Volume 9 Number 10 1981 Nucleic Acids Research

Mammalian tRNA genes: nucleotide sequence of rat genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$

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Received 30 March 1981

ABSTRACT

A cloned 2.1 kb fragment of rat DNA hybridized to purified $tRNA^{Asp}$ has been sequenced. The result revealed that in addition to the putative gene for $tRNA^{Asp}_{GAU(C)}$, the fragment contained the $tRNA^{Gly}_{GGA(G)}$ and $tRNA^{Glu}_{GAG}$ genes. The genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$ have the same polarity, are arranged in this order and are regularly separated by DNA regions of about 450 bp. These rat genes contain neither intervening sequences nor the CCA sequence expected in the 3'-end of the mature tRNA. As observed in lower eukaryotic tRNA genes, the 5'-flanking regions of the three rat genes do not have any significant sequence homology as a regulatory element. In the 3'-flanking region, the sequences CTTTTTG and CTTTTG are present 11 bp downstream from the 3'-end of the genes for $tRNA^{Asp}$ and $tRNA^{Gly}$, respectively. The same CTTTTG sequence is repeated twice in regions 47 and 60 bp away from the $tRNA^{Glu}$ gene. The short T cluster common to the three genes might be the transcription termination site as in lower eukaryotic tRNA genes.

INTRODUCTION

Recent development of the molecular cloning technique (1) has mad it possible to isolate and study eukaryotic tRNA genes. Convenient DNA sequencing methods (2,3) have made it possible to elucidate the detailed structures of a number of lower eukaryotic tRNA genes; the genes for $tRNA^{Tyr}$ (4), $tRNA^{Phe}$ (5) and $tRNA^{Leu}_3$ (6) in yeast, the $tRNA^{Phe}$ gene in *Neurospora* (7), the genes for $tRNA^{Arg}$ and $tRNA^{Ile}$, three genes for $tRNA^{Lys}$ and for $tRNA^{Asn}$ (8) and five genes for $tRNA^{Glu}$ (9) in *Drosophila*, and the genes for $tRNA^{Met}_1$ (10,11), $tRNA^{Phe}$ and $tRNA^{Tyr}$ (12) in *Xenopus* and $tRNA^{Ala}_2$ in *Bombyx* (13,14). In addition, genes producing dimeric tRNA precursors have been found in yeast, the genes for $tRNA^{Ser}$-$tRNA^{Met}$ (15) and $tRNA^{Arg}_3$-$tRNA^{Asp}$ (16). Sequence analysis has also revealed the presence of intervening sequences in some of the tRNA genes described above (4-7,12-15).

Although information on the structure (4-16), the arrangement (8,9,12, 17,18), and even the expression (11, 13-15,19-22) of lower eukaryotic tRNA

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genes has accumulated, almost nothing is known about mammalian tRNA genes. To study the genes for tRNA in mammalian cells, we have analyzed a DNA fragment cloned from rat genome DNA. DNA fragments obtained by digestions of rat DNA with several restriction endonucleases were separated by agarose gel electrophoresis and subjected to blot hybridization by the method of Southern (23). When they were probed by purified rat $tRNA^{Asp}$, $tRNA^{Phe}$, $tRNA^{Val}$ or $tRNA^{His}$ labeled with $[^{125}I]$iodine, only $tRNA^{Asp}$ gave a detectable hybridization product on the autoradiogram.

We decrided to clone and analyze the DNA fragment hybridized to $tRNA^{Asp}$. In this paper, the nucleotide sequence of cloned rat DNA of 2.1 kb containing the genes for $tRNA^{Asp}_{GAU(C)}$, $tRNA^{Gly}_{GGA(G)}$ and $tRNA^{Glu}_{GAG}$ is described and some features of the genes are discussed.

MATERIALS AND METHODS

Enzymes and reagents

Restriction endonucleases were purchased from New England Biolabs, Bethesda Research Laboratories or Boehringer-Mannheim. All reactions with restriction endonucleases in this work were performed at 37°C for 16 hrs in 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl and 1 mM dithiothreitol. T_4-Polynucleotide kinase and *E. coli* DNA polymerase I were from Boehringer-Mannheim and bacterial alkaline phosphatase (BAPF) and deoxyribonuclease I were from Worthington. T_4-DNA ligase was prepared according to the published procedure (24). $[^{32}P]$Orthophosphate, $[^{125}I]$NaI and $[\alpha$-$^{32}P]$dTTP purchased from New England Nuclear or Amersham. $[\gamma$-$^{32}P]$ATP and $[5'$-$^{32}P]$pCp were prepared by published methods (25,26).

