we prepare an M13 subclone bank from a lambda clone, select the M13 clones which contain tRNA coding regions by plaque screening with labelled tRNA and sequence them by the dideoxy chain termination procedure. These initial sequences rarely contain an entire tRNA coding region and its flanking loci. Therefore, as a novel feature of the protocol, we iodinate the single-stranded DNA of the sequenced M13 subclone and use it as a probe to detect other M13 clones which partially overlap and also contain DNA sequences contiguous to those sequenced initially. In addition, the iodinated probe is used to detect an M13 clone containing the same DNA fragment cloned in the opposite orientation, allowing the sequence of the complementary DNA strand to be de-

termined. Thus, by using specific iodinated single-stranded M13 probes, it is possible to "walk" along a DNA molecule and rapidly and accurately determine its sequence on both strands.

In this paper we report the nucleotide sequence of a mouse lysine tRNA gene and its flanking regions obtained by the above protocol.

MATERIALS AND METHODS

Preparation of M13 Subclones of Lambda Clone λMt3.

A mouse genomic DNA-λcharon 4A recombinant library was prepared and screened with iodinated mouse tRNA as described (1). DNA was isolated from clone λMt3 and two M13 subclone banks were prepared as follows:

M13 subclone bank A. One ug of λMt3 DNA was incubated with 10 units of AluI in 10 ul of 5 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 50 mM NaCl for two hours at 37°. The DNA was ethanol precipitated and suspended in 10 ul of 0.1M glycine buffer (pH 9.5), 1 mM $MgCl_2$, 1 mM $ZnSO_4$. One ug (0.1 unit) of calf intestine alkaline phosphatase was added, incubated for 30 min at 37°, phenol extracted twice and the DNA was precipitated with ethanol. About 200 ng of the DNA was ligated to 30 ng of HincII cut M13mp7 RF DNA (2), as described in ref. 1. The ligated DNA was transfected into E. coli JM103 and plated in the presence of IPTG and X-Gal (2).

M13 subclone bank H. One ug of λMt3 DNA was cut with 10 units of HinfI. as described above. The sticky ends of the DNA generated by HinfI digestion were filled in by adding 300 uM of each dNTP and 0.1 unit of the Klenow fragment of DNA polymerase I and incubating for 30 min at 37°. The DNA was treated with calf intestine alkaline phosphatase and ligated with M13mp7 as for subclone bank A.

Screening of M13 Phage Plaques.

DNA Filter Preparation. The plates containing M13 plaques were incubated overnight at 37°, dried without a cover for 1 hr at 37° and chilled on ice. Plaques were transferred to autoclaved cellulose nitrate filters (Schleicher and Schuell, BA 85) as described (3). Duplicate filters were made from each plate. The filters were denatured on Whatman 3 MM paper with 0.5M NaOH, 1.5M NaCl for 3 min and neutralized with 1M Tris-HCl (pH 7.4), 3 M NaCl for 5 min. The filters were air dried and baked for 2 hrs in vacuo.

Hybridization and Autoradiography. The filters were washed in 2X SET (1X SET is 0.15M NaCl, 2mM EDTA, 30 mM Tris-HCl, pH 7.5), 0.1% sodium dodecyl sulfate (SDS) and prehybridized with 2 ml/filter of 50% (V:V) deionized formamide, 4X SET, 0.1% SDS, 0.2% (W:V) each, Ficoll-400, polyvinyl pyrolidone-

360 and Bovine serum albumin, 100 ug/ml polyadenylic acid, and 50 ug/ml M13mp7 single-stranded DNA carrier overnight at 43°. Hybridization was performed in the above solution containing 1 x 10^7 CPM of iodinated mouse tRNA at 37° or 1 x 10^6 CPM of iodinated single-stranded M13 DNA probe at 43°. The filters were incubated in the hybridization solution lacking probe at either 37° (tRNA hybridization) or 43° (M13 DNA hybridization) and washed twice with 500 ml of 4X SET, 0.1% SDS for 30 min with shaking at 37°, and twice with 500 ml of 2X SET, 0.1% SDS for 30 min. The filters were blotted, covered with plastic wrap and exposed to X-ray film for several hours with an intensifying screen at -70°. Occasional filters with high backgrounds were further washed in 0.1X SET at 68° for 1 hr and autoradiographed again.

