JOURNAL OF BACTERIOLOGY, Feb. 1977, p. 1141-1143

Vol. 129, No. 2
Printed in U.S.A.

NOTES

Genetic Characterization of the Temperature-Sensitive and Suppression Phenotypes of *Escherichia coli* Mutant N4316

M. B. HERRINGTON[1] AND M. C. GANOZA*

Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario M5G1L6, Canada

Received for publication 29 August 1976

Escherichia coli mutant N4316 is temperature sensitive and exhibits temperature-dependent suppression. These phenotypes are due to separate genes, as shown by reversion and mapping studies. The suppressor mutation was mapped and lies near *argF*.

Escherichia coli mutant N4316 is a starvation temperature-sensitive (*sts*) mutant that exhibits temperature-sensitive growth (11). It was isolated by a procedure that enriches for mutations affecting translation (2, 10). In the starvation temperature-sensitive selection technique, the number of ribosomes is reduced by growing mutagenized cells in minimal medium (starvation). Colonies that do not survive "starvation" at 43°C are then selected by replica-plating as potential protein synthesis mutants (10). Protein synthesis in the mutant strain N4316 is indeed defective at the nonpermissive temperature (43 to 45°C), both in whole cells (unpublished data) and in cell-free extracts (4, 9, 11). Protein synthesis in extracts is temperature sensitive only when natural messenger ribonucleic acids are used (6, 12), and it can be restored by adding a new protein factor isolated from the ribosome-free supernatant of wild-type cells (4, 9, 11, 12).

In addition, strain N4316 suppresses the nonsense codons UAA and UGA at 36°C but not at 31°C (11). This temperature-dependent suppression (*sut*) of codons involved in protein chain termination suggested that the mutant was defective at this stage of protein synthesis (11). Although there is a moderate effect of the mutation on chain termination (4, 9, 11), the major effect appears to be on earlier events (6, 12). This suggested that the different phenotypes of strain N4316 might not be pleiotropic effects of one mutation as orginally thought (11). We examined this problem by genetic mapping and reversion studies.

[1] Present address: Department of Biological Sciences, Concordia University, Sir George Williams Campus, Montreal, Quebec H3G 1M8, Canada.

The *metB*$^+$ locus from strain RG11 (see Table 1 for strain descriptions) was introduced into strain N4316 by transduction mediated by P1Cm (obtained from R. Grant). The MetB$^+$ recombinants were tested for their ability to suppress (Table 2, experiment 1), and 11% had the donor phenotype, suggesting that *metB* and *sut* are linked. In this cross 27% of the *metB*$^+$ recombinants grew at the nonpermissive temperature (45°C). However, this was likely an artifact of the method used to measure temperature sensitivity, because in no other cross were *metB* recombinants also recombinant for *sts*. We have since found that the most reliable measure of temperature sensitivity is the number of viable cells at permissive and nonpermissive temperatures.

We concentrated on mapping *sut* because of the problems we had in measuring temperture sensitivity. We found that *sut* was cotransduced at a frequency of 31% with *metA* (Table 2, experiment 2) and 74% with *argE* (Table 2, experiment 3). This indicates that *sut* is located close to *argE*, which is near 88 min on the *E. coli* chromosome (3).

In all crosses suppression was tested using the T4 UGA mutant strain eL1P12. Several of the Arg$^+$ Sut$^+$ recombinants (Table 2, experiment 3) were tested for their ability to suppress T4 UAA mutant strain eL5 (Table 3). All recombinants had a (plaque-forming units per milliliter)/(plaque-forming units per milliliter on strain N4316) ratio much higher than the nonsuppressing parental strain AB1115, indicating that they suppress UAA as well as UGA. This implies that the suppression of UGA and UAA is due to the same mutation.

Growth of these recombinants at nonpremis-

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

Strain	Genotype[a]	Source and reference
AB1115	*thi-1 argE3 his-4 proA2 thr-1 leu-6 mtl-1 xyl-5 ara-4 galK2 lacY1 supE44*	CGSC[b]
AB1927	Hfr *metA28 argH1 purF1 xyl-7 supE44*?	CGSC (7)
D10[c]	*metB thy rna*	S. Phillips (5)
MH126[d]	*thy rna sts sut*$^-$	Met$^+$ transduction from AB1115 to N4316
N4316[d]	*metB thy rna sts sut*$^-$	S. Phillips (11) This mutant was induced with nitrosoguanidine
RG11	*his rpsL (chlD-pgl)* λ^s	R. Grant

[a] Symbols are those used by Bachmann et al. (3).
[b] CGSC, Coli Genetic Stock Center, Yale University (B. J. Bachmann, Curator).
[c] Strain D10 is the parent of strain N4316.
[d] The symbol *sts* is used to refer to temperature-sensitive growth, and the new symbol *sut* is used to refer to temperature-dependent suppression. Suppressing strains are Sut$^-$, whereas the wild-type nonsuppressing strains are Sut$^+$.

TABLE 2. *Cotransduction of sut with metB, metA, and argE*[a]

Expt	Donor	Recipient	Selected marker	Unselected marker	No.	Frequency (%)
1	RG11 (*sut*$^+$)	N4316 (*sut*$^-$)	MetB	Sut$^-$	89	89
				Sut$^+$	11	11
2	MH126 (*sut*$^-$)	AB1927 (*sut*$^+$)	MetA	Sut$^-$	11	31
				Sut$^+$	25	69
3	N4316 (*sut*$^-$)	AB1115 (*sut*$^+$)	ArgE	Sut$^-$	56	74
				Sut$^+$	20	26

[a] P1Cm transductions were performed as described by Miller (8). Recombinants were partially purified by picking onto selective agar. Overnight cultures in broth medium (1) were used as host cells for plating of T4 UGA mutant strain eL1P12 (obtained from G. Streisinger) as described (11). A recombinant was Sut$^+$ if the number of plaque-forming units per milliliter on it at 36°C was similar to that on N4316. At 36°C the number of plaque-forming units per milliliter on suppressing strains was at least 1,000-fold higher than on nonsuppressing strains, whereas at 31°C there was essentially no difference between strains.