Preparation and screening of rat chromosomal DNA fragments carrying tRNA genes

Nuclei were isolated from rat (Donryu strain) liver and DNA was extracted by the method of Marmur (27). Purified DNA (2 mg) was digested with 5,000 units of *Eco*RI and the digests were fractionated by RPC-5 column (0.5 x 30 cm) chromatography as described by Tilghmann *et al.* (28). DNA fragments distributed in ten fractions were analyzed by electrophoresis on 1 % agarose gel followed by blot hybridization by the method of Southern (23). The first two fractions were found to contain a unique DNA fragment hybridized to purified rat $tRNA^{Asp}$ labeled with $[^{125}I]$iodine and they were combined. Iodination of tRNA was performed as described by Prensky (29).

By this fractionation, the fragment hybridized to $tRNA^{Asp}$ was enriched about 5 times. Five μg of the *Eco*RI fragments thus obtained were digested with 5 units of *Bam*HI at 37°C. Three reaction mixtures (40 μl each) were incubated for 5, 10 and 20 min, respectively. Then the reaction was stopped by heating at 65°C for 5 min and the mixtures were combined. The resulting DNA fragments (15 μg) carrying *Eco*RI and *Bam*HI restriction sites at the two termini were ligated to 15 μg of plasmid vector pBR322 (30) previously cleaved by *Eco*RI and *Bam*HI. The T_4-DNA ligase reaction and transformation of *E. coli* χ1776 (31) using ligated DNA were performed as described previously (32). Ampicillin resistant and tetracyclin sensitive transformants were screened by the colony hybridization method of Grunstein and Hogness (33) using rat $tRNA^{Asp}$ labeled with [5'-^{32}P]pCp at the 3'-terminus as a probe. Labeling of tRNA with [5'-^{32}P]pCp was performed as described by Bruce and Uhlenbeck (34).

Filter hybridization with labeled tRNA

Baked nitrocellulose filters carrying denatured DNA were prepared by the method of Southern (23) or that of Grunstein and Hogness (33). The filters were moistened with hybridization mixture containing 1,000 counts/min of labeled tRNA per cm^2 of filter in 5 x SSC, 50 % formamide and 0.5 % SDS, wrapped in a sheet of polyvinylidene film and incubated at 37°C for 16 hrs. They were rinsed three times at room temperature in 6 x SSC for 15 min and then briefly in 2 x SSC and autoradiographed.

In vitro labeling of DNA and hybridization to chromosomal DNA

Purified DNA insert was nick-translated as described by Rigby *et al.* (35). Five μg of rat chromosomal DNA was digested with 20 units of *Eco*RI or a mixture of *Eco*RI (20 units) and *Bam*HI (20 units). Digests were separated by electrophoresis on 1 % agarose gel and transferred to nitrocellulose filters by the method of Southern (23). Hybridization was carried out at 42°C for 16 hrs in 0.8 M NaCl, 100 mM Piperazine-N,N'-bis(2-ethane sulfonate) (PIPES) (pH 6.8), 100 μg/ml heat-denatured salmon testis DNA, 0.01 % N-lauroylsarcosine sodium salt, 5 x Denhardt's, 50 % formamide, 10 % (w/v) dextran sulfate and 10^5-10^6 counts/min of nick-translated DNA. The filter was washed 4 times with 0.2 x SSC at 50°C for 30 min.

Gel electrophoresis of DNA and DNA sequence analysis

Fractionation of DNA fragments by electrophoresis on agarose or polyacrylamide gels and elution of DNA from gels were carried out as described previously (32). Labeling of DNA fragments and DNA sequencing were also performed as described previously (32) by the method of Maxam and Gilbert(2).