Preparation of Iodinated Single-Stranded M13 Probes.

Isolation of single-stranded M13 DNA. E. coli JM103 was infected with an M13 clone of interest, grown overnight at 37° and single-stranded DNA was isolated from phage particles obtained from the culture supernatant as described (2). The single-stranded DNA preparation was treated with 50 ug/ml of pancreatic RNase (heated for 10 min at 80° prior to use) in 50 mM sodium acetate (pH 6) for 30 min at 37° followed by phenol extraction and ethanol precipitation. Alternatively, the DNA was chromatographed on a Sepharose 4B (Pharmacia) column (1 x 30 cm) in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. DNA was eluted in the void volume and ethanol precipitated. The purified DNA was suspended in water at a concentration of 10 mg/ml and stored frozen at -20°.

Iodination. DNA was iodinated according to a procedure modified from ref. 4. In a small Eppendorf microfuge tube (100 ul volume), 10 ug of single-stranded M13 DNA in 1 ul of water was mixed with 4 ul of 250 mM sodium acetate (pH 4.5) and 50 mM thalic acetate (Kodak). Within a fume hood, 15 ul of 125-I sodium iodide (Amersham code IMS 30, 100 mCi/ml) was added and the tube was capped and sealed tightly with parafilm. The tube was incubated for 30 min at 60°. After chilling on ice, the sample was transferred to 150 ul of ice-cold stop mix containing 100 mM Tris-HCl, pH 8.0, 10 mM EDTA, 10 mM Na_2SO_3 and 100 ug/ml of polyadenylic acid. The sample was chromatographed on a Sephadex G-50 column (1 x 20 cm) in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. The DNA peak, well separated from free iodine, was pooled and incubated at 60° for 10 min. The DNA was ethanol precipitated, dissolved in 10mM Tris-HCl, pH 8.0, 1 mM EDTA and stored frozen at -20°. The recovery of DNA was about 60%. The specific activity of the DNA was c. 0.8-1 x 10^8 cpm/ug.

The labelled DNA could be used as a hybridization probe for at least two months after iodination.

DNA Sequencing.

M13 plaques which hybridized with the probe were picked from the plate and single-stranded template DNA was prepared as described above. M13 clones were sequenced by the dideoxy chain termination procedure (5).

Dot Blots.

Dot blots were prepared by spotting 1 ul of M13 culture supernatant (prepared by centrifugation at 17,000 x g for 10 min) onto dry nitrocellulose filters followed by denaturation and neutralization as described above for DNA filter preparation.

RESULTS AND DISCUSSION

Sequencing Strategy.

Lambda clone λMt3 was obtained from a mouse genomic DNA-λ charon 4A library screened with iodinated tRNA. As shown in Fig. 1, Southern blot analysis of λMt3 indicates that it contains a c. 11 kb EcoRI fragment which hybridizes with iodinated tRNA (lanes A and C). An EcoRI-XbaI double digest contains two hybridizing fragments of c. 5 and 1.8 kb, indicating that λMt3 contains at least two tRNA genes (Fig. 1, lanes B and D). Sequence analysis of the single tRNA gene (coding for lysine tRNA) in the 1.8 kb fragment is presented here.

In order to sequence the tRNA genes in λMt3, we first prepared M13 subclone bank A from an AluI digest of the phage DNA (see Materials and Methods). Sequence analysis of one of the M13 subclones which hybridized with iodinated tRNA, termed subclone A1, indicated that it contained a 278 bp mouse DNA insert which begins at position 10 of a lys tRNA coding region and includes 213 bp of 3' flanking sequence (see Fig. 2 and below).

