FRAMESHIFT SUPPRESSION BY *THYA* MUTANTS OF *ESCHERICHIA COLI* K-12

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ABSTRACT

We have extended our previous study on the suppression of frameshift mutants by *Escherichia coli thyA* mutants by assaying suppression of 15 *rIIB* frameshift mutants of bacteriophage T4 on one of our suppressing *thyA* mutant strains. The majority of insertion mutants were suppressible, whereas none of the deletion mutants tested was suppressible. Frameshift suppression could be inhibited by adding thymidine to the assay medium, but was not affected by the presence of a restrictive *rpsL* mutation in the host strain. We suggest that the frameshift suppression event occurs at a nonsense codon generated by the frameshift mutation.

SUPPRESSION of frameshift mutants by extragenic suppressors has been extensively studied in *Salmonella typhimurium* and in *Saccharomyces cerevisiae*. Many of the suppressors studied have been shown (RIDDLE and CARBON 1973; CUMMINS, DONAHUE and CULBERTSON 1982; GABER and CULBERTSON 1982) or are thought (ROTH 1974; BOSSI, KOHNO and ROTH 1983; GABER *et al.* 1983) to affect tRNA genes. Frameshift suppressor tRNAs that have been characterized have an extra nucleotide in the anticodon region of the tRNA (RIDDLE and CARBON 1973; CUMMINS, DONAHUE and CULBERTSON 1982; GABER and CULBERTSON 1982) and may suppress either by four-base reading (RIDDLE and CARBON 1973) or by interfering with normal translocation to cause a reading frame shift (KURLAND 1979). Mutants of *S. typhimurium* affecting the *supK* gene lack a tRNA methylase and suppress UGA and frameshift mutants (ATKINS and RYCE 1974). ATKINS and RYCE (1974) proposed that in these strains undermodified tRNAs are responsible for suppression. Some mutant strains of *S. cerevisiae* and *Podospora anserina* that suppress frameshift mutants are thought to have altered ribosomes (COPPIN-RAYNAL 1977; SMIRNOV *et al.* 1978; SHERMAN 1982; GABER *et al.* 1983).

In *Escherichia coli*, apart from the classic study of intragenic suppression of phage T4 *rIIB* frameshift mutants (BARNETT *et al.* 1967; CRICK *et al.* 1967), little work has been done on frameshift suppressor mutants. However, it has been shown in *E. coli* that the normal cell makes a certain level of reading frame errors during translation. These reading frame errors are reflected in the leakiness of *lacZ* frameshift mutants (ATKINS, ELSEVIERS and GORINI 1972;

FOX and WEISS-BRUMMER 1980), and they appear to be required for the expression of specific minor proteins in MS2 and T7 phages (KASTELSTEIN *et al.* 1982; DUNN and STUDIER 1983) and translation of RF-2 in *E. coli* (CRAIGEN *et al.* 1985). This normal frameshifting can be perturbed by conditions that perturb the accuracy of translation, such as the presence of restrictive *rpsL* (streptomycin-resistant) or error-enhancing *ram* mutations (ATKINS, ELSEVIERS and GORINI 1972), the error promoting antibiotic neomycin (BRAKIER-GINGRAS and PHOENIX 1984), or the limitation of some aminoacyl tRNA species in relaxed (*relA*) strains (WEISS and GALLANT 1983).

We have shown that some *thyA* mutants of *E. coli* suppress nonsense mutations and a frameshift mutation in phage T4. This suppression acts at the level of translation and may result from thymine stress (CHEUNG and HERRINGTON 1982; HERRINGTON, KOHLI and LAPCHAK 1984). In our earlier study, we only tested three frameshift mutants (CHEUNG and HERRINGTON 1982), so we have extended our examination of frameshift suppression by assaying some of the T4 *rIIB* frameshift mutants isolated by CRICK *et al.* (1967) for their elegant demonstration of the triplet code. More recently, PRIBNOW *et al.* (1981) sequenced the 5′ region of the *rIIB* gene and located on this sequence many of the frameshift mutants. We show here that a suppressing *thyA* strain. N4316, suppressed plus (+) frameshifts, but did not suppress any of the minus (−) frameshift mutants tested. We have also shown that other Thy$^-$ strains suppress frameshifts. We propose that the *thyA* suppression of frameshift mutants occurs by an alteration in translation, such that a reading frame shift occurs at the barrier sequence.