RESULTS

Isolation of a rat DNA fragment containing tRNA genes (TR1dge)

DNA from rat liver was cleaved with restriction endonuclease *Eco*RI and the fragment hybridized with [^{125}I]labeled rat $tRNA^{Asp}$ was partially purified by RPC-5 column chromatography. The *Eco*RI fragments thus prepared were further digested with a limited amount of *Bam*HI. The resulting DNA was inserted into plasmid vector pBR322 using the restriction sites for *Eco*RI and *Bam*HI and the recombinant plasmids were used to transform *E. coli* χ1776. About 4,000 transformants (ampicillin resistant-tetracycline sensitive) were screened by the colony hybridization procedure of Grunstein and Hogness (33) using rat $tRNA^{Asp}$ labeled at the 3'-end with [^{32}P]pCp as a probe and four clones carrying tRNA genes were obtained. Since the inserts in all four clones showed the same restriction profile, only one of them, designated as pRT1dge, was studied further. As shown in Fig. 1, digestion of pRT1dge with a mixture of *Eco*RI and *Bam*HI gave the 2.1 kb insert besides the vector DNA. The insert was designated as RT1dge.

Identification of the cloned DNA RT1dge in rat chromosomal DNA

Rat genome DNA cleaved with *Eco*RI or a mixture of *Eco*RI and *Bam*HI was subjected to electrophoresis on agarose gel, transferred to a nitrocellulose filter and hybridized with nick-translated RT1dge. The result shown in Fig. 1 indicates that RT1dge hybridized to an *Eco*RI fragment of 3.3 kb. When hybridization of RT1dge to *Eco*RI-*Bam*HI fragments was analyzed, a single radioactive DNA with the same chain length as that of RT1dge was observed (Fig. 1). Therefore, the presence of the cloned DNA fragment RT1dge on rat chromosomal DNA was evident.

Nucleotide sequence of RT1dge

The cloned fragment RT1dge was cleaved with several restriction endonucleases. Cleavage maps of RT1dge with the restriction endonucleases

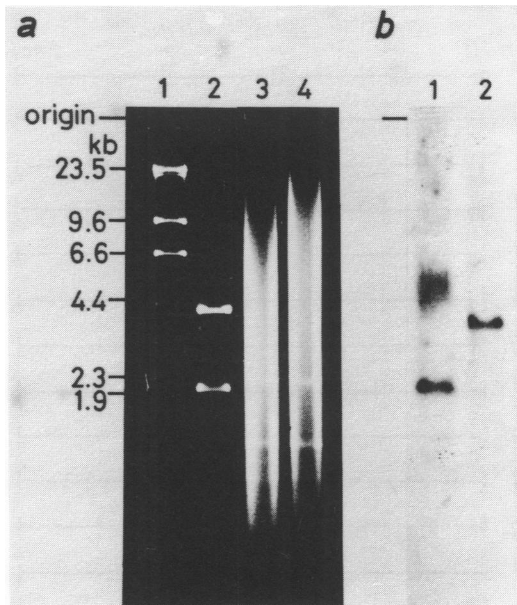

Fig. 1. The cloned DNA fragment, RT1dge, carrying the genes for rat tRNA. (a) Separation of DNA fragments by electrophoresis on 1 % agarose gel. Lane 1, phage λ DNA digested with *Hin*d III, lane 2, pRT1dge digested with a mixture of *Eco*RI and *Bam*HI, lanes 3 and 4, rat chromosomal DNA digested with a mixture of *Eco*RI and *Bam*HI (lane 3) and with *Eco*RI alone (lane 4). (b) Blot hybridization. Rat chromosomal DNA fragments in the agarose gel shown in (a) (lanes 3 and 4) were transferred to a nitrocellulose filter and hybridized with nick-translated RT1dge by the method of Southern (23). Lanes 1 and 2 correspond to lanes 3 and 4 in (a), respectively.

used for sequence analysis are summarized in Fig. 2. The DNA sequence of total 2,109 bp for RT1dge was determined by the method of Maxam and Gilbert (2). As shown in Fig. 3, RT1dge contained three putative tRNA genes, those for $tRNA^{Asp}_{GAU(C)}$, $tRNA^{Gly}_{GGA(G)}$ and $tRNA^{Glu}_{GAG}$ in this order from the *Bam*HI terminus. The region separating the $tRNA^{Asp}$ and $tRNA^{Gly}$ genes and that separating the $tRNA^{Gly}$ and $tRNA^{Glu}$ genes were 464 and 429 bp, respectively. The DNA sequence also revealed that the three tRNA genes on RT1dge had the same polarity.