TABLE 3. *Lack of linkage between sut and sts*

Strain	Sut[a]	(CFU/ml at 45°C)/(CFU/ml at 31°C)[b]	(PFU/ml on strain)/(PFU/ml on strain N4316)[c]
N4316	−	4.1×10^{-4}	1.0
AB1115	+	1.09	8.5×10^{-3}
1	−	1.14	1.6
2	−	1.32	1.4
3	−	1.00	1.4
4	−	1.26	0.95
6	−	1.41	1.5
7	−	1.45	1.1
8	−	1.05	1.2
10	−	0.48	1.1
11	−	1.19	1.2
12	−	0.82	1.3

[a] Suppression characteristic of the recombinants as determined with strain eL1P12.
[b] Colony-forming units (CFU) per milliliter were determined on broth agar (1) at 31 and 45°C.
[c] Plaque-forming units (PFU) per milliliter on different strains were determined at 36°C as described in Table 2, except that the T4 UAA mutant eL5 (obtained from G. Streisinger) was used.

sive and permissive temperatures was measured (Table 3). All resembled strain AB1115, the non-temperature-sensitive parent, indicating that recombinants for *sut* are not recombinant for *sts*.

When we examine non-temperature-sensitive revertants of strain N4316 (Table 4), we find that they resemble strain D10 (parent of strain N4316) in their growth characteristics but strain N4316 in their support of growth of strain eL1P12. Since recombinants for *sut* are not recombinant for *sts*, and revertants from Sts$^-$ to Sts$^+$ are still Sut$^+$, we can conclude that the temperature-sensitive growth of strain N4316 and the temperature-dependent suppression are due to separate genes.

We wish to thank Martha Doherty for technical assistance.

This work was supported by a grant from the Medical Research Council of Canada. M.B.H. was a recipient of a Medical Research Council of Canada Postdoctoral Fellowship and the Hume-McEachern Fellowship.

TABLE 4. *Characteristics of parental, mutant, and revertant strains*

Strain[a]	(CFU/ml at 45°C)/ (CFU/ml at 31°C)[b]	(PFU/ml on strain)/ (PFU/ml on strain N4316)[c]
N4316	$<10^{-4}$	1.0
D10	0.9	6.4×10^{-4}
R3	0.76	0.64
R5	0.54	0.64
R6	0.73	0.67
R8	0.64	0.62
R31	0.94	0.59
R32	1.22	0.67
R44	0.69	0.57
R45	1.00	1.1
R46	1.00	0.74
R50	0.83	0.41
R52	0.81	0.77

[a] Non-temperature-sensitive revertants (prefixed R) were selected by plating strain N4316 on broth agar at 45°C. Colonies were selected and purified. Overnight cultures in broth were assayed for colony-forming units (CFU) per milliliter as in Table 3. Of the 59 putative revertants tested, only 20 had a ratio (CFU per milliliter at 45°C)/(CFU per milliliter at 31°C) greater than 0.5, but many of the others had a ratio greater than that of strain N4316 but less than 0.5. Only those with a ratio greater than 0.5 were considered non-temperature sensitive.

[b] Determined as described in Table 3, except that strain eL1P12 was used in the phage assays.

[c] PFU, plaque-forming units.

LITERATURE CITED

1. **Apirion, D.** 1966. Altered ribosomes in a suppressor strain of *Escherichia coli*. J. Mol. Biol. 16:285–301.
2. **Apirion, D., S. L. Phillips, and D. Schlessinger.** 1969. Approaches to the genetics of *E. coli* ribosomes. Cold Spring Harbor Symp. Quant. Biol. 34:117–128.
3. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. 40:116–167.
4. **Ganoza, M. C., J. P. Van der Meer, N. Debrecini, and S. L. Phillips.** 1973. Mechanism of protein chain termination: further characterization of a mutant defective in a new protein synthesis factor. Proc. Natl. Acad. Sci. U.S.A. 70:31–35.
5. **Gesteland, R. F.** 1966. Isolation and characterization of ribonuclease I mutants of *Escherichia coli*. J. Mol. Biol. 16:67–84.
6. **Herrington, M. B., M. J. Doherty, and M. C. Ganoza.** 1975. Translation of natural mRNAs *in vitro* by extracts of a mutant in protein synthesis. Nature (London) 256:678–679.
7. **Howard-Flanders, P., R. P. Boyce, and L. Theriot.** 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics 53:1119–1136.
8. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Phillips, S. L.** 1971. Termination of messenger RNA translation in a temperature-sensitive mutant of *Escherichia coli*. J. Mol. Biol. 59:461–472.
10. **Phillips, S. L., D. Schlessinger, and D. Apirion.** 1969. Mutants in *Escherichia coli* ribosomes—a new selection. Proc. Natl. Acad. Sci. U.S.A. 62:772–777.
11. **Phillips, S. L., D. Schlessinger, and D. Apirion.** 1969. Temperature dependent suppression of UGA and UAA codons in a temperature sensitive mutant of *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 34:499–503.
12. **Van der Meer, J. P., and M. C. Ganoza.** 1975. Purification and characterization of a new factor which restores protein synthesis in a conditionally lethal mutant of *Escherichia coli*. Eur. J. Biochem. 54:229–237.