The sequence of A1 contains a HinfI site just to the 3' side of the lys tRNA coding region. In order to isolate an M13 subclone which contains the 5' end of the lys tRNA coding region and the 5' flanking region we prepared M13 subclone bank H from a HinfI digest of λMt3 DNA. About 50 of the c. 5000 M13 plaques gave strong signals on duplicate filters, as shown in Fig. 3A. Eleven of the M13 clones were picked at random and the location of T residues determined by the dideoxy chain termination procedure (5). These data indicated that the single-stranded DNA of four of the 11 clones contained the lys tRNA coding region and its 5' flanking region. The mouse DNA insert of one of these, termed H1, was fully sequenced. It comprises a 182 bp HinfI

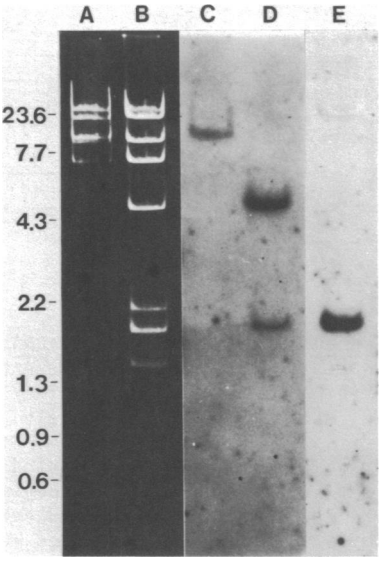

Figure 1. Southern Blot Analysis of Clone λMt3.
Lanes A and B, EcoR1 and EcoR1-XbaI digests of λMt3 DNA, respectively, resolved on a 1% agarose gel and stained with ethidium bromide. Sizes in kb of HindIII digested λ and Hae III digested ϕX174 DNA fragments are given on the left. Lane C, autoradiograph of a Southern blot of lane A hybridized with iodinated mouse tRNA. Lane D, Southern blot of lane B hybridized with mouse tRNA. Lane E, the labelled tRNA was eluted from the filter in D which was then hybridized with iodinated, single-stranded clone A1 DNA (see fig. 2). Filters C and D were hybridized with 10^6 CPM of iodinated tRNA overnight at 37° in 50% formamide, 4X TES. Filter E was hybridized with 10^6 CPM of A1 DNA overnight at 68° in 4X TES.

fragment, as shown in Fig. 2. Five of the 11 M13 clones contained the HinfI fragment adjacent to H1 in the 3' direction and were not further sequenced because the 3' sequence had already been determined from clone A1. Two of the clones did not contain a sequence contiguous to that of clone A1 or H1 and presumably contained two or more HinfI fragments which were ligated randomly during construction of subclone bank H.

We also used iodinated, single-stranded A1 DNA to isolate an M13 phage in which the A1 sequence was cloned in the opposite orientation. This was accomplished by washing the iodinated tRNA probe from the filter originally used to screen subclone bank A and rehybridizing with iodinated, single-stranded A1 DNA. This identified, and we subsequently sequenced, clone A2, the single-stranded DNA of which contained the sequence complementary to that of A1. Similarly we used iodinated single-stranded H1 DNA to isolate

