Isolation and nucleotide sequence of a mouse histidine tRNA gene

Jang H.Han and John D.Harding*

922 Fairchild Center, Department of Biological Sciences, Columbia University, New York, NY 10027, USA

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ABSTRACT

We have sequenced a 1307 base pair mouse genomic DNA fragment which contains a histidine tRNA gene. The sequence of the putative mouse histidine tRNA differs from the published sequence of sheep liver histidine tRNA by a single base change in the D-loop. It does not contain an unpaired 5' terminal G residue, as reported for *Drosophila* and sheep histidine tRNAs. The gene does not contain introns. The 3' flanking region contains a typical RNA polymerase III termination site of 6 consecutive T residues. 523 residues after the 3' end of the his tRNA coding region, the mouse DNA contains a sequence 72% homologous to part of the consensus sequence of the B1 (alu) family.

INTRODUCTION

Eucaryotes contain many families of reiterated genes. In mammals, the individual members of most reiterated gene families are dispersed in the genome rather than clustered in tandemly repeated units (reviewed in Ref. 1). The ubiquity of dispersed repetitive families implies that elucidation of their structure, modes of regulation and evolutionary history will be important for understanding the molecular biology of eucaryotic cells.

Eucaryotic tRNA genes are reiterated and generally dispersed in the genome. Individual members of some families are widely separated in humans (2), yeast (3-5), silkworms (6) and *Drosophila* (7). Other *Drosophila* tRNA gene families are organized in a few clusters at different chromosomal loci. Genes of different families are sometimes present in the same cluster and sometimes not (8-11). A number of *Xenopus* tRNA genes are present in a large tandemly repeated DNA sequence which, however, does not contain regular repeating sequences near the tRNA genes (12).

We have recently isolated several cloned fragments of the mouse genome which contain tRNA genes in order to obtain detailed information about mouse tRNA gene organization. In this paper we report the nucleotide sequence of a 1307 bp segment of mouse DNA which contains a single histidine tRNA gene

and a truncated B1 (alu) family member.

MATERIALS AND METHODS

Preparation of a Mouse Recombinant DNA Library

The recombinant DNA library was prepared according to Refs 13 and 14. The components of the library are shown in fig. 1. High molecular weight DBA-2 mouse liver DNA was partially digested with EcoRI under a number of reaction conditions and fractionated by sucrose density gradient centrifugation (14). Phage λ charon 4A DNA (15) was circularized, ligated and restricted with EcoRI. The 31 kb lambda "arms" were separated from the nonessential internal DNA sequences by sucrose density gradient centrifugation. Eleven micrograms of fractionated mouse genomic DNA (c. 12-20 kb in size) (fig. 1, lane 3) was incubated with 20 μ g of the 31 kb λ charon 4A arms (fig. 1, lane 2) and 8 units of T4 ligase (Bethesda Research Labs) at 12° for 15 hours in 66mM Tris HCl (pH 7.6), 6.6mM MgCl₂, 10mM dithiothreitol, 1mM ATP, 1mM spermidine and 250 μ g/ml of autoclaved gelatin. The resulting DNA molecules (fig. 1, lane 4) were packaged in vitro and transduced into E. coli DP50 SupF. Packaging extracts were prepared by modifications of published methods (16). The bacteria were plated onto NZYDT plates and the phage were recovered from the top agar and stored as described (13). Greater than 99% of the phage were recombinant as judged by the XG plate assay (15).

Screening the Recombinant Library

Phage from the recombinant library were spread on a lawn of E. coli DP50 SupF grown on NZYDT agar. The resulting plaques from NZY top agarose were transferred to cellulose nitrate filters by the method of Benton and Davis (17). After washing with 4X SET (1X SET is 0.15M NaCl, 2mM EDTA, 30mM Tris HCl, pH 7.5) each filter was prehybridized with 4X SET, 50% (v:v) deionized formamide, 0.2% (w:v) each, Ficoll-400, Polyvinyl Pyrrolidone-360, and Bovine serum albumin, 0.1% (w:v) Sodium dodecyl sulfate, 100 μ g/ml PolyA, and 100 μ g/ml E. coli tRNA for 2 hours at 37°C. Each filter was hybridized with 1 ml of the above solution containing 2×10^7 cpm of iodinated mouse liver tRNA for 24 hours at 37°. Following hybridization the filters were washed for one hour at 37° in hybridization buffer lacking probe and then with 4X SET, 50% formamide for three hours at 37°. Autoradiographs of duplicate filters made from each plate were compared in order to distinguish signals from background spots. Phage plaques were picked and successively replated until more than 90% of individual plaques hybridized with the tRNA probe. In order to verify the fact that a plaque-purified phage harbored a tRNA gene, phage DNA was