MATERIALS AND METHODS

Bacterial strains: The *E. coli* K12 strains used are listed in Tables 1 and 2. Strain constructions are described below. *E. coli* strain B, which was used as the permissive host for the *rIIB* frameshift mutants, was obtained from S. P. CHAMPE.

Bacteriophage strains: Bacteriophage T4 frameshift mutants affecting the *rIIB* cistron were obtained from J. GALLANT (FC47, FC151, A31, 370, FC10 and FC0 (also obtained from S. P. CHAMPE)) and from B. SINGER (FC301, FC49, FC73, FC32, FC1, FC33, FC105, FC54, FC41). Further information about these strains can be found in BARNETT *et al.* (1967) and PRIBNOW *et al.* (1981). The T4 frameshift mutants J42 and J44 affecting the T4 lysozyme gene were obtained from J. OWEN. The transducing phage P1CM was obtained from E. B. NEWMAN. λCI Ind$^-$ (JACOB and CAMPBELL 1959) was obtained from the American Type Culture Collection. λCI14 was obtained from M. BELFORT.

Media: The AB medium used for suppression assays contained casamino acids and nutrient broth (APIRION 1966; CHEUNG and HERRINGTON 1982). It was supplemented with thymidine where indicated and with streptomycin (100 μg/ml) to isolate streptomycin-resistant transductants. Minimal medium A (MILLER 1972), which was supplemented with amino acids (40 μg/ml) and thymidine (50 μg/ml) where appropriate, was used for selection of recombinant strains. λ phages were assayed on TB agar (SHLEIF and WENSINK 1981).

Phage techniques: Suppression assays and preparation of T4 lysates were previously described (CHEUNG and HERRINGTON 1982). λ was assayed as described (SHLEIF and WENSINK 1981). Tests for λ sensitivity were done by streaking cells across a line of λCI14 phage on AB plates containing 50 μg/ml thymidine.

Strain construction: Strain D10Thy$^-$ was isolated by trimethoprim selection (BER-

TABLE 1

***Escherichia coli* K-12 strains**

Strain	Genotype	Source and reference[a]
D10	*metB rna* (λ)	M. C. GANOZA (1)
D10Thy$^-$	*metB rna thyA* (λ)	This study
K12rpsL2	*leu thr thi lac supE rpsL2*	L. BRAKIER-GINGRAS (2)
KL14	Hfr *thi-1 relA1* λ$^-$	B. BACHMANN, CGSC[b]
MA50	*thr-1 leuB6 thi-1 cys-46 lysA24 lacY1 malA1 mtl-2 xyl-7 ara-13 gal-6 tonA2* λ^R, λ$^-$ *supE44*	B. BACHMANN, CGSC
MH167	HfrC *Δ(lacZW4680) thyA723*	(3)
N4316	*metB rna thyA(Ts) sts* (λ)	M. C. GANOZA (4)
263	HfrH *thi lac thyAΔ2*	M. BELFORT (5)
264	HfrH *thi lac thyAΔ64*	M. BELFORT (5)

[a] References: (1) GESTELAND (1966); (2) PHOENIX, MELANCON and BRAKIER-GINGRAS (1983); (3) HERRINGTON, KOHLI and LAPCHAK (1984); (4) PHILLIPS, SCHLESSINGER and APIRION 1969; (5) BELFORT and PEDERSEN-LANE (1984).

[b] CGSC-coli Genetic Stock Center (B. BACHMANN, curator) Yale University.

