LOCALIZED MUTAGENESIS FOR THE ISOLATION OF TEMPERATURE-SENSITIVE MUTANTS OF *ESCHERICHIA COLI* AFFECTED IN PROTEIN SYNTHESIS

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ABSTRACT

Two variations of the method of localized mutagenesis were used to introduce mutations into the 72 min region of the *Escherichia coli* chromosome. Twenty temperature-sensitive mutants, with linkage to markers in this region, have been examined. Each strain showed an inhibition of growth in liquid medium at 44°, and 19 of the mutants lost viability upon prolonged incubation at this temperature. A reduction in the rate of *in vivo* RNA and protein synthesis was observed for each mutant at 44°, relative to a control strain. Eleven of the mutants were altered in growth sensitivity or resistance to one or more of three ribosomal antibiotics. The incomplete assembly of ribosomal subunits was detected in nine strains grown at 44°. The characteristics of these mutants suggest that many of them are altered in genes for translational or transcriptional components, consistent with the clustering of these genes at this chromosomal locus.

PROTEIN biosynthesis in prokaryotes is an extraordinarily complex process that requires the participation of more than 130 different macromolecules. A substantial amount of biochemical research has defined the essential components and steps involved in protein formation (reviewed by LENGYEL 1974). However, a clear understanding of the specific functions and interactions of many of the components is still lacking. Physical and chemical alterations of ribosomes and protein synthesis factors have suggested possible interactions (KURLAND 1977), and various biochemical assays have indicated specific functional roles for certain protein synthesis factors (LENGYEL 1974). Protein omission experiments with reconstituted 30S subunits have also suggested functional roles for certain groups of ribosomal proteins (NOMURA 1973).

An alternative approach to a better understanding of the complexities of protein synthesis is the isolation of protein synthesis mutants. Certain antibiotic-resistant mutants and strains with temperature-sensitive elongation factors have been described (JASKUNAS, NOMURA and DAVIES 1974). Several ribosomal subunit assembly defective mutants have also been isolated and analyzed (GUTHRIE, NASHIMOTO and NOMURA 1969; ROSSET *et al.* 1971; BRYANT and SYPHERD 1974). Recently, several reports have described the isolation of temperature-sensitive

mutants of *E. coli* containing altered ribosomal proteins (BERGER *et al.* 1975; ISONO, KRAUSS and HIROTA 1976; ISONO *et al.* 1977; KUSHNER, MAPLES and CHAMPNEY 1977). Studies on many of these mutants have primarily emphasized the characteristics of the structural alteration of the protein synthesis component, but have not examined the effects of the mutational alteration on the process of protein synthesis.

A recent review by SCHLESSINGER (1974) has particularly emphasized the importance of a genetic approach in the dissection of the complexities of protein synthesis. He discussed the necessity of acquiring a collection of mutants affected in many of the macromolecular components of protein synthesis. These strains should be useful not only in the genetic analysis of the affected component, but more importantly could be used to study the mechanism of protein synthesis.

The genes for over half of the ribosomal proteins and several protein synthesis factors have been shown to be clustered near 72 min on the *Escherichia coli* chromosome (JASKUNAS *et al.* 1975; JASKUNAS, LINDAHL and NOMURA 1975). This enrichment of the genes for many translational macromolecules suggested that the technique of localized mutagenesis (HONG and AMES 1971; MURGOLA and YANOFSKY 1974) could be used specifically to isolate conditionally lethal mutants affected in different protein synthesis components. We have recently obtained a collection of temperature-sensitive mutants by two variations of this method. One strain was defective in a ribosomal protein processing activity (KUSHNER, MAPLES and CHAMPNEY 1977), and certain characteristics of 20 additional mutants are the subject of this communication.

MATERIALS AND METHODS

Bacterial strains and growth conditions: The *E. coli* strains used for localized mutagenesis were derivatives of either W3110 (DOOLITTLE and YANOFSKY 1968) or AB2834 (*aroE* 353, PITTARD and WALLACE 1966). The origin of the control strain (SK901) has been described (KUSHNER, MAPLES and CHAMPNEY 1977). Cells were grown in tryptone (T) broth (1.3% tryptone, 0.7% NaCl) or Luria (L) broth (KUSHNER, MAPLES and CHAMPNEY 1977), as indicated. Viable cell numbers were determined for each strain at a permissive (30°) and restrictive (44°) temperature. Cells growing at 30° in 10 ml of T broth were sampled by periodically removing 0.1 ml and plating a 10^{-6} dilution in duplicate at 30° on nutrient agar plates. At a cell density of 2 to 4×10^8/ml, the cultures were shifted to 44°, sampled and plated as before. Cells from the cultures were also spread directly on plates and incubated at 44° to determine the frequency of appearance of temperature-insensitive colonies. The cell density was measured by taking Klett readings (No. 66, red filter) throughout the growth experiment.