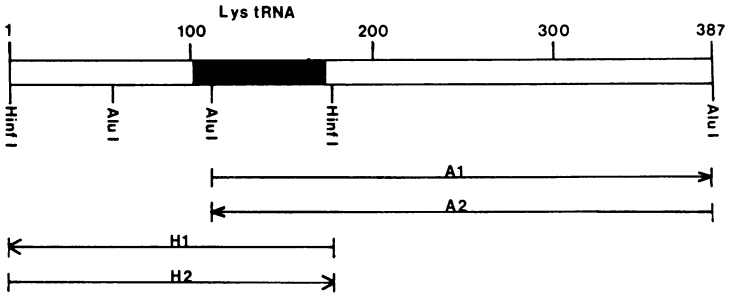

Figure 2. M13 Subclones Used to Sequence the Lys tRNA Gene.
The upper bar represents a 387 bp sequence present in λMt3 which contains a lys tRNA coding region (filled-in area). The 5' end, relative to the non-coding (tRNA-like) DNA strand, is to the left. Relevant restriction sites are indicated.
A1, A2, H1 and H2 are the M13 subclones, isolated as described in the text, used to determine the 387 bp sequence. The beginning of the mouse DNA insert relative to the DNA sequencing primer is indicated by a vertical line. The arrow head followed by a vertical line indicates the end of the sequence read from the gel and the end of the mouse DNA insert. Arrows pointing to the right indicate that the coding strand (relative to the lys tRNA gene) was sequenced. Left arrows indicate that the non-coding strand was sequenced.

and sequence clone H2 (Fig. 2).

As shown in Fig. 2, clones A1, A2, H1 and H2 define a 387 bp region which has been sequenced completely on both strands. As described above, this sequence was determined by using the initial clone (A1) as a probe and "walking" in the 5' direction.

In order to determine which EcoRI-XbaI fragment of λMt3 DNA contains the lys tRNA gene, the iodinated tRNA probe was eluted from the Southern filter shown in Fig. 1D and the filter was rehybridized with iodinated, single-stranded A1 DNA. As shown in Fig. 1E, the 1.8 kb fragment hybridizes. We also note that the iodinated probe gives an intense autoradiographic signal with very low background when hybridized with a cloned DNA fragment. We are currently determining whether the probes can also be used to detect specific sequences in blots of genomic DNA.

The question of whether the 1.8 kb EcoRI-XbaI fragment contains any other tRNA genes was examined as follows: We isolated the 1.8 kb fragment by preparative agarose gel electrophoresis and digested it with HinfI. The HinfI fragments were resolved on a formamide-agarose gel (6), blotted to nitrocellulose and probed with iodinated tRNA. A single HinfI fragment of c. 180 bp hybridized (data not shown). The lys tRNA gene is on a 182 bp fragment (Figs. 2 and 4). These observations are consistent with the con-

clusion that the 1.8 kb fragment of λMt3 contains a single tRNA gene.

Further Analysis of Single Stranded M13 Probe Hybridization.

The autoradiograph shown in Fig. 3B indicates that iodinated single-stranded M13 probes can also be used to detect specific lambda phage clones by the Benton-Davis procedure (3). We prepared a plate containing approximately equal numbers of phage λMt1 (which contains a his tRNA gene and no lys tRNA genes; ref. 1 and unpublished observations) and λMt3. Duplicate filters were hybridized with labelled single-stranded clone A1 DNA. An autoradiograph of one of the filters is shown in Fig. 3B. Intense signals which matched on duplicate filters were obtained from about one-half the plaques, as expected. Furthermore, the M13 probe hybridizes specifically to a mouse DNA fragment in λMt3 and not to λ vector sequences (Fig. 1E). It should be noted, however, that M13mp7 contains part of the E. coli lac z gene (2). The probe will therefore hybridize with non-recombinant λ charon 4A phage, which also contain the lac z gene (7). Recombinant phage, such as those in Fig. 3B, do not contain the segment of lac z gene present in M13 and will hybridize only if they contain a sequence homologous to the insert in the M13 probe.

The autoradiograph in Fig. 3C indicates that iodinated probes can also be used in dot blot experiments, and in addition, illustrates a procedure for reducing non-specific background. Dots 1 and 2, containing single-stranded M13mp7 and clone A1 DNA, respectively, give a very weak signal in comparison to dot 3, which contains single-stranded clone A2 DNA. The low level of background hybridization in dots 1 and 2 can be eliminated completely by addition of unlabelled, single-stranded M13 vector DNA to the hybridization, as shown for dots 4 and 5. Dot 6, which contains clone A2 DNA, still hybridizes intensely. The autoradiographs of Figs. 3A and 3C are overexposed. The level of non-specific background can often be reduced simply by varying the exposure time.

Concluding Remarks on the Sequencing Protocol.