TABLE 2

Strains constructed by P1CM-mediated transductions

Strain	Donor parent	Recipient parent	Selected phenotype	Relevant genotype
MH192	MH128	N4316	Thy$^+$	*thyA(+) lysA*
MH128	MA50	D10 Thy$^-$	Thy$^+$	*thyA(+) lysA*
MH209	K12rpsL2	N4316	Str^R	*thyA(Ts) rpsL2*
MH427	MH167	MH128	Lys$^+$	*thyA723*
MH432	N4316	MH128	Lys$^+$	*thyA(Ts)*
MH460	263	MH128	Lys$^+$	*thyAΔ2*
MH461	264	MH128	Lys$^+$	*thyAΔ64*

TINO and STACEY 1966; HERRINGTON, KOHLI and LAPCHAK 1984). The strains described in Table 2 were constructed by P1CM-mediated transduction (MILLER 1972). Strains N4316 (λ^s) and D10 (λ^s) were isolated as described (LEDERBERG and LEDERBERG 1953). Strains N4316 (λCI Ind$^-$) and D10 (λCI Ind$^-$) were isolated from cells growing in the centers of plaques of λCI Ind$^-$ formed on strains N4316 (λ^s) and D10 (λ^s). Strains were tested for their sensitivity to ultraviolet light by spotting aliquots of cells on AB plates containing 50 μg/ml thymidine and then irradiating for different times. To test if λ prophage could be induced by ultraviolet light, cells were spotted on TB plates seeded with KL14 and then were irradiated.

RESULTS

Suppression of frameshift mutants by strain N4316: Seventeen frameshift mutants were assayed on the suppressor strain N4316, on the nonpermissive host D10 and on the permissive host B (*rIIB* mutants) or on strain D10 on lysozyme supplemented plates (lysozyme mutants). The results are given in Table 3 as suppression indices (the efficiency of plating on N4316 relative to D10), which range from 1.0 to 36,000. We arbitrarily chose to interpret a

TABLE 3

Suppression of frameshift mutants by strain N4316

Phage	Type	Suppression index[a]	Efficiency of plating[b]
J42[c]	–	58	7.5×10^{-2}
FC1[c]	–	3.0	7.5×10^{-6}
FC151	–	1.4	9.5×10^{-7}
A31	–	1.1	1.2×10^{-8}
FC10	–	4.4	1.3×10^{-5}
FCO[d]	+	1.0	1.7×10^{-6}
FC47	+	6.5	4.3×10^{-5}
370	+	1.4	5.0×10^{-6}
J44[c]	+	390	14.3
FC105	+	400	1.0×10^{-4}
FC41	+	520	1.6×10^{-2}
FC33	+	1,000	9.7×10^{-3}
FC49	+	6,500	6.3×10^{-2}
FC301	+	10,000	3.2×10^{-2}
FC54	+	17,000	1.1×10^{-2}
FC73	+	33,000	7.8×10^{-2}
FC32	+	36,000	3.1×10^{-2}

[a] The suppression index is the number of PFU/ml on strain N4316 divided by the number of PFU/ml on strain D10.

[b] The efficiency of plating is the number of PFU/ml on strain N4316 divided by the number of PFU/ml on strain B.

[c] Data for FC1, J42 and J44 were taken from CHEUNG (1981) and CHEUNG and HERRINGTON (1982).

[d] Data presented for FC0 were obtained using phage obtained from J. GALLANT. Similar results were obtained with a stock obtained from S. P. CHAMPE (CHEUNG 1981; CHEUNG and HERRINGTON 1982).

suppression index of greater than a hundred as indicating suppression (CHEUNG and HERRINGTON 1982; HERRINGTON, KOHLI and LAPCHAK 1984). This is a useful cutoff point because most of the nonsuppressed mutants give suppression indices tenfold- to 100-fold lower, whereas most suppressible mutants give values at least tenfold higher. Using this criterion, none of the five (−) frameshift mutants tested were suppressed, whereas nine of the 12 (+) frameshift mutants tested were suppressed. The range of suppression observed with the frameshift mutants was somewhat narrower than that observed with suppression of nonsense mutants, which gave suppression indices ranging from 490 to 1.4×10^6 (CHEUNG 1981; CHEUNG and HERRINGTON 1982). When we compared the efficiency of plating of the *rIIB* frameshift mutants on strain N4316 relative to strain B, we found that the suppressible mutations had high efficiencies of plating, whereas the nonsuppressible mutants had low efficiencies (Table 3). The lysozyme frameshift mutants were plated on strain D10 on lysozyme-supplemented plates. When the titers on strain N4316 (on AB medium) were compared to the titers on lysozyme-supplemented plates, both J42 and J44 had relatively high plating efficiencies. This was partially due to high levels of revertants in our lysates; however, we have been unable to obtain