purified, digested to completion with EcoRI, electrophoresed on a 0.6% agarose gel in 40mM Tris HCl, 5mM sodium acetate, 1mM EDTA (pH 7.7) transferred to nitrocellulose (18) and hybridized with iodinated mouse tRNA (fig. 1, lanes 5,6).

M13 Shotgun Sequencing

Target DNA preparation: Lambda phage λ Mt1 contained a 1307 bp XbaI fragment which hybridized with iodinated mouse tRNA (fig. 2). This XbaI fragment was isolated from an agarose gel by electroelution and circularized by incubation with T4 DNA ligase as described below for sticky end ligations. The DNA was cleaved with Sau3A, AluI or HaeIII.

Vector DNA preparation: Phage M13mp7 (19) was digested with BamHI (for cloning Sau3A cut target fragments) or HincII (for AluI or HaeIII targets). Control experiments indicated that restricted vector preparations contained less than 5% uncleaved DNA and that restricted-religated preparations gave less than 2% white plaques when plated on indicator plates.

Ligation and plating: For sticky end ligation of Sau3A fragments to BamHI digested M13mp7, 25 ng of vector DNA was mixed with 75ng of target DNA and incubated at 12°C overnight in 20 μ l of 66mM Tris HCl, pH 7.5, 6.6mM MgCl₂, 10mM dithiothreitol, 66 μ M ATP and 0.5 units of T4 DNA ligase (BRL). For blunt end ligation of AluI or HaeIII fragments to HincII cut M13mp7, the same amount of vector and target DNAs were incubated at 4° overnight in 10 μ l of the above solution containing 500 μ M ATP and 1 unit of T4 DNA ligase. The ligated DNA was transfected into CaCl₂ treated JM103 and plated on X-Gal plates (19).

Subcloning the entire 1307 bp XbaI fragment into M13mp7: The sticky ends of the XbaI fragment was filled in by incubating 75ng of DNA in 5 μ l of T₄ ligase buffer containing 50 μ M each dNTP and 0.1 unit of T4 DNA polymerase at 37°C for 30 min. ATP (final concentration of 500 μ M) and vector DNA (HincII cleaved M13mp7, 25ng) were added and the volume was adjusted to 10 μ l with T₄ DNA ligase buffer. The mixture was incubated at 4°C overnight. The ligated DNA was transfected into JM103 as described above.

Sequencing: About 25 white plaques were picked from the several hundred arising from each ligation and partially sequenced by the T-screen procedure (20). Based on the T-screen data, several clones from the Sau3A, AluI and HindIII digestions as well as a clone containing the entire XbaI fragment were sequenced by the dideoxy chain termination method (21) using 8% or 6% thin sequencing gels (22). The sequences of the various subclones were assembled into the complete sequence of the 1307 bp XbaI fragment using the

computer programs of Staden (23).

RESULTS AND DISCUSSION

Isolation of Mouse tRNA Genes

In order to isolate mouse tRNA genes, we (with our colleague, James Looney) prepared a recombinant DNA library from DBA2 mouse liver DNA and the vector, λ charon 4A, as described in Materials and Methods. Fig. 1 (lanes 1-4) shows an agarose gel of the components of the library. The recombinant DNA library was screened with iodinated mouse liver tRNA and several clones were purified to homogeneity. The clone characterized in this paper is termed λ Mt1. As shown in Fig. 1, lane 5, EcoRI digestion of λ Mt1 yields 3 fragments, the middle of which is a c. 15 kb mouse genomic DNA insert which hybridizes with iodinated mouse tRNA (lane 6). Further Southern blot analysis of λ Mt1 indicated that a XbaI fragment c. 1300 bp in size hybridized with the tRNA probe (fig. 2). This fragment was isolated by preparative agarose gel electrophoresis and sequenced by the M13 shotgun method, as described in Materials and Methods.

