Kinetoplast Minicircles Carrying Guide RNA Genes of *Leishmania tarentolae*

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The nucleotide sequences of the minicircles from the kinetoplast DNA network of Leishmania tarentolae have been determined. Each minicircle encoded a single unique guide RNA (gRNA) gene located approximately 150 base pairs from the conserved region which contains the replication origins for both strands. Out of seven minicircle-encoded gRNA genes identified, three were involved in RNA editing of the blocks I, II and V of the mitochondrial unidentified reading frame 4 gene (MURF4), four in RNA editing of the blocks II, V, VII and VIII of ribosomal protein S12 gene. Sequence comparison of the surrounding of the genes showed some similarity in the 5' flanking region which may contain signal sequences for transcriptional initiation of gRNAs

Keywords: kinetopalstid protozoa/RNA editing/mitochondria

Several mitochondrial mRNA in kinetoplastid protozoa such as *Crithidia*, *Leishmania* and *Trypanosoma* are extensively edited after transcription. The location of editing blocks, number of editing sites within a single editing domain, and number of U residues to be added or deleted at each editing site are very specific to individual mRNAs. The information for insertion and deletion of the uridine residues in the primary sequence of other mitochondrial RNA transcripts , so called guide RNAs (gRNA). The molecules have a length of 50-70 nucleotides, contain posttranscriptionally added 3' oligo(U) extensions and function as templates in the editing reaction by base complementarity to editing domains of the mRNAs (1,2).

The guide RNAs are transcribed from the maxicircle and the minicircle DNA molecules present in the kinetoplast DNA network (kDNA). The minicircle component of *L. tarentolae* consists of approximately 10^4 molecules 850-880 base pairs (bp) in size. Each minicircle is organized into a conserved region of 170-180 bp, which contains the replication origins for both strands, GGGCGT and GGGGTTGTGTGTAAA and a bent DNA region (see Figure 1), and a variable region of approximately 700 bp which defines the sequence class.

Unit-length linearized minicircles were released from networks by treatment with each of restriction enzymes *Bam*HI, *Bgl*I, *Eco*RI, *Kpn*I *Sma*I and *Xba*I. A recognition site of *Sma*I is located within the conserved region of all minicircles sequenced so far. In practice, however, some network DNA remained uncut by this enzyme. The unit-length linearized minicircle molecules were isolated from agarose gels and ligated into pUC118 or pUC119. The total sequence of each minicircle clone was obtained with the universal and reverse primers for

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Scope of Research

With emphasis on regulatory mechanisms of gene expression in higher organisms, the research activity has been focused on analysis of signal structures at the regulatory regions of transcriptional initiation and of molecular mechanisms involved in post-transcriptional modification by the use of eukaryotic systems appropriate for analysis. As of December 1994, studies are concentrated on the molecular mechanism of RNA editing in mitochondria of kinetoplastids.5

