dnaB125, a *dnaB* Nonsense Mutation

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A temperature-sensitive *dnaB* mutation, *dnaB125*, was shown to be a suppressed amber mutation. The effects of inserting different amino acids at the mutated site via amber suppressors were examined for both *Escherichia coli* and bacteriophage λ growth. In addition, the *dnaB125* amber allele was shown to be different from the previously described *dnaB* amber allele, *dnaB266*. The extent of residual deoxyribonucleic acid synthesis observed in a *supF*(Ts) *dnaB125* strain at high temperature revealed that the *dnaB* protein was present in excess and that deoxyribonucleic acid synthesis could continue for several generation equivalents without further production of *dnaB* protein.

The isolation of nonsense mutations in essential genes may be accomplished in several ways. The most general method is by using a parent strain containing a temperature-sensitive nonsense suppressor. A second, and usually unintentional, method has been to use a *sup* parent and screen temperature-sensitive mutants to find those that absolutely require presence of the nonsense suppressor for survival. A *dnaB* amber allele, *dnaB266*, was the serendipitous product of the latter method and has been described by several laboratories (6, 13). One of the temperature-sensitive *dnaB* alleles in our collection was also found to be an amber mutation and is characterized here. This mutation, *dnaB125*, was produced by a milder mutagenesis treatment than used to obtain *dnaB266*, and is different from *dnaB266* as shown by the phenotypic characteristics of the two alleles when combined with the various suppressors.

During the construction of a set of isogenic *dnaB* strains, it was noticed that the *dnaB125* allele could not be transduced into a *sup*⁺ strain but was transduced into a *supE* strain. CR34 was the parent of the *dnaB125* mutant and contains *supE44* (19). Although the two strains were of different genetic backgrounds, this observation suggested that the *dnaB125* allele might be an amber nonsense mutation. Corroboration of this inference was obtained when the screening of spontaneous temperature-resistant revertants of E125 (*dnaB125*) showed that some of these contained a second amber suppressor in addition to the originally present *supE*.

To confirm these initial observations we attempted to cotransduce tetracycline resistance (Tet^r) conferred by the *zyb-504::Tn10* insertion and *dnaB125* into a set of nearly isogenic recipients carrying different amber suppressors (Table 1). Cotransduction of temperature sensitivity with the selected Tet^r was observed when RS101 (*supD60*) or RS102 (*supE44*) was the recipient but not with RS100 (*sup*⁺) or RS103 (*supF66*). P1 lysates of Tet^r transductants of RS100 and RS103 were then used to transduce RS102 (*supE*) to Tet^r. No temperature-sensitive transductants were recovered when P1 lysates of the Tet^r derivatives of RS100 were used. This is expected if *dnaB125* is an amber mutation as the *dnaB125 sup*⁺ combination would be lethal. The fact that several of the P1 lysates on the RS103 (*supF*) series produced temperature-sensitive transductants of the *supE* recipient demonstrated that the insertion of tyrosine at the amber codon by *supF* results in an active, temperature-insensitive *dnaB* product. Transduction of *supF* into the original mutant to produce a *dnaB125 supE supF* strain (RS110) also conferred temperature resistance. This result indicates that like *dnaB*⁺ (18), the *supF dnaB125* product confers a temperature-resistant phenotype on the cell, even when the cell is also producing the temperature-sensitive product of the *supE* suppressed *dnaB125* mutation. This is a minor but necessary consideration because *supF*(Ts), used in some of the experiments below, can insert glutamine (as does *supE*) in addition to tyrosine (5).

