Restoration by Ribosomal Protein S1 of the Defective Translation in a Temperature-Sensitive Mutant of Escherichia coli K-12: Characterization and Genetic Studies

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A temperature-sensitive mutant of Escherichia coli was isolated that had a temperature-sensitive defect in ribosomal-wash protein(s) required for translation in vitro of E. coli endogenous messenger ribonucleic acid. It was found that 30S ribosomal protein S1 rescued the defect in the ribosomal-wash protein(s) of the mutant and that the complete restoration to the wild-type level was attained when 1 mol of protein S1 was added to 1 mol of 70S ribosome. The mutation, tss, causing such a defect was mapped at 21 min and was closely linked to the pyrD locus, the region of which was entirely different from that of the other genes coding for the many ribosomal proteins of E. coli. These results indicate that the gene specified by this mutation is involved in the function of the 30S ribosomal protein S1.

Temperature-sensitive mutations that affect bacterial protein synthesis were very important tools for the elucidation of in vivo roles of the translational machinery and also for analyses of the genetic control of protein synthesis. Such mutations have been described for many of the aminocacyl-transfer ribonucleic acid (RNA) synthetases (26), for the elongation factors EF-G, EF-Ts, and EF-Tu (17, 23, 32), and for the ribosomal proteins S4 and S12 (16, 27). However, none of the temperature-sensitive mutants that are defective in initiation and release factors or in many of the ribosomal proteins have been isolated until now.

As reported in this paper, we isolated a mutant of Escherichia coli K-12 temperature sensitive for in vivo translation and for in vitro protein synthesis. In the course of characterizing a protein factor from the wild-type strain, which complements the synthesis defect in this mutant, we identified this factor as the 30S ribosomal protein S1. From the genetic analyses described in this paper, we found that a mutation determining the phenotype of the temperature-sensitive growth of the mutant was located at 21 min and that it was closely linked to the pyrD locus on the E. coli chromosome. These results indicate that the gene specified by this mutation is involved in the function of the 30S ribosomal protein S1.

MATERIALS AND METHODS

Bacterial strains. E. coli strains used in this study are described in Table 1.

Media. Nutrient broth contained per liter of distilled water: 10 g of tryptone (Difco); 5 g of yeast extract; and 10 g of NaCl. Tris(hydroxyethyl)amino(methane (Tris)-glucose minimal medium (TGM medium) was composed of the following components per liter: 6 g of Tris (pH 7.4, adjusted with concentrated HCl); 1 g of (NH₄)₂SO₄; 0.1 g of MgSO₄·7H₂O; 272 mg of KH₂PO₄; 0.5 mg of FeSO₄·7H₂O; 0.5 g of trisodium citrate·2H₂O; and 2 g of glucose. This medium was supplemented with 200 mg of L-threonine, 100 mg of L-arginine, 40 mg of L-leucine, 40 mg of L-isoleucine, 20 mg of L-histidine, and 1 mg of thiamine hydrochloride (each per liter). An enriched medium used for the penicillin selection was prepared by supplementing the TGM medium with Casamino Acids (Difco, vitamin-free) at 2 g/liter, L-tryptophan at 50 mg/liter, nucleobases, and a vitamin mixture. The nucleobases were adenine, guanine, uracil, and cytosine at concentrations of 25 mg/liter and thymine at a concentration of 200 mg/liter. The vitamin mixture was added at the following concentrations (milligrams per liter): thiamine hydrochloride, 1.0; riboflavin, 1.0; pyridoxine hydrochloride, 1.0; calcium panthothenate, 1.0; nicotinic acid, 1.0; p-aminobenzoic acid, 1.0; folic acid, 0.10; biotin, 0.010; and cyanocobalamin, 0.015.

Agar in plates was used at a concentration of 1.5% (wt/vol).

