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supN Ochre Suppressor Gene in *Escherichia coli* Codes for $tRNA^{Lys}$

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We describe the cloning and nucleotide sequence of a new $tRNA^{Lys}$ gene, *lysV*, in *Escherichia coli*. An ochre suppressor allele of this gene, *supN*, codes for a $tRNA^{Lys}$ with anticodon UUA, presumably derived by a single base change from a wild-type UUU anticodon. The sequence of the *supN* $tRNA^{Lys}$ is identical to the sequence of ochre suppressor tRNAs encoded by mutant alleles at the *lysT* locus. This locus, which contains the two previously known $tRNA^{Lys}$ genes of *E. coli*, is located far from the *lysV* locus on the chromosome.

The *supN* ochre suppressor gene is known to map at 52 min on the *Escherichia coli* chromosome (1, 3, 4). No other suppressor or tRNA gene has been mapped in this region (M. J. Fournier and H. Ozeki, Microbiol. Rev., in press). The amino acid insertion mediated by this suppressor has not been determined. In this report we describe the cloning and nucleotide sequence analysis of the *supN* gene.

The *supN* gene was isolated from a λ607 (10) clone bank of a *supN23*-containing strain, *E. coli* AB2547 (3), by selection for suppression of the *ilvD188* ochre mutation in lysogens of strain GE884 (16). DNA from a λ607 *supN23* phage was cleaved with *Sau*3A and cloned into the *Bam*HI site of M13mp2*Bam*HI (7). M13 clones carrying active suppressor genes were detected by suppression of a *lacZ* amber mutation in strain CSH39 (8). Inserts carrying the suppressor were sequenced by the dideoxy method (14). The sequence obtained is shown in Fig. 1.

***SupN* codes for a $tRNA^{Lys}$.** The *supN* gene is seen to encode a $tRNA^{Lys}$ with anticodon 5′UUA3′. The nucleotide sequence of the corresponding *supN* tRNA differs from that of the wild-type $tRNA^{Lys}$ (2) by only a single base change in the anticodon (UUU in the wild-type form). In the short sequenced 5′-flanking region no likely initiation signals for RNA polymerase were detected. However, a possible rho-independent terminator is seen downstream of this region (positions 111 through 130 in Fig. 1). Clearly, the present data do not permit any conclusions to be drawn about the size of the *supN* transcription unit or about the number of genes it may contain.

The primary sequence of the *supN* $tRNA^{Lys}$ is identical to the sequence of the ochre suppressor tRNA found in strains carrying the su_β^+, *supG*, and *supL* mutations (11, 12). These suppressors map at the *lysT* locus at 16.5 min on the chromosome (1–5, 11, 12). This locus has recently been analyzed and found to contain two $tRNA^{Lys}$ genes (now designated *lysT*α and *lysT*β [Fournies and Ozeki, in press]) and a gene for $tRNA_1^{Val}$, *valT* (17). The three genes are in a single transcription unit which is organized as follows: promoter-*lysT*α-132 base pairs-*valT*-2 base pairs-*lysT*β. The 3′ and 5′ flanking sequences of the *lysT*α and *lysT*β genes are different from those of the *supN* gene shown in Fig. 1. The gene symbol *lysV* will be used for this gene (*supN23* = *lysV123*).

***LysV* (*supN*) and *lysT* suppressors compared.** The su_β^+ mutation has been shown to be an anticodon mutation (UUU-UUA) of the *lysT*β gene (17), but it is not known which of these genes is mutated in *supG* or *supL* strains. In a recent study, Murgola and Pagel (9) described a missense suppressor which maps at the *lysT* locus. The suppressor mutation has been shown to cause a single-base subsitution in the amino acid acceptor stem of $tRNA^{Lys}$ (12a). It was inferred that the suppressor mutation causes misacylation of $tRNA^{Lys}$, probably by glycine or alanine. Genetic observations indicated that ochre suppressors at the *lysT* locus and the new mutation are mutually exclusive. This was interpreted to mean that the wild-type function of at least one of the *lysT* genes is essential for the cell and that there are probably only these two $tRNA^{Lys}$ genes in the cell. Although a third $tRNA^{Lys}$ gene, *lysV*, has now been found, it is possible that it does not by itself produce sufficient $tRNA^{Lys}$ for efficient cell growth. The finding that the amount of $tRNA^{Lys}$ is approximately doubled in strains that are diploid for the *lysT* region (6, 9) suggests that the *lysV* locus contributes less than the *lysT* locus to the $tRNA^{Lys}$ pool of the cell. However, it should be pointed out that suppression by *supN* is, by phenotypic criteria, of similar efficiency as suppression by *supL* or *supG* (3, 4; G. Eggertsson and S. Thorbjarnardóttir, unpublished data). In a study of 24 independently isolated suppressors of this phenotype (3), 19 were mapped at the *lysV* locus and only 5 were mapped at the *lysT* locus. This might suggest that there is more than one copy of the $tRNA^{Lys}$ gene at the *lysV* locus.

In a study of tRNA precursors which accumulate in a temperature-sensitive RNase P mutant of *E. coli*, Sakano and Shimura (13) detected two precursors of $tRNA^{Lys}$. One of them had a chain length of approximately 180 bases and also contained the sequence of $tRNA_1^{Val}$. The other was monomeric, with a chain length of approximately 120 bases. It has been suggested that the monomeric precursor was formed by cleavage of a transcript of the *lysT* operon (17). In light of the present results, we must also consider the

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          10         20         30         40         50
5'-GATCCCGTCA TCACCCACCA CTTCGGGTCG TTAGCTCAGT TGGTAGAGCA

          60         70         80         90        100
GTTGACTTTA AATCAATTGG TCGCAGGTTC GAATCCTGCA CGACCCACCA

         110        120        130        140
ATGTAAAAAA CGCCCTAAAG GCGCTTTTTT ACTATCTGCG ATACTCAAA
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FIG. 1. Nucleotide sequence of the *supN* allele of *lysV*. The anticodon is underlined.

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possibility that this precursor represents a transcript of the wild-type *lysV* gene.

Mutations which reduce the efficiency of the *supL* suppressor have recently been described (15). Three different classes of such mutations (*asuD*, *asuE*, and *asuF*) were found. Mutations at the *asuD* locus (mapping at 61.9 min) were shown to inhibit suppression by *supL* without affecting suppression by *supN* or other ochre suppressors. The function of the *asuD* locus is not known, but the *asuD* mutants had only 43% of the lysyl-tRNA synthetase activity found in the "wild-type" strain. It seems doubtful that this reduction alone can account for the inhibition of *supL*-suppression, and the reason why these two $tRNA^{Lys}$ ochre suppressors, *supL* and *supN*, respond differently to the *asuD* mutations remains unknown.

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