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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

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ABSTRACT

A λ clone carrying a rat DNA fragment of 11.9 kb was isolated from a rat gene library with total rat tRNA as a probe. Nucleotide sequence analysis revealed that the DNA fragment contained six tRNA genes, three for $tRNA^{Pro}$ and three for $tRNA^{Lys}$. Of the six genes all but one $tRNA^{Pro}$ gene have the same polarity. Each tRNA gene is separated by a DNA region of 0.1 to 3.6 kb. The 5'-flanking regions of the six rat genes in the cluster do not have any significant sequence homology, but in the 3'-flanking region, each gene has a short T cluster, which is supposed to be a transcription termination signal.

INTRODUCTION

About 60 species of tRNA in eukaryotic cells are known to be transcribed from corresponding genes by RNA polymerase III. Each gene exists in multiple copies. In the haploid genome of yeast (1,2) and *Drosophila* (3), about 10 genes are present for each tRNA species, while the copy number in *Xenopus* is about 200 (4,5). In both *Drosophila* and *Xenopus* (6-8), several tRNA genes are present in clusters, but the tRNA genes in yeast seem to be scattered throughout the chromosome (1,2).

In mammalian cells, the gene for each tRNA species is also reiterated and its copy number has been estimated as 20 in humans (9) and 100 in mice (10). The copy number in rats is suggested to be similar to that in humans (11). Recent results by us (12,13) and others (11) indicated that, like *Drosophila* and *Xenopus* genes, rat tRNA genes are present as small clusters on the chromosome.

In this work, we obtained a rat DNA fragment of 11.9 kb containing six tRNA genes from a rat gene library. We analyzed the nucleotide sequence of the genes and the surrounding regions, finding that three genes for $tRNA^{Pro}$ and the same number of the genes for $tRNA^{Lys}$ are in clusters.

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MATERIALS AND METHODS

DNA clones: λRT2-1 was isolated from a rat gene library using [^{125}I] labeled total rat tRNA. The gene library constructed with partial *Eco*RI digests of liver DNA from a Sprague-Dawley rat was provided by T. Sargent (14). *Eco*RI fragments carrying tRNA genes in λRT2-1, A (4.3 kb), C (2.3 kb), D (1.2 kb) and F (0.7 kb) were ligated to the *Eco*RI cleavage site of pBR322 and subcloned in *E. coli* HB101. The plasmid clones thus obtained were pRT2-1A, pRT2-1C, pRT2-1D and pRT2-1F.

Filter hybridization with labeled nucleic acids: Digestion of DNA with restriction endonucleases and separation of the products by electrophoresis on 1 % agarose gel were performed as described previously (12). DNA on the gel was transferred to nitrocellulose filters by the method of Southern (15) and hybridized on the filters with [^{125}I] labeled total rat tRNA or [^{32}P] labeled DNA fragments as described previously (12).

DNA sequence analysis: Fractionation of DNA fragments and their labeling with [^{32}P]phosphate were performed as described previously (12). DNA sequences were determined by the method of Maxam and Gilbert (16).

RESULTS

λ Clone RT2-1 and plasmid subclones: λRT2-1 contains six *Eco*RI fragments of rat DNA: E (0.9 kb), C (2.3 kb), B (2.5 kb), A (4.3 kb), D (1.2 kb) and F (0.7 kb) in this order, as shown in Fig. 1a. When digests of λRT2-1 with *Eco*RI were separated by electrophoresis on 1 % agarose gel and then subjected to Southern hybridization (15), fragments A, C, D and F were labeled with [^{125}I] total rat tRNA (Fig. 1b). For detailed analysis of the regions hybridized to tRNA, fragments A, C, D and F were subcloned and the plasmid clones obtained were designated as pRT2-1A, pRT2-1C, pRT2-1D and pRT2-1F.

Nucleotide sequence analysis of subcloned fragments: pRT2-1C and pRT2-1A were digested with several restriction endonucleases (Fig. 2a and 3a) and the resultant smaller fragments were separated by polyacrylamide gel electrophoresis. Among them, fragments which hybridized with [^{125}I] labeled total rat tRNA were subjected to nucleotide sequence analysis. The results in Fig. 2b and 3b show that both RT2-1C and RT2-1A carried two tRNA genes, one for tRNA^{Pro} (Pro1 or 2) and the other for tRNA^{Lys} (Lys1 or 2). On RT2-1C, Lys1 and Pro1 have the opposite polarity and are separated by a region of 108 bp. On RT2-1A, two genes (Pro2 and Lys2) separated by a DNA region of about 2.8 kb are transcribed in the same direction.