TABLE 4

***thyA* allele and suppression**

Strain	*thyA* allele	Suppression index of			
		FC73	FC301	FC41	FC54
D10	*thyA(+)*	1.0	1.0	1.0	1.0
MH192	*thyA(+)*	1.1	0.96	0.93	0.14
MH128	*thyA(+)*	2.6	0.46	0.08	0.39
N4316	*thyA(Ts)*	33,000	10,000	520	1,600
MH432	*thyA(Ts)*	6,300	20,000	1,300	3,300
MH429	*thyA723*	10,000	13,000	1,700	3,300
MH460	*thyAΔ2*	2,600	3,500	160	1,600
MH461	*thyAΔ64*	8,400	10,000	170	7,600

better lysates. The much higher efficiency of plating of J44 is consistent with our conclusion that it was suppressed while J42 was not suppressed.

All of the suppressible *rIIB* frameshift mutants gave very small plaques on strain N4316. In contrast, the suppressible nonsense mutations in the *rIIB* gene produce normal-sized plaques on strain N4316 (M. B. HERRINGTON and P. K. F. CHEUNG, unpublished results) indicating that the nonsense mutants might be more efficiently suppressed. The lysozyme mutant J44 made normal-sized plaques on strain N4316. This difference may reflect the levels of *rIIB* protein and lysozyme needed to produce plaques, or could be related to the numbers of phage released per infected cell.

***thyA* and suppression:** Suppression of nonsense mutants and the frameshift mutant J44 by strain N4316 requires the presence of a *thyA* mutation (CHEUNG and HERRINGTON 1982), and newly isolated *thyA* mutants have suppressor activity (HERRINGTON, LAPCHAK and KOHLI 1984). We have assayed four of the suppressible *rIIB* frameshift mutants on various strains (Table 4). The three Thy^+ strains, D10, MH192 and MH128, do not suppress. Strain MH192 was a Thy^+ transductant isolated from a cross using strain MH128 as the donor and N4316 as the recipient and, thus, has an identical background to strain N4316, except for the *thyA-lysA* region. These results indicate that frameshift suppression by strain N4316 requires the *thyA* mutant allele. Strain MH128 was a Lys^- derivative of strain D10, which was used as the recipient parent in the construction of the four new Thy^- strains in Table 4. All of the Thy^- strains suppressed. Strain MH432 had the same *thyA* allele as strain N4316, but in the MH128 background, thus eliminating the possibility that suppression is the result of a cryptic mutation (or the *sts* mutation) in N4316 that is expressed in a Thy^- background. This was a particular concern since strain N4316 was isolated after nitrosoguanidine mutagenesis (PHILLIPS, SCHLESSINGER and APIRION 1969), which often causes multiple mutations (MILLER 1972).

The other three Thy^- strains have different *thyA* alleles in the MH128 background. We assayed suppression by these strains on AB plates containing 20 μg/ml thymidine. This concentration does not interfere with suppression (see below) or affect the plating efficiency of the *rIIB* mutants on strain D10,

but does allow better growth of the host strains. Strain N4316, which has a temperature-sensitive mutation in *thyA*, grew well on AB medium without thymidine. The differences in growth probably reflects the amount of active thymidylate synthase in the different mutant strains. The Thy$^-$ strains all suppressed the four *rIIB* frameshift mutants, indicating that suppression is a property of Thy$^-$ strains. In each case, as for strain N4316, we observed small plaques. In contrast, the plaques formed on the Thy$^+$ strains were similar to wild-type T4 plaques, as would be expected of revertants.

There was as much as a tenfold variation in the suppression indices for a frameshift mutant on various Thy$^-$ hosts (Table 4). We do not have an explanation for this, but we have seen similar variations with the nonsense suppression (HERRINGTON, LAPCHAK and KOHLI 1984).