DISCUSSION

Arrangement of the tRNA genes in RT1dge

In this work using the molecular cloning technique, we isolated a DNA

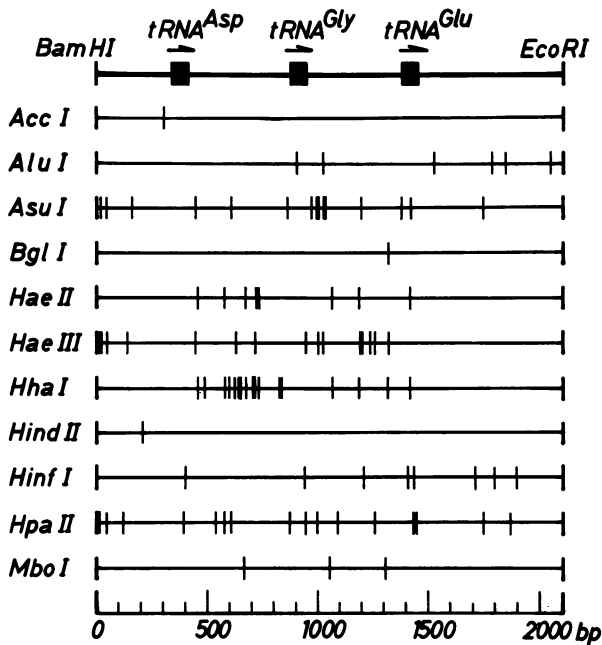

Fig. 2. Restriction map of the cloned rat DNA fragment, RTldge. Cleavage sites on the DNA fragment were mapped for several restriction endonucleases used for DNA sequence analysis. Positions of the genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$ and their polarities are indicated on top.

fragment RTldge hybridized to rat $tRNA^{Asp}$ from restricted rat chromosomal DNA.

In eukaryotes, tRNA genes exist in multiple copies (8,9,18,36-47). In the case of mammalian cells, each gene for 40-60 major tRNA species is reiterated about 20 (human) (36) to 100 (mouse) (40) times. From this point of view, we studied the reassociation kinetics of nick-translated RTldge in rat DNA. The result suggested that in the haploid rat genome, there might be about 10 regions hybridizing to the cloned fragment (data not shown). The arrangment of the reiterated sequence is not clear yet.