The total nucleotide sequences of RT2-1D and RT2-1F were determined using smaller restriction fragments (Fig. 4a). The nucleotide sequences of the tRNA genes and surrounding regions are shown in Fig. 4b. As described below, the *Asu*I fragment (620 bp) of λRT2-1 carrying the genes for both tRNA^{Lys} and tRNA^{Pro} was obtained from the junction between fragments D and F.

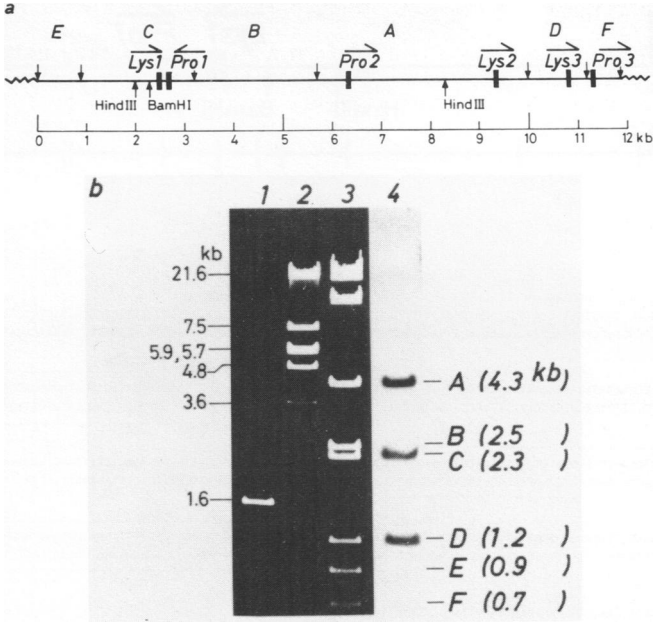

Fig. 1. (a) Structure of a rat DNA fragment of 11.9 kb in λRT2-1. Rat DNA carrying six tRNA genes is indicated by a solid line. The wavy lines represent λDNA. Positions and polarities of genes for $tRNA^{Lys}$ (Lys1, 2 and 3) and $tRNA^{Pro}$ (Pro1, 2 and 3) are indicated. Downward arrows indicate RI cleavage sites. (b) Analysis of λRT2-1 with *Eco*RI were separated by electrophoresis on 1 % agarose gel (lane 3). As size markers, *Hin*fI fragments of pBR322 (lane 1) and *Eco*RI fragments of λDNA (lane 2) were separated on the same gel. *Eco*RI fragments of λRT2-1 in lane 3 were transferred to a nitrocellulose filter and hybridized with [^{125}I] labeled total rat tRNA (lane 4).

Therefore, the nucleotide sequences shown in Fig. 4b must be contiguous. Fragments D and F contain one gene for $tRNA^{Lys}$ (Lys3) and for $tRNA^{Pro}$ (Pro3), respectively, and these genes have the same polarity and are separated by a region of 340 bp.

Total arrangement of tRNA genes: From complete digests of λRT2-1 with *Hin*dIII, DNA fragments of 6.1 and 8.8 kb were isolated. The 6.1 kb DNA carrying *Eco*RI fragment B and portions of fragments A and C was further digested partially with *Eco*RI or completely with *Bam*HI. The products were analyzed by Southern hybridization using nick-translated *Eco*RI fragments A to F and also nick-translated *Alu*I fragments from pRT2-1C (66 and 183 bp fragments as probes for $tRNA^{Lys}$ and $tRNA^{Pro}$ genes, respectively). The results revealed the directions of fragments C and A, that is, the polarities of Lys1, Pro1, Pro2 and Lys2 to be as shown in Fig. 1a. When DNA of 8.8 kb