The speed with which tRNA gene sequences cloned in λ phage can be determined by the "walking" protocol described above is limited mainly by the first step -- sequencing part of an identifiable tRNA coding region from an initial M13 subclone bank. This sequence must be within c. 400 bp of the end of the DNA fragment inserted proximal to the sequencing primer site of M13mp7 in order to be read from a sequencing gel. This is often the case if a 4-cutter restriction enzyme is used to prepare the initial M13 subclone bank. The particular restriction enzyme to be used can sometimes be selected

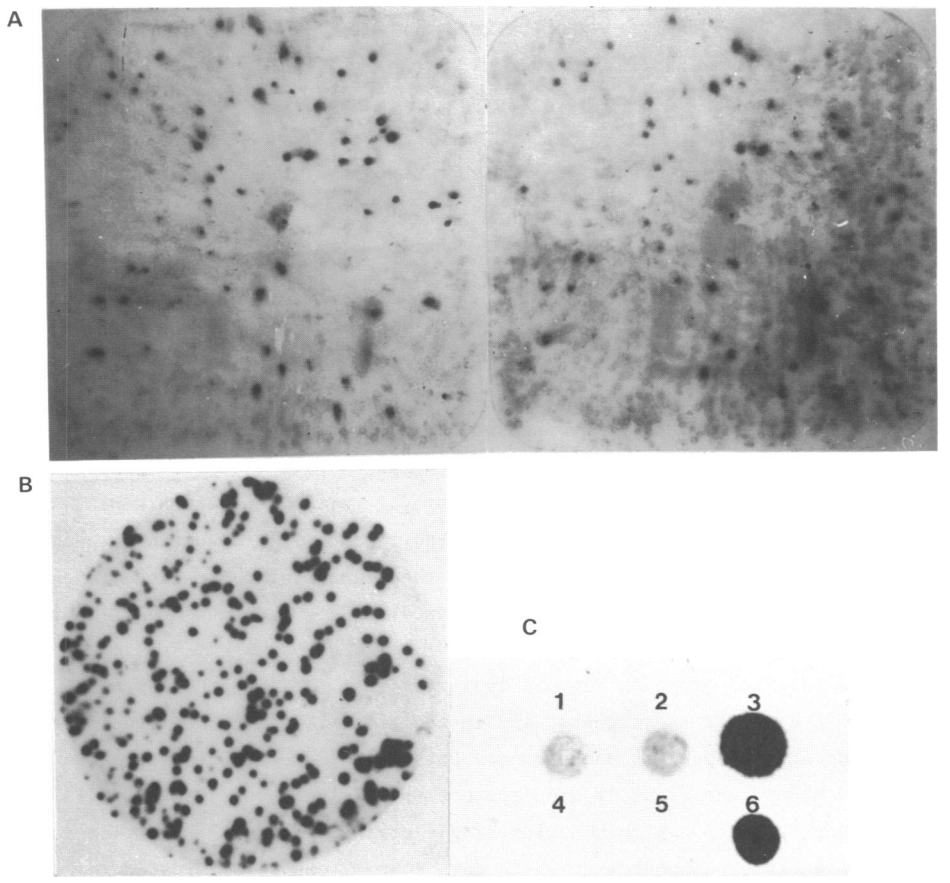

Figure 3. Hybridization of Iodinated Single-Stranded M13 DNA Probes.

Part A. Duplicate nitrocellulose filters were prepared from a plate containing c. 5000 plaques from subclone bank H (see text) and hybridized with 10^6 CPM of iodinated single-stranded A1 DNA overnight at 37° in 50% formamide, 4X TES, 10µg/ml of M13 mp7 single-stranded DNA carrier, washed and exposed to X-ray film for 5 hrs at room temperature without an intensifying screen. Autoradiographs of the filters are shown.

Part B. Duplicate nitrocellulose filters were prepared from a plate containing c. 500 plaque forming units each of lambda clone λMt1 (1) (which does not contain a lys tRNA gene) and λMt3. The filters were hybridized with 10^6 CPM of iodinated single-stranded A1 DNA in 4X TES overnight at 68°, washed and exposed to X-ray film for 2 hrs at -70° with an intensifying screen. Autoradiographs of the filters, one of which is shown, contained identical spot patterns.