Some *dnaB* mutants exhibit severely depressed plating efficiencies for λ bacteriophage at the permissive temperature. This GroP⁻ phenotype is characteristic of the original *dnaB125*

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TABLE 1. *E. coli* K-12 strains^a

Strain	Relevant genotype	Source or reference
CR34	<i>thyA6 deoC1 rpsL supE44</i>	(20)
JW165	<i>thyA36 deoC2 rpsL malB45 sup+</i>	<i>metE</i> ⁺ transductant of JG123 (12)
E125	CR34 <i>dnaB125</i>	(19)
RS100 ^b	<i>trpA9761 metF159 ilvD188 thyA99 deoC56 rpsL179</i>	This paper
RS101	RS100 <i>supD60</i>	Transductant-CR63 (2) as donor
RS102	RS100 <i>supE44</i>	Transductant-CR34 as donor
RS103	RS100 <i>trpA</i> ⁺ <i>supF66</i> (<i>tyrT66</i>)	Transductant- <i>trp</i> ⁺ <i>supF66</i> <i>supF66</i> from CA275 (4)
RS106 ^c	RS100 <i>supF</i> (Ts)	Transductant-MB204 (16) as donor
RS108 ^d	RS101 <i>zjb-504::Tn10 dnaB125</i>	Transductant-RS73 as donor
RS109 ^d	RS102 <i>zjb-504::Tn10 dnaB125</i>	Transductant-RS73 as donor
RS110 ^d	E125 <i>zch-506::Tn10 supF66</i>	Transductant- <i>supF66</i> from CA275 (4)
RS125 ^d	RS103 <i>zjb-504::Tn10 dnaB125</i>	Transductant-RS73 as donor
RS126 ^d	RS106 <i>zjb-504::Tn10 dnaB125</i>	Transductant-RS73 as donor
RS73 ^d	E125 <i>zjb-504::Tn10</i>	See text
RS149 ^d	CR34 <i>zjb-504::Tn10 dnaB266</i>	Transductant- <i>dnaB266</i> from Q1610 (6)
RS123	RS100(P1 <i>bac</i>)	Lysogen
RS124 ^d	RS123 <i>zjb-504::Tn10 dnaB125</i>	Transductant-RS73 as donor

^a The presence of particular amber suppressors was determined by the ability to plate T4 amber mutants. T4M619 is suppressed by *supF* but not by *supE*; T4B17 is suppressed by *supE* but not by *supF*; both are suppressed by *supD* (16).

^b 594, a *sup*⁺ *rpsL197* strain (21), was obtained from J. S. Parkinson. A *metF159* amber (from J. S. Parkinson), *trpA9761* amber (from C. Yanofsky), *ilvD188* ochre derivative was constructed by sequential transduction by using linked Tn10 insertions and curing the transposon at each step (3). The *thyA* and *deoC* spontaneous mutations were selected by using trimethoprim, and the presence of the *deoC* mutation was verified by growth criteria (1, 17).

^c *supF*(Ts) is the allele termed suA2(P) in reference 16.

^d Tn10 insertions were isolated by selecting for cotransduction of Tet^r and another marker by using P1 lysates grown on a mixed pool of insertions as described in reference 8. Genetic backcrosses verified the locations of the insertions.

supE44 mutant. The influence of the various amber suppressors on the GroP character and on temperature sensitivity is summarized in Table 2.

P1 *bac* is a mutant of bacteriophage P1 that is derepressed for the *dnaB* analog protein encoded by the viral *ban* gene. As P1 *bac* lysogens of *dnaB266* amber *sup*⁺ are viable, the *ban* protein can substitute for *dnaB*; these strains are, however, cryosensitive (fail to grow at 25°C) and nonpermissive for λ bacteriophage (6, 13). Using a P1 lysate of RS73 (*dnaB125 zjb-504::*

Tn10), we selected Tet^r transductants of RS123(*sup*⁺ P1 *bac*) at 37°C. Transductants that were cryosensitive and nonpermissive for λ were obtained at the frequency expected (~12%) for cotransduction of *dnaB* and *zjb-504::Tn10*.

