JOURNAL OF BACTERIOLOGY, Jan. 1984, p. 126–129
0021-9193/84/010126-04$02.00/0

Vol. 157, No. 1

Suppression by Thymidine-Requiring Mutants of *Escherichia coli* K-12

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Thymidine-requiring strains of *Escherichia coli* isolated by trimethoprim selection often simultaneously acquire the ability to suppress bacteriophage T4 nonsense mutations. Suppression is lost in Thy$^+$ revertants and recombinants, but is sometimes retained in *thyA* plasmid-bearing transformants. Suppression is restricted in Str^r derivatives of the Thy$^-$ mutants, indicating that suppression occurs at the level of translation.

The *thyA*(TS) *Escherichia coli* strain N4316 suppresses a variety of bacteriophage T4 mutants, including one frameshift mutant, many UAA and UGA mutants, and a few UAG mutants (7, 17). This strain requires thymine at 43°C but not at 31°C and no longer suppresses when it is converted to a thymine-nonrequiring strain by reversion or recombination (7). Suppression by strain N4316 is inhibited in the presence of high levels of thymine or thymidine in the assay medium (7).

We obtained similar suppressor mutants by trimethoprim selection for thymine-requiring mutants (5, 22). These mutants, like strain N4316, no longer suppress when they are made thymine independent by replacing the mutant allele with the wild-type allele.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The *E. coli* K-12 strains used in this work are listed in Table 1. *E. coli* B was obtained from S. P. Champe. The *E. coli* B derivative 301 (*thyA101 deoB*) was obtained from G. R. Greenberg. Bacteriophage T4 lysozyme mutants eL1P12, J44, L3, and M103 have been described (7). P1CM was obtained from E. B. Newman.

Media. AB medium is the broth medium described by Apirion (2) and was solidified with 15 g of agar per liter for plates and 6.5 g/liter for soft agar. This medium was used for growing cells and for suppression assays. Minimal medium A (16) was used when a defined medium was required. When required, amino acids were added at a final concentration of 40 μg/ml, and thymidine was added at 50 μg/ml. Trimethoprim medium was minimal medium A containing methionine, thymidine, and trimethoprim (5 μg/ml). Superbroth (14) was used to prepare lysates of P1CM.

Isolation of Thy$^-$ mutants. Thy$^-$ mutants were selected from strains D10, DS4680A, MH142, CR63, and χ342 by plating cells on minimal medium containing trimethoprim and thymidine and incubating them at 31, 37, or 43°C. Mutants were found at a frequency of 10^{-6} to 10^{-7}.

Suppression assays. Suppression was assayed rapidly (7) by using 24-well cluster dishes (Costar). Phage and bacteria were mixed in a well, and 1 ml of AB soft agar was added. Each bacterial strain was tested with four concentrations of phage. A mutant was scored as suppressing if the number of PFU was at least 100 times higher than on the parent strain. Since many of the Thy$^-$ mutants we tested did not grow well on AB medium unless it was supplemented with thymidine, we usually added thymidine at 50 μg/ml for growing cells and at 25 μg/ml in the suppression assay.

Suppression indices (see Table 3) were derived as follows. The efficiency of plating of the T4 nonsense mutants on the Thy$^+$ parent strains and their Thy$^-$ derivatives was determined relative to that on the appropriate permissive host (strain CR63 for M103, strain CA165 for L3, and strain CAJ64 for eL1P12). The efficiency of plating of the frameshift mutant J44 was determined relative to that of strain D10 on lysozyme-supplemented plates. The efficiency of plating on a Thy$^-$ mutant was then divided by that on its Thy$^+$ parent to give the suppression index. If this index was greater than 100, we concluded that the Thy$^-$ mutant suppressed the T4 mutant.

Transductions. Lysates of P1CM were prepared by heat induction of lysogenic cells (16). Transductions were carried out as described by Miller (16).

Construction of partial diploids. The recombinant plasmid pBTA contains the *E. coli thyA*$^+$ gene inserted into the *Hin*dIII site of pBR322 (4). This plasmid complements Thy$^-$ mutants. Plasmid DNA was prepared from strain Reu10(pBTA) and transformed into appropriate recipients by standard methods (8, 15). Ampicillin-resistant transformants were selected and then tested for their thymine requirement. The presence of pBTA in Thy$^+$ transformants was confirmed by isolating plasmid DNA by the rapid plasmid isolation method (6) and comparing its size with that of pBTA by agarose gel electrophoresis.

Chemicals. Common chemicals were reagent grade and were generally obtained from Fisher Scientific Co. Biochemicals were obtained from Sigma Chemical Co.