Nucleotide sequence analysis revealed that the cloned DNA RTldge contained the genes for $tRNA^{Asp}_{GAU(C)}$, $tRNA^{Gly}_{GGA(G)}$ and $tRNA^{Glu}_{GAG}$ in this order from the *Bam*HI restriction site. The three genes are regularly separated by DNA regions of about 450 bp. Since coding sequences for the tRNAs are present on the same DNA strand, each gene would be transcribed in the same direction, if the genes are functional.

```
                                                                                                  100
5' GATCCCCGGCCTGCAGGCCCCTGTCTGTCCCCACAGCACGCAGGGCGGCCCGGGTGTGTGGCACCAGCCTCTCGTCCTGACACGGCGAGTGTTGCCCGCA
3' CTAGGGGCCGGACGTCCGGGGACAGACAGGGGTGTCGTGCGTCCCGCCGGGCCCACACACCGTGGTCGGAGAGCAGGACTGTGCCGCTCACAACGGGCGT

                                                                                                  200
ACCACTCGCACTCGGCACCGGTGGCTTAGAGGCCGGGTCTCCAGCCCGGCGGGACGGGGGGCCGGCGACACGGTGGTCTGGCGGAACTGCGAATCGGAGT
TGGTGAGCGTGAGCCGTGGCCACCGAATCTCCGGCCCAGAGGTCGGGCCGCCCTGCCCCCCGGCCGCTGTGCCACCAGACCGCCTTGACGCTTAGCCTCA

                                                                                                  300
CCTGGTGTTAACACCGAATTTCGGAGTCTTTTGGGTTGCGGTGCAGTGCAGAGCAAGAGGTGGTCGTTGGGGGAGACGGACGCCTGTGGTTCTTCCCTAG
GGACCACAATTGTGGCTTAAAGCCTCAGAAAACCCAACGCCACGTCACGTCTCGTTCTCCACCAGCAACCCCCTCTGCCTGCGGACACCAAGAAGGGATC

                                                                                                  400
CGTGGTAGACGGGCACGGAGTGCGTGTGTTTGTGGCTGTCGTCGTCCTCGTTAGTATAGTGGTGAGTATCCCCGCCTGTCACGCGGGAGACCGGGGTTCG   tRNA^Asp
GCACCATCTGCCCGTGCCTCACGCACACAAACACCGACAGCAGCAGGAGCAATCATATCACCACTCATAGGGGCGGACAGTGCGCCCTCTGGCCCCAAGC

                                                                                                  500
ATTCCCCGACGGGGAGACATGATGTCCTTTTTGGACACCTAGGTGGCCCGCGGGCGCTAGCCCTTCCACCCACGCCCCACGCGCAGACCCAGACCCACGT
TAAGGGGCTGCCCCTCTGTACTACAGGAAAAACCTGTGGATCCACCGGGCGCCCGCGATCGGGAAGGTGGGTGCGGGGTGCGCGTCTGGGTCTGGGTGCA

                                                                                                  600
CAGCCCGCCGAGCCCCATAATCTCCAGCAAAGCCTCTCCGGAGCCCTCAAACCCCTTTTCTGCCGACTGCACCGGCGCCCGGTCCGCTCAGGGTGGCGCC
GTCGGGCGGCTCGGGGTATTAGAGGTCGTTTCGGAGAGGCCTCGGGAGTTTGGGGAAAAGACGGCTGACGTGGCCGCGGGCCAGGCGAGTCCCACCGCGG

                                                                                                  700
TACCGGAGCGGTCCCTGGCGCACTGGCCTTGGGCTAGCGCGCCGTCTTGCAGCAGCCAGGAGACGGGAGCGCCCCATTGGAAAGGCGTGAACATGTGAGA
ATGGCCTCGCCAGGGACCGCGTGACCGGAACCCGATCGCGCGGCAGAACGTCGTCGGTCCTCTGCCCTCGCGGGGTAACCTTTCCGCACTTGTACACTCT

                                                                                                  800
GGCGCGCGTGGGAGGCCGAGGAAGAGCGCGGAGAGGAGGAGCCGTGGGTGTGTTCGTGGTTGGGGCGTCCCCCGTGCGTGCGTGCGGTGCGCAGGCAAGT
CCGCGCGCACCCTCCGGCTCCTTCTCGCGCCTCTCCTCCTCGGCACCCACACAAGCACCAACCCCGCAGGGGGCACGCACGCACGCCACGCGTCCGTTCA

                                                                                                  900
AAGAGGTAGGCGGCGTCGAGGCGCTGGGCGCCGTGTGGTGGCAGTCGGCGGCGACGTGGGGGTGTGGGTCCGGCTGTGCGGCGTTGGTGGTATAGTGGTG   tRNA^Gly
TTCTCCATCCGCCGCAGCTCCGCGACCCGCGGCACACCACCGTCAGCCGCCGCTGCACCCCCACACCCAGGCCGACACGCCGCAACCACCATATCACCAC

                                                                                                 1000
AGCATAGCTGCCTTCCAAGCAGTTGACCCGGGTTCGATTCCCGGCCAACGCAGCGTGCCCAACTTTTGCCAAGGTCCTCACCCAGGACGCGCCCACCCGG
