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Genetic Location of the *Bacillus subtilis sup-3* Suppressor Mutation

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The *sup-3* suppressor mutation of *Bacillus subtilis* has been located between the *aroI* and *mtlB* loci by PBS-1 phage transduction.

Relatively little information is available on the location of the genes coding for tRNA in *Bacillus subtilis*. By hybridizing newly replicated DNA made during spore outgrowth with ^{32}P-labeled bulk tRNA and rRNA, Oishi et al. (11) and Smith et al. (15) were able to identify two regions of the chromosome as possible sites for these genes. A major region was identified near the origin of chromosome replication, and a second, minor region was determined to be near the terminus. These conclusions were based on the assumption that *B. subtilis* contained a linear chromosome which replicated in a unidirectional fashion. More recent evidence has shown that the chromosome is circular and that it replicates in a bidirectional manner (6, 9, 20). Thus, the tRNA-coding regions initially located by hybridization studies (11, 15) could be on either side of the chromosome origin.

A number of tRNA genes have been located on the chromosome of *Escherichia coli* by mapping of suppressor tRNA mutations (16). These genes are scattered around the *E. coli* chromosome. In *B. subtilis*, three mutations (*sup-1*, *sup-3*, and *sup-44*) have been shown to suppress nonsense mutations (i.e., polypeptide chain termination) (4, 10, 12). The assumption has been that they mediate these effects via an altered tRNA (1, 2, 14, 19). Although all the data are consistent with this interpretation, the only direct evidence in favor of this mechanism is a preliminary observation by D. Novelli and M. Mandel (cited in reference 19) that the *sup-3*-bearing strain of *B. subtilis* may contain one different isoacceptor species of serine-accepting tRNA. We have mapped the *sup-3* suppressor mutation by PBS-1 phage transduction in an effort to locate the chromosomal position of this presumptive tRNA gene in *B. subtilis*.

The bacterial strains used in this study are listed in Table 1. Both the *metB5* and the *thrA5* mutations are suppressed in the *sup-3* background (4). Motile strains for use in PBS-1 phage transduction were selected on water-agar plates (13). Procedures for PBS-1 phage propagation and transduction have been described (18). Stocks of phage SPO1 and SPO1 *sus-5* were prepared from lysates of strain 168 or the *sup-3* strain grown in Penassay medium (Difco). SPO1 phage titers were determined by using a soft agar overlay. For phage SPO1 *sus-5*, plaque-forming units per milliliter were determined on both strain *sup-3* and strain 168. Only lysates which had efficiencies of plating of less than 10^3 plaque-forming units per ml on strain 168 and greater than 10^8 plaque-forming units per ml on strain *sup-3* were used to test for suppression (18). A minimal glucose medium with appropriate supplements was used to select for prototrophic recombinants (17). Tryptose-blood-agar base (Difco) was used to select for Dal$^+$ (D-alanine) recombinants. Susceptibility to lincomycin was determined according to the procedure of Goldthwaite et al. (5). All recombinants were cloned once on the same media on which they were isolated. They were then tested for secondary markers by replica plating (8).

The linkage of the *sup-3* mutation with the *lin-2* locus by PBS-1 transduction was shown with strains DH21 (donor, *sup-3 metB5 thrA5*) and DH29 (recipient, *metB5 thrA5 lin-2*). The presence of the *sup-3* marker was scored by examining the transductants for suppression of the *metB5* mutation and was confirmed by testing the Thr$^+$ phenotype. All Met$^+$ transductants resulted from the presence of the *sup-3* marker, because both the donor and the recipient carried the *metB5* mutation. To confirm that suppression had occurred, a second, unlinked suppressible mutation, *thrA5*, was examined; 120 of the 122 Met$^+$ transductants were also Thr$^+$. Because the *thrA5* mutation is unlinked to both the *metB5* and the *sup-3* loci, it can be concluded that the Thr$^+$ Met$^+$ transductants resulted from the integration of donor DNA carrying the *sup-*

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TABLE 1. *B. subtilis strains*

