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Modification of the suppressor phenotype of thymine requiring strains of *Escherichia coli*

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Summary

Thymine requiring strains of *Escherichia coli* suppress nonsense and frameshift mutations during translation. Strains with different genetic backgrounds exhibited different nonsense suppression spectra and showed differences in the apparent suppression efficiency. Part of this strain difference is due to a presumably novel gene (*tsmA*) mapping near 39 min. This gene affects the spectrum and apparent efficiency of suppression, and appears to affect the utilization of thymidine.

1. Introduction

Thymidylate synthase catalyses the formation of thymidylate from 2′-deoxyuridylate. Mutant cells lacking this activity can grow if they can be supplied exogenously with thymidylate or can convert exogenous thymine or thymidine to thymidylate. However, if the exogenous source of thymidylate is limited cells exhibit enhanced mutagenesis, increased recombination and ultimately may die (thymineless death) (Barclay *et al.* 1982). Thymine requiring (Thy^-) strains of *Escherichia coli* exhibit a novel phenotype. They suppress many nonsense and frameshift mutations of bacteriophage T4. Although suppression occurs in media containing sufficient thymine or thymidine to support cell growth, it may require thymidylate limitation since it is inhibited by the addition of relatively high concentrations of thymine or thymidine (Cheung & Herrington, 1982; Herrington *et al.* 1984).

When Thy^- cells are made streptomycin resistant by the introduction of a restrictive *rpsL* allele they no longer suppress nonsense mutations, implying that the suppression event occurs on the ribosome (Herrington *et al.* 1984). It is not obvious how mutations which prevent the biosynthesis of a DNA precursor can effect the accuracy of translation.

Some of our observations suggest that there may be genetic factors which effect suppression by Thy^- strains. Newly isolated Thy^- derivatives of strain D10 suppressed the three T4 mutants tested. In contrast, Thy^- derivatives of strain DS4680A suppressed the amber (UAG) mutant, but not the opal (UGA) or ochre (UAA) mutants. This difference in suppression spectrum could result from differences in the genetic background of the two strains. Alternatively, it could result from differences in the *thyA* mutations since the strains were independently isolated Thy^- mutants (Herrington *et al.* 1984).

In this study we constructed Thy^- derivatives of strains D10 and DS4680A containing the same *thyA* alleles. The isoallelic strains have different suppression spectra indicating that genetic differences between the strains affect suppression. We have identified one gene which affects suppression and which appears to affect the way the cells utilize thymidine.

2. Materials and methods

(i) *Bacteriophage and bacterial strains*

The generalized transducing phages P1CM and Plvir were obtained from E. B. Newman. The transposition vector lambda::TN10 (Δatt cI857ts) was obtained from J. Wood. The T4 mutants have been previously described (Cheung & Herrington, 1982). The *E. coli* K12 strains used in this study are described in Table 1.

(ii) *Suppression assays*

Suppression was assayed by measuring the number of plaque forming units detected in phage lysates plated

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Table 1. E. coli *K12 strains*