RESULTS

Suppression by Thy$^-$ mutants. Independently isolated trimethoprim-resistant strains derived from strains D10, DS4680A, MH142, and χ342 were tested to determine whether they suppressed either of the T4 mutants M103 (UAG) or eL1P12 (UGA) at 37°C. They were also tested for their thymidine requirement at 31 and 43°C, since many mutants isolated by trimethoprim selection have a temperature-sensitive requirement for thymidine (1).

A few of the trimethoprim-resistant mutants were Thy$^+$ (Table 2). They probably have a mutation which makes dihydrofolate reductase resistant to inhibition by trimethoprim and were not further characterized. The remainder of

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

Strain	Genotype	Source and reference
CR63	F⁺ *supD60* λˢ	M. C. Ganoza (3)
D10	*metB rna*	M. C. Ganoza (9)
DB313	HfrC *metB proC*	B. Hall
DS4680A	HfrC Δ*(lacZW)4680*	B. Hall (11)
K12*rpsL2*	*leu thr thi lac supE rpsL2*	L. Brakier-Gingras (18)
K12*rpsL60*	*leu thr thi lac supE rpsL60*	L. Brakier-Gingras (18)
MH142	HfrC *metB lacX90*(UAA)	P1CM transduction[a]
MH164	*metB rna thyA721*(Ts)	TM/D10/43[b]
MH165	*metB rna thyA722*	TM/D10/43[b]
MH167	HfrC Δ*(lacZW)4680 thyA723*	TM/DS4680A/43[b]
MH168	HfrC Δ*(lacZW)4680 thyA724*	TM/DS4680/43[b]
MH169	F⁺ *supD60 thyA725*(Ts) λˢ	TM/CR63/43[b]
MH186	*metB rna thyA728*(Ts)	TM/D10/43[b]
N4316	*metB rna thy*(Ts) *sts*	M. C. Ganoza (7, 17)
Rue10(pBTA)	F⁻ *hsdS20 hsdR⁻ hsdM⁻ recA13 ara14 proA2 lacY1 galK12 rpsL20 xyl-5 mtl-1 supE44 thy* λ⁻ (pBTA)	M. Belfort (4)
χ342	*proC29 metB1 relA1* λ⁻ *spoT1*	B. Bachmann, CGSC (4515)[c]
525	*lacX90*(UAA)	M. C. Ganoza

[a] Donor strain 525, recipient strain DB313, selected for Pro⁺ Lac⁻.

[b] Strains selected on trimethoprim plates from strain D10, DS4680A, or CR63 at 43°C.

[c] Coli Genetic Stock Center, B. Bachmann, curator; number is their strain designation.

the strains required thymidine at 43°C. Of these, 45% were Thy(Ts) and did not require thymidine at 31°C. Such Thy(Ts) mutants were found even when the selection was done at 31°C, which is consistent with our observation that strain N4316 [Thy(Ts)] is resistant to trimethoprim at 31°C (7). The distribution of suppressor phenotypes among Thy⁻ mutants was determined by the fast suppression assay (Table 2). Most of the Thy⁻ mutants of strains D10 and DS4680A suppressed, whereas few mutants from strains MH142 and χ342 did so. We have no explanation for this difference. Suppressing derivatives of strain D10 suppressed the UGA mutant eL1P12 (other T4 mutants were not tested in this experiment), whereas the other suppressing strains suppressed the UAG mutant M103 but not eL1P12. Both Thy⁻ and Thy(Ts)⁻ mutants had suppressor activity.

Several suppressor strains derived from strains D10 and DS4680A were selected for further characterization. Also, a suppressing Thy(Ts) derivative of strain CR63 was characterized. These strains were assayed for their ability to suppress the T4 nonsense mutants eL1P12 (UGA), M103 (UAG), and L3 (UAA) and the frameshift mutant J44 at 31 and 37°C (Table 3). Strain N4316 was included for comparison, since it suppresses these four T4 mutants at 37°C but not at 31°C (7). The three strain D10 derivatives, MH164 [Thy(Ts)], MH165 (Thy⁻), and MH186 (Thy⁻), suppressed mutants M103 and L3 at both temperatures and eL1P12 at 37°C. Strain MH186 suppressed the frameshift mutant J44, whereas strains MH164 and MH165 did not. The strain DS4680A derivatives MH167 (Thy⁻) and MH168 (Thy⁻) suppressed M103 only at 37°C and did not suppress the other nonsense mutants. Strain MH167 suppressed the frameshift mutant at 37°C. The strain CR63 derivative MH169 [Thy(Ts)] suppressed L3 and eL1P12 at both temperatures but did not suppress the frameshift mutant. It suppressed the UAG mutant M103 because it carries the *supD* suppressor tRNA (Table 1). This strain grows normally and shows suppression patterns similar to those of other Thy⁻ strains. Thus the simultaneous presence of two suppressor activities causes no obvious problems.