Nucleotide Sequence

The sequence of the XbaI fragment was determined from the overlapping sequences of 21 randomly chosen M13 subclones derived from Sau3a, AluI and HaeIII digests, as diagrammed in Fig. 3. In addition, part of one end of the sequence was determined from a M13 subclone of the entire fragment (X3). Each part of the 1307 bp sequence was derived from at least 2 different overlapping M13 subclones, over 80% from 3 or more clones. Both strands of the sequence were determined with the exception of residues 920-1000. The redundancy inherent in the shotgun technique helped assure the accuracy of the sequence determination. The ends of the sequence were determined from subclone H2 which contained an internal XbaI site and overlapped subclones which were on opposite ends of the sequence (X3 at the 3' end relative to the orientation of the tRNA coding sequence and several AluI clones at the 5' end, fig. 3). This was expected since we self-ligated the purified XbaI fragment prior to restriction and subcloning.

Two technical comments concerning the sequencing may be of interest to others using the shotgun technique. First, we have modified the blunt end ligation reaction conditions to give very efficient ligation (see Materials and Methods). Second, the subclones are distributed relatively uniformly over the entire 1307 bp sequence. However, we never detected a subclone of the entire XbaI fragment in the orientation opposite to that of X3.

Figure 1. Isolation of clone λ Mt1. Lanes 1-4, an Ethidium Bromide stained 0.6% agarose gel showing components of the recombinant DNA library: Lane 1, EcoRI digested phage λ charon 4A DNA, lane 2, the 31kb, ligated λ arms, lane 3, fractionated mouse genomic DNA (c. 12-20kb in size), lane 4, DNA isolated after ligation of the arms and the mouse genomic DNA. Lane 5, EcoRI restricted λ Mt1. Lane 6, an autoradiogram of a Southern blot of lane 5 hybridized with iodinated mouse tRNA.

The Histidine tRNA Gene Coding Sequence. The complete nucleotide sequence of the 1307 bp XbaI fragment from λ Mt1 is shown in fig. 4. In order to identify the tRNA gene(s) in the sequence we searched it for GTTC and GATC tetranucleotides, one of which is present in the T-pseudo U stem and

Figure 2. Southern blot analysis of λ Mt1. Left lane; agarose gel profile of XbaI digested λ Mt1. The size in kb of HindIII digested λ DNA fragments and HaeIII digested ϕ X-174 fragments run in parallel lanes are given at the left. Right lane, an autoradiogram of a Southern blot of the XbaI digest hybridized with iodinated mouse tRNA.

Figure 3. M13 Subclones used to Sequence the 1307 Base Pair XbaI Fragment of λ Mt1.

The upper bar represents the 1307 bp sequence with relevant restriction sites indicated. The filled in region is the histidine tRNA coding region which is transcribed from left to right. The sequences of M13 subclones are indicated below the bar. Subclones marked S, A, and H were derived from Sau3A, AluI and HaeIII digests, respectively, of the XbaI fragment. The sequence X3 was obtained by cloning the fragment directly into M13. The beginning of the mouse DNA insert relative to the M13 sequencing primer is indicated by a vertical line; the arrowhead indicates 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 subclone was sequenced. Arrows pointing to the right indicate that DNA corresponding to the coding strand (as defined by the orientation of the histidine tRNA gene) of the 1307 bp fragment was sequenced, arrows pointing to the left indicate that the non-coding (tRNA-like) strand was sequenced.

loop of most eucaryotic tRNAs (24), and then constructed a tRNA coverleaf structure from the surrounding residues. This analysis indicated that the sequence contained a single tRNA gene, located at residues 462-533 (solid box in fig. 4). The cloverleaf structure encoded by these residues is shown in fig. 5. It contains the histidine anticodon, 5'-GTG-3' and the invariant residues characteristic of eucaryotic tRNAs (24). This sequence differs in only two respects from the published sequence of the sheep liver major histidine tRNA isoacceptor (25). It contains a G residue at position 16 in the D loop (underlined in fig. 5) rather than a U as in the sheep tRNA. In addition, both sheep liver (25) and *Drosophila* (26) histidine tRNAs contain an unpaired 5' terminal G residue. The DNA sequence of fig. 4 contains a C at this position and we have not included this in fig. 5. Based on the correspondence of the mouse sequence presented here with that of the sheep liver tRNA, we conclude that λ Mt1 can encode a biologically active histidine tRNA.

