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Mapping of the *supP* ($Su6^+$) Amber Suppressor Gene in *Escherichia coli*

ÁSTRÓS ARNARDÓTTIR, SIGRÍDUR THORBJARNARDÓTTIR, AND GUDMUNDUR EGGERTSSON*

Institute of Biology, University of Iceland, Reykjavik, Iceland

The *supP* ($Su6^+$) amber suppressor gene has been mapped on the clockwise side of the *valS* locus near min 95 on the *Escherichia coli* chromosome.

The *supP51* (formerly $Su6^+$) amber suppressor was originally described by Chan and Garen (3) and shown to cause the amber triplet UAG to code for leucine. Fractionation of leucyl tRNA from $supP^+$ (wild type) and *supP51* strains (5) revealed two fractions of UUG-binding leucyl-tRNA in the $supP^+$ strain, whereas only one of these fractions was found in the *supP51* strain. In the *supP51* strain the other fraction had been replaced by UAG-binding leucyl tRNA (suppressor tRNA). Thus, the *supP51* mutation appears to have transformed one of two UUG-binding leucyl tRNA fractions normally present in *Escherichia coli* into amber suppressor tRNA.

The map position of the *supP* gene has not been known, but it has been reported to be located in the region covered by the F′ factor F14 (9). Recently an amber suppressor gene, *supJ*, has been mapped in the corresponding region of the *Salmonella typhimurium* chromosome, closely linked to the *cya* locus (11).

Little is known about the distribution of leucyl-tRNA genes on the *E. coli* chromosome. However, approximate mapping of tRNA genes in *E. coli* (6) has indicated the presence of leucyl tRNA genes in 3 widely separated chromosomal regions: min 66–69, 82–85, and 90–93.

After having obtained mapping results which indicated that the *supP* gene is not located in the F14 region, we carried out conjugation experiments to determine its approximate map postion. These experiments showed that *supP* is located in the clockwise neighborhood of the *purA* gene, which is near min 93.5 on the *E. coli* map (1). Further mapping was done by P1-mediated transduction involving the *purA*, *pyrB*, *argI*, and *valS* genes. The last three genes are closely linked to each other near min 95 on the map (1). Their order has been determined in *E. coli* B and found to be as shown above (8). The recipients all carry the amber mutations *his-85* and *trpA9605*. Selection and scoring for *supP51* transductants was carried out on minimal media without histidine and tryptophan.

Our transduction mapping (Table 1 and Fig.

TABLE 1. *Mapping of supP51* ($Su6^+$) *by transduction with P1*[a]

Recipient strain	Selection	No. of transductants scored	Unselected marker	No. of cotransductants	% Cotransduction
GE554	*supP51*	295	$argI^+$	140	47.5
			$purA^+$	1	0.34
	$purA^+$	300	$argI^+$	6	2.0
			supP51	0	0
GE556	*supP51*	272	$pyrB^+$	100	36.8
			$purA^+$	1	0.37
	$purA^+$	282	$pyrB^+$	14	5.0
			supP51	1	0.35
GE557	*supP51*	250	$valS^+$	211	84.4
			$purA^+$	0	0
	$valS^+$	233	*supP51*	169	72.5
			$purA^+$	4	1.7

[a] Strain CGSC4487 (strain S26, $Su6^+$, of A. Garen; Hfr *P02A phoA4 relA1 tonA22 supP51* = $Su6^+$) was used as donor in all experiments. All recipient strains are $supP^+$ and carry the amber mutations *his-85* and *trpA9605*. Other characteristics of the recipient strains: GE554 F^- *purA54 argF58 argI61 thr-25 rpsL190 tsx-84*; GE556 F^- *purA54 pyrB9 argF58 rpsL190 tsx-84*; GE557 F^- *purA54 valS7 argF58 rpsL190 tsx-84*. Selection and scoring for *supP51* were carried out on minimal medium without tryptophan and histidine; $valS^+$ transductants were selected on L-agar at 42°C. Selection for $pyrB^+$ in strain GE556 resulted in preferential recovery of $purA^+$ $supP^+$ cotransductants (data not shown). Transduction procedures were as described by Thorbjarnardóttir et al. (10).