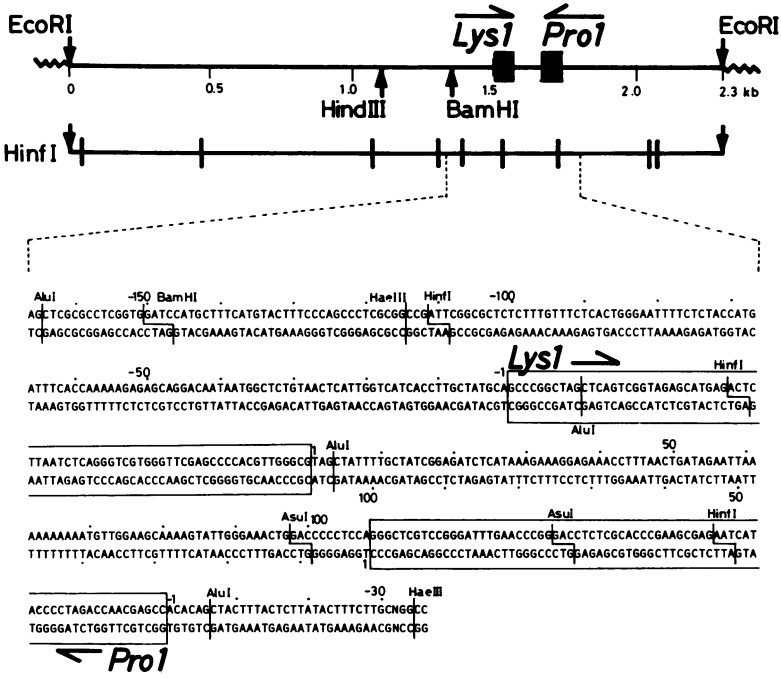

Fig. 2. Structure of RT2-1C (a) and nucleotide sequences of the genes for $tRNA^{Lys}$ (Lys1) and $tRNA^{Pro}$ (Pro1) and the surrounding regions (b).

carrying half fragment A and fragments D and F, together with a portion of λDNA, was digested with *Asu*I and subjected to Southern hybridization, a fragment of 620 bp carrying genes for both $tRNA^{Pro}$ and $tRNA^{Lys}$ was obtained from the junction of fragments D and F, indicating the directions of Lys3 and Pro3 (Fig. 1a).

DISCUSSION

Arrangement of the tRNA genes in RT2-1: On the cloned rat DNA of 11.9 kb, we identified six tRNA genes, three for $tRNA^{Lys}$ (Lys1, 2 and 3) and three for $tRNA^{Pro}$ (Pro1, 2 and 3), as summarized in Fig. 1a. The genes are arranged in the order Lys1-Pro1-Pro2-Lys2-Lys3-Pro3. Of the six genes, all but the Pro1 gene have the same polarity in the direction from Lys1 to Pro3. Unlike in the rat tRNA gene cluster containing the genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$ (12), the six genes are irregularly separated by DNA regions of 0.1 to 3.6 kb. At least in the clone analyzed, we did not find any incomplete tRNA genes, such as those found in repeating units of the tRNA

