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Genetic Analysis of an *Escherichia coli* Syndrome

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A mutant strain of *Escherichia coli* that fails to recover from prolonged (72 hr) starvation also fails to grow at 43 C. Extracts of this mutant strain show an increased ribonuclease II activity as compared to extracts of the parental strain, and stable ribonucleic acid is degraded to a larger extent in this strain during starvation. Ts^+ transductants and revertants were tested for all the above-mentioned phenotypes. All the Ts^+ transductants and revertants tested behaved like the Ts^+ parental strain, which suggests that all the observed phenotypes are caused by a single *sts* (starvation-temperature sensitivity) mutation. The reversion rate from sts^- to sts^+ is rather low but is within the range of reversion rates for other single-site mutations. Three-point transduction crosses located this *sts* mutation between the *ilv* and *rbs* genes. The properties of sts^+/sts^- merozygotes suggested that the Ts^- phenotype of this mutation is recessive.

In classical microbial genetics, great strides in our understanding of basic biological processes were achieved with the use of auxotrophic mutants. To apply the microbial genetic approach to indispensable functions, some kind of conditional mutants had to be used. Some such mutants could be extremely pleiotropic, especially those involved with the production or regulation of key macromolecules. Here we shall describe the analysis of a bacterial syndrome apparently caused by such a single mutation.

Recently, we isolated a class of temperature-sensitive mutants of *Escherichia coli* that also show an increased sensitivity to carbon starvation, (designated *sts* mutants, for starvation-temperature sensitivity; see references 3 and 14). These mutants cannot recover from starvation and are also temperature-sensitive (grow at 30 C but not at 43 C). Among the members of this class, we identified strains altered in their protein synthetic machinery, in ribosomal (3, 14) as well as in supernatant components (15). It seemed reasonable to assume that strains with increased messenger ribonuclease (mRNase) activity would also fail to recover from a period of extended starvation if, for instance, the level of a messenger ribonucleic acid (mRNA) molecule required for the turning over processes should fall below a critical level, since ribonucleic acid (RNA) synthesis is reduced under such conditions (10). We therefore tested sonic extracts of 130 such *sts* strains and found 8 strains with an increased ribonuclease activity. The studies reported here were carried out with one of these strains, designated N4752. This strain seems to have a modified ribonuclease activity when grown at the nonpermissive temperature (Lennette, Gorelic, and Apirion, *manuscript in preparation*). The modified ribonuclease activity seems to be ribonuclease II (Gorelic and Apirion, *manuscript in preparation*). In this strain, stable RNA is degraded to a larger extent than in the parental strain during starvation (Lennette and Apirion, *manuscript in preparation*).

In the experiments described here, we deal with four phenotypes of this strain: temperature sensitivity, failure to recover from prolonged starvation, increased ribonuclease activity, and increased degradation of stable RNA during starvation.

The results suggest that all these phenotypes are caused by a single mutation which maps between the genes *ilv* and *rbs* (19) on the *E. coli* map.

MATERIALS AND METHODS

With the exception of tetrazolium indicator agar (13), all growth media were prepared as described by Apirion (2). Yeast-RNA-soft agar contained per liter, 25 g of yeast RNA and 7 g of agar and was in 0.1 M ethylenediaminetetraacetate and tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride, 0.5 M, *p*H 7.0 to 7.5.

Strains. Strains used and their characteristics are shown in Table 1. Most of the work described here was carried out with strain N4752. This strain, like its parental strain 112-130, can grow on minimal medium supplemented with L-cysteine and L-leucine, or on rich medium at 30 C, but it fails to grow on both media at 42 C whereas the parental strain can grow on both media at 42 C. This strain fails to recover from pro-

longed (72 hr) carbon source starvation at 42 C whereas the parental strain can recover. (Recovery after starvation at 30 C was not tested.) This strain has an increased ribonuclease activity in extracts, which is easily manifested only when cultures are transferred to 42 C for about 0.5 hr. In this strain, degradation of stable RNA is increased during starvation for a carbon source but, again, is readily observable only when cultures are starved at 42 C.