Effect of temperature on frameshift suppression: Strain N4316 does not suppress nonsense mutants or J44 at 31° (PHILLIPS, SCHLESSINGER and APIRION 1969; CHEUNG and HERRINGTON 1982). Three of the four *rIIB* frameshift mutants tested were not suppressed at 31° by strain N4316: FC301, FC41 and FC54 gave suppression indices of 0.92, 0.65 and 0.16, respectively. In contrast, FC73 was suppressed almost as well at 31° as at 37° (suppression index of 10,000). Under our conditions, the plating efficiencies of mutant phage strains on strain D10, and wild-type phage on strains D10 and N4316, were the same at 31° as at 37°.

Effect of *rpsL* on frameshift suppression: Mutations in *rpsL* often restrict suppression, and this is true of the *thyA*-dependent nonsense suppression (HERRINGTON, KOHLI and LAPCHAK 1984). We assayed four frameshift mutants on strain MH209, a streptomycin-resistant derivative of strain N4316, that carries one of the two restrictive *rpsL* alleles we used to show the restriction of nonsense suppression by Thy$^-$ strains (HERRINGTON, KOHLI and LAPCHAK 1984). Strain MH209 suppressed FC73, FC301, FC41 and FC32 to the same extent as strain N4316, indicating that the *rpsL* allele does not restrict the frameshift suppression.

Effect of amino acids and thymidine on suppression: Frameshift suppression has been observed when *E. coli* cells (particularly relaxed strains) are starved for certain amino acids (WEISS and GALLANT 1983). Although the medium we used to assay suppression has a high content of amino acids, it may have very low concentrations of specific amino acids. We asked whether supplementing the medium had any effect on frameshift suppression of FC73 by strain N4316. We added asparagine, glutamine, serine, alanine or proline, or a mixture of the five (each of 40 μg/ml); there was no effect on the plating efficiency or the plaque morphology. We have also tested the effect of adding all common amino acids, singly, or in various combinations, to assays of nonsense mutants and have observed no effects on nonsense suppression by strain N4316 (CHEUNG and HERRINGTON 1982; M. B. HERRINGTON, unpublished results).

Thymidine, when added at concentrations greater than 50 μg/ml, inhibited suppression of nine of the 12 nonsense mutants previously tested and resulted in smaller plaques in the other three. It also completely inhibited suppression

of the lysozyme mutant J44 (CHEUNG and HERRINGTON 1982). When thymidine was added at 50 μg/ml, the suppression indices for FC73, FC301, FC41 and FC54 on strain N4316 ranged from 0.42 to 2.4, indicating that suppression of these mutants was inhibited. These results suggest that thymidylate might be limiting in the infected cells, although the host cells do not appear to be limited for thymidylate, and T4 codes for its own thymidylate synthase (WOOD and REVEL 1976).

Is suppression really thymineless mutagenesis? Since limitation of Thy$^-$ mutants for thymidylate is known to be mutagenic (BARCLAY *et al.* 1982), and we appear to limit thymidylate under our suppression assay conditions, we checked that we were observing suppression rather than enhanced mutagenesis by testing the phenotype of phage in plaques formed on strain N4316. This was done as previously described (HERRINGTON, LAPCHAK and KOHLI 1983). Three mutants, FC32, FC54 and FC73, were tested. In each case the phage from the small plaques formed on N4316 formed plaques on strain B and did not make plaques on strain D10, indicating that they had the rII phenotype. From this we can conclude that our conditions do not lead to extensive mutagenesis.

Does λ induction play a role? The λ prophage can be induced by thymine starvation (KORN and WEISSBACH 1962) as well as by ultraviolet light (LEDERBERG and LEDERBERG 1953). Mutants (Ind$^-$) in the CI gene of λ cannot be induced by either treatment (JACOB and CAMPBELL 1959; KORN and WEISSBACH 1962). To determine if induction affected suppression, we constructed derivatives of strains N4316 and D10 carrying a λCI Ind$^-$ prophage. These strains were as resistant to ultraviolet light as their λ-sensitive parents, whereas strains N4316 and D10 were more sensitive than either their sensitive derivatives or the λCI Ind$^-$ lysogens. The prophage in strains N4316 and D10 could be induced by ultraviolet light, whereas the λCI Ind$^-$ prophage in strains N4316 (λCI Ind$^-$) and D10 (λCI Ind$^-$) was not induced, although spontaneous phage production was detectable with all four strains.