TABLE 2. Distribution of suppressing and nonsuppressing strains among trimethoprim-resistant mutants

Parent strain	Selection temp (°C)	No. of strains:				
		Suppressing		Nonsuppressing		
		Thy⁻	Thy(Ts)	Thy⁻	Thy(Ts)	Thy⁺
D10	43	1	9	0	0	0
D10	31	5	12	1	0	1
DS4680A	43	1	7	0	0	0
DS4680A	31	7	0	2	0	0
MH142	43	2	0	18	2	0
MH142	37	1	1	17	3	0
χ342	37	0	3	0	7	4

These results indicate that Thy⁻ and Thy(Ts) mutants of *E. coli* K-12 often simultaneously acquire the ability to suppress mutants of phage T4. We have also assayed suppression by a Thy⁻ mutant of *E. coli* B, strain 301, which carries the *thy-101* mutation and requires thymine at 37°C but not at 25°C (19). The suppression indices of eL1P12 on strain 301 were 2.5×10^3 at 31°C and 7.4×10^3 at 37°C, indicating that this previously described Thy(Ts) derivative of *E. coli* B suppresses.

Suppression in the presence of high levels of thymidine. Suppression by strain N4316 is inhibited when large amounts of thymidine are added to AB medium (7). We tested the effect of adding thymidine at 500 μg/ml in fast suppression assays of the new suppressor mutants. In these assays, and those in the following sections, T4 mutant eL1P12 was tested with strain D10 and CR63 derivatives and T4 mutant M103 was tested with strain DS4680A derivatives. Suppression by mutant strains MH164, MH165, MH167, MH168, and MH186 was inhibited at high levels of thymidine, but suppression by mutant strain MH169 was not inhibited.

Suppression by Thy⁺ derivatives. Strain N4316 loses the ability to suppress when it is made Thy⁺ by either reversion

TABLE 3. Suppression by Thy⁻ strains

Strain	Phenotype	Temp (°C)	Suppression index with mutant phage:			
			M103 (UAG)	L3 (UAA)	eL1P12 (UGA)	J44 (FS)[a]
N4316	Thy(Ts)	31	1.3	1.0	0.26	0.82
		37	4.2×10^4	2.0×10^5	1.6×10^5	460
MH164	Thy(Ts)	31	3.8×10^3	6.6×10^4	1.9	1.0
		37	6.5×10^3	4.9×10^4	2.9×10^3	32
MH165	Thy⁻	31	2.0×10^4	5.2×10^6	15	1.2
		37	7.1×10^3	6.7×10^4	1.3×10^3	Var[b]
MH186	Thy⁻	31	120	3.5×10^3	2.8	0.0068
		37	2.9×10^4	7.3×10^4	1.2×10^5	320
MH167	Thy⁻	31	0.03	0.07	1.3	0.74
		37	614	7.2	2.0	220
MH168	Thy⁻	31	0.12	0.07	0.03	Var
		37	1.1×10^3	6.5	0.29	83
MH169	Thy(Ts)	31	0.32	3.1×10^5	6.6×10^3	0.85
		37	0.53	Var	129	Var

[a] FS, Frameshift mutation.

[b] Var, Results varied at least 100-fold when assayed three to four times.

or recombination (7). We attempted to select spontaneous revertants of the mutant strains MH164, MH165, MH167, MH168, MH169, and MH186 by plating them on minimal medium at 43°C. Spontaneous revertants could not be isolated from mutant strain MH165, MH167, or MH169 and were infrequent (10^{-10} to 10^{-11}) from mutant strains MH164, MH168, and MH186. Treatment with aminopurine did not induce reversion. We constructed Thy$^+$ recombinants by P1CM-mediated transductions, using strain K12*strA2* as the donor and selecting on minimal medium at 43°C. None of the Thy$^+$ derivatives suppressed.

The recombinant plasmid pBTA (*thyA*$^+$) was introduced into strain N4316 and the six new mutant strains by transformation, and the transformants were selected for ampicillin resistance. The transformants were Thy$^+$ and plasmid DNA the same size as pBTA could be isolated from them. Some of these partial diploids [strains N4316(pBTA) and MH169(pBTA)] suppressed as well as their parent strains; some [strains MH165(pBTA) and MH168(pBTA)] suppressed less efficiently than their parents; and the others [strains MH164(pBTA), MH167(pBTA), and MH186(pBTA)] did not suppress. There is no apparent pattern to these results.