Mutagenesis. Mutagenesis was carried out as described by Apirion (2).

Prolonged mating. Prolonged mating was carried out as described by Curtiss et al. (5).

Preparation of P1kc phage and transduction. Preparation of P1kc phage and transduction was carried out as described by Apirion (2), with the following modifications. After the phage lysed the donor strain, 3 to 4 ml of nutrient broth was pipetted on top of the soft agar overlay and allowed to sit at room temperature for 3 to 5 hr. (This was suggested by C. Yanofsky.) The broth was transferred to a tube containing 0.3 ml of chloroform. The contents were mixed vigorously and centrifuged at 9,000 × *g* for 15 min at room temperature. The aqueous layer containing the phage was removed.

The lysate of P1kc grown on the desired donor was diluted 1:10 with dilution buffer. A 1.5-ml amount of the diluted lysate was pipetted into a small glass petri dish and subjected to ultraviolet irradiation (with shaking) for 25 sec, about 14 inches (36 cm) from a 15-w General Electric mercury lamp. (About 95% of the plaque-forming units are inactivated under these conditions.) The ultraviolet irradiation is carried out just before the transduction experiment.

To isolate Ts$^+$ transductants, infected recipient cells were plated on broth-agar plates and incubated at 30 C for 6 to 8 hr before incubation at 42 C. To isolate transductants for amino acid markers, infected recipients were plated on minimal agar plates supplemented with the amino acids required by the recipient strain, except for the one that the selection was carried out for. The plates were incubated at 30 C for 48 hr. Transductants from each experiment were isolated, purified, and tested for their markers.

Plate assay for Ts and Sts phenotypes. Master plates of the strains to be tested were prepared and grown at 30 C on broth agar plates for 24 hr. To test for the Ts phenotype, the clones were replicated to another broth-agar plate prewarmed to 42 C. The plate was incubated for 24 hr at 42 C. Temperature-sensitive (Ts$^-$) strains would not grow under these conditions. To test for the Sts phenotype, the clones on the master plate were replicated to phosphate-agar plates and incubated at 42 C for 3 days or longer. The clones were then transferred from the phosphate plate to a fresh broth-agar plate by replication and incubated at 30 C. The wild-type control can recover even after 5 to 6 days of starvation on the phosphate plate, but the starvation-sensitive strains (sts$^-$) cannot.

Labeling of stable RNA. For labeling of stable RNA, final concentration of 5 μCi/ml of ^{3}H-uracil was added to an exponentially growing culture (optical den-

TABLE 1. *List of strains*

Strain	Markers[a]	Mating type and direction of transfer	Source[b]
112-130	*cys leu rns-19 gal str*	F$^-$	A19 × 112-123
N141	*thi ilv arg-3 his pro mal lac gal xyl*	F$^-$	Ng. AB774
N485	*aroE thi spc*	F$^-$	Sp. AT2472
N486	*metA aroE spc rns-19*	F$^-$	Tr. N485 → D10
N1126	*ilv trp tna phoS lac xyl str*	F$^-$	CGSC 4520
N1127	*thi ilv arg metE his trp proA mtl gal mal*	F$^-$	CGSC 3509
N1129	*rbs thi*	Hfr P055	CGSC 4531
N1130	*trp phoS rbs lac xyl str*	F$^-$	Tr. N1129 → N1126
N3615	*arg met his trp leu recA mtl xyl malA gal lacY str*	F′ 133 ilv$^+$ argE$^+$ (←)	CGSC 4265
N4752	*cys leu rns-19 gal str sts-4752*	F$^-$	Ng. 112-130
N5152	*cys leu rns-19 gal str sts-4752 spc*	F$^-$	Tr. N486 → N4752
N5352	*cys sts-4752 argE str spc*	F$^-$	AB492 × N5152
N3	*metB ade xyl lac gal mal*	Hfr P012	Sp. JC12
AB492	*thi arg lac mal mtl xyl str*	Hfr P044	E. A. Adelberg (Yale Univ.)