We assayed FC73, FC301, FC41 and FC54 on the sensitive derivatives and on the λCI Ind$^-$ lysogens of strains N4316 and D10. The titers of the *rIIB* mutants on strains N4316 (λ^s) and D10 (λ^s) were similar to those observed on strain B, and the plaques were large, indicating as expected that these strains were permissive for *rII* mutants. The titers on strains D10 and D10 (λCI Ind$^-$) were similarly low, indicating that these strains were nonpermissive. When strain N4316 (λCI Ind$^-$) was used as host, small plaques and relatively high titers were observed. The suppression indices ranged from 4200 to 43,000. These were similar to those observed on strain N4316, thus indicating that if induction occurs under our suppression assay conditions, it does not affect suppression.

DISCUSSION

Comparison of nonsense and frameshift suppression: In this study we demonstrated that strain N4316 suppressed many (+) frameshift mutants of phage T4. This suppression was similar to that observed with nonsense mutants

TABLE 5

Summary of T4 mutants tested for suppressibility

	No. of mutants			
	Suppressed		Not suppressed	
Type	*rII* or *e*	Other	*rII* or *e*	Other
Nonsense				
UAG	3	1	4	9
UAA	5	1	2	0
UGA	3	2	2	2
Frameshift				
(−)	0	0	5	0
(+)	10	0	3	0
Missense	0	0	6	0
Deletion	0	0	1	0

(CHEUNG and HERRINGTON 1982). It was temperature-dependent, required the presence of a *thyA* allele and was inhibited by thymidine. The inhibition of suppression by thymidine suggests that suppression results from thymine limitation. We have shown that the frameshift and nonsense suppression are not a result of enhanced mutagenesis or prophage induction caused by thymine limitation.

We have summarized in Table 5 the types of mutants that we have tested. It is clear that all three types of nonsense mutants and (+) frameshift mutants can be suppressed. Although the majority of mutants tested affected either the rII region or the lysozyme gene, a variety of other mutants were also tested. Of these, mutations affecting gene *23* (coat protein), gene *34* (tail fiber) and gene *43* (DNA polymerase) mutants are suppressible (CHEUNG and HERRINGTON 1982), indicating that the suppression by strain N4316 is not limited to *rII* and lysozyme mutants. We have not observed suppression of bacterial mutants (M. B. HERRINGTON, unpublished results), so we do not know if suppression is limited to T4 mutants.

Nature of the suppression by *Thy*⁻ strains: Suppression of a mutation can occur by functionally bypassing it (HARTMAN and ROTH 1973). Such suppression is generally gene-specific. Suppression by strain N4316 is allele-specific rather than gene-specific, so it is difficult to envisage a mechanism of functional suppression.

Allele-specific suppression generally occurs at the level of translation (HARTMAN and ROTH 1973), but could occur during transcription. Restriction of suppression by an *rpsL* mutation has been used as an indication that suppression is occurring during translation (WEISS and GALLANT 1983). We have demonstrated that an *rpsL* mutation restricts nonsense suppression by strain N4316 (HERRINGTON, KOHLI and LAPCHAK 1984) but that it does not restrict frameshift suppression. However, since the frameshift and nonsense suppression by strain N4316 are otherwise similar, we are assuming that they both occur at the translational level.

Model for *thyA* suppressor activity: A useful model has to explain the broad suppression spectrum of the suppressor strains, the role of mutations affecting thymidylate synthetase in causing the suppression and the patterns of inhibition of suppression by thymidine and by the restrictive *rpsL* mutation.