Strain	Genotype	Source and reference[a]
CAG12122	*zea*-3125::Tn10*kan*	M. Singer (1)
CAG12133	*zhd*-3171::Tn10*kan*	M. Singer (1)
CAG18518	*zdi*-3123::Tn10*kan*	M. Singer (1)
CAG18561	*zda*-51::Tn10*kan*	M. Singer
CAG18578	*zdj*-3124::Tn10*kan*	M. Singer (1)
D10[b]	*metB*1 *relA*1 *spoT*1 *rna*-10	M. C. Ganoza (2)
DS4680A[b]	▲(*lacZ*4680 (= *lacZ*39)) *relA*1 *spoT*1 *rpsE* λ^-	B. Hall (3)
MA50	*thr*-1 *leuB*6 *thi*-1 *cys*-46 *lysA*24 *lacy*1 *malA*1 *mtl*-2 *xyl*-7 *ara*-13 *gal*-6 *tonA*2 λ^R λ^- *supE*44	CGCS[c]
MH128	*metB*1 *relA*1 *spoT*1 *rna*-10 *lysA*24	(4)
MH164[b]	metB1 relA1 spoT1 rna-10 *thyA*721(*Ts*)	(5)
MH167	▲(*lacZ*4680 (= *lacZ*39)) *relA*1 *spoT*1 *rpsE thyA*723 λ^-	(5)
MH420	▲(*lacZ*4680 (= ▲ *lacZ*39)) *lysA*24 *relA*1 *spoT*1 *rpsE* λ^-	This study
MH421	▲(*lacZ*4680 (= ▲ *lacZ*39)) *relA*1 *spoT*1 *rpsE thyA*721(TS) λ^-	This study
MH423	▲(*lacZ*4680 (= ▲ *lacZ*39)) *relA*1 *spoT*1 *rpse thyA*723 λ^-	This study
MH427	*metB*1 *relA*1 *spoT*1 *rna*-10 *thyA*721(TS)	(4)
MH429	*metB*1 *relA*1 *spoT*1 *rna*-10 *thyA*723	This study
MH617	▲(*lacZ*4680 (= ▲ *lacZ*39)) *relA*1 *spoT*1 *rpsE thyA*723 zeb::Tn10 λ^-	This study

[a] (1) Singer *et al.* (1989); (2) Gesteland (1966); (3) Hall (1974); (4) Herrington *et al.* (1986); (5) Herrington *et al.* (1984).
[b] Detailed genotypes and genealogies of strains D10 and DS4680A were provided by B. Bachmann (personal communication).
[c] Coli Genetic Stock Center, B. Bachmann, curator.

on different host strains or by using the fast assay which provides a crude estimate of the plating efficiency (Cheung & Herrington, 1982). A strain suppressed a phage mutation when there were at least a hundred times more plaque-forming units detected on the strain than on a non-permissive strain (Herrington *et al.* 1984). This cut-off point was arbitrarily chosen based on our observations with different phage mutants. Mutants which are not suppressed usually give about the same number of plaques on a Thy$^-$ strain as on a Thy$^+$ strain. However, particularly in the fast assays the numbers can be variable. In contrast, most suppressible mutants produce at least several thousand more plaques on the Thy$^-$ host than on the Thy$^+$ host (Cheung & Herrington 1982; Herrington *et al.* 1986).

(iii) *Media*

AB, superbroth and minimal media have been described (Cheung & Herrington 1982; Herrington *et al.* 1984, 1986). Thymidine was routinely added to AB medium at 20 μg/ml for suppression assays, and at 50 μg/ml for cell growth. Tryptone broth contained 10 g tryptone, 2·5 g sodium chloride, and 0·12 g magnesium sulphate per litre. Tetracycline plates contained 10 g tryptone, 5 g yeast extract, 5 g sodium citrate, 1 g glucose, 50 mg of thymidine and 15 g agar per litre. Tetracycline was dissolved in methanol and added after autoclaving to give a final concentration of 12 μg/ml. R plates used for P1CM plate lysates are described in Miller (1972).

(iv) *Conjugations and transductions*

Conjugations and transductions were carried out as described by Miller (1972).

(v) *Construction of Thy$^-$ strains*

Thy$^+$ Lys$^-$ derivatives of strain D10Thy$^-$ [a spontaneous Thy$^-$ mutant isolated by trimethoprim selection (Herrington *et al.* 1984)] and the DS4680A Thy$^-$ strain MH167 were obtained by transduction using P1CM lysates (Miller, 1972) prepared on strain MA50. The resulting Lys$^-$ strains (MH128 and MH420) were

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then transduced to Lys⁺ Thy⁻ using P1CM lysates prepared on strains MH164 and MH167.