[a] Markers are designated by letters and allele numbers, when known, according to Demerec et al. (7); *lac xyl gal mal mtl rbs* refer, respectively, to the inability to ferment lactose, xylose, galactose, maltose, mannitol, and ribose; *spc* and *str* indicate resistance to spectinomycin and streptomycin; *rns* refers to a lack of ribonuclease I; *phoS* designates constitutivity for alkaline phosphatase; and the other markers indicate auxotrophy for the L-amino acids and a requirement for thiamine (*thi*).

[b] A19 and D10 were from Gesteland (9); 112-123 was from Apirion (2); Ng. AB774 and Sp. JC12 were from Apirion and Schlessinger (4); Sp. AT2472 was from Pittard and Wallace (16); and CGSC 4520, 3509, 4531, and 4265 were sent to us by B. Bachmann, Coli Genetic Stock Center, Yale University. Abbreviations: Tr., transduction; Ng., isolated after treatment with nitrosoguanidine of the indicated strain; sp., spontaneous mutant from the indicated strain.

sity at 560 nm, 0.1) in minimal or glycerol medium. The cells were grown for at least three generations before the experiment was started.

Starvation. An exponentially growing culture in glycerol medium (optical density at 560 nm, 0.5 to 0.6) was centrifuged at 9,000 $\times$ *g* at room temperature for 5 min, washed once in base medium, and resuspended in the original volume of base medium. The starving culture was incubated at either 30 C or at 42 C as indicated.

Harvesting of cells. Cells were harvested by pouring the culture on crushed ice. The chilled culture was centrifuged at 5 C for 5 min at 9,000 $\times$ *g*. The pellet was washed once in the desired buffer. The final cell pellet was frozen and stored at −20 C. The pellet can be resuspended in the same buffer and frozen in a three- to fivefold concentrated suspension.

Preparation of crude extracts. The frozen cell suspension was thawed and disrupted by sonic treatment for 30 to 40 sec (Bronwill Biosonik). Deoxyribonuclease was added to the broken cells at a final concentration of 10 μg/ml. The cell debris was removed by centrifugation at 10,000 $\times$ *g* for 20 min at 5 C. The supernatant fluid is the crude extract.

Determination of protein in crude extracts. Crude extract from a cell suspension with 5 $\times$ 10^9 cells/ml was prepared by sonic disruption as described. The protein concentration of the crude extract was measured by the method of Lowry et al. (11). The optical density of the crude extract at 260 nm was also measured; about 75 μg of crude extract protein per ml corresponds to an optical density of 1.0 at 260 nm. Routinely, the protein concentration of crude extract was estimated from its optical density at 260 nm with the conversion factor of 75 μg per ml per optical density (at 260 nm) unit of 1.0.

Ribonuclease assays. Assays for ribonuclease II were done as described by Spahr and Schlessinger (18). The enzyme sources were extracts prepared by sonic treatment of cells in TM4. A 50-μliter amount of the assay mixture contained 2 mmoles of Tris-hydrochloride (*p*H 7.6), 0.4 mmoles of magnesium acetate, 5 mmoles of KCl, 1 μg of ^{3}H-polyuridylic acid (poly U), and 10 μg of protein. The mixture was incubated at 42 C for 10 min, and the reaction was stopped with 1.0 ml of ice-cold 95% ethanol, followed by the addition of 50 μliters of yeast carrier RNA. The reaction tubes were centrifuged at 6,000 $\times$ *g* for 15 min at 5 C. A 0.5-ml amount of the supernatant fluid was pipetted into a scintillation vial containing 10 ml of Bray's solution and counted.

Reagents. The reagents used were TM2 buffer, TM4 buffer, carrier RNA, scintillation fluid, and Bray's solution. TM2 buffer consisted of 0.01 M Tris-hydrochloride (*p*H 7.6), and 0.01 M $MgCl_2$. TM4 buffer consisted of 0.01 M Tris-hydrochloride (*p*H 7.6) and 0.1 mM $MgCl_2$. Carrier RNA was: 0.1 M Tris-hydrochloride (*p*H 7.6), 0.6 M NaCl, 5 mg of yeast RNA per ml, and 0.05 M $CaCl_2$. Scintillation fluid consisted of: 1 liter of toluene, 3.97 g of PPO (2,5-diphenyloxazole), and 0.1 g of POPOP (1,4-bis-[2-(5-phenyloxazolyl)]-benzene). Bray's solution contained: 1 liter of dioxane (1,4-diethylene dioxide), 60 g of naphthalene, 100 ml of absolute methanol, 20 ml of ethylene glycol, 4 g of PPO, and 0.2 g of POPOP.