Mutagenesis. Mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine was performed according to the method of Adelberg et al. (1). Growth studies. Liquid cultures were grown aerobically in Monod tubes with shaking in a reciprocating water bath. Growth was measured turbidimetrically in a Klett-Summerson-type photoelectric colorimeter at 660 nm. One turbidity unit (Klett unit) corresponds to 8 × 10⁶ cells/ml.
Measurements of RNA and protein synthesis. The rate of RNA and protein synthesis was examined in exponentially growing culture and starved culture by measuring the incorporation of \(^{3}H\)uridine and \(^{14}C\)leucine into trichloroacetic acid-insoluble material. The sample used to measure uracil incorporation was kept at 4°C for at least 20 min, placed in a boiling water bath for 15 min, and, after cooling, filtered through membrane filters (Millipore HAWP, 0.45-\(\mu\)m pore size). The sample used to measure uracil incorporation was filtered without heating. Each filter was washed with ice-cold 5% trichloroacetic acid, dried, and counted in a liquid scintillation counter. Details for labeling conditions are specified in the figure legends.

Preparations of crude lysates. Procedures for preparing crude lysates were as described by Cozzone and Stent (3), except that all the steps were done in the presence of 1 mM dithiothreitol. Cells were grown at 30°C in a medium at pH 7.6 which contained the following components per liter: 12 g of Tris; 2 g of KCl; 2 g of \(\text{NaH}_{2}\text{CO}_{3}\); 0.5 g of \(\text{MgCl}_{2}\cdot 6\text{H}_{2}\text{O}\); 0.02 g of \(\text{Na}_{2}\text{SO}_{4}\); 14.7 mg of \(\text{CaCl}_{2}\cdot 2\text{H}_{2}\text{O}\); 358 mg of \(\text{Na}_{2}\text{HPO}_{4} \cdot 12\text{H}_{2}\text{O}\); and 2 g of glucose. This medium was supplemented with 50 mg of each required amino acid per ml and 2 mg of thiamine hydrochloride per ml. One hundred milliliters of a log-phase culture (4 x 10\(^8\) cells/ml) was poured over an equal volume of crushed ice. Cells were pelleted by centrifugation and resuspended in 12 ml of sucrose buffer solution containing 0.5 M ribonucleic acid-sucrose, 0.016 M Tris-hydrochloride buffer (pH 8.1), 0.05 M KCl, and 0.001 M dithiothreitol. Then 1.5 ml of lysozyme solution (10 mg/ml in sucrose buffer solution) and 0.3 ml of 10% disodium ethylenediaminetetraacetic acid (adjusted to pH 8.0 with NaOH) were added to produce protoplasts. The suspension was stirred for 4 min, and 0.3 ml of 1 M MgCl\(_2\) was added to stop lysozyme action. Protoplasts were pelleted by centrifugation and resuspended in 0.7 ml of freshly prepared lysing medium (0.05 M NH\(_4\)Cl, 0.01 M MgCl\(_2\), 0.01 M Tris, pH 7.8) containing 0.5% Brij-58, 0.5% sodium deoxycholate, 0.001 M dithiothreitol, and 5 \(\mu\)g of ribonuclease-free deoxyribonuclease per ml. The resultant lysate, after being centrifuged at 15,000 x g for 12 min to remove cell debris, was divided into portions, rapidly frozen in liquid nitrogen, and stored at -70°C until use.

Cell-free protein synthesis. The cell-free system was a modification of that described by Matthaei and Nirenberg (25). The complete reaction mixture contained the following components per milliliter: 100 \(\mu\)mol of Tris (pH 7.8); 8 \(\mu\)mol of magnesium acetate; 50 \(\mu\)mol of KCl; 1 \(\mu\)mol of dithiothreitol; 1 \(\mu\)mol of disodium adenosine 5'-triphosphate; 0.5 \(\mu\)mol of trithium guanosine 5'-triphosphate; 5 \(\mu\)mol of phosphoenolpyruvate (sodium salt); 20 \(\mu\)g of pyruvate kinase; 0.05 \(\mu\)mol of each of 20 L-amino acids except leucine; 1.5 \(\mu\)Ci of L-[\(^{14}\)C]leucine (342 mCi/mmol); and 100 \(\mu\)l of lysate. The mixture was incubated at the indicated times at either 30 or 42°C. Samples were withdrawn from the reaction mixture, spotted on filter-paper disks, and processed according to the method of Mans and Novelli (24). The radioactivity of material insoluble in hot trichloroacetic acid was counted by immersing filters into toluene-based scintillation fluid.