(vi) *Construction of tsmA⁺ derivatives of MH423*

Approximately 5×10^8 MH429 cells were infected with lambda::TN10 at a multiplicity of infection of 2. Tetracycline-resistant colonies were selected on tetracycline plates. Approximately 20000 colonies were pooled, diluted in tryptone broth containing 25 μg/ml tetracycline, 5 mg/ml sodium citrate and 50 μg/ml thymidine, and grown to late exponential phase. These cells were used to prepare a P1CM plate lysate which was used to transduce strain MH423 to tetracycline resistance. Approximately 1000 transductants were screened for their ability to suppress the T4 phage eL1P12 by cross-streaking. This phage is not suppressed by strain MH423. Putative suppressing strains were then tested using the fast phage assay. Approximately 80% of the strains did not suppress eL1P12 indicating that the cross-streaking assay was not very reliable. Ten of the 23 suppressing strains were used as donors in transductions with strain MH423 as the recipient. The frequency of cotransduction of the Tn10 and the ability to suppress eL1P12 ranged from 0 to 67%. One donor strain (MH617), which gave a co-transduction frequency of 46%, was further characterized.

(vii) *Thymidylate synthase assays*

Crude extracts were prepared by sonicating cells grown at 37 °C in minimal medium containing glucose (2%), thymidine (50 μg/ml), lysine (40 μg/ml) and methionine (40 μg/ml). Thymidylate synthase activity in crude extracts was assayed by the tritium-release assay (Roodman & Greenberg, 1971). The reaction was carried out in a 1·5 ml Eppendorf tube and contained 50 μM [5-³H]dUMP (specific activity of 418 MBq/mmol). All reactions were incubated at 31 °C. The reaction was stopped by the addition of charcoal (Norit A, 12% in 0·01 N-HCl) and the aqueous phase was separated from the charcoal by centrifuging for 10 min in an Eppendorf microfuge. The samples were counted in 5 ml of Bray's scintillation fluid (Bray, 1960) using an LKB scintillation counter. A unit of activity was defined as the amount of enzyme required to convert 1 μmol of dUMP to dTMP per min. Under the conditions used, this assay was sensitive enough to detect approximately 0·005 munits. Assays on crude extracts were generally done several times and data from two or three extracts were pooled. The variability on replicate assays was less than 15%. The quantity of thymidylate synthase in crude extracts was determined by the FdUMP-binding assay (Moore *et al.* 1984). The reaction mixture for the formation of the ternary complex was the same as for the enzyme assay, except that 50 nM [6-³H]FdUMP (specific activity of 666 GBq/mmol) was used instead of [5-³H]dUMP. The reaction was carried out for 1 h at 31 °C and then was stopped by adjusting each sample to a final concentration of 20% trichloroacetic acid. Precipitates were collected on 0·45 μm nitrocellulose filters (type HAWP, Millipore), washed several times with 5% trichloroacetic acid and once with 50% ethanol. The dried filters were counted in Bray's scintillation fluid (Bray, 1960). Assays were done several times using two extracts for each strain and growth temperature. The data were averaged and replicate assays did not vary more than 15%.

3. Results

(i) *Suppression by Thy⁻ strains*

Two *thyA* alleles were transferred into strains MH128 (a Lys⁻ D10 derivative) and MH420 (a Lys⁻ DS4680A derivative) by transduction. The D10 derivatives suppressed both T4 phage mutants. In contrast neither strain DS4680A derivative suppressed the opal mutant eL1P12, whereas both suppressed the amber mutant M103 although the efficiency of suppression was lower than on strain D10 derivatives (Table 2).

Since suppression was affected by the strain background, we assayed eight phage T4 nonsense mutants on the isoallelic strains MH423 (a DS4680A derivative) and MH429 (a D10 derivative) (Table 3). The suppression spectrum of strain MH423 was more restricted than that of strain MH429 but it did suppress both UAG and UGA mutants indicating that even in this background the suppression acts on a variety of mutations.

(ii) *Identification of a modifier gene*

We asked if we could identify a gene in strain MH429 which when transferred to strain MH423 would allow it to suppress eL1P12. To do this random transposon Tn10 insertions were made into the genome of strain MH429. The tetracycline-resistant derivatives were then pooled and used to prepare a transducing lysate which was used to transduce strain MH423 to tetracycline resistance. The resulting transductants were then screened for their ability to suppress the T4 mutant eL1P12. This mutant is not suppressed by strain MH423. One strain (MH617) which suppressed the T4 phage eL1P12 was further characterized.