Radioisotopes. Radioisotopes used were: ^{3}H-poly U, 2.8 μCi/mg, from Miles Laboratories; ^{3}H-uracil, 20.5 Ci/mmole, from Schwarz Bioresearch, Inc.; and ^{14}C-uracil, 54.8 mCi/mmole, from Schwarz Bioresearch, Inc.

RESULTS

Transduction frequency of the ts gene. If the Ts^- mutation in N4752 is a single-site mutation, its transduction frequency should be similar to that of another marker. We compared the transduction frequencies of the Ts^+ marker with that of a *spc* marker (1, 6). A P1kc transducing phage lysate was prepared from the donor strain N486 (Ts^+ Spc-R), and it was used to infect a culture of N4752. Separate selections were carried out for Ts^+ and for Spc-R transductants. The transduction frequency was similar for both markers. In a typical experiment, the numbers obtained were 247 Ts^+ and 382 Spc-R transductants from about 10^8 recipient cells. No cotransduction between the two genes was observed.

Reversion rate and frequency from Ts^- to Ts^+. Two hundred and seven 1-ml cultures in nutrient broth, started from individual colonies of N4752, were grown overnight at 30 C. The final cell density was about 5 $\times$ 10^9 cells/ml. A 0.4-ml amount of each culture was plated on broth-agar plates and incubated at 42 C. Of the 207 cultures, 121 had no Ts^+ revertants, 76 had 1 to 3 revertants, 6 had between 4 and 10, and 4 had between 10 and 50. The total number of revertants was 282. The reversion rate, calculated from the zero revertant class, was 1.8 $\times$ 10^{-10} (12), whereas the revertant frequency was 6.9 $\times$ 10^{-10}.

Phenotypes of Ts^+ transductants and revertants. If the four observed phenotypic differences between N4752 and its parental strain 112-130 are due to a single mutation, the change of one characteristic to that of the parental strain should be accompanied by corresponding changes in the three other characteristics. To test this possibility, Ts^+ transductants and revertants were tested for each of the remaining three characters.

Figure 1 shows the distribution of 44 Ts^+ transductants and 87 Ts^+ revertants with respect to ribonuclease II activity. Similarly, Fig. 2 shows the distribution of 44 Ts^+ transductants and 45 Ts^+ revertants with respect to long-labeled RNA degradation during starvation. All of the Ts^+ transductants and revertants tested resembled the parental strain 112-130 and not the mutant strain N4752 with respect to these two

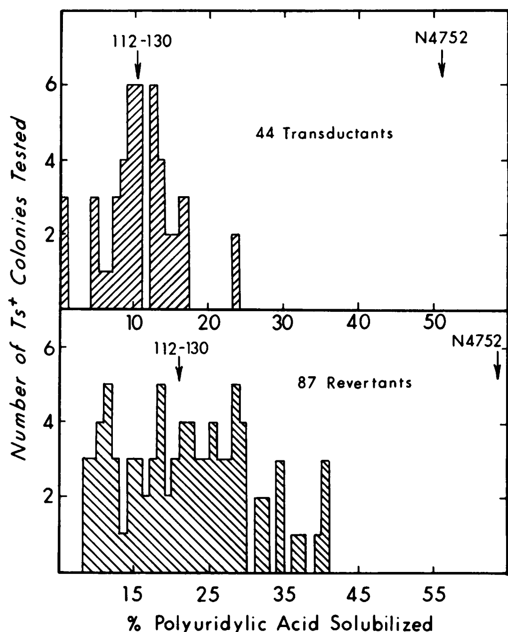


FIG. 1. *Ribonuclease II activity: Ts^+ transductants and revertants. Forty-four Ts^+ transductants and 87 Ts^+ revertants were tested. Cultures were grown to 30 ml each in nutrient broth at 30 C to an optical density at 560 nm of 0.5 and then incubated for 30 min at 42 C. Cell extracts were prepared and assayed for ribonuclease II activity. The arrows indicate the values obtained for 112-130 and N4752 cell extracts in the same experiment. One hundred per cent hydrolysis of ^3H-poly U represents the solubilization of 3,000 counts per min.*