In common with most other eucaryotic tRNA genes, the DNA sequence shown

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100      200      300      400      500      600
TCTAGAGTGC ATCAGCTACA GTAAAGACTT GAAGGGCGAA GAAAGCTTCA TGCTGTCTAG
700      800      900      1000     1100     1200
CAAAAGGGGA GGGGGGCACA CGTGTGAACA CAAAGAAAAC GGAGTCTAAT ACAGGGAATC
1300     1400     1500     1600     1700     1800
GAACTTTTGA CTTCTCTAAC ACAAAGCAAC CTCACTACCC AGCTGAACCA CAGAAGAAAT
1900     2000     2100     2200     2300     2400
GAGTGTGATT GTTCTCTATC GCAGCATAAA CAAGTCTCTA CATCAGTGCC AGTTGCAGCC
2500     2600     2700     2800     2900     3000
ACAGCCACAA CCTTTGCGTC ACAGACCTCT CAGACGCTC GGAATAAAC ATCGGATCAA
3100     3200     3300     3400     3500     3600
TCCTTGCTGG GCTCACTTTA TCCCCGACGC AGCAGCGCGT AGCCGAGTTA CTGCGCAGGC
3700     3800     3900     4000     4100     4200
ATCCGACAGA AAGCTAACAT CGATGTCAGG TCCAAGCAGA AGTGAATTA AGGCTCAGCT
4300     4400     4500     4600     4700     4800
GACAGCGGGA GAGCTTAAAT TTGCTATCTA GTGTGTGGTC GCCGTGATC GTATAGGGT
4900     5000     5100     5200     5300     5400
TAGTACTCTG CGTTGTGGCC GCAGCAACCT CGGTTTCAAT CCGAGTCACG GCAATTATCCT
5500     5600     5700     5800     5900     6000
CTGGTCACTT TTTTGTCTCA CTCTCTCTCT GATGAACCTT TCCCTACAGA TCTACCCGCT
6100     6200     6300     6400     6500     6600
TCCGCTCATC GTCACCCAGG AAGCGTGGGA AGTGCTTGCT CTCTCCCAAG CTGTTTGGCA
6700     6800     6900     7000     7100     7200
GGAAATGGGA ATGAACCTTT AATGCTTTG AATCCATCCT GCTGCAGCGG GCTGTCAGCA
7300     7400     7500     7600     7700     7800
GTCAGCATTC ACCTTCTTCA TATTTGTATG CATATTGTAA TAAACATACC GAAGCATCTT
7900     8000     8100     8200     8300     8400
ACAATAAAAT GGTTTTGAAG AGTCAACACC TGGACCAGGT TACTGTGAAA TTTCTCATC
8500     8600     8700     8800     8900     9000
CGTCTGTGAG GGGAGGGGTG GAGAGGGAGG AGGGACAGGG AACCATGGTG CATCCTAGAA
9100     9200     9300     9400     9500     9600
GGTCAGAGGA AAATTTTCAG GAATTGAAGG AACTTGGCTT TACCAGGCTT TCAGGGCAAG
9700     9800     9900     10000    10100    10200
TCCTCCCACC CACCTTAAAG AGGTGCGGCC CACTGGCTTT TTCTGTGTTG CTTGTTTTTG
10300    10400    10500    10600    10700    10800
ACAGGGTCTC ACGCTGTCCT GTAACCTTGA CTCTGATCC TCTGCTTCA GACCTGAGT
10900    11000    11100    11200    11300    11400
ACTAGGATTA CAGACATTAG CTAACAAGCC AGGCTGAGTT TTACTCTTTG ATTCTCATGA
11500    11600    11700    11800    11900    12000
GTGAATGTGC CTCCGCTGGC CCACCAGGTT TTTCCATAGC TTTCTGCGCT TGGGAATCTG
12100    12200    12300    12400    12500    12600
TCCACTTTGG TGTGGTTATC ACTCCTTCGT TTGATTTCCT TTTGGTTTGA GTGGGAGGGC
12700    12800    12900    13000
GATATGTATG TGTCTATCA CACTGGATTG CATCTTGGGG GTCTAGA

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Figure 4. Nucleotide Sequence of the 1307 bp XbaI Fragment of λ Mt1.