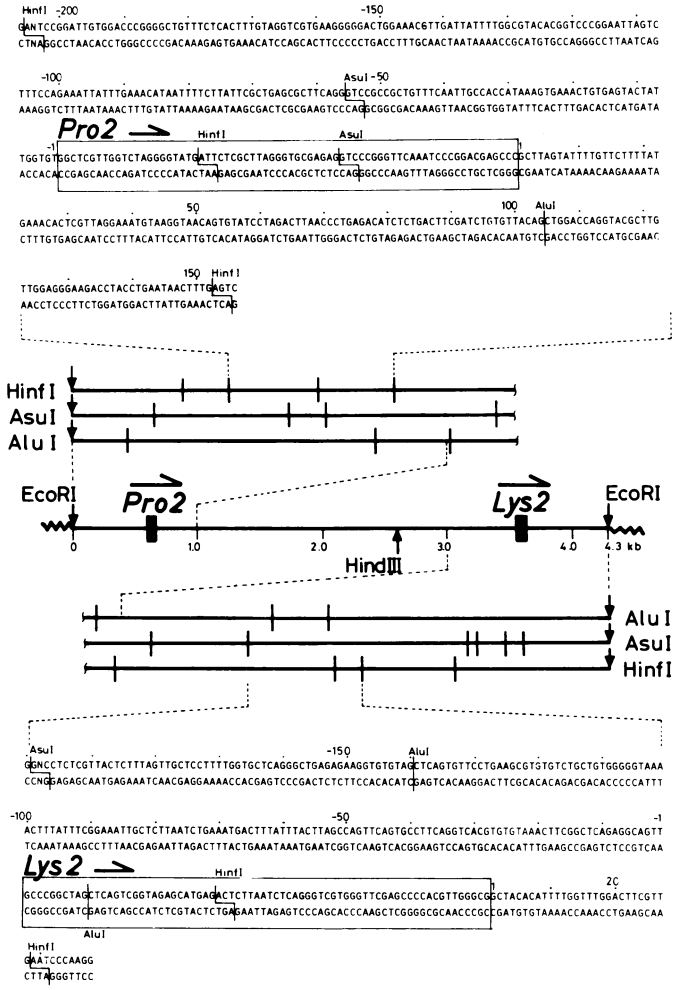

Fig. 3. Structure of RT2-1A (a) and nucleotide sequences of the genes for $tRNA^{Pro}$ (Pro2) and $tRNA^{Lys}$ (Lys2) and the surrounding regions (b).

gene cluster containing the genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$ (13). All six tRNA genes in λRT2-1 are actively transcribed in crude HeLa cell extracts (T. Sekiya, unpublished data).

Sequences flanking the tRNA genes: The 5'- and 3'-flanking sequences for the six tRNA genes are compared in Fig. 5. As expected from the fact that the signals for transcription initiation by RNA polymerase III exist within the coding region of the eukaryotic tRNA gene (17-20), the 5'-flanking

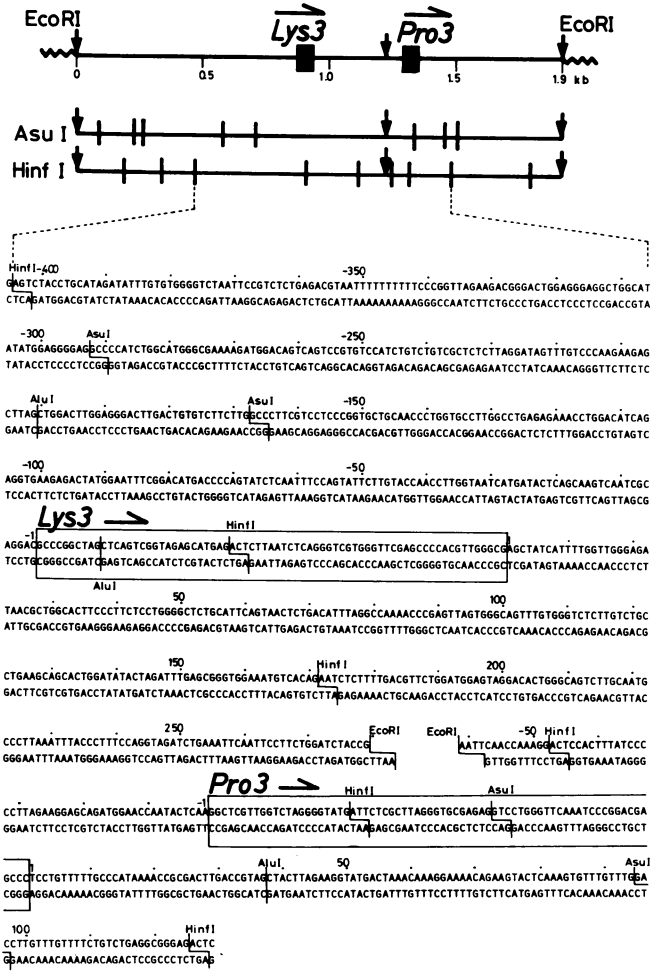

Fig. 4. Structure of RT2-1D (0-1.2 kb) and F (1.2-1.9 kb) (a) and nucleotide sequences of the genes for $tRNA^{Lys}$ (Lys3) and $tRNA^{Pro}$ (Pro3) and the surrounding regions (b).

regions have no significant sequence homologies. However, the 3'-flanking regions all contain short T clusters, which are supposed to be the signals for transcription termination. As with the rat genes for $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Glu}$ (12), the Prol gene has the CTTTTG sequence. All other genes, except Pro3 (TTTTTG), are followed by a sequence of ATTTTG.