Estimation of the ribosomal content of lysate. About 50 \(\mu\)l of lysate or about 2 OD\(_{500}\) (optical density at 500 nm) units of salt-washed ribosomes was dissolved in water, mixed with an equal volume of cold 10% trichloroacetic acid at 4°C, and fractionated according to the Schmidt-Thannhauser-Schneider method (35). The RNA fractions were analyzed for RNA by the orcinol colorimetric assay at OD\(_{500}\) or by ultraviolet absorption assay at OD\(_{260}\). Using salt-washed ribosomes as a standard and assuming that 80% of total RNA in lysate is ribosomal RNA, the content of ribosomes in lysate was calculated and expressed as OD\(_{500}\) of ribosomes per milliliter. The
lysates used in this study ordinarily contained 30 to 60 OD units of ribosomes per ml.

Preparation of ribosomal-wash proteins. Either the mutant or wild-type strain was grown under vigorous aeration at 30°C in a medium containing 1% tryptone (Difco), 0.1% yeast extract, 0.8% NaCl, and 0.1% glucose. All subsequent operations were carried out at 4°C. Cells (15 g) were ground with two times their weight of alumina. The paste was suspended in 30 ml of buffer A (0.02 M Tris-hydrochloride [pH 7.8], 0.03 M NH₄Cl, 0.01 M magnesium acetate, and 0.01 M 2-mercaptoethanol). Alumina and cell debris were removed by centrifugation at 30,000 × g for 20 min. The supernatant fluid was treated with 2 µg of ribonuclease-free deoxyribonuclease per ml and centrifuged at 30,000 × g for 20 min. After centrifugation for 13 h at 30,000 rpm in a Hitachi RP65T rotor, the ribosomal pellets were re-suspended and washed for 16 h in 20 ml of 1 M NH₄Cl (in buffer A). The wash was freed of ribosomes by centrifugation at 50,000 rpm for 3 h in a Hitachi RP65T rotor. The precipitate of the 30 to 80% ammonium sulfate fraction of the wash was dissolved in 3 ml of buffer A, dialyzed overnight against buffer A, and stored at −70°C until use. The ribosomal-wash fraction thus prepared contained 5.3 to 6.1 mg of protein per ml.

Protein concentrations were determined as described by Lowry et al. (22), using crystalline bovine serum albumin as standard.

Conjugation methods. Matings of Hfr and F− strains were carried out according to the method of Taylor and Thomas (30). In interrupted mating experiments, the parental cultures were grown in nutrient broth to log phase (4 × 10⁸ to 8 × 10⁸ cells/ml) at 37°C and then mixed at 2 × 10⁹ donor and 4 × 10⁹ recipient per ml in a final volume of 4 ml in a 200-ml Erlenmeyer flask and shaken gently at 37°C. The interruption of mating was accomplished by diluting the mating mixture (1:10 with buffer) and shaken vigorously, using a Vortex mixer, for 60 s. Samples of the mating mixtures were plotted on selective media.