1) shows that the *supP* gene is cotransducible at high frequency with the *pyrB*, *argI*, and *valS* genes and at very low frequency (0.2 to 0.3%) with the *purA* gene. The order *purA*-(*pyrB*, *argI*, *valS*)-*supP* is established, placing the *supP* gene near 95 min on the map. This map position of the *supP* gene indicates that it may be very closely linked to the *ts-210* mutation which has recently been mapped by Isono and Kitakawa (7). One tRNA locus, *glyV*, has previously been mapped within the region shown in Fig. 1 (1, 4, 6). However, its exact position has not been reported. According to the approximate mapping referred to above (6), a leucyl tRNA locus, *leuV*, lies in the counterclockwise neighborhood of *purA*. The *leuV* locus is known to specify a CUG-binding leucyl-tRNA, $tRNA_1^{Leu}$ (2, 6).

It can be concluded that the *supP* locus is neither identical to the *supJ* locus of *S. typhimurium* nor to any previously mapped leucyl tRNA locus in *E. coli.*

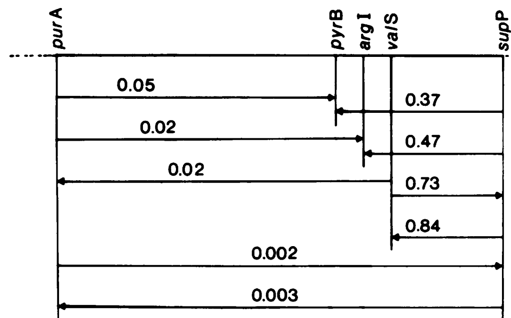

FIG. 1. *Transduction map of the purA-supP region. Cotransduction frequencies are all derived from Table 1. Arrows point from selected marker to unselected marker.*

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LITERATURE CITED

1. **Bachmann, B.J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40:**116-167.
2. **Blank, H. U., and D. Söll.** 1971. Purification of five leucine transfer ribonucleic acid species from *Escherichia coli* and their acylation by heterologous leucyl-transfer ribonucleic acid synthetase. J. Biol. Chem. **246:** 4947-4950.
3. **Chan, T., and A. Garen.** 1969. Amino acid substitutions resulting from suppression of nonsense mutations. IV. Leucine insertion by the *Su6*+ suppressor gene. J. Mol. Biol. **45:**545-548.
4. **Fleck, E. W., and J. Carbon.** 1975. Multiple gene loci for a single species of glycine transfer ribonucleic acid. J. Bacteriol. **122:**492-501.
5. **Gopinathan, K., and A. Garen.** 1970. A leucyl transfer-RNA specified by the amber suppressor gene *Su6*+. J. Mol. Biol. **47:**393-401.
6. **Ikemura, T., and H. Ozeki.** 1977. Gross map location of *Escherichia coli* transfer RNA genes. J. Mol. Biol. **117:** 419-446.
7. **Isono, K., and M. Kitakawa.** 1979. Cluster of ribosomal protein genes in *Escherichia coli* containing genes for proteins S6, S18, and L9. Proc. Natl. Acad. Sci. U.S.A. **73:**6163-6167.
8. **Jacoby, G. A.** 1971. Mapping the gene determining ornithine transcarbamylase and its operator in *Escherichia coli* B. J. Bacteriol. **108:**645-651.
9. **Smith, J. D.** 1972. Genetics of transfer RNA. Annu. Rev. Genet. **6:**235-256.
10. **Thorbjarnardóttir, S. H., R. Á. Magnúsdóttir, G. Eggertsson, S. Kagan, and Ó. S. Andrésson.** 1978. Mutations determining generalized resistance to aminoglycoside antibiotics in *Escherichia coli.* Mol. Gen. Genet. **161:**89-98.
11. **Winston, F., D. Botstein, and J. H. Miller.** 1979. Characterization of amber and ochre suppressors in *Salmonella typhimurium.* J. Bacteriol. **137:**433-439.