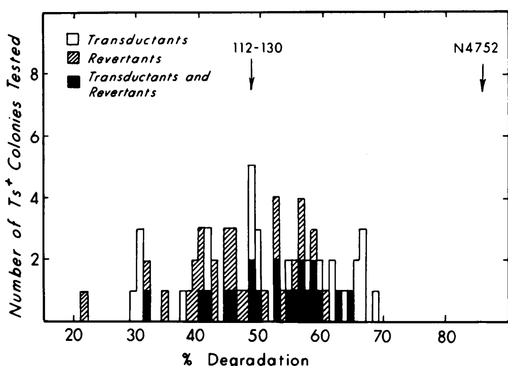


FIG. 2. *Degradation of stable RNA: Ts^+ transductants and revertants. Cultures of 44 Ts^+ transductants and 45 Ts^+ revertants were grown, labeled, and starved. Duplicate samples of the cultures were withdrawn at zero time and after 8 hr of starvation. The cold trichloroacetic acid-precipitable material before and after starvation was measured. The value obtained for each strain at time zero was the 100% value for that strain. The fraction of long-labeled RNA degraded after 8 hr of starvation is plotted along the abscissa.*

phenotypes.

Of the 700 Ts^+ and 100 Ts^+ revertants tested by the plate assay for recovery from prolonged starvation at 43 C, all recovered like the parental strain 112-130. Therefore, we conclude that all these four characteristics are pleiotropic effects of a single mutation.

Mapping of ts by conjugation and transduction. To map the *sts* 4752 mutation on the *E. coli* linkage map, a prolonged mating experiment was carried out.

For the prolonged mating experiment, the F^- strain N5152, a Spc-R (spectinomycin-resistant) derivative of N4752, was used. The Hfr strain used was N3 (see Table 1). Two classes of recombinants were selected from this cross: Str-R Ts^+, and Spc-R Leu^+. The results of the analysis of the segregation of unselected markers are shown in Table 2.

Of 158 Str-R Ts^+ recombinants, 63% showed the Hfr characteristics, i.e., Xyl^- Met^-. Among the 160 Spc-R Leu^+ recombinants, about 40% of the Ts^+ colonies carried also the *met* and *xyl* alleles of the Ts^+ parental strain. These results indicated close linkage between *sts* and *xyl*, as well

TABLE 2. *Analysis of segregation of unselected markers*[a]

Hfr N3: + + xyl^- $metB^-$ +
—O—O—O—O—O—

F^- N5152: —O—O—O—O—O—
spc^r str^r + + leu^-

Selection	No. of colonies tested	Segregation of unselected markers (%)		Minimal no. of cross-overs if *ts* located between *xyl* and *metB*
Str-R Ts^+	158	Xyl^- Met^-	(62.9)	2
		Xyl^+ Met^-	(14.5)	2
		Xyl^- Met^+	(18.2)	2
		Xyl^+ Met^+	(4.4)	4
Spc-R Leu^+	160	Xyl^- Met^- Ts^-	(0.0)	4
		Xyl^- Met^- Ts^+	(29.7)	2
		Xyl^+ Met^- Ts^-	(9.4)	2
		Xyl^+ Met^- Ts^+	(30.6)	2
		Xyl^+ Met^+ Ts^-	(18.2)	2
		Xyl^+ Met^+ Ts^+	(1.2)	4
		Xyl^- Met^+ Ts^-	(0.9)	4
		Xyl^- Met^+ Ts^+	(10.0)	4