We proposed a model for suppression by strain N4316 in which an indirect effect of the *thyA* mutation led to an imbalance in the tetrahydrofolate pools (CHEUNG and HERRINGTON 1982). This imbalance would affect the extent of modification of tRNA so that several species of tRNA would be abnormally modified. Some of these tRNAs could have the potential of interacting with nonsense codons. Depending on the anticodon-codon interaction and the context, the nonsense codon would be read as a triplet (suppression of a nonsense mutant), not be read at all (termination) or be read, with an adjacent nucleotide, as a quartet (suppression of frameshift mutants). Frameshifting events might also occur when an undermodified tRNA reads a sense codon. Alternatively, an undermodified tRNA might be able to initiate translation at an internal codon. If the fragment of the protein produced by such an event were active, we would observe suppression. The temperature-dependent suppression as seen with strain N4316, and to a lesser extent with some of the other *thyA* suppressor strains, could be a function of the effect of temperature on the level of thymidylate synthetase, on tetrahydrofolate pools in mutant strains or on the decoding activity of the abnormally modified tRNAs. The different effects of the restrictive *rpsL* mutation on nonsense and frameshift mutations may reflect an action of S12 protein in checking codon-anticodon mismatches. If the frameshift event involves an initial mispairing of an abnormally modified tRNA, followed by a slipping by one nucleotide to generate a correct codon-anticodon interaction, the frameshift event might not be detected as an incorrect pairing, but the triplet interaction required for nonsense suppression would be. Finally, thymine could exert its inhibitory effects on suppression by restoring the tetrahydrofolate balance in the cell, thereby allowing normal tRNA modification to proceed. A precedent for suppression of both nonsense and frameshift mutants by abnormally modified tRNAs comes from studies on *supK* mutants of *S. typhimurium* that are defective in methylation of tRNA. However, there is no evidence that tetrahydrofolate is directly involved in tRNA modification in *E. coli*, although it has been shown to be involved in other systems (DELK *et al.* 1976).

Examination of sequences: The DNA sequences of both the lysozyme gene (OWEN *et al.* 1983) and a portion of the *rIIB* gene coding for the N-terminal part of the *rIIB* protein (PRIBNOW *et al.* 1981) have been determined, and the locations of the frameshift mutants used in this study are known to within a few base pairs (OKADA *et al.* 1966; PRIBNOW *et al.* 1981; OWEN *et al.* 1983). We have examined these sequences to determine if they can provide information about the mechanism of *thyA*-dependent suppression.

As indicated above, translational reinitiation could explain suppression. The lysozyme mutant, J44, which was suppressed by strain N4316, generates a UAA barrier at position 134 of the sequence of the lysozyme gene (OKADA *et al.* 1966; OWEN *et al.* 1983). Inspection of the nucleotide sequence of the

lysozyme gene (OWEN *et al.* 1983) indicates that the first potential reinitiation codon after the J44 barrier is a UGG codon located at nucleotides 296-298. The product of initiation at this site would be a C-terminal fragment containing only 40% of the amino acids of active lysozyme (GRUTTER *et al.* 1983) and would clearly not be active. Thus, translational reinitiation cannot explain suppression of J44.

NAPOLI, GOLD and SINGER (1981) have identified two in-frame UUG codons within the *rIIB* coding sequence that do not serve as initiation codons in wild-type *E. coli*. If the Thy$^-$ strains enhance initiation at either of these codons, we would expect that any (+) or (−) frameshifts that generate a barrier upstream from these codons would be suppressible. Mutants FC0, FC1, FC10 and A31 are upstream from the potential reinitiation codons (PRIBNOW *et al.* 1981) and are not suppressible (Table 3). Thus, it is unlikely that translational reinitiation plays a role in suppression by Thy$^-$ strains.

Suppression could occur if the reading frame is shifted back to the 0 frame at random sites within the gene. In this case we would expect Thy$^-$ strains to suppress all frameshift mutants at a low efficiency, since high levels of random reading-frame shifts would interfere both with correction of frameshift mutants and with synthesis of normal proteins. Since we do not observe suppression of all frameshifts, we can exclude random reading-frame shifts.

Frameshift suppression could occur by four-base reading or sloppy translocation at a sense codon. We would expect such "shifty sites" to be limited to one or a few codons, because if most codons allow frameshifting, then all (+) frameshift mutations would be suppressible. We have examined the *rIIB* and lysozyme sequences for codons occurring near the frameshift mutations and conclude that there are no obvious sense codons at which frameshifting might be occurring.

Since all of the frameshift mutants tested generate downstream nonsense codons (barriers), and since Thy$^-$ strains are clearly able to suppress nonsense codons, we suggest that the frameshift suppression occurs by a shift in the reading frame at the barrier.

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