Sequence of a tRNA gene cluster in *Trypanosoma brucei*

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Submitted June 20, 1991

EMBL accession no. X57045

A recent analysis of tRNA genes in *Trypanosoma brucei* has revealed an unusual genomic organisation where several genes encoding tRNAs and other small RNAs are very closely clustered (1). Genes encoding tRNA^{lys} (anticodon CUU) and tRNA^{arg} (ACG) were found tightly linked to genes encoding a U snRNA (U RNA B, ref. 2) and a 275 nt RNA X of unknown function. At a separate genomic locus genes encoding tRNA^{tyr} and tRNA^{thr} were found to be closely linked to the U6 snRNA gene. We now report the identification of several other potential tRNA genes, encoding tRNA^{arg} (UCU), tRNA^{gln} (UUG) and tRNA^{asn} (GUU) (Figure 1), following further sequence analysis of the region 5' to the tRNA^{lys} (UUU) gene (1). This cluster of tRNA genes also displays the tight linkage seen in previously described trypanosome loci (1, 3, 4), with similar spacings between head-to-head genes (between 95 and 102 bp) and, to a lesser extent, between head-to-tail genes (60–90 bp). We believe this may reflect the closest distance that tRNA genes can be arranged in the genome to maximize efficiency of transcription (1). All four tRNA sequences contain the invariant and semi-invariant bases typical of tRNAs as well as the consensus Box A and Box B intragenic polymerase III control sequences. The tRNA^{gln} gene, however, may be a pseudogene as it has two apparent mismatches, one U-U pair in the acceptor stem and one C-C pair in the anticodon stem.

The tRNA^{arg} (UCU) gene isolated in this study is only 57% homologous to the *T. brucei* tRNA^{arg} (ACG) gene previously described but is 81% homologous to a bovine tRNA^{arg} (CCU) (5), suggesting that it is a member of a second tRNA^{arg} gene family. This is confirmed by the Southern blot analysis (1) which showed that there are at least two other copies similar or identical to the tRNA^{arg} (ACG) gene, none of which are closely linked to the other copies of the tRNA^{lys} (CUU) gene.

ACKNOWLEDGEMENTS

This work was supported by the Wellcome Trust. JDB is a Wellcome Trust Senior Lecturer.

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1  GATCCGCATC CTTGCAAGTT GTAGTGCCGA TTTTGACGCA GCATTCCATC
51  GGACTGCCTT TTTTCCTTTC CTAACGCACC ACTTACCAAT TTACAAAGGT
101  AAAAAAACA CAAAAGTCA AATGGTGCT AAAAGGGCTT TAATCCGAC
151  TTGTTGCTT TATCGTGGCA GAACACGAAC AAGCAACATG GTGGCAGTCA
201  CCGGAAAGGT GTTGCCAGCG ACGGTCGCCA CTCCGACCGA AACGAAGCAG

      tRNAasn →
251  TAGCGGCTAC TAAGGCAGAG ACGCTCTGGC ACTCGGTTCC TCCCTGGCGC
301  AGTCGGCAGC GCGTTAGGCT GTTAACCATC AGGTGTTGGT TCGAATCCAA
351  CGGGAGGAGT CTTTTTGCTG CCACTCGAAA TTCTGGAGGT ACTTTGCTAC
401  AAAGGAAGAA CTGAAAGCTG TTGCAAAAC ATGGACGAGT AGGGGTTTGA
451  ACCCTAGGCC TCCGTATGCA AAACAGGCGC CTCTACCACT GAGGTACAGC
501  CCGTTCGCGC CTGCTAAATC CCTAATACCA CATAAGGTTG TTTAGCAACC
      ← tRNAgln
551  GTTGTGTAAC ATCAGTGAA GTGGTCTGCT GTGATTGGCG AGAGGGCGTG

      tRNAarg →
601  GCGGTGCTCC AGTGGCCTAA TGGATATGGC TTTGGACTTC TAATCCAAAG
651  GTTGGCGGTT CGAGTCCCGT CTGGAGTACG TTTTCATGTC CAAATATTAT

      tRNAlys →
701  TCATATTTGT TCGTTTCTAG TTGTTTGCCG GAAGAGCAGC CTTCTAGCT
751  CAGTGGTAGA GCGCAGGCT TTTAACCGTG TGGTCGTGGG TTCGATCCCC
801  ACGGAAGGCG CTTTCTTACA CTGCCGCTTA AGCTAATTTT TTTGCTGGGT
851  ACGCCATGTA AGCGTTAGAG GCTTGCTTGG GGGAGGGTTG GCGGTGCCCG
901  TAGCCCAATG GATAAGGC

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Figure 1. Nucleotide sequence of a *T. brucei* tRNA gene cluster. tRNA sequences are underlined, with sizes based on inferred secondary structure. The direction of transcription is indicated by the arrows. The tRNA^{gln} gene is complementary to the underlined sequence as only one strand is shown. Anticodons are over-dotted.