[a] Prolonged mating: N3 × N5152. The mating conditions were as described by Curtis et al. (5). For selection of Str-R Ts^+ recombinants, the mating mixture was plated on broth-agar plates containing 200 μg of streptomycin per ml, and the plates were incubated for 4 hr at 30 C prior to transfer to 42 C. Spc-R Leu^+ recombinants were selected on minimal medium, 200 μg of spectinomycin, 50 μg of L-cysteine, and 50 μg of L-methionine per ml. The plates were incubated at 30 C.

as *sts* and *metB*. Furthermore, the pattern of the segregation of the unselected markers was compatible with the possibility that *sts* is located between *xyl* and *metB*. This was inferred from the good match between the low frequency of recombinants in certain classes of segregants and the high number of cross-overs required if *sts* were located between *xyl* and *metB* (see Table 2).

Markers *xyl* and *metB* are separated by 6.3 min on the *E. coli* chromosome (19); *xyl* is located at 70.3 min and *metB* at 76.6 min. There are four markers situated at the center of this region. They are *phoS*(73.6), *rbs* (73.8), *ilv* (74.0), and *metE* (74.6).

Possible linkage to *ilv* was first studied because it is located at the middle of the region in question. In the first transduction, N4752 (ilv^+ sts^-) was the donor and N141 (ilv^- sts^+) was the recipient. Of the 168 ilv^+ transductants isolated, 23% were Ts^-; *sts* is therefore linked to *ilv*.

Therefore three-point transduction crosses were carried out to determine the position of *sts* with respect to *ilv* and neighboring markers.

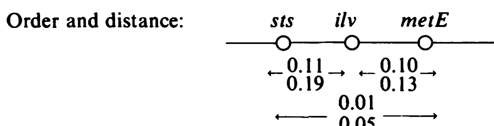
In the next transduction, N4752 (sts^- ilv^+ $metE^+$) was again the donor and N1127 (sts^+ ilv^- $metE^-$) the recipient (Table 3). Two classes of transductants were selected: ilv^+ and met^+. The segregation of the unselected markers in

TABLE 3. *Transduction with N4752 as donor and N1127 as recipient*

N4752	Donor	sts^- ilv^+ $metE^+$	
N1127	Recipient	+ − −	

Selection for	No. of colonies tested	Segregation of unselected markers[a]	Percentage
Ilv^+	94	Ts^+ Ilv^+ Met^-	73.6
		Ts^- Ilv^+ Met^-	13.7
		Ts^+ Ilv^+ Met^+	7.4
		Ts^- Ilv^+ Met^+	5.3
Met^+	91	Ts^+ Ilv^- Met^+	90.1
		Ts^+ Ilv^+ Met^+	8.8
		Ts^- Ilv^+ Met^+	1.1
		Ts^- Ilv^- Met^+	0.0

Order and distance:



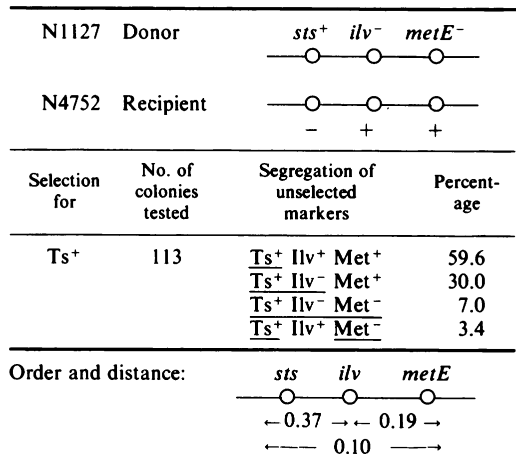
[a] In this and the following tables, the phenotypes characteristic of the donor strain found in the transductants are underlined. From the segregation of markers and the cotransduction frequencies, the order and the distances (cotransduction frequencies) between markers are inferred.

both classes suggested the order *sts ilv metE*, with a similar distance between *ilv* and both flanking markers.

In the reciprocal transduction, when N1127 was the donor and N4752 was the recipient (Table 4), the results again were consistent only with the suggested order *sts ilv metE*. However, the frequencies of cotransduction between the same pair of markers were higher than in the previous transduction.