Both *dnaB125* and *dnaB266* are temperature sensitive when suppressed by *supD* or by *supE* and temperature resistant when suppressed by *supF*. Also, both amber mutations map within a small region of the *dnaB* gene (Sciafani and Wechsler, submitted for publication). That the two mutations are not at the same site is shown by the following observations. (i) A *dnaB125 supD* strain is GroP⁻, whereas a *dnaB266 supD* strain is GroP⁺ in strains of similar genetic background (13; Table 2). (ii) The *dnaB266 supE* phenotype is affected by the state of the *rpsL* (*strA*) locus; *rpsL*⁺ derivatives are GroP⁺, and *rpsL* (streptomycin-resistant) derivatives are GroP⁻ (13). On the other hand, all *dnaB125 supE* strains, whether *rpsL*⁺ or *rpsL*, are GroP⁻. This distinction was verified by transducing *dnaB266* into CR34 to produce RS149. RS149 and RS73 are identical, except for the particular *dnaB* allele, and exhibited different GroP phenotypes (Table 2). (iii) Phage P1 *bac* lysogenized a *dnaB125 supF* strain as efficiently as it lysogenized the *dnaB*⁺ *supF* parent, and both lysogens grew equally well over the 25 to 42°C range (data not shown), whereas P1 *bac* lysogens of *dnaB266 supF* are difficult to obtain and grow poorly at 42°C (13).

The fact that the two mutations map close together and both are temperature sensitive with *supE* (insertion of glutamine) or *supD* (insertion of serine) and temperature resistant with *supF* (insertion of tyrosine) may reflect the structural constraints operating on this section of the protein. It is quite possible that tyrosine was the original amino acid specified by the codon mutated to *dnaB125*, since the *supF* suppressed protein cannot be distinguished from the wild type by any of the phenotypic criteria employed. Similar arguments do not apply to *dnaB266*, since *supF* suppression results in a strain which is poorly lysogenized by P1 *bac* and the rare lysogens obtained are unstable (13).

It is unlikely that the phenotypes of either amber mutant are dependent on the level of suppression as both *supE* derivatives cease DNA synthesis immediately at the restrictive temperature (9, 19) and the *dnaB* protein purified from a *supE dnaB266* strain is temperature sensitive (10).

In an attempt to determine the relative amount of *dnaB* protein in a cell, we monitored the in vivo DNA-labeling kinetics of the *dnaB125 supF*(Ts) strain, RS126, after a shift to 42°C.

TABLE 2. Phenotypic characterization of suppressed *dnaB* amber

Strain	<i>dnaB</i> allele	Amber suppressor	Amino acid insertion	Growth at 42°C	GroP ^a
RS108	125	<i>supD60</i>	Ser	—	—
RS109	125	<i>supE44</i>	Gln	—	—
RS125	125	<i>supF66</i>	Tyr	+	+
RS110	125	<i>supF66 supE44</i>	Tyr, Gln	+	+
RS126	125	<i>supF(Ts)</i>	Tyr, Gln	—	+
RS73	125	<i>supE44</i>	Gln	—	—
RS149	266	<i>supE44</i>	Gln	—	+
	125	<i>sup⁺</i>	None	Not viable	Not viable
RS124	125 (P1 <i>bac</i>)	<i>sup⁺</i>	None	+ ^b	— ^b

^a Plating of λ c160 at 30°C (except RS124 at 38°C). —, Efficiency of plating of $\sim 10^{-3}$ and pinpoint plaques; +, efficiency of plating of 1 and normal plaques.

^b P1 *bac*-suppressed *dnaB125* is cryosensitive and nonpermissive for λ at all temperatures.

The ability of *supF(Ts)* to suppress is inactivated almost immediately upon incubation at 42°C (16). The *dnaB125 supF(Ts)* strain had an efficiency of plating at 42°C of 10^{-5} to 10^{-6} , whereas the *dnaB125 supF* strain, RS125, plates equally (efficiency of plating = 1) at 42 and 30°C. Thus, temperature sensitivity of RS126 [*dnaB125 supF(Ts)*] results from lack of production of an otherwise wild-type protein at 42°C rather than inactivation of a defective *dnaB* protein as is the case for *dnaB(Ts)* mutants (11, 19).