Strain MH617 had a suppression spectrum intermediate between those of its parents. It differed from strain MH423 by suppressing eL1P12 and by suppressing M103 more efficiently. It differed from strain MH429 by suppressing eL1P12 and opC105 less efficiently and not suppressing JC1922 or opC100 (Table 3).

These results suggest that there may be several genetic differences between the parent strains. We call these modifying genes *tsm* (for thymine suppressor modifier) and have arbitrarily assigned the Tsm⁺

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Table 2. *Suppression by Thy⁻ derivatives of D10 and DS4680A*

Strain	Derivative of strain	*thyA* allele	Suppression index[a] of eLIP12	Suppression index[a] of M103
MH429	D10	723	4300	10000
MH423	DS4680A	723	1·9	3400
MH427	D10	721(TS)	1000	8800
MH421	DS4680A	721(Ts)	2·3	2300

[a] The suppression index is the number of plaque-forming units per ml on the Thy⁻ strain divided by the number of plaque-forming units on the Thy⁺ parent. When the suppression index is greater than 100 the phage mutant is suppressed (Herrington, Kohli & Lapchak, 1984).

Table 3. *Spectrum of suppression of isoallelic strains*

Phage[a]	Mutation	Gene[b]	Suppression[c] by strain MH429	MH423	MH617
M103	UAG	e	++	+	++
JC1912 (B22)	UAG	43	−	−	−
JC1917 (B17)	UAG	23	−	−	−
JC1922 (eL2)	UAA	e	+	−	−
eLIP12	UGA	e	++	−	+
opC23	UGA	34	+	+	+
opC100	UGA	23	+	−	−
opC105	UGA	37	++	+	+

[a] Phage strains prefixed JC were obtained from A. J. Clark, and are given his designations. Their original designations are given in parentheses (Templin *et al.* 1978).
[b] e is the lysozyme gene, 23 is a head component gene, 34 and 37 are tail fibre genes, and 43 is the DNA polymerase gene (Wood & Revel, 1976).
[c] Suppression was assayed semi-quantitatively using the fast assay. − indicates that the phage mutant was not suppressed; + and ++ indicate that the mutant was suppressed with the ++ indicating about 10-fold more plaques than +.

phenotype to strain MH429. This does not assume that alleles from strain MH429 are wild-type alleles. The *tsm* gene which was transferred from strain MH429 to strain MH423 to make strain MH617 was designated *tsmA*.

(iii) *Mapping the tsmA gene*

The frequency of cotransduction of the Tn10 and *tsmA*⁺ allele from strain MH617 was 46%. The transposon was mapped near 40 min by conjugations (data not shown). Several kanamycin resistant strains from the P1 mapping kit (Singer *et al.* 1989) were used as donors in P1 mediated transductions with MH617 as the recipient (Table 4). Kanamycin-resistant recombinants were screened for tetracycline resistance (all crosses) and for suppression of eL1P12 in one cross. The data from this cross indicates that the Tn10*kan* located at 39·5 min is located between the TN10 and the *tsmA* gene (Table 4).

Cotransduction frequencies (Table 4) were converted to minutes (Wu 1966; Singer *et al.* 1989). The *tsmA* gene was located at approximately 39·4 min and the TN10 in strain MH617 was located at approximately 40·9 min.

(iv) *Effects of thymidine on cell growth and suppression*

The Tsm⁺ strains MH429 and MH617 did not grow as well as the Tsm⁻ strain MH423 and Thy⁺ strains on AB agar medium containing 20 μg/ml thymidine. They grew better when more thymidine was added.