To establish the relationship of the *sts* marker to *phoS*, the following transduction was carried out. The donor was N1126 ($phoS^-$ sts^+ ilv^-) and the recipient was N4752 ($phoS^+$ sts^- ilv^+). Ts^+ transductants were selected and tested for the unselected markers *phoS* and *ilv*. The results (Table 5) showed that *sts* was located between these two markers, closer to *ilv*. With N4752 used as donor and N1126 as recipient, 48 Ts^+

TABLE 4. *Transduction with N1127 as donor and N4752 as recipient*



N1127	Donor	sts^+ ilv^- $metE^-$	
N4752	Recipient	− + +	

Selection for	No. of colonies tested	Segregation of unselected markers	Percentage
Ts^+	113	Ts^+ Ilv^+ Met^+	59.6
		Ts^+ Ilv^- Met^+	30.0
		Ts^+ Ilv^- Met^-	7.0
		Ts^+ Ilv^+ Met^-	3.4

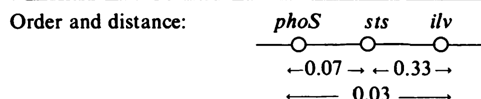
Order and distance:

TABLE 5. *Transduction with N1126 as donor and N4752 as recipient*

N1126	Donor	$phoS^-$ sts^+ ilv^-	
N4752	Recipient	+ − +	

Selection for	No. of colonies tested	Segregation of unselected markers	Percentage
Ts^+	99	$PhoS^+$ Ts^+ Ilv^+	63.0
		$PhoS^+$ Ts^+ Ilv^-	30.0
		$PhoS^-$ Ts^+ Ilv^+	4.0
		$PhoS^-$ Ts^+ Ilv^-	3.0

Order and distance:



Ilv$^+$ transductants were selected, of which 45 were PhoS$^-$ and only 3 were PhoS$^+$, again indicating unequivocally that *sts* is located between *phoS* and *ilv*.

To locate *sts* 4752 with relation to the last known marker in this region (*rbs*), a strain was constructed that carried *rbs* together with *phoS*. This was done by crossing strain N1129 (donor) and N1126 (recipient). The results of this cross suggested that *rbs* is located between *phoS* and *ilv*, as shown by Taylor and Trotter (20). One of the *phoS rbs*$^-$ transductants, N1130, was used as the donor in the final transduction, and the recipient was N4752 (Table 6); Ts$^+$ transductants were selected. Segregation of the unselected markers unambiguously placed *sts* clockwise from both *phoS* and *rbs*. The *sts* marker was cotransduced with *rbs* with a frequency of 0.53 and with *phoS* with a frequency of 0.17. Only two of 136 Ts$^+$ transductants were PhoS$^-$ Ts$^+$, consistent with the order *phoS rbs sts*, as four cross-overs were necessary to obtain such a class assuming this order.

A genetic map composed from all the data obtained during the transduction analyses is shown in Fig. 3.

Although there was considerable variation among experiments in the cotransduction frequencies between markers, in no case was there an inconsistency with respect to the relative order of the markers in this region. Therefore the location of *sts* 4752 between *rbs* and *ilv* is firmly established.

Is sts dominant or recessive? To test for dominance, N5352, a *sts*$^-$ *argE*$^-$ derivative of N4752, and a merozygote carrying an episome covering these two markers were mixed under mating conditions, and 127 Arg$^+$ colonies were isolated. Of these, 106 were Arg$^+$ Ts$^+$ and 21 were Arg$^+$ Ts$^-$. Three of the Arg$^+$ Ts$^+$ colonies were tested for segregation of the *sts* allele. Two of the three segregated Ts$^-$ cells. One hundred Ts$^-$ segregants from each of the merozygotes were isolated, purified, and tested for arginine requirement. All Ts$^-$ segregants tested were also Arg$^-$. Therefore, the *sts*$^-$ allele is recessive in merozygotes at least with regard to growth at 42 C.