Although the *supF(Ts)* allele used here [this allele was termed suA2(P) in reference 16] is only 20 to 30% as efficient as *supF66* at the permissive temperature (7, 16), suppression of *dnaB125* by *supF(Ts)* was sufficient to produce *dnaB* in amounts suitable for normal cellular and λ growth. This result at low temperature suggests either that *dnaB* product is normally present in sufficient excess that the decreased level of translation with *supF(Ts)* is irrelevant or that the cell compensates by increasing production so that the gene product is present in wild-type amounts. This latter situation has been observed in *rpoB* amber *supF(Ts)* strains (7).

DNA synthesis in RS126 [*dnaB125 supF(Ts)*] at 42°C should decrease and eventually halt as the amount of *dnaB* protein in the cell at the time of the shift is diluted out or otherwise becomes insufficient for continued replication. The *dnaB125 supF(Ts)* strain maintains a normal rate of synthesis for three generations after the shift to 42°C (Fig. 1). (Note that the cells were not fully prelabeled before the experiment.) The rate of DNA synthesis subsequently decreases and finally halts. The total amount of DNA made at the high temperature represents approximately a 16-fold increase over that in the cells at the time of the temperature shift. Cell division continued for slightly more than five

doublings after the shift, and then the cells became elongated (data not shown).

The fact that in the *dnaB125 supF(Ts)* strain DNA synthesis continued for the equivalent of more than four generations at 42°C (Fig. 1) indicates that the protein is stable, that continual synthesis of *dnaB* product is not required for continued DNA synthesis, and that the *dnaB* product is present in excess of the immediate cellular requirement. Assuming that the total *dnaB* activity in the *supF(Ts) dnaB125* cell is equal to or less than that in the wild-type strain, then *dnaB* protein must also be in excess in *dnaB⁺* strains.

The demonstration that DNA synthesis continued unabated for three doublings after the shift to high temperature (Fig. 1) means that there is at least an eightfold excess of *dnaB* protein. In fact, the additional synthesis beyond the three doublings, which could reflect either all cells replicating at a reduced rate or a fraction replicating at the normal rate and some not replicating at all, suggests that the excess may be as great as 20-fold. Furthermore, these estimates assume that the *dnaB* activity in the *supF(Ts) dnaB125* strain is equal to the activity in a *dnaB⁺* strain; if the *dnaB* activity in the mutant is less than that in a wild-type cell, these estimates would be low. Since the cells were growing rapidly (generation time of 30 min) replication must have been dichotomous (14, 15). We assume that each chromosome is undergoing two simultaneous cycles of replication at all times, and with two replication forks per cycle this means that there were four forks per chromosome. If one *dnaB* multimer were required at each fork, each cell must have contained 32 to 80 *dnaB* multimers per replicating template chromosome. If the actual number of *dnaB* multimers required at a replication fork is greater than one, this estimate would need to be multiplied accordingly.

FIG. 1. Kinetics of DNA synthesis in *dnaB125 supF(Ts)* and *dnaB125 supF66* strains. Cells were grown in labeling medium (19), containing thymine (10 $\mu\text{g}/\text{ml}$) to 1×10^8 to $2 \times 10^8/\text{ml}$ at 30°C , and then diluted to 2×10^8 to 4×10^8 cells per ml into identical medium plus [^3H]thymine at 4 $\mu\text{Ci}/\mu\text{g}$. Two-milliliter cultures were grown for 60 min at 30°C and then transferred to 42°C . Samples (50 μl) were taken every 30 min, starting with the dilution into media containing radioactive isotope, and were processed to determine incorporation of radioactive label as previously described (19). ●, RS126 [*dnaB125 supF(Ts)*] after shift to 42°C ; ○, RS126 at 30°C , not shifted to 42°C ; △, RS125 (*dnaB125 supF66*) after shift to 42°C (RS110 and RS106 kinetics were identical to RS125 kinetics). Generation time at 42°C was ~28 min.