Strain	Genotype[a]	Relevant phenotype[b]	Origin
168	*trpC2*		C. Anagnostopoulos
QB928	*aroI926 purB33 dal-1 trpC2*		J.-A. Lepesant
PG594	*mtlB1 metC3 trpC2*		J.-A. Lepesant
VB132	*cysA14 lin-2*		D. Dubnau
Mu8u5u5	*leuA8 metB5 thrA5*	Mot^-	N. Sueoka
DH20	*leuA8 metB5 thrA5*	Mot^+	Spontaneous motile derivative of Mu8u5u5
sup-3	*leuA8 [metB5] [thrA5] sup-3*	$Leu^- Sup^- Mot^-$	C. P. Georgopoulos
*sup-3*M	*leuA8 [metB5] [thrA5] sup-3*	$Leu^- Sup^- Mot^+$	Spontaneous motile derivative of *sup-3*
DH21	*leuA8 [metB5] [thrA5] sup-3*	Sup^-	DH20 by transformation; *sup-3* donor
DH29	*leuA8 metB5 thrA5 lin-2*		DH20 by transformation; VB132 donor
DH30	*leuA8 [metB5] [thrA5] sup-3 lin-2*	Sup^-	DH20 by transformation; *sup-3* donor
DH32	*trpC2 purB33 dal-1 mtlB1*		QB928 by transformation; PG594 donor
DH33	*metB5 purB33 dal-1 mtlB1*		DH32 by transformation; *sup-3* donor

[a] Nomenclature in accordance with Demerec et al. (3). Markers enclosed in brackets are suppressed.
[b] Sup^- denotes the presence of the *sup-3* suppressor.

3 locus. A co-transduction frequency of 36% was found between the *lin-2* and *sup-3* (44 of the 122 Met^+ transductants were Lin^s) loci, but the orientation of *sup-3* with respect to the *lin-2* locus could not be deduced from this two-factor cross. The linkage of the *sup-3* locus with the *dal-1* and *purB33* loci by PBS-1 transduction was shown with strains DH30 (donor, *sup-3*) and QB928 (recipient, *dal-1 purB33*). Among the 400 transductants selected for Pur^+, 6 were $Sup^- Dal^+$, 1 was $Sup^- Dal^-$, 29 were $Sup^+ Dal^+$, and 364 were $Sup^+ Dal^-$ (Sup^- denotes presence of *sup-3* suppressor). In this case the presence of the *sup-3* mutation was verified by checking the Dal^+ transductants for sensitivity to SPO1 *sus-5* phage. Analysis of the crossover classes indicated that the order is *sup-3–dal-1–purB33*. The linkage of *sup-3* with *mtlB1*, *dal-1*, and *purB33* was also analyzed by PBS-1 transduction with donor *sup-3M* (*sup-3 metB5*) and recipient DH33 (*dal-1 mtlB1 purB33 metB5*). Selection was for Dal^+, and the presence of the *sup-3* mutation was scored as suppression of the *metB5* mutation and confirmed by sensitivity to SPO1 *sus-5* phage. Of the 37 Dal^+ transductants, 16 were $Sup^+ Mtl^+$, 11 were $Sup^+ Mtl^-$, 8 were $Sup^- Mtl^+$, and 2 were $Sup^- Mtl^-$. Analysis of the crossover classes indicated that the order of the loci is *sup-3–mtlB1–dal-1–purB33*. A composite of the data is shown in Fig. 1. Calculated linkage distances were compared with published values (9). The data indicated that the *sup-3* mutation should map near the *narB* marker.

If the *sup-3* mutation is in a tRNA gene, as presently suspected, these results place the location of at least one *B. subtilis* tRNA gene in a proximal region of the right chromosome arm. This area is relatively near a region enriched in ribosomal protein genes (7), and the location is consistent with the initial observations of Oishi et al. (11) and Smith et al. (15).