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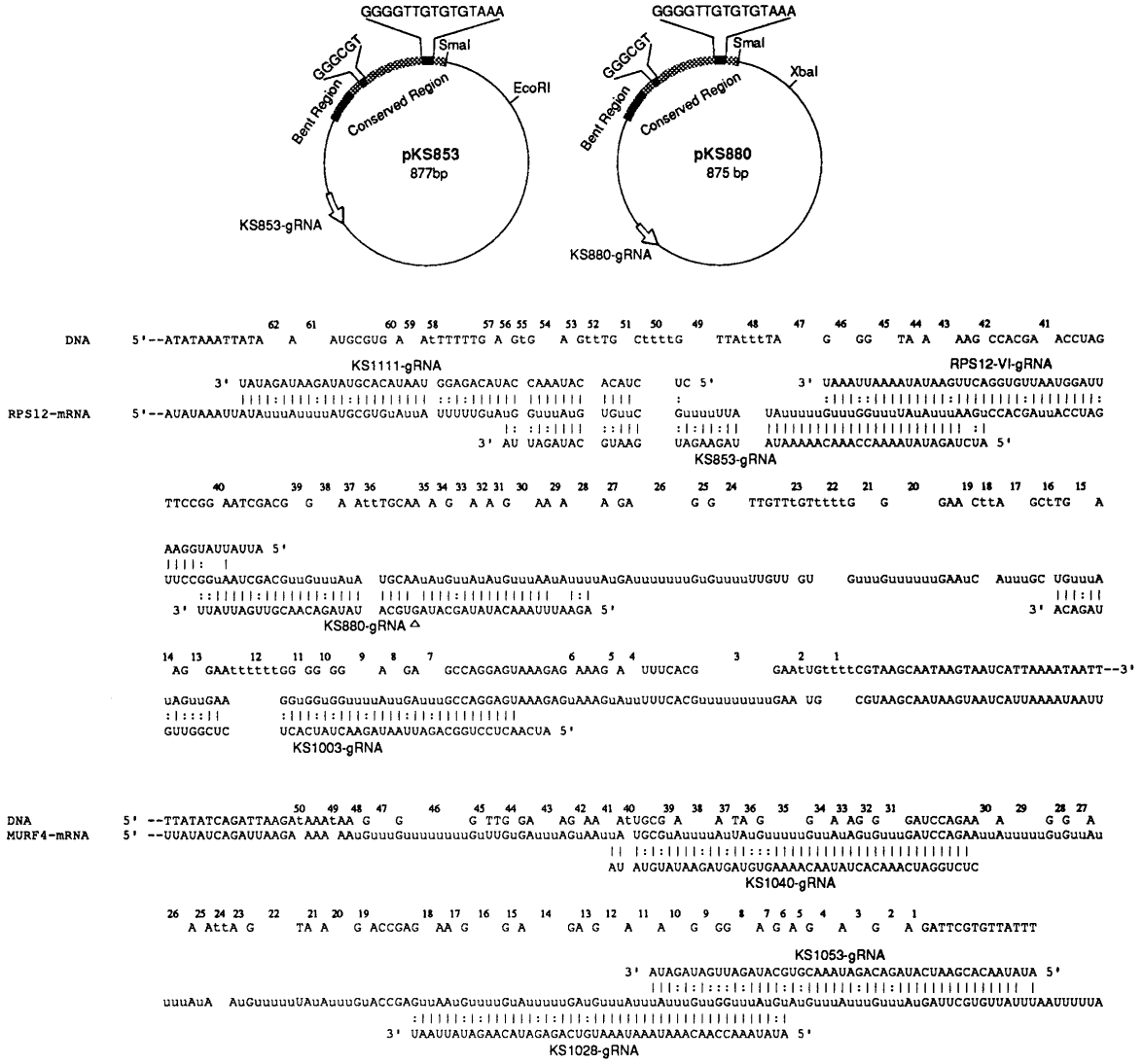

Figure 1. gRNAs mediating the RNA editing reactions of the MURF4 and RPS12 genes
The 5' portion of the mature edited MURF4 mRNA and the entire mature edited RPS12 mRNA are shown with the editing sites numbered. Edited nucleotides are indicated as lowercase u's in mRNA sequence for insertion and lowercase t's in DNA sequences for deletion. The complementary sequences are shown with G-C and A-U base pairs indicated by parallel lines and G-U base pairs by colons. Maxicircle-encoded RPS12 VI-gRNA is also shown.

the pUC sequencing system using the Applied Biosystems DNA sequencing system. Ninety nine sequences were determined from the *Sma*I library, two from the *Xba*I library and each one from the *Bam*HI, *Bgl*I, *Eco*RI, and *Kpn*I libraries.

The minicircle sequences were analyzed for potential gRNA sequences involved in the known editing of the cytochrome oxidase subunit III, mitochondrial unidentified reading frame 4 (MURF4) or ribosomal protein S12 gene (RPS12) mRNA, using the local alignment program. Each minicircle encoded a single unique gRNA located approximately 150 bp from the conserved region. Out of nine kinetoplast minicircle-encoded genes identified, minicircle-encoded gRNAs from pKS1053, pKS1028, and pKS1040 was involved in the RNA editing reaction for the blocks I, II and V, respectively, of the MURF4 gene, those from pKS1003, pKS880, pKS853, and pKS1111 for the blocks II, V, VII and VIII, respectively, of the RPS12 gene(Fig. 1), the remaining two for the blocks of unassigned genes.

The molecular mechanisms of transcriptional initiation and posttranscriptional addition of oligo(U) extension for gRNAs are interesting problems. When the sequences of the 5' flanking region the gRNA genes were compared with each other, two conserved blocks, AT(A/T)(G/T)T and AA(A/T)(G/T)T were located approximately -43 and -20 downstream from the 5' terminals of the gRNA genes. The sequences would be promoters which recognized by mitochondrial RNA polymerase of kinetoplastids. Sequence comparison of the surrounding regions of the 3' terminals of the gRNA genes which would contain the signal sequences responsible for posttranscriptional addition of oligo(U) extension showed little similarity. Specific secondary structures surrounding the 3' terminals of the transcription products from the gRNA genes may requited for the processing.

References

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