Part C. Dot blots were prepared as described in Materials and Methods from M13mp7 culture supernatant (Dots 1 and 4), clone A1 supernatant (Dots 2 and 5), clone A2 supernatant (Dots 3 and 6). Dots 1-3 were hybridized with 10^6 cpm of iodinated A1 DNA in 4X TES for 5 hrs at 68°. Dots 4-6 were hybridized as for dots 1-3 except that 50µg/ml of unlabelled single-stranded M13mp7 DNA was included. The autoradiograph was exposed for 3 hrs at -70° with an intensifying screen.

on the basis of preliminary restriction mapping data. However, one of the strengths of the procedure is that detailed restriction mapping is not necessary. The above limitation is not as serious if sequences comprising a larger proportion of the DNA inserted into the lambda phage (or other) vector are being determined.

Single-stranded probes can also be used to isolate M13 phage containing the same sequence cloned in opposite orientations. Of particular convenience is the fact that one set of phage plates and nitrocellulose filters can be used to isolate different M13 clones containing complementary inserts, as shown above. Other procedures for identifying complementary clones have been described (Refs. 8, 9 and J. Looney, J. Han and J. Harding, submitted).

Iodinated, single-stranded probes can replace conventionally prepared, nick-translated double-stranded plasmid or phage probes for most applications. A significant advantage of our procedure is that expensive and time-consuming cesium chloride density gradient centrifugation is not required for purification of single-stranded M13 DNA.

A procedure for preparing ^{32}P labelled strand-specific M13 probes has also been published (9). We suggest that for most applications iodinated probes are probably more useful than ^{32}P labelled probes. Iodinated probes are less expensive to prepare, do not require a specific primer for labelling, are not inactivated by melting and have comparable specific activities and a significantly longer half-life than ^{32}P-labelled probes.

Nucleotide Sequence of a Mouse Lysine tRNA Gene.

Coding Region. The 387 bp sequence determined as described above is shown in Fig. 4. We examined it for GTTC and GATC tetranucleotides which are diagnostic for tRNA coding regions, and identified a single tRNA gene (boxed in Fig. 4) which codes for a lys tRNA, anticodon UUU. The cloverleaf structure of the putative tDNA is shown in Fig. 5. In common with most other eucaryotic tRNA genes, the 3' terminal CCA residues of the mature tRNA are not encoded in the DNA. The tDNA sequence differs in five positions (underlined in Fig. 5) from rabbit liver lys tRNA (10) and a human lys tRNA gene sequence (11). All five positions can be variable in eucaryotic tRNAs (12). We therefore suspect that the 387 bp sequence can encode a biologically active lys tRNA.

Flanking Regions. The 5' and 3' flanking regions of the mouse lys tRNA gene have little homology with cognate regions of human (11) or Drosophila (13) lys tRNA (anticodon UUU) genes. One exception is that both the mouse and human genes contain a short homologous sequence adjacent to the 5' end

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        1Ø          2Ø          3Ø          4Ø          5Ø          6Ø
GATTCTGTCA CCTTTTCAGG GCAAGAATTA GCCTATGCTA GTGCTGAAAA TGGTAGCTAT
        7Ø          8Ø          9Ø         1ØØ         11Ø         12Ø
CAATTTTTTT TCTCTATGTG TAACAAACAT GCTATGTGAG AGCCTGGATA GCTCAATTGG
       13Ø         14Ø         15Ø         16Ø         17Ø         18Ø
TAGAGCATCA GACTTTTAAT CTGAGGGTTC AGGGTTCAAG TCCCTGTTCA GGCGCTGATT
       19Ø         2ØØ         21Ø         22Ø         23Ø         24Ø
CTCAAACTTT GAAAGTTAAA CTTGATAGTA AGGTGGACTT TTATTCTACT CCAGCAAGAT
       25Ø         26Ø         27Ø         28Ø         29Ø         3ØØ
AGCAGTTTTA AAATTACTCT TCCGGAGGAA CATGGTATGA AAAACTTACA GAGCACAGAG
       31Ø         32Ø         33Ø         34Ø         35Ø         36Ø
CATCCTTCCG TTCTAGGTTG GTTTCAAATT ATTGAAATAT TTACTATGGC TTAGATTTAG
       37Ø         38Ø          3          13          23          33
ATTTAACTGT CTTTCCATCT TATAGCT
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Figure 4. The 387 bp Sequence from λMt3 which Contains a lys tRNA Gene.
The non-coding (tRNA-like) strand is shown with the 5' end to the left The lys tRNA coding region is boxed. Two 11 bp direct repeats which flank the coding region are underlined.