Transduction methods. The procedure used was based on that described by Lennox (20). Phage lysates for transduction were obtained in L broth by using phage Plvir (11). Lysates of temperature-sensitive donor strains were prepared at 30 to 35°C. The phage stocks were cycled through a given strain at least twice before use and sterilized with chloroform. Transductions were carried out by mixing 1 ml of a solution 10 mM in CaCl₂ and 5 mM in MgCl₂, containing 10⁶ log-phase recipient cells, with 0.1 ml of the appropriate dilutions of phage lysate. This suspension was incubated for 20 min at 37°C and then plated on selective media. For this purpose, K10 medium (6) was used. After the plates were incubated for 2 to 3 days at 30°C, the transductants were counted, purified, and scored for the inheritance of nonselective markers. Where the temperature-insensitive transductants were scored K10 medium supplemented with 0.5% Casamino Acids (Difco, vitamin-free) was used as selective medium and incubated overnight at 42°C.

Ribosomal protein S1. Ribosomal protein S1 of E. coli MRE600 was purified from the 1.0 M NH₄Cl ribosomal wash by T. Yokota and K. Arai (Institute of Medical Science, University of Tokyo), essentially according to the method of Wahtba et al. (36), and kindly supplied to us. They confirmed that the protein S1 used here gave only one band co-migrating with the S1 band of unfraccionated 30S ribosomal proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (over 90% purity) and that it also gave only one band at the same position as that of subunit I of Qβ replicase on isoelectric gel electrofocusing (K. Arai, personal communication).

Materials. Materials were obtained from the following sources: phosphoenolpyruvate (monosodium salt), pyruvate kinase (10 mg/ml of suspension in ammonium sulfate), adenosine 5′-triphosphate (dissodium salt), guanosine 5′-triphosphate (trilithium salt), and lysozyme from Boehringer Mannheim Ltd., Mannheim, Germany; deoxyribonuclease (pancreate, electrophoretically pure) from Worthington Biochemicals Co., Freehold, N.J.; penicillin G and streptomycin from Meiji Seika Ltd., Kawasaki, Japan; Brij-58 from Atlas Chemical Industries, Wilmington, Del.; sodium deoxycholate from Difco Laboratories, Detroit, Mich.; ribonuclease-free sucrose from Nakarai Chemicals Ltd., Kyoto, Japan; and yeast extract from Daigo Eiyo Kagaku Co., Osaka, Japan. Other chemicals used were of reagent grade.

RESULTS

Isolation of temperature-sensitive mutants. To obtain a collection of mutant strains that stop protein synthesis at 42°C, we attempted to select by the following procedure, those strains that were temperature sensitive at 42°C but not lethal even after a sufficient period of incubation at this temperature.

The mutagenized cells of E. coli K-12 strain CP78 were grown in an enriched medium to midlog phase at 30°C and shifted to 42°C. At that time penicillin G (2,000 U/ml) was added, and the cells were incubated for a further 120 min. The cells were washed, resuspended in the same medium, and grown at 30°C overnight. When this penicillin selection procedure was repeated three times, 70 to 80% of the colonies isolated on nutrient broth agar plates at 30°C were temperature sensitive as judged from their growth on nutrient broth agar plates incubated at 30 and 42°C. Each of these temperature-sensitive strains was examined for growth in nutrient broth at 30 and 42°C. One of these mutants, strain HO-108, was selected for further investigation.

Growth characteristics of strain HO-108. Growth of strain HO-108 stopped very rapidly...
as measured by the OD increase when the temperature was raised from 30 to 42°C, whereas growth of its parent strain, CP78, did not (Fig. 1). When the temperature was returned to 30°C 90 min after the temperature shift, strain HO-108 immediately resumed growth at a rate similar to that of the preshifted culture. These results suggested that strain HO-108 was non-lethal at 42°C and that its temperature sensitivity was possibly caused by reversible inactivation of a factor(s) required for growth.