Suppression by streptomycin-resistant derivatives. Mutations affecting *rpsL*, which make the cell streptomycin resistant, often restrict suppressors acting at the ribosomal level (10). We constructed streptomycin-resistant derivatives of mutant strains N4316, MH164, MH165, MH167, MH168, and MH169 by P1CM-mediated transductions, using strains K12*rpsL2* and K12*rpsL60* as donors. All Str^r derivatives were Thy$^-$. The streptomycin-resistant derivatives of mutant strains N4316, MH164, MH165, MH167, and MH168 did not suppress. The streptomycin-resistant derivatives of mutant strain MH169 did suppress. Although suppression by strain MH169 was not inhibited when thymidine (500 μg/ml) was added in the assay, suppression by the Str^r derivative was inhibited.

DISCUSSION

We have shown that many Thy$^-$ and Thy(Ts) derivatives of *E. coli* K-12 acquire the ability to suppress some mutations in phage T4. These strains probably carry mutations in *thyA*, since extensive mapping of other Thy$^-$ mutants isolated by trimethoprim selection indicates that their mutations all map in the *thyA* gene (1), and the thymidine requirement of our strains was complemented by a plasmid carrying the wild-type *thyA* gene.

Suppression by the Thy$^-$ and Thy(Ts) mutants probably results directly from certain types of *thyA* mutations rather than from two mutations, one affecting *thyA* and the other affecting a suppressor gene, since the suppressor phenotype is frequent among the mutants and is lost when mutants are reverted to Thy$^+$. Both Thy(Ts) and Thy$^-$ mutants suppress, indicating that different types of *thyA* mutants are capable of producing the suppressor phenotype. This is further supported by the differences in level and pattern of suppression of independently isolated mutants derived from the same strain and their response to the plasmid.

The genetic background of a strain influences both the frequency with which Thy$^-$ suppressors can be isolated and their suppression patterns. The available information about the genotypes of the strains used here does not provide any clues to genetic differences which could influence the production of a suppressor phenotype.

Suppression by Thy$^-$ and Thy(Ts) mutants can be inhibited by adding thymidine, suggesting that suppression results when intracellular levels of thymidylate are low. When *E. coli* cells infected with phage T4 are starved for thymidylate, there is an increase in the rate of mutation of T4 (20). Although apparent suppression by the mutant strains might actually represent enhanced reversion of our T4 tester strains, we do not think this is likely since we have excluded it as an explanation of suppression by the similar mutant strain N4316 (13).

Suppression by strain N4316 and the newly isolated mutant strains occurs at the level of translation, since five of the six strains examined showed no suppression when they were made streptomycin resistant by the introduction of restrictive *rpsL* alleles. Although streptomycin-resistant derivatives of the sixth strain, MH169, were able to suppress, their suppression, unlike that of their parent (strain MH169), could be inhibited by thymidine. This implies that the *rpsL* mutations also affect the suppression phenotype of strain MH169. We proposed that suppression by strain N4316 results from alterations in tetrahydrofolate-mediated modification of tRNA (7) caused by the mutation which makes the cell Thy$^-$. The altered tRNAs may more readily slip through the ribosomal screens (10) for noncognate codon-anticodon interactions than do normally modified tRNAs, allowing suppression. Since mutations affecting *rpsL* enhance the ability of the ribosome to detect codon-anticodon mismatches (10, 21, 23), they restrict suppression. These results indicate a link between thymidylate synthesis and the fidelity of translation.

We previously reported that the suppressor in strain N4316 mapped near *metB* (12). This was based on transductions in which strain N4316 was used as both recipient and donor. Recombinant clones with suppressor activity were purified from transductant colonies without loss of the suppressor activity and were stored for several years at −20°C in glycerol. When these strains were recovered from storage, they no longer suppressed. We have now attempted to reconstruct similar recombinants, using many of the same crosses as well as different ones. Although a few recombinants appeared to suppress on preliminary assay, none suppressed when purified. We cannot account for this discrepancy between the previous results (12) and our present work.

ACKNOWLEDGMENTS

We thank L. Brakier and especially E. B. Newman for their helpful comments during the writing of this paper. We also thank summer research assistants A. Guindi, N. Gavrilopoulos, P. K.-F. Cheung, and W. Hodge.

This research was supported by grant A6727 from the Natural Sciences and Engineering Council of Canada.

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