of the coding region (TGAGA in the mouse gene, TGAAGA in the human gene). If these genes are transcribed like other eucaryotic tRNA genes (13,14), these residues may encode part of the primary transcript.

The 5' flanking regions of the mouse, human (11) and Drosophila (13) lys tRNA genes (anticodon UUU) are moderately A-T rich (61-67%). In addition, each gene contains a localized area of higher A-T content. This region comprises residues 58-71 in Fig. 4, 30-44 bp from the coding region. Thirteen of 14 base pairs in this sequence are A-T. A human lys tRNA gene contains an 18 bp region comprised of 15 A-T base pairs 14-31 bp from the

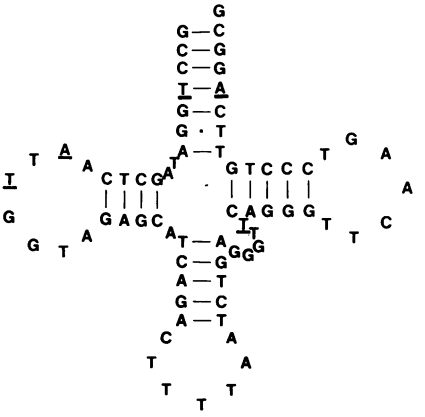

Figure 5. The Cloverleaf Structure of the lys tDNA encoded by the 387 bp Sequence.
Residues which differ from those of a sequenced rabbit liver lys tRNA (anticodon UUU, Ref. 10) are underlined.

coding region (11). A Drosophila lys tRNA gene contains an 18 bp region containing 14 A-T base pairs 21-38 residues from the coding region (13). The A-T rich regions of the three genes are, however, of completely different sequence. Indik and Tartof (15) have noted that several other eucaryotic tRNA genes contain localized A-T rich regions situated 37±8 bp from the coding region. These regions are often shorter than those noted above. In addition, the sequence GGCAGTTTTTG has been demonstrated by DeFranco et al. to affect the efficiency of transcription of Drosophila lys tRNA genes (anticodon CUU) in vitro, depending on its precise position relative to the coding sequence (16). It will be of interest to determine whether localized regions of high AT content in the 5' flanking regions of other tRNA genes also affect expression.

In the 3' flanking region, the most likely transcription termination site, comprising at least four consecutive T residues on the non-coding strand (17), begins at position 219 (Fig. 4). This is unusually far from the 3' end of the coding region (45 bp). A survey of 25 Drosophila and 14 vertebrate tRNA gene sequences (ref. 1 and refs. within and refs. 13-15, 18-20), indicates that termination sites are usually within 20 bp of the coding region. An exception is a rat glu tRNA gene which has a likely termination site 48 bp into the 3' flanking region (19).

The sequence of Fig. 4 contains a number of short direct repeats in the flanking regions. The longest of these, consisting of an 11 bp sequence with one mismatch, are underlined in the figure. These repeats flank the tRNA coding region and the 5' repeat directly abuts the short 5' sequence conserved in the mouse and human lys tRNA genes (see above). The presence of flanking direct repeats is characteristic of transposable elements in bacteria (21) and eucaryotic alu family sequences and small nuclear RNA pseudogenes, which may be transposable (22-25). It will be necessary to sequence more mouse lys tRNA genes to determine whether the 11 bp repeats are related to dispersal of tRNA coding regions in the mouse genome.