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LITERATURE CITED

- Ahmad, S. I., and R. H. Pritchard. 1969. A map of four genes specifying enzymes involved in catabolism of nucleosides and deoxynucleosides in *Escherichia coli*. *Mol. Gen. Genet.* 104:351-359.
- Appleyard, R. K., J. F. MacGregor, and K. M. Baird. 1956. Mutation to extended host range and the occurrence of phenotypic mixing in the temperate coliphage lambda. *Virology* 2:565-574.
- Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames. 1980. Positive selection for loss of tetracycline resistance. *J. Bacteriol.* 143:926-933.
- Brenner, S., and J. Beckwith. 1965. Ochre mutants, a new class of suppressible nonsense mutants. *J. Mol. Biol.* 13:629-637.
- Celis, J. E., M. L. Hooper, and J. D. Smith. 1973. Amino acid acceptor stem of *E. coli* suppressor tRNA^{Tyr} is a site of synthetase recognition. *Nature (London)* New Biol. 244:261-264.
- D'Ari, R., A. Jaffe-Brachet, D. Touati-Schwartz, and M. Yarmolinsky. 1975. A *dnaB* analog specified by bacteriophage P1. *J. Mol. Biol.* 94:341-366.
- Glass, R. E., M. Goman, L. Errington, and J. Scaife. 1975. Induction of RNA polymerase synthesis in *Escherichia coli*. *Mol. Gen. Genet.* 143:79-83.
- Kleckner, N., J. Roth, and D. Botstein. 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. *J. Mol. Biol.* 116:125-159.
- Kohiyama, M., D. Cousin, A. Ryter, and F. Jacob. 1966. Mutants thermosensibles d'*Escherichia coli* K-12. *Ann. Inst. Pasteur* 110:465-486.
- Lanka, E., M. Mikolajczyk, M. Schlicht, and H. Schuster. 1978. Association of the prophage P1 *ban* protein with the *dnaB* protein of *Escherichia coli*. *J. Biol. Chem.* 253:4746-4753.
- Lark, K. G., and J. A. Wechsler. 1975. DNA replication in *dnaB* mutants of *E. coli*: gene product interaction and synthesis of 4S pieces. *J. Mol. Biol.* 92:145-163.
- Monk, M., M. Peacey, and J. D. Gross. 1971. Repair of damage induced by ultraviolet light in DNA polymerase-defective *Escherichia coli* cells. *J. Mol. Biol.* 58: 623-630.
- Ogawa, T. 1975. Analysis of the *dnaB* function of *Escherichia coli* K12 and the *dnaB*-like function of P1 prophage. *J. Mol. Biol.* 94:327-340.
- Oishi, M., H. Yoshikawa, and N. Sueoka. 1964. Synchronous and dichotomous replications of the *Bacillus subtilis* chromosome during spore germination. *Nature (London)* 204:1069-1073.
- Pritchard, R. H., and K. G. Lark. 1964. Induction of replication by thymine starvation at the chromosome origin in *E. coli*. *J. Mol. Biol.* 9:288-307.
- Smith, J. D., L. Barnett, S. Brenner, and R. L. Russell. 1970. More mutant tyrosine transfer ribonucleic acids. *J. Mol. Biol.* 54:1-14.
- Stacey, K. A., and E. Simson. 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. *J. Bacteriol.* 90:554-555.
- Wechsler, J. A. 1973. Complementation analysis of mutations at the *dnaB*, *dnaC* and *dnaD* loci, p. 375-383. In R. D. Wells and R. B. Inman (ed.), *DNA synthesis in vitro*. University Park Press, Baltimore.
- Wechsler, J. A., and J. D. Gross. 1971. *Escherichia coli* mutants temperature-sensitive for DNA synthesis. *Mol. Gen. Genet.* 113:273-284.
- Wechsler, J. A., and W. H. Kurtz. 1979. Characterization of arithmetic deoxyribonucleic acid synthesis at restrictive temperature in a *dnaE* mutant of *Escherichia coli* K-12. *J. Bacteriol.* 138:201-206.
- Weigle, J. 1966. Assembly of phage lambda *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* 55:1463-1466.