RNA and protein synthesis of strain HO-108. To determine why strain HO-108 stopped growing at high temperature, synthesis of RNA and protein was measured by the incorporation of radioactive precursors during the first 60 min after temperature shift-up. Net incorporation of [14C]leucine into protein was not detected at 42°C (Fig. 2), and accumulation of RNA at 42°C was severely restricted compared with that at 30°C (Fig. 3a). Therefore, we think that the defect in RNA synthesis at 42°C may be due to the stringent control of stable RNA synthesis, which is dependent on protein synthesis (5), since strain HO-108 is a rel+ strain (Table 1). In fact, strain HO-109, a rel− derivative of strain HO-108 (Table 1), retained the ability to accumulate RNA at 42°C at the same rate as at 30°C (Fig. 3b). Under these conditions, strain HO-109 as well as strain HO-108 stopped protein synthesis completely as measured by leucine incorporation (data not shown).

Multiplication of bacteriophage MS2 in the presence of rifampin. Although stable RNA synthesis was found to be normal as indicated above, it remained possible for strain HO-108 to

\[ \text{Fig. 1. Effect of a temperature shift on growth of strains CP78 and HO-108. Each strain was grown aerobically at } 30^\circ\text{C in nutrient broth to about 20 Klett units and shifted up to } 42^\circ\text{C at the times indicated. The cultures were further aerated by shaking at } 42^\circ\text{C. After 1.5 h, the culture of strain HO-108 was returned to } 30^\circ\text{C and further aerated by shaking at } 30^\circ\text{C. Cell density (Klett units) was followed in a spectrophotometer. Symbols: } \bullet, \text{CP78; } \bigcirc, \text{HO-108.} \]

\[ \text{Fig. 2. Incorporation of } [14C]\text{leucine into hot trichloroacetic acid-insoluble material in strain HO-108. Strain HO-108 was grown at } 30^\circ\text{C in TGM medium to a density of } 1.5 \times 10^8 \text{cells/ml, and } 5 \mu\text{Ci of L-}[14C]\text{leucine was added to } 5 \text{ml of the culture } 30 \text{min before temperature shift. At zero time, indicated by the arrow, the culture was divided into two portions. One portion was further aerated at } 30^\circ\text{C (})\bullet, \text{and the other was aerated at } 42^\circ\text{C (}}\bigcirc. \text{Aliquots of } 0.2 \text{ml were withdrawn from each culture at the times indicated, mixed with } 3 \text{ml of } 5% \text{trichloroacetic acid, and treated as described in Materials and Methods.} (a) HO-108 (rel+); (b) HO-109 (rel−).} \]

\[ \text{Fig. 3. Incorporation of } [3H]\text{uridine into cold trichloroacetic acid-insoluble material in strain HO-108 and its relaxed derivative strain HO-109. The strains were grown at } 30^\circ\text{C in TGM medium to a density of about } 2 \times 10^8 \text{cells/ml, and each culture was divided into three portions; one was further aerated at } 30^\circ\text{C (}}\bullet, \text{another at } 42^\circ\text{C (}}\bigcirc, \text{and the third at } 42^\circ\text{C in the presence of } 100 \mu\text{g of chloramphenicol/ml (Δ). A } 1.7-\mu\text{Ci amount of } [3H]\text{uridine (49 mCi/mmol) was added to } 1 \text{ml of each portion at zero time, and } 0.1-\text{ml aliquots were withdrawn at the times indicated, mixed with cold } 3 \text{ml of } 5\% \text{trichloroacetic acid, and treated as described in Materials and Methods. (a) HO-108 (rel+); (b) HO-109 (rel−).} \]

block specifically the synthesis of messenger RNA, resulting in no protein synthesis at 42°C. If this was the case, it was expected that RNA phage growth, which is independent of cellular messenger RNA synthesis, would occur even at 42°C. The intracellular phage particle forma-
tion of RNA phage MS2 within cells of strain F15/HO-108, an F-prime derivative of strain HO-108, did not occur at 42°C, whereas at 37°C it did occur at the same rate as in an F-prime derivative of the parental strain, CP78 (F15/CPT8) (Fig. 4). From these results, it appears that the defect in protein synthesis at 42°C in strain HO-108 cannot be explained only by the synthesis or degradation of messenger RNA itself, but that it can be explained by the inability to translate messenger RNA.