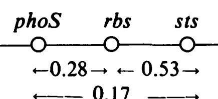
TABLE 6. *Transduction with N1130 as donor and N4752 as recipient*

N1130 Donor: *phoS*$^-$ *rbs*$^-$ *sts*$^+$

N4752 Recipient: + + −

Selection for	No. of colonies tested	Segregation of unselected markers	Percentage
Ts$^+$	136	PhoS$^+$ Rbs Ts$^+$	45.5
		PhoS$^+$ Rbs$^-$ Ts$^+$	38.0
		PhoS$^-$ Rbs$^-$ Ts$^+$	15.0
		PhoS$^-$ Rbs$^+$ Ts$^+$	1.5

Order and distance:



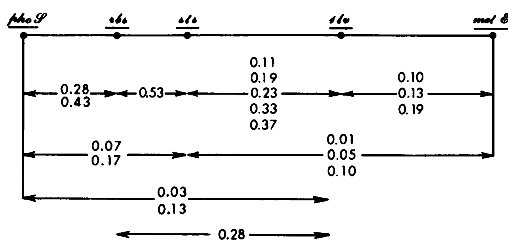


FIG. 3. *Genetic map of the phoS metE region of the E. coli chromosome. Numbers between arrows represent the cotransduction frequencies obtained in all the experiments presented in the text and in Tables 3 to 6.*

DISCUSSION

Experiments were carried out to answer four questions. (i) Is *sts* 4752 a single-site mutation? (ii) Are all the phenotypic differences between the parental and mutant strain caused by a single mutation? (iii) What is the position of *sts* on the *E. coli* linkage map? (iv) Is the mutation recessive or dominant?

Temperature sensitivity was the phenotypic difference most amenable to genetic analyses. Hence all the genetic experiments were carried out with Ts$^+$ used as a selective marker.

The transduction frequency from Ts$^-$ to Ts$^+$ was compared to that from Spc-S to Spc-R. About the same number of Ts$^+$ and Spc-R transductants were obtained when a Ts$^+$ Spc-R strain was the donor and a Ts$^-$ Spc-S strain was the recipient. Since *spc* mutations are very likely single-site mutations (1, 6), it is likely that the temperature sensitivity in N4752 is caused by a single mutation.

This suggestion was further strengthened by the fact that spontaneous Ts$^+$ revertants were obtained with a rate and frequency of 1.8×10^{-10} and 7×10^{-10}, respectively. This reversion frequency is comparable to the spontaneous forward mutation frequency observed for Spc-R (5×10^{-11}; reference 17) which is most likely due to mutations in several sites in the *spc* gene (Funatsu, Nierhaus, and Wittman-Liebold, *personal communication*). The reversion rate of *sts* 4752 is in perfect agreement with forward mutation rates in a single base pair in *E. coli* (8). If *sts* 4752 is located in a single site, the low reversion frequency could indicate that the revertants

are true revertants. This is also suggested by the fact that they behaved very similarly to the parental strain in all tests to which they were subjected.

When Ts^+ transductants and revertants were tested for their sensitivity to starvation, their ribonuclease II activities, after growth at 42 C (Fig. 1) and for the degradation of stable RNA during starvation at 42 C (Fig. 2), all showed the parental wild-type phenotype. Thus, reversion of Ts^- to Ts^+ was accompanied by a simultaneous reversion of all other tested mutant characteristics. Therefore it is likely that all these phenotypic differences between strains 112-130 and N4752 are due to a single-point mutation.

Extensive three-point transduction crosses showed that *sts* is located between *rbs* and *ilv* on the linkage map (Tables 2 to 6). The ordering of the markers, as inferred from the cotransduction frequencies and their segregation pattern, was unambiguously determined as ***phoS rbs sts ilv, metE***. Using the positions of *rbs* and *ilv* on Taylor's map (20), *sts* was placed at 73.8 min on the linkage map. We inferred, from cotransduction frequencies to physical distances on the *E. coli* chromosome (20), that the distance between *sts* and *rbs* contains about six genes that can code for proteins of about 400 amino acids in length. Therefore it is evident that even this region of the chromosome is far from being saturated with markers.

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