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REFERENCES

1. Han, J.H. and Harding, J.D. (1982) Nucleic Acids Res. 10, 4891-4900.
2. Messing, J., Crea, R., and Seeburg, P. (1981) Nucleic Acids Res. 9, 309-321.
3. Benton, W.D. and Davis, R.W. (1977) Science 196, 180-182.
4. Prensky, W. (1976) in Meths. in Cell Biology, Prescott, D., ed. Vol. XIII, pp. 121-152, Academic Press, New York.
5. Sanger, F., Coulson, A.R., Barrell, B., Smith, A.J. and Roe, B.A. (1980) J. Mol. Biol. 143, 161-178.
6. Sun, Y.L., Xu, Y.Z. and Chambon, P. (1982) Nucleic Acids Res. 10, 5752-5764.
7. Blattner, F.R., Williams, B.G., Blechl, A.E., Thompson, K.E., Faber, H.E., Grimwald, D.J., Kiefer, D.O., Moore, D.D., Schumn, J.W., Sheldon, E.L., and Smithies, O. (1980) Science 208, 161-169.
8. Gardner, R.C., Howarth, J., Hahn, P., Brown-Luedi, M., Sherpher, R.J. and Messing, J. (1981) Nucleic Acids Res. 9, 2871-2888.
9. Hu, N. and Messing, J. (1982) Gene 17, 271-277.
10. Raba, M., Limburg, K., Burghagen, M., Katze, J.R., Simsek, M., Heckman, J.E., Rajbhandary, U.L., and Gross, M.J. (1979) Eur. J. Biochem. 97, 305-318.
11. Roy, K.L., Cooke, H. and Buckland, R. (1982) Nucleic Acids Res. 10, 7313-7322.
12. Sprinzel, M. and Gauss, D.H. (1982) Nucleic Acids Res. 10, r1-r55.
13. DeFranco, D., Burke, K.B., Hayashi, S., Tener, G.M., Miller, Jr., R.C., and Soll, D. (1982) Nucleic Acids Res. 10, 5799-5808.
14. Sharp, S., DeFranco, D., Silberklang, M., Hosbach, H.A., Schmidt, T., Kubli, E., Gergen, J.P., Wensick, P.C. and Soll, D. (1981) Nucleic Acids Res. 9, 5867-5882.
15. Indik, Z. and Tartof, K. (1982) Nucleic Acids Res. 10, 4159-4172.
16. DeFranco, D., Sharp, S. and Soll, D. (1981) J. Biol. Chem. 256, 12424-12429.
17. Bogenhagen, D.F. and Brown, D.D. (1981) Cell 24, 261-270.
18. Addison, W.R., Astell, C.R., Delaney, A.D., Gillam, I.C., Hayashi, S. Miller, R.C., Rajput, B., Smith, M., Taylor, D.M., and Tener, G.M. (1982) J. Biol. Chem. 257, 670-677.
19. Shibuya, K., Noguchi, S., Nishimura, S. and Sekiya, T. (1982) Nucleic Acids Res. 10, 4441-4448.
20. Sekiya, T., Nishizawa, R., Matsuda, K., Taya, Y. and Nishimura, S. (1982) Nucleic Acids Res. 10, 6411-6419.
21. Calos, M.P. and Miller, J.M. (1980) Cell 20, 579-595.
22. Schmid, C.W. and Jelinek, W. (1982) Science 216, 1065-1070.
23. Grimaldi, G., and Singer, M.F. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 1497-1500.
24. Jagadeeswaran, P., Forget, B.G. and Weissman, S.M. (1981) Cell 26, 141-142.
25. Van Arsdell, S.W., Denison, R.A., Bernstein, L.B., Weiner, A.M., Manser, T. and Gesteland, R.F. (1981) Cell 26, 11-17.