Suppression by Thy⁻ strains is inhibited by high concentrations of thymine or thymidine (Cheung & Herrington, 1982). We tested inhibition of suppression of the UAG mutant M103 by adding varying amounts of thymidine. The highest concentration of thymidine tested (500 μg/ml) reduced the size of plaques but not the number of plaques on the Tsm⁺ strains MH429 and MH617. In contrast, a lower level of thymidine (100 μg/ml) reduced both the plaque size and numbers on strain MH423. Thus, suppression by strain MH423 appeared to be more sensitive to inhibition by thymidine. Both the poorer growth in AB medium containing 20 μg/ml thymidine and the lower sen-

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Table 4. *Mapping the* tsmA *gene*

Donor[a]	Number of Kan^R recombinants	Phenotype[b]	&
CAG18578 (39·5)[c]	142	Tet^R Tsm$^+$	0·7
		Tet^S Tsm$^+$	9·9
		Tet^R Tsm$^-$	87
		Tet^S Tsm$^-$	2·1
CAG18518 (38·25)	100	Tet^S	0
CAG12122 (40·25)	100	Tet^S	4
CAG18561 (40·75)	100	Tet^S	30

[a] Plvir lysates prepared on the indicated strains (Kan^R Tet^S Tsm$^-$) were used to transduce kanamycin resistance to strain MH617 (Kan^S Tet^R Tsm$^+$).
[b] The Tsm phenotype was assayed by testing suppression of the opal mutation in phage strain eLIP12. Tsm$^+$ cells suppressed this mutation whereas Tsm$^-$ cells did not suppress.
[c] The number in parentheses indicates the map position of the Tn10*kan* in the strain (Singer *et al.* 1989).

Table 5. *Levels of thymidylate synthase in different strains*

Strain	Parent	*thyA* allele	TS activity[a]	FdUMP binding[b]
MH128	D10	WT	270	0·52
MH420	DS4680A	WT	280	0·35
MH427	MH128	721(Ts)	8·6	0·045
MH421	MH420	721(Ts)	5·9	0·038
MH429	MH128	723	2·2	< 0·019
MH423	MH420	723	1·6	< 0·019

[a] Thymidylate synthase tritium release activity in pmol per min/mg protein.
[b] FdUMP binding in fmol/mg protein.

sitivity to thymidine inhabitation were seen in Tsm$^+$ strains. This suggested that when these strains are growing in AB medium they do not take up exogenous thymidine or utilize it as effectively as the Tsm$^-$ strains.

Mutations in several genes can affect the phenotype of Thy$^-$ strains either rendering them unable to use thymine or altering the level of thymine they require (Mollgaard & Nauhard 1983). We assayed the thymine and thymidine requirement of strains MH423 and MH429 in minimal medium. Both strains grew to the same extent with adequate amounts of either thymine or thymidine. Neither strain grew when 3 or 15 μM thymine was added but grew well when 150–600 μM thymine was used. This growth response is typical of *thyA* or *thyA*, *deoA*, *cytR* mutants (Møllgaard & Neuhard, 1983).

These apparent differences in the ability to utilize exogenous thymidine could result if the *thyA* mutation was leakier in one strain than the other. We measured thymidylate synthase activity using the tritium release assay (Roodman & Greenberg, 1971) and thymidylate synthase levels using the FdUMP-binding assay (Moore *et al.* 1984). There were no differences in the amount or activity of thymidylate synthase in strains with the same *thyA* allele in the different backgrounds (Table 5).

(v) *Effect of the* rpsE *mutation*

Strain MH423 is spectinomycin resistant because of an *rpsE* mutation. Since ribosomal mutations can affect the fidelity of translation and affect the efficiency of suppression (Gorini, 1974; Piepersberg *et al.* 1975; Cabezón *et al.* 1976) we tested whether the *rpsE* mutation in strain MH423 affected suppression. When the *rpsE* allele was transduced from strain MH423 to strain MH429, the phage mutant eL1P12 had the same plating efficiency on the resulting spectinomycin-resistant transductants as on strain MH429. Strain MH423 does not suppress this mutation. We also transduced strains MH423 and MH617 to spectinomycin sensitivity using a strain (CAG12133) from the P1 mapping kit as donor. Spectinomycin-sensitive derivatives of strain MH423 had the same suppression pattern as the spectinomycin-resistant derivatives. Phage mutants eL1P12 and opC105 had a higher plating efficiency on the spectinomycin sensitive derivatives of strain MH617 than the resistant derivatives. Phage mutant M103 plated with the same efficiency on both strains.

