The tyrT Locus of Escherichia coli B

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The tyrT (tRNA\textsubscript{\textsc{tyr}}\textsuperscript{T\textsuperscript{\textsc{v}}}\textsc{v}) locus of Escherichia coli B differs structurally from that of K-12 strains by the absence of 2 of 3.14 terminal repeat sequences.

The tyrT locus in Escherichia coli K-12 consists of two tandem copies of the minor tRNA\textsubscript{\textsc{tyr}}\textsuperscript{T\textsuperscript{\textsc{v}}}\textsc{v} isoacceptor gene, transcribed from a single promoter. The coding sequences are separated by a 208-bp spacer and followed by a 178-bp sequence that is repeated 3.14 times (4). The major rho-dependent transcription terminator is in the second repeat following the tRNA sequences, with a weaker terminator in the third repeat (7). In K-12 strains, mutations in the anticodon of either of the tRNA sequences, with a weaker terminator in the third repeat (7). In K-12 strains, mutations in the anticodon of either of the tRNA\textsubscript{\textsc{tyr}}\textsuperscript{T\textsuperscript{\textsc{v}}}\textsc{v} genes can give rise to both ochre (su\textsubscript{1}) and amber (su\textsubscript{2}) translation termination codon suppressors at this locus (5, 6).

It was recently reported (3) that in the E. coli B strain WU3610 (tyrA14 leu-308) (9), the ochre tyrA14 mutation could be suppressed by mutations in an unknown gene. These slowly growing suppressor-containing mutants were detected under conditions of starvation but could also be shown to occur in growing bacteria if appropriate experimental conditions were applied. In contrast to what has been observed with E. coli K-12, nonsense suppressors have not been observed at the tyrT locus of E. coli B (8), and it was thought possible that the slowly growing mutants might be tyrT suppressor mutants that had escaped detection with conventional methodology.

PCR primers were designed, by using E. coli K-12 sequence data, to amplify a 1,393-bp product, including both tyrT tRNA genes and the terminal repeat sequences. Both 5' (TTT256; GT TATCATGTCGATCATACCTACAC) and 3' (TTT1625; AT TTTAGTTCACATAGACCTCCTCAGTACCGTCTC) primers were synthesized with and without 5' biotinylation to enable solid-phase sequencing of both strands (6). Sequencing reactions were performed by using the Sequenase Kit (Amersham) according to the manufacturer's instructions. Six slowly growing tyrosine-independent mutants of WU3610 were sequenced, but no mutations in the tRNA\textsc{tyr}\textsuperscript{T\textsuperscript{\textsc{v}}}\textsc{v} genes were seen.

However, while amplification of control K-12 DNA resulted in a PCR product of the anticipated size, the product from the B strain indicated that there was a deletion of approximately 350 to 400 bp somewhere in the amplified region. The entire length of the wild-type E. coli B PCR product, from WU3610, was subsequently sequenced, confirming a deletion of 357 bp from the terminal repeat sequences at the tyrT locus. Sequencing primers were synthesized according to information obtained by primer walking along the PCR fragment in both directions. At least two independent PCR clones were sequenced with each primer. Southern blot analysis of genomic DNA and probing with the full-length PCR product from E. coli K-12 verified that the tyrT region from E. coli B carried the observed deletion.

Comparison of the wild-type sequences from E. coli K-12 and the B strain WU3610 indicated that there were 10 base pair substitutions in the 987 bases sequenced, 8 transitions and 2 transversions, as well as one base pair deletion and one base pair insertion. The base pair deletion and insertion both occurred in the intervening sequence between the two tRNA genes, maintaining the net 208-bp spacing between them. The two tRNA gene coding sequences were identical to the K-12 sequences; however, there was a complete absence of two of the terminal repeat sequences at the B locus.

The structures of the B and K-12 tyrT loci are indicated in Fig. 1. PCR amplification of DNA from two primary-source B strains (one obtained from Tikvah Alper ca. 1957 and one, identified as CSH, obtained from John Donch ca. 1969) indicates that the deletion is probably present throughout the B family. The sequence corresponding to the major and minor rho termination signals in the K-12 tyrT gene (CAATCAA) contains two mutations in E. coli B, changing the sequence to TAATCAA. It is possible that this sequence may no longer function as the termination signal for transcription of tyrT in E. coli B, although this has not been verified experimentally. The 33-amino-acid basic polypeptide (Tpr), coded for by the first terminal repeat in E. coli K-12 (1), would also appear to be absent from E. coli B, since a termination codon is present 10 residues into the equivalent coding sequence.

It has previously been noted that nonsense suppressors have not been observed at the tyrT locus of E. coli B (8), despite the isolation of both ochre and amber suppressors at this site in K-12 strains. The structure of this locus in WU3610 may provide an explanation for their absence. Our sequencing data are consistent with two possibilities. (i) The terminal repeat sequences may provide crucial stability to the nascent transcripts prior to processing. In K-12 strains both gene copies are processed to mature tRNA, allowing a mutation to a suppressor genotype in one copy while maintaining wild-type recognition by the other copy. In E. coli B strains the locus may be functionally monocistronic; either the transcript is degraded before processing is complete, requiring the presence of two wild-type tRNA genes for viability, or the mutations noted affect the processing sites required for generation of mature tRNA from one or both tRNA genes. (ii) The absence of suppressors may be an indirect consequence of the lack, in B strains, of tRN\textsc{A} or the basic polypeptide Tpr, which appears to modulate the cellular response to amino acid starvation (2) and which may affect the expression of suppressors at the tyrT locus under certain conditions.

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Nucleotide sequence accession number. The sequence of the tyrT locus of the E. coli B strain WU3610 can be accessed from the EMBL database under accession number X90989.

REFERENCES