TCGTATCGACGGAAGGTTCGTCAACTGGGCCCAAGCTAAGGGCCGGTTGCGTCGCACGGGTTGAAAACGGTTCCAGGAGTGGGTCCTGCGCGGGTGGGCC

                                                                                                 1100
GCCCAGCCTGGCTCCGCCGAGCTGGGCCCAGGCTCTACTGTAGGCAGGGAAGATCGGAAAGCGCCAGACCTTTTCAGGTAGTGGGCACCGGGAGGGAAGC
CGGGTCGGACCGAGGCGGCTCGACCCGGGTCCGAGATGACATCCGTCCCTTCTAGCCTTTCGCGGTCTGGAAAAGTCCATCACCCGTGGCCCTCCCTTCG

                                                                                                 1200
GGCAGGGGCAAGTTTCTAGTCCCCGAAGTCGCACGGTGCTGGCTGTGGAAGCGGCAGAGGCCAGGGGGCAAGTGGCAGCCCAGCGCCAGGCCAGCCAGGG
CCGTCCCCGTTCAAAGATCAGGGGCTTCAGCGTGCCACGACCGACACCTTCGCCGTCTCCGGTCCCCCGTTCACCGTCGGGTCGCGGTCCGGTCGGTCCC

                                                                                                 1300
CCAAGAGAGAATCGAGAACGCCAGCCTCCTAAGGCCTTAGCATGCATCAGGCGGCCGGGTATGAGGAGACGGGCAGGCGGGCAGGTCGGCAGGCAGGCAG
GGTTCTCTCTTAGCTCTTGCGGTCGGAGGATTCCGGAATCGTACGTAGTCCGCCGGCCCATACTCCTCTGCCCGTCCGCCCGTCCAGCCGTCCGTCCGTC

                                                                                                 1400
CATGATCTGCGCCTTTGTGGCCGAGAGGGTGTGTGTCTGTCTGTTTCCAGGAGTTGATAGCCGTGAGCATGGGACCCACGCCCTTGGTGGTCTAGTGGT   tRNA^Glu
GTACTAGACGCGGAAACACCGGCTCTCCCACACACAGACAGACAAAGGTCCTCAACTATCGGCACTCGTACCCTGGGTGCGGGAACCACCAGATCACCA

                                                                                                 1500
TAGGATTCGGCGCTCTCACCGCCGCGGCCCGGGTTCGATTCCCGGTCAGGGAAGCCTCTCTTCTTAGTCCCTCCACTTCCCACCTCACCACCCTGACCCC
ATCCTAAGCCGCGAGAGTGGCGGCGCCGGGCCCAAGCTAAGGGCCAGTCCCTTCGGAGAGAAGAATCAGGGAGGTGAAGGGTGGAGTGGTGGGACTGGGG

                                                                                                 1600
TTTTGAGTGACCCTTTTGTTTGTACATGAGCTACACAGACCAAGACCACAACTCCACATCTCCCCTCCATATTTCTCTCTCTCTCTCTCTCTCTCTCT
AAAACTCACTGGGAAAACAAACATGTACTCGATGTGTCTGGTTCTGGTGTTGAGGTGTAGAGGGGAGGTATAAAGAGAGAGAGAGAGAGAGAGAGAGA

                                                                                                 1700
CTCTCTCTCTCTCTCTCTCTCTCTCTCACTCTCCCTCCACCCCCTTTCCCTTTTCCTCTTTCTTTCCAGAAACCCATCACCCAGTACCACAGAACCGTCT
GAGAGAGAGAGAGAGAGAGAGAGAGAGTGAGAGGGAGGTGGGGGAAAGGGAAAAGGAGAAAGAAAGGTCTTTGGGTAGTGGGTCATGGTGTCTTGGCAGA

                                                                                                 1800
TCTGCCTAAGAGTCCATGGCTGCACCATGCCTACCTTAACATCCCGGACCAGCCTCTCTGACACCACACGCCAACTTTGCACTTCCCCAGCTCAGACTCC
AGACGGATTCTCAGGTACCGACGTGGTACGGATGGAATTGTAGGGCCTGGTCGGAGAGACTGTGGTGTGCGGTTGAAACGTGAAGGGGTCGAGTCTGAGG

                                                                                                 1900
TTGCTTGCCCTGGAGCCCAGACCTCTCACCTGCCACCATCACAAGCCGAGCTCTCTCACACACCGCCCGGATCACAAACACTCGTCCACCGAGGGAATCT
AACGAACGGGACCTCGGGTCTGGAGAGTGGACGGTGGTAGTGTTCGGCTCGAGAGAGTGTGTGGCGGGCCTAGTGTTTGTGAGCAGGTGGCTCCCTTAGA

                                                                                                 2000
CAACACCACTCCATCTCCTGATCGCCTGCTCTTACCTCTCCAACAAACCCTCTGGCTAGCCCGCTCCTCCTGTCGCACCTACCTCGAATGCATAACCTGT
GTTGTGGTGAGGTAGAGGACTAGCGGACGAGAATGGAGAGGTTGTTTGGGAGACCGATCGGGCGAGGAGGACAGCGTGGATGGAGCTTACGTATTGGACA

                                                                                                 2100
ATCACCTGATTGCACGCCTAGCGTGCATCGCGTGCAAGACAGGGTGGTCCCAGTGAGCTTTCCGAAATAGTCACAAAGGAGCGTCCAGTTCAAACATCGG
TAGTGGACTAACGTGCGGATCGCACGTAGCGCACGTTCTGTCCCACCAGGGTCACTCGAAAGGCTTTATCAGTGTTTCCTCGCAGGTCAAGTTTGTAGCC

TAGCGAATT 3'
ATCGCTTAA 5'
```