Temperature-sensitive defect in cell-free synthesis of protein. Crude lysates containing polysomes were prepared from strains HO-202 and HO-201. These strains were the temperature-sensitive and -insensitive transductants with P1 lysates, respectively, prepared from the original temperature-sensitive mutant, HO-109 (Table 1). The incorporation of radioactive leucine into trichloroacetic acid-precipitable material by the lysates was measured at 30 or 42°C as a function of time. When assayed at 30°C, the incorporating abilities of the lysates from strains HO-201 and HO-202 were nearly similar, at least during the first 30 min of incubation (Fig. 5). When incubated at 42°C, however, the incorporation of leucine into mutant HO-202 stopped more rapidly than into the wild-type strain HO-201, and after 60 min of incubation the incorporation into the former strain was about 30% of that into the latter.

These results suggest that the reduced level of incorporation into the mutant lysate may be due to the defect(s) in the component(s) essential for protein synthesis. Therefore, such a defect(s) in the mutant lysate would be expected to be rescued by adding the wild-type cell extract or its fractionated component(s). First, the S30 extract of the wild-type strain HO-201 was fractionated into S100, salt-washed ribosomes, and ribosomal-wash proteins, and the fractions were then tested for ability to rescue the defect in the mutant lysate. It was observed that both S100 and salt-washed ribosomes were less able to restore the reduced incorporation in the mutant lysate than were ribosomal-wash proteins. In this test, an equivalent volume of each fraction that was derived from the same volume of the original S30 extract was used (data not shown). Therefore, each of the ribosomal-wash proteins prepared from strain HO-201 or HO-202 was added to the mutant HO-202 lysate and was used to measure the ability to stimulate leucine incorporation at 42°C. Addition of a saturating amount of ribosomal-wash proteins of the wild-type strain resulted in about a threefold stimulation of leucine incorporation, whereas addition of those proteins derived from the mutant HO-202 stimulated it only slightly (Fig. 6). When similar experiments were done using lysates prepared from the wild-type strain HO-201 or two independently isolated revertants able to grow at 42°C, instead of the mutant lysates, such a differential stimulation by the ribosomal-wash proteins between the mutant HO-202 and the wild-type strain HO-201 was not observed (data not shown).

These results show that the reduced incorporating activity of the mutant lysate (Fig. 5 and 6) truly reflects the genetic alteration of a factor
that is essential for maximum in vitro protein synthesis. In addition, most of this factor appears to be present in the protein fraction released from ribosomes by a 1 M NH₄Cl wash procedure.

Properties of a factor restoring the temperature-sensitive defect. A factor from the wild-type strain HO-201 that complements the defect in the mutant lysate can be assayed as the stimulation factor as described above. Partial purification of the factor from ribosomal-wash proteins of strain HO-201 was attempted by using this assay system, in parallel with assays for the well-known initiation factors IF-1 and IF-2.

Step 1. Ribosomal wash proteins (10 mg in 2 ml) were loaded onto a diethylaminoethyl-cellulose column (1.0 by 10 cm; Brown). After washing with 20 ml of buffer L (0.02 M Tris-hydrochloride, pH 7.6, 0.02 M NH₄Cl, 0.0002 M magnesium acetate, 0.01 M 2-mercaptoethanol, 5% glycerol), the protein was eluted with a linear gradient of NH₄Cl in buffer L (40 ml, 0.02 to 0.35 M). A rather broad peak of the factor activity, centering around 0.25 M NH₄Cl, was observed. These fractions were pooled, concentrated, dialyzed against buffer L, and used for the next step.

Step 2. The factor obtained in step 1 was loaded onto a phosphocellulose column (1.0 by 10 cm; Whatman P-11) and eluted with buffer L. The unadsorbed fraction having all the activity of the factor was pooled, concentrated, dialyzed against buffer L without 5% glycerol, and used for the next step.