4. Discussion

We show here that differences in suppression patterns of Thy$^-$ derivatives of different strains (Herrington *et al.* 1984) are due to differences in the genetic background of the strains.

The parents (D10 and DS4680A) of Thy$^-$ strains which exhibit the different suppression patterns are derived from the same ancestral strain (W6). Strain D10 was constructed in four steps which included an ultraviolet irradiation step and a treatment with nitrosoguanidine. Strain DS4680A was constructed in eight steps including two ultraviolet treatments (Bachmann, 1987; Gesteland, 1966; Hall & Hartl, 1974; B. J. Bachmann, personal communication). Both strains could easily have accumulated additional mutations during their isolation and subsequent propagation.

The difference in suppression spectrum and efficiency that was observed could be due to one or more genetic differences between the strains. We transferred one allele (*tsmA*$^+$) from the D10 derivatives MH429 to the DS4680A derivative, MH423. The effect of this is to broaden the suppression spectrum of strain MH423. However, neither the spectrum nor the apparent efficiency of suppression of the recombinant strain are identical to that of strain MH429. This indicates that there are probably other loci which differ between

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strains D10 and DS4680A and which affect suppression.

Since the *tsmA*$^+$ allele is not sufficient to make the suppression phenotype of strain MH423 the same as that of strain MH429, we asked if the *rpsE* mutation found in strain MH423 had any effect on the suppressions. The *rpsE* gene codes for the ribosomal protein S5. Some mutations of *rpsE* have a ribosomal ambiguity (Ram) phenotype but do not make the cell resistant to spectinomycin. Others, like that in strain MH423, make the cell spectinomycin resistant. Mutants of this type do not have a Ram phenotype (Piepersberg *et al.* 1975; Cabezón *et al.* 1976). The only effect we observed when we compared suppression by strains which were spectinomycin resistant with those that were sensitive was that sensitive derivatives of strain MH617 (the *tsmA*$^+$ DS4680A derivative) had a slightly higher efficiency of suppression. Thus, the effect of the *rpsE* mutation is to slightly restrict the suppression by some Thy$^-$ strains.

The mechanism of suppression by thymine requiring strains is not known although it does appear to occur during translation (Cheung & Herrington, 1982; Herrington *et al.* 1986).

We proposed a model to explain how *thyA* mutations might affect translational fidelity (Cheung & Herrington, 1982; Herrington *et al.* 1986). The thymidylate synthase reaction is the only folate-dependent reaction in which the co-factor is oxidized to dihydrofolate (O'Donovan & Neuhard, 1970) suggesting that this reaction may regulate folate biosynthesis or the distribution of folate cofactors. We propose that thymine requiring strains have different levels or distributions of folates than wild-type cells. This folate imbalance may then lead to changes in tRNA modification patterns so that thymine requiring strains produce tRNAs which are more prone to misreading and frameshifting than those wild-type cells. The broad spectrum of mutations that can be suppressed by Thy$^-$ cells could then be explained if modification of several different species of tRNA were affected. This model provides a number of sites at which environmental or genetic factors could act to modify suppression. Thus, the *tsmA* gene has many possible functions.

The *tsmA* gene could code for a component of the translational apparatus which is important for translational accuracy. Alternatively, it could be important for folate biosynthesis, modification of tRNAs or the physiological effects of *thyA* mutations which result in suppression.

Two observations suggest that the *tsmA* gene affects the physiological consequences of a *thyA* mutation. The growth of the Tsm$^+$ strains on rich medium supplemented with moderate levels of thymidine is less abundant than that of the Tsm$^-$ strains and suppression is less sensitive to inhibition by thymidine. These observations suggest that the net intracellular concentration of thymine nucleotides is affected by *tsmA*. The intracellular concentration of thymine nucleotides is a function of the transport, breakdown and utilization of exogenous thymidine; the availability of deoxyribose-1-phosphate; and the level of endogenous thymidylate synthesis. In addition, phage infection contributes to the nucleotide pools since the host cell DNA is degraded and the phage encodes thymidylate synthase. Since the growth difference between the Tsm$^+$ and Tsm$^-$ cells was observed even in the absence of phage infection we have not considered this contribution further in this discussion.