Fig. 3. Total nucleotide sequence of the cloned rat DNA fragment, RT1dge. Nucleotides are numbered from the *Bam*HI cleavage site. The positions of the genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$ are indicated by boxes. The polarities of the tRNA genes are shown by arrows.

The percentage of guanine and cytosine (GC) in rat liver DNA is about 42 % (48), whereas in RTldge it is unexpectedly high, being 63 %. In lower eukaryotes, especially yeast and *Drosophila*, the regions flanking tRNA genes so far sequenced are relatively AT rich (4,6,8,9). This type of base distribution is not the case in the regions around the three rat tRNA genes in RTldge.

Sequences flanking the tRNA genes

The 5'- and 3'-flanking sequences for the three tRNA genes are compared in Fig. 4. The 5'-flanking regions contain no significant sequence homologies which might be candidates as regulatory elements for gene recognition by RNA polymerase III or its factors. Similar observations have been made on tRNA genes of lower eukaryotes. This feature of the 5'-flanking regions could be explained by recent reports that regulatory sequences for correct transcription initiation by RNA polymerase III occur within tRNA (11,19,21,49), 5S RNA (50-53) and Adenovirus VA1 RNA (54) genes.

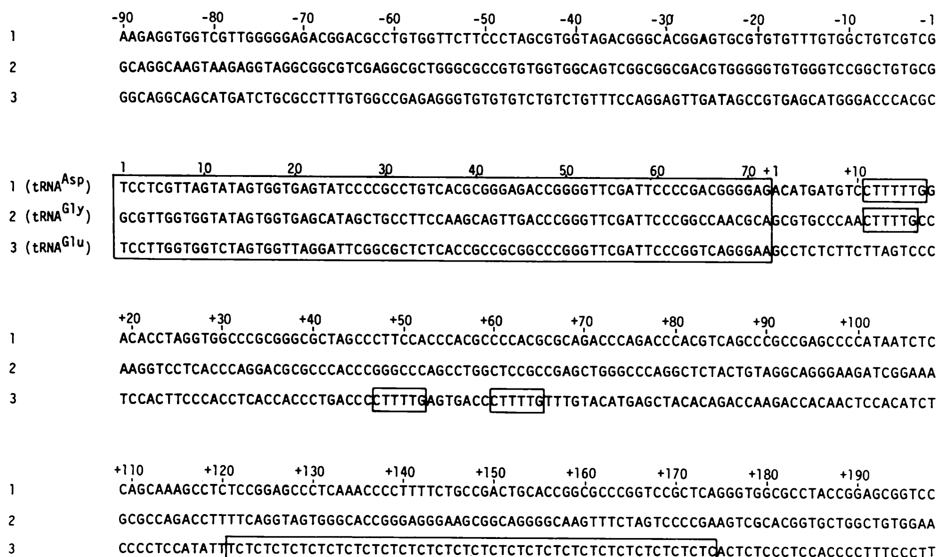

Fig. 4. Nucleotide sequences of the flanking regions of the genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$. Nucleotide sequences of the coding and flanking regions of the three rat tRNA genes are compared using the antisense strand. The coding regions for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$ are shown in a box. The common CTTTT(T)G sequences and the repeating TC sequence are also indicated by small boxes.

However, it has been suggested that the 5'-flanking sequences also contain some signals required for active expression of tRNA genes (21).

The 3'-flanking regions of the three rat tRNA genes (Fig. 4) contain a common sequence CTTTT(T)G. This sequence appears 11 bp away from the coding regions of both the $tRNA^{Asp}$ and $tRNA^{Gly}$ genes. On the other hand, in the case of the $tRNA^{Glu}$ gene, the CTTTTG sequence is repeated 47 bp and 60 bp downstream from the 3'-end of the coding region. A similar $C(T)_{3-5}G$ sequence has been reported to be present in the corresponding region of the *Xenopus* genes for $tRNA^{Met}_{1}$, $tRNA^{Phe}$ and $tRNA^{Tyr}$ (10-12). A run of three to eight T residues has also been found adjacent to the 3'-end of tRNA genes in other eukaryotes (4-9,13-16). Accumulated evidence indicates that the short T clusters must be the site for transcription termination (13-15,21). Therefore, the sequence of CTTTT(T)G in the 3'-flanking regions of the rat tRNA genes could be the signal, if these genes are functional. It should be pointed out that in the region 121 bp downstream from the 3'-end of the $tRNA^{Glu}$ gene, a dinucleotide sequence TC is repeated 27 times. However, the biological meaning of this peculiar sequence is unknown.