Step 3. Samples obtained in step 2 were layered over linear 5 to 20% glycerol gradients in buffer L and centrifuged for 38 h at 40,000 rpm in a Spinco SW50L rotor. The peak fractions, which were centered around a molecular weight of 80,000 as judged from the positions of the marker protein, bovine serum albumin, were pooled and used for further analyses. The "rescue" factor obtained in this way was completely free from the activities of both IF-1 and IF-2 as judged from the ability to support the trinucleotides ApUpG-dependent N-formyl-methionyl-transfer RNA binding to 70S ribosomes. Although the assay for another initiation factor, IF-3, was not performed here, it seemed unlikely that the rescue factor would be identical to it, because IF-3 had a molecular weight of 21,000 to 23,000, strongly adsorbed to phosphocellulose even in the presence of 0.25 M
NH₄Cl, and then eluted at 0.5 M NH₄Cl (2, 10, 19). These experiments indicated that this factor may be identical to ribosomal protein S1 since: (i) most of the ribosomal protein S1 was washed off from ribosomes by 1 M NH₄Cl; (ii) this protein adsorbed to diethylaminoethyl-cellulose and eluted at 0.25 M NH₄Cl from it, but not to phosphocellulose under similar conditions (15, 18); and (iii) this protein had a molecular weight of 74,000 (9, 36). Then, electrophoretically pure ribosomal protein S1 of E. coli MRE600 (a generous gift of T. Yokota and K. Arai) was examined for ability to rescue the protein synthesis defect in the mutant lysate. Addition of the protein S1 to the mutant lysate completely rescued its synthesis defect (Fig. 7). Saturation of stimulation was found to be attained when 1 mol of protein S1 was added to 1 mol of 70S ribosomes in the mutant lysate. This fact, therefore, strongly suggests that the protein synthesis defect in the mutant lysate is due to a functional defect of ribosomal protein S1. Since the defect in the lysate of the mutant HO-202 was responsible for the temperature-sensitive growth phenotype of this strain as shown above, it appears that the temperature-sensitive mutation of strain HO-202 is directly or indirectly related to the function of ribosomal protein S1.

Genetic studies. To determine whether a mutation responsible for the temperature-sensitive growth phenotype of strain HO-108 is like the already known genes that are related to the translational machinery, we attempted to map the mutation as follows. Since strain HO-108 was able to grow normally at 37°C as well as 30°C but not 42°C, an interrupted mating of HfrH with HO-108 str was performed under standard conditions at 37°C. Transfer of the temperature-insensitive allele (ts⁺) by HfrH began about 20 min after the initiation of thr⁺/leu⁺ transfer (Fig. 8). In addition, this allele was not transferred with an early marker in a cross of HO-108 str with HfrC or Hfr KL99 (data not shown). These data suggest that the temperature-sensitive phenotype of strain HO-108 is specified by a locus near the origin of transfer of Hfr KL99, at about 20 min on the E. coli genome.
coli chromosome. Based on these experiments, this locus was tentatively designated as tss: tss\textsuperscript{-} for the temperature-sensitive mutation and tss\textsuperscript{+} for its temperature-insensitive allele (Table 1).

The results described above led us to test for cotransduction of tss with the various loci in the region around 20 min on the chromosome. Phage P\textvisiblespace{}1vir lysates grown on strains carrying a mutation in the pyrD, pdxC, or tss locus were used to transduce each of these strains to the wild-type phenotype, and all the possible combinations of transduction consisting of three reciprocal transductions were made. The tss gene was cotransducible with pyrD at frequencies of 54 to 72% and also with pdxC at frequencies of 10 to 15%, whereas pyrD and pdxC showed only 2.9 to 4.1% cotransduction (Table 2). Cronan et al. (4) reported a nearly similar value for the frequency of cotransduction of pdxC with pyrD. The cmlB locus previously reported by Reeve and Doherty (28) is expected to be very close to the tss locus. Therefore, we concluded that the map order consistent with the above data was pdxC-cmlB tss-pyrD (Fig. 9).