The tRNA-like (non-coding) strand is shown with the 5' end at the left. The his-tRNA coding region (residues 462-533) is enclosed in the solid box; sequences 7 bp or larger repeated in the tRNA coding region are underlined (some of these are on the coding strand). The sequence homologous to part of a B1 family consensus sequence is enclosed in the dashed box (residues 1057-1113).

in fig. 4 does not encode the 3' terminal CCA residues found in the mature tRNA. The gene does not contain an intervening sequence.

Flanking Sequence. The DNA sequences flanking the histidine tRNA coding region have little obvious homology with other eucaryotic tRNA genes. Two common features are, however apparent. Sixteen residues after the end of the coding region are 6 consecutive T residues, which, in analogy with other genes copied by RNA polymerase III, is probably a transcription termination region (27). The 1307 bp sequence also contains 6 regions of 7 or more consecutive nucleotides which are repeated in the tRNA coding region, as underlined in

Figure 5. Cloverleaf Structure of the Histidine tRNA Encoded by Residues 462-533. The single residue in the D-loop which differs from the published sequence of sheep liver his tRNA (25) is underlined.

fig. 4. Three of these are on the coding strand and 4 on the non-coding strand. Similar repeats have been noted near other tRNA genes; their significance, if any, is unknown (2,7).

Sequence of a B1 (alu) Family Fragment. Analysis of the sequence of fig. 4 for other known genes suggests that residues 1057-1113 (enclosed in the dashed box) are related to one end of a B1 family sequence. The B1 family is dispersed and highly repeated in the mouse genome (28) and is related

	986	1085	1025	1045
Mt1:	CGGCCCACTGGCTTTTCTGTTTCTGTTTTCACAGGGCTCACGCTGTCTGTAAC			
	.	.	.	.
B1:	AGACAGGGTTTCTCTGTAGCCCTGGTGTCTGGAAGTCACTCTGAAGACCGGCTGGCCT			
	1	20	40	60
		1065	1084	1104
Mt1:	TTGAACTCCTGATCCTCCTGCCTCAGAC	CCTGAGTACTAGGATTACAGACATTAGCTAA		
	.	.	.	.
B1:	CGAACTCAGAAATCCGCCTGCCTCTGCCTCCCGAGTGTCTGGGATTAAAGGCATGCACCA			
		80	100	120
	1113			
Mt1:	CAAGCCAGG			
	..			
B1:	CATGCCCGG			
	129			

Figure 6. Homology Between Residues of the 1307 Base Pair XbaI Fragment and a B1 Family Consensus Sequence.

Residues marked Mt1 are from fig. 4. Residues marked B1 are the complement of the B1 family consensus sequence in ref. 28.

to similar families in other mammals, usually termed alu families (29). A comparison of a B1 consensus sequence (28) with residues 986-1113 is shown in fig. 6. Residues 1057-1113 of the 1307 bp sequence share 72% homology with residues 72-129 of the B1 consensus sequence if a single nucleotide gap is introduced to maximize the homology. The homology region can perhaps be extended to include the hexanucleotide GAACTC at residues 1048-1053 which is also present in the B1 sequence (residues 62-67). Beyond this, the two sequences exhibit little homology. Therefore, the 1307 bp sequence appears to contain a truncated B1 family member which has perhaps accumulated point mutations. This sequence does not contain the internal residues shown to be necessary for in vitro transcription of alu family sequences by RNA polymerase III (30). More extensive analysis of murine B1 sequences is necessary to determine whether this sequence has a function or any significance relative to the histidine tRNA gene.

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* To whom to address correspondence.

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