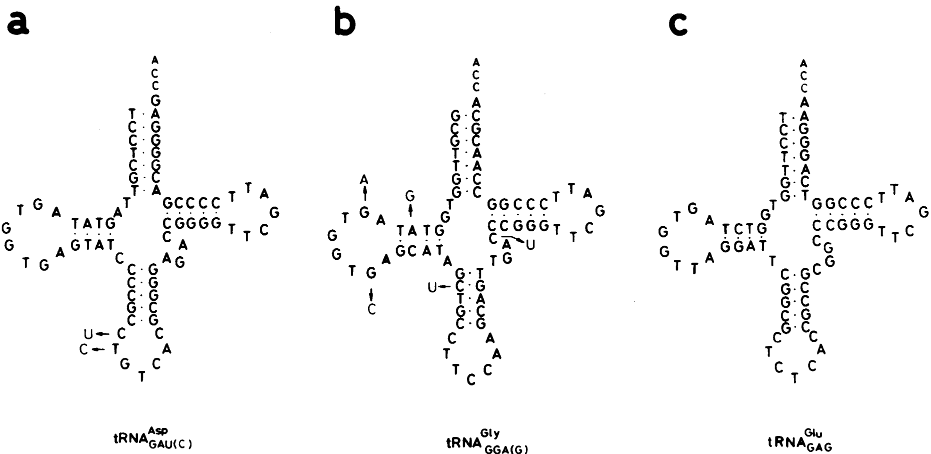

Fig. 5. Secondary structures of rat tRNAs proposed from the DNA sequences. Possible clover-leaf structures for $tRNA^{Asp}_{GAU(C)}$ (a), $tRNA^{Gly}_{GGA(G)}$ (b) and $tRNA^{Glu}_{CAG}$ (c) are shown. The positions different from the known RNA sequences of the major $tRNA^{Asp}$ from rat liver (Kuchino, Y., Shindo-Okada, N., Ando, N., Watanabe, S. and Nishimura, S., unpublished data) and *Bombyx* $tRNA^{Gly}_{2}$ (55) are indicated by arrows.

Structure of tRNA genes

The DNA sequences of the three tRNA genes in RT1dge can be arranged in clover-leaf secondary structures as shown in Fig. 5. The sequences revealed that these genes contain neither an intervening DNA sequence nor the sequence corresponding to the 3'-terminal CCA expected in mature tRNAs.

The RNA sequence of the major $tRNA^{Asp}$ from rat liver has been determined in our laboratory (Kuchino, Y., Shindo-Okada, N., Ando, N., Watanabe, S. and Nishimura, S., unpublished data). The sequence differs from that of DNA at the two positions adjacent to the 5'-end of the anticodon, as indicated in Fig. 5a. This implies that, if the putative gene for $tRNA^{Asp}$ is functional, it might code an unknown minor species or that some processing of the gene product to alter the CU sequence to UC might occur.

Although the RNA sequences of rat $tRNA^{Gly}_{GGA(G)}$ and $tRNA^{Glu}_{GAG}$ have not been determined, the secondary structures arranged from DNA sequences (Fig. 5b and c) seem to contain enough characteristics to be functional tRNA. The sequence of the $tRNA^{Gly}$ gene shows very high similarity to that of *Bombyx mori* $tRNA^{Gly}_{2}$ (55). As indicated in Fig. 5b, they differ in only 5 positions.

This paper is the first description of the fine structure of tRNA genes from a mammalian genome. The features of the structure of these mammalian tRNA genes, including the surrounding regions, seem to be essentially the same as those found in lower eukaryotic genes.

ACKNOWLEDGEMENTS

We are grateful to Dr. S. Yokoyama and Mr. D. Kouda of Tokyo University for help with computer analysis of the DNA sequence. This work was partly supported by Grants-in-Aid for Cancer Research and Scientific Research from the Ministry of Education, Science and Culture of Japan.

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