**DISCUSSION**

The data presented above indicate that the mutation designated as tss is responsible for the protein synthesis defect in E. coli. The defect is restored in vitro by adding 30S ribosomal protein S1. Protein S1 has been reported to be required for the binding of 30S ribosomal subunit to messenger RNA and for the formation of the 30S initiation complex (29, 34). These facts are consistent with our data indicating that protein S1, which complements the synthesis defect in the mutant lysate, is required for the maximum protein synthesis attained in the wild-type lysate. Furthermore, it is important to mention that protein S1 may be essential for cell growth and also for in vitro translation, since the protein synthesis defect of the mutant in vitro appears to be due to the functional defect of protein S1. However, a definite conclusion awaits direct demonstration of the genetically altered protein S1 of the mutant.

Recently, ribosomal protein S1 was found to be identical to an interference factor (i-factor) (12, 36), which was assayed as the inhibition of protein synthesis programmed with either natural or synthetic messenger RNAs (8, 15, 18). In our assay using lysates containing polystyrene, such inhibition was also exhibited, only at the higher molar ratio of protein S1 to ribosomes, and the saturation of stimulation was attained when 1 mol of S1 was added to 1 mol of 70S ribosomes (Fig. 7). This type of stimulation by protein S1 was also discovered by other

![Fig. 9. Location of tss on the genetic map of Escherichia coli. Hfr origins and relevant genetic markers of strains used in this study are inserted according to Taylor and Trotter (31). Numbers at the bottom are percent cotransduction frequencies. The pdxC-tss, tss-pyrD, and pdxC-pyrD frequencies are averaged from the data of Table 2. The other frequencies are from Cronan et al. (4) and from Reeve and Doherty (28). The order of tss and cmlB illustrated in this figure is expressed only as a matter of convenience.](http://jb.asm.org/)

**Table 2. Transductional mapping of the tss locus**

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Transductants selected</th>
<th>Colonies cotransduced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Character</td>
<td>No.</td>
</tr>
<tr>
<td>HO-109 tss</td>
<td>HO-200 pyrD34</td>
<td>pyrD\textsuperscript{+}</td>
<td>93</td>
</tr>
<tr>
<td>HO-200 pyrD34</td>
<td>HO-202 tss</td>
<td>tss\textsuperscript{+}</td>
<td>57</td>
</tr>
<tr>
<td>HO-109 tss</td>
<td>AT3145 pdxC3</td>
<td>pdxC\textsuperscript{+}</td>
<td>96</td>
</tr>
<tr>
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<td>HO-202 tss</td>
<td>tss\textsuperscript{+}</td>
<td>86</td>
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<td>pdxC\textsuperscript{-}</td>
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</table>
It is possible that any other factors present in the ribosomal-wash fraction could rescue the synthesis defect in the mutant lysate and that any other minor substances able to contaminate the purified protein S1 preparation used in this study could also do so. But these possibilities seem unlikely, since purified S1 at roughly a 1:1 molar ratio to ribosomes resulted in the complete restoration of the mutational reduction of in vitro amino acid incorporation. However, we cannot rule out the possibility that some unknown substance other than nucleic acids or proteins could form the 1:1 molar complex with protein S1 during the course of its purification and exhibit the effect now ascribed to protein S1.

The genetic data show that the pdcC-ppyRD region containing the tss mutation at 21 min is clearly different from the other chromosomal genes coding for the many ribosomal proteins (14). It is worth noting that within the pdcC-ppyRD region such a mutation involved in protein synthesis has never been discovered except the mutation cmIB, which causes partial resistance to chloramphenicol (28). Therefore, it should be of considerable interest to study the biochemical and genetic relationships between this region and the other ribosomal protein structural genes. For that purpose, it must be confirmed that the gene specified by the tss mutation codes for the polypeptide structure of ribosomal protein S1. Purification and analyses of the mutant S1 are in progress.

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LITERATURE CITED