Coding sequences of tRNA genes: The clover-leaf secondary structures deduced from the DNA sequences are shown in Fig. 6. The anticodon of Prol is

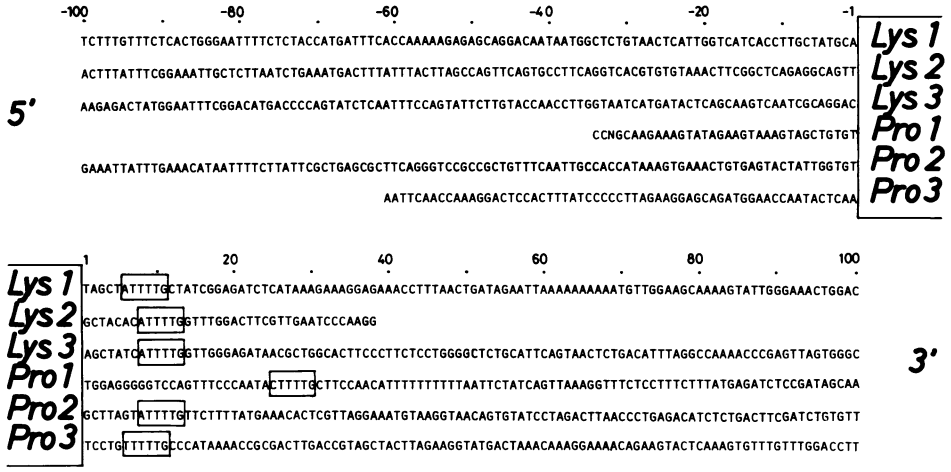

Fig. 5. Comparison of the 5'- and 3'-flanking sequences of the six tRNA genes. In the 3'-flanking regions, common short T clusters are shown in boxes.

CGG while that of Pro2 or Pro3 is AGG. Pro2 differs from Pro3 in only one nucleotide in the TψCG stem region; all other nucleotides sequences of the three $tRNA^{Pro}$ genes are identical (Fig. 6a). Of the $tRNA^{Lys}$ genes, Lys1, 2 and 3 share the same nucleotide sequence. Although the RNA sequences for rat $tRNA^{Pro}$ and $tRNA^{Lys}$ have not been determined, those of mouse $tRNA^{Pro}$ (21) and rabbit and mouse $tRNA^{Lys}$ (22) are available for comparison with the rat genes. The nucleotide sequences of Pro1 and Pro2 correspond exactly to those of mouse $tRNA_2^{Pro}$ and $tRNA_1^{Pro}$, respectively, both of which have been identified

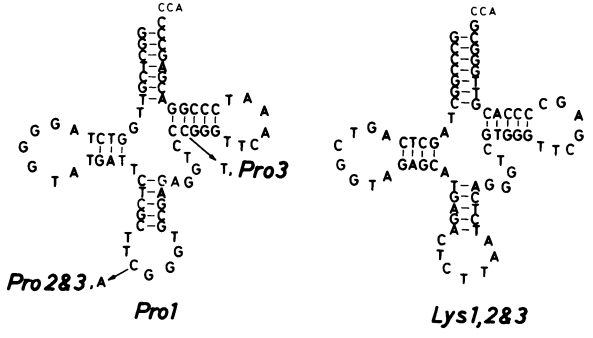

Fig. 6. Clover-leaf structures deduced from DNA sequences of the tRNA genes. (a) The genes for $tRNA^{Pro}$ (Pro1, 2 and 3). Nucleotide differences among genes are indicated by arrows. (b) The genes for $tRNA^{Lys}$ (Lys1, 2 and 3).

as the primer for Moloney murine leukemia virus (21). Therefore, among the three $tRNA^{Pro}$ genes analyzed, at least Pro1 and Pro2 must be functional on the rat chromosome.

The nucleotide sequence of the rat $tRNA^{Lys}$ gene is exactly the same as that of rabbit $tRNA_2^{Lys}$ or mouse fibroblast $tRNA^{Lys}$ (22). This tRNA species of mice is known to be abundant in mouse mammary tumor virus particles, although it does not function as a primer for virus DNA synthesis (23).

The present results provide direct evidence for the idea that in the rat chromosome some tRNA genes are clustered in a short region. The features of the structures of rat tRNA genes and surrounding regions seem to be essentially the same as those found in *Drosophila* and *Xenopus*.

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