None of the known genes involved in transport (*nupC*, *nupG* and *tsx*) or utilization of exogenous thymidine (*deo*A, B, C, and R; *cyt*R; *udp*; *dut*; *dcd*; and *nrd*A) (Møllgaard & Neuhard, 1983; Munch-Petersen & Mygind, 1983; Bachmann, 1990) map near the *tsmA* gene. In addition, the growth response of our strains to thymine and thymidine in minimal medium were not consistent with the presence of mutations in known genes which affect the utilization of exogenous thymine or thymidine.

If the *thyA* mutations in the suppressing strains are leaky a strain which expresses the gene at a higher level than another strain would contain more thymidylate synthase activity. We did not detect an influence of the *tsmA* gene on the expression of thymidylate synthase. Furthermore, a mutant strain with a deletion in *thyA* has the same phenotype as the point mutations described here (M. B. Herrington and M. Faraci, unpublished observations).

The *tsmA* gene mapped at 39·4 min on the *E. coli* chromosome. Very few genes have been identified in this region (Bachmann, 1990; Kohara, 1990). Four genes could affect suppression although it is difficult to see how three of them could affect the intracellular thymine nucleotide pools. The *rnd* gene codes for a ribonuclease that appears to be specific for RNAs which resemble tRNA precursors. Although loss of this activity is not detrimental to the cell, overexpression of it results in slower growth (Zhang & Deutscher, 1988). If this enzyme is involved in tRNA processing variations in the amount of enzyme made could affect tRNA pools and thus affect the competition between suppressing tRNAs and release factors. Mutations in the *argS* gene coding for the arginyl-tRNA synthetase could perturb the distribution of charged tRNA and affect the accuracy of translation. The *pabB* gene codes for a subunit of *p*-aminobenzoate synthase (Goncharoff & Nichols, 1984). Alterations in this gene could affect folate biosynthesis and thereby modify the postulated effect of the *thyA* mutation on folate distributions. The third gene in this region, *dnaI* gene was identified by a temperature-sensitive mutation which affects DNA synthesis (Beyersmann *et al.* 1974). Such a defect might alter the intracellular concentration of thymine nucleotides sufficiently to affect the suppression. However, the DNA defect in this strain was later reported to result from a *dnaA* mutation (Von

Meyenburg & Hansen, 1987). From this report it was not evident whether there is a second temperature sensitive mutation mapping near 39 min or if the mapping data (Beyersmann *et al.* 1974) is incorrect. Further characterization of the *tsmA* gene is necessary before we can determine if it is a new gene or one of these previously described genes. An understanding of the function of this gene will assist us to understand the mechanism of suppression by Thy⁻ strains of *E. coli*.

We were the first to demonstrate that mutations affecting a gene involved in DNA metabolism confer a suppression phenotype. Suppressors which, like the *thyA* mutants, suppress several types of mutations have been isolated in other microorganisms (for a review see Herrington, 1989). Some of these affect components of the translational apparatus but in many cases the function of the affected gene is not known. Recent work on the omnipotent suppressor, *sup45*, of the yeast *Saccharomyces cerevisiae* suggests that the *SUP45* gene product is involved in nucleic acid metabolism and that suppression may occur via a pathway similar to that which we proposed for *thyA* mutants. Mutations which render *S. cerevisiae* resistant to the drug novobiocin affect the *SUP45* gene. Novobiocin is known to inhibit the activity of topoisomerases but it is not known yet whether *SUP45* codes for a topoisomerase (Pocklington *et al.* 1990). The *ADE3* gene in *S. cerevisiae* codes for a trifunctional enzyme C_1 tetrahydrofolate synthase which catalyses folate interconversions. Mutations in *ADE3* act as antisuppressors when combined with mutations affecting *SUP45* (Song & Liebman, 1989).

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