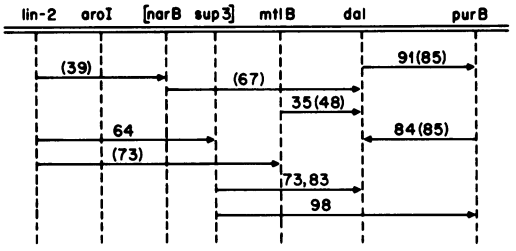

FIG. 1. *Linkage map of the sup-3 region. Data derived from the text are expressed as percent recombination (1 – cotransfer frequency) in PBS-1-mediated transduction. The head of each arrow designates the selected marker. Values in parentheses are from published work (9).*

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LITERATURE CITED

1. **Baptist, J. N., M. J. Tevethia, M. Mandel, and C. R. Shaw.** 1974. Altered proteins with triosephosphate isomerase activity in suppressor-containing strains of *Bacillus subtilis*. J. Bacteriol. **119**:976–985.
2. **Camacho, A., F. Moreno, J. L. Carrascosa, E. Vinuela, and M. Salas.** 1974. A suppressor of nonsense mutations in *Bacillus subtilis*. Eur. J. Biochem. **47**: 199–205.
3. **Demerec, M. E., E. Adelberg, A. J. Clark, and P. E. Hartman.** 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61–76.
4. **Georgopoulos, C. P.** 1969. Suppressor system in *Bacillus subtilis* 168. J. Bacteriol. **97**:1397–1402.
5. **Goldthwaite, C., D. Dubnau, and I. Smith.** 1970. Genetic mapping of antibiotic resistance markers in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S.A. **65**:96–103.
6. **Harford, N.** 1975. Bidirectional chromosome replication

in *Bacillus subtilis* 168. J. Bacteriol. **121**:835–847.
7. **Hoch, J. A.** 1978. Developmental genetics at the beginning of a new era, p. 119–121. *In* G. Chambliss and J. C. Vary (ed.), Spores VII. American Society for Microbiology, Washington, D.C.
8. **Lederberg, J., and E. M. Lederberg.** 1952. Replica plating and indirect selection of bacterial mutants. J. Bacteriol. **63**:399–406.
9. **Lepesant-Kejzlarova, J., J.-A. Lepesant, J. Walle, A. Billault, and R. Dedonder.** 1975. Revision of the linkage map of *Bacillus subtilis* 168: indications for circularity of the chromosome. J. Bacteriol. **121**:823–834.
10. **Mellado, R. P., E. Venuela, and M. Salas.** 1976. Isolation of a strong suppressor of nonsense mutations in *Bacillus subtilis*. Eur. J. Biochem. **65**:213–223.
11. **Oishi, M., A. Oishi, and N. Sueoka.** 1966. Location of genetic loci of soluble RNA on *Bacillus subtilis* chromosome. Proc. Natl. Acad. U.S.A. **55**:1095–1103.
12. **Okubo, S., and T. Yanagida.** 1968. Isolation of a suppressor mutant in *Bacillus subtilis*. J. Bacteriol. **95**: 1187–1188.
13. **Ordal, G. W., and D. J. Goldman.** 1975. Chemotaxis away from uncouplers of oxidative phosphorylation in *Bacillus subtilis*. Science **189**:802–805.
14. **Shub, D. A.** 1975. Nature of the suppressor of *Bacillus subtilis* HA101B. J. Bacteriol. **122**:788–790.
15. **Smith, I., D. Dubnau, P. Morell, and J. Marmur.** 1968. Chromosomal location of DNA base sequences complementary to transfer RNA and to 5S, 16S, and 23S ribosomal RNA in *B. subtilis*. J. Mol. Biol. **33**:123–140.
16. **Smith, J. D.** 1976. Transcription and processing of transfer RNA precursors. Prog. Nucleic Acid Res. Mol. Biol. **16**:25–73.
17. **Spizizen, J.** 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Natl. Acad. Sci. U.S.A. **44**:1072–1078.
18. **Steinberg, W., and C. Anagnostopoulos.** 1971. Biochemical and genetic characterization of a temperature-sensitive, tryptophanyl-transfer ribonucleic acid synthetase mutant of *Bacillus subtilis*. J. Bacteriol. **105**: 6–19.
19. **Tevethia, M. J., J. N. Baptist, and M. Mandel.** 1974. Pleiotropic effects of suppressor mutations in *Bacillus subtilis*. J. Bacteriol. **119**:961–975.
20. **Wake, R. G.** 1973. Circularity of the *Bacillus subtilis* chromosome and further studies on its bidirectional replication. J. Mol. Biol. **77**:569–575.