Isolation of temperature-sensitive mutants: Two methods were used to introduce mutations into the *aroE-rpsL* (72 min) region of the *E. coli* chromosome. The procedures for hydroxylamine mutagenesis of phage P1 *vir* (propagated on strain JC158) and for the isolation of high temperature (44°)-sensitive mutants by cotransduction with *aroE*+ prototrophy have been described (KUSHNER, MAPLES and CHAMPNEY 1977). A spectinomycin-resistant derivative of AB2834 was used as a recipient strain in the isolation of mutants SK1218 through SK1226. The remaining temperature-sensitive mutants were isolated by treatment of a *trpS* strain (10330, DOOLITTLE and YANOFSKY 1968) with nitrosoguanidine (100 μg/ml at 30° for 30 min) and selection for *trpS*+ revertants at 30°. Cells unable to grow in replica plates at 42° were tested for linkage to *aroE* by P1 transduction. A collection of mutants with temperature sensitivity linked to *aroE* were isolated in this manner by MARTIN MARINUS (University of Massachusetts) and were kindly provided to us.

Measurements of RNA and protein synthesis rates: Cells were grown in 10 ml of tryptone broth in a shaking water bath at 30° and shifted to 44° at a density of about 2×10^8 cells per ml. The kinetics of RNA or protein synthesis at 44° were measured by labeling the shifted cells with ^{3}H-uracil (2 μCi/ml and 5 μg/ml) or with ^{3}H-leucine (2 μCi/ml and 2 μg/ml) for two to three hours after the shift. Samples of 0.1 ml were removed periodically and precipitated with 1 ml of 10% trichloroacetic acid (TCA). The precipitates were collected on GF/A filters, dried, and counted in a Beckman LS 355 scintillation counter in a toluene-PPO (0.25%) solution.

Determination of antibiotic sensitivity: All temperature-sensitive mutants and the control strain were tested for growth sensitivity or resistance to four different ribosome-specific antibiotics by the method of APIRION, PHILLIPS and SCHLESSINGER (1969). Strains were grown overnight in L broth at 30° and then diluted by 10^{-2} into a series of tubes containing different concentrations of erythromycin, kanamycin, spectinomycin or streptomycin in L broth. The tubes were incubated at 37° for 18 hours, and the final absorbance at 600 nm was recorded.

Analysis of ribosomal subunit assembly: Cells were grown at 30° in 10 ml of T broth and shifted to 44° at a density of about 3×10^8/ml. The cells were labelled with 2 μCi of ^{3}H-uracil per ml in the presence of 5 μg of carrier uracil per ml immediately following the temperature shift. After two hours at the restrictive temperature, the cultures were poured over ice and collected by centrifugation at 4° (10,000 rpm for 15 min). The cell pellet was resuspended in 0.2 ml of S buffer (10 mM tris-HCl, pH 7.6, 0.5 mM Mg acetate, 50 mM NH_4Cl, 6 mM mercaptoethanol) and 10 μl of lysozyme (3 mg/ml) was added. After 10 to 15 min at room temperature, 2 μl of DNAase I (5 mg/ml) was added, and the cells were kept at 4° for five min. The cell debris was removed by centrifugation at 10,000 rpm for 10 min, and the lysates were layered on 5 to 20% sucrose gradients in S buffer. The gradients were centrifuged for two hours at 45,000 rpm (SW 50.1 rotor). About 35 fractions were collected, diluted to 1 ml with water and the absorbance at 260 nm measured. An equal volume of 10% TCA was added along with 100 μg of bovine serum albumin, and the precepitated materials were collected on filters and counted as described above.

Biochemicals and radioisotopes: Biochemicals were obtained from the following sources: DNAase I, CalBiochem.; lysozyme, Miles Labs; erythromycin, kanamycin and streptomycin, Sigma Chemical Co.; spectinomycin, a gift from the Upjohn Company. Radioisotopes (^{3}H-uracil, 40 Ci/mM; ^{3}H-leucine, 15 Ci/mM) were purchased from Schwarz-Mann.

RESULTS

We have employed two variations of the technique of localized mutagenesis for the isolation of temperature-sensitive mutants in the location of the ribosomal gene cluster at 72 min on the *E. coli* chromosome (BACHMANN, LOW and TAYLOR 1976). Mutagenesis of bacteriophage P1, followed by the transduction of AB2834 (*aroE*) to *aroE*$^+$, with the subsequent selection of linked temperature-sensitive cells, has given us a collection of mutant strains. One of these (*rimE*) has been described previously (KUSHNER, MAPLES and CHAMPNEY 1977) and six others are discussed below. We have other strains with mutations in this same region that were generated by selecting nitrosoguanidine-induced revertants of a *trpS* strain. Temperature-sensitive mutants were found among the revertants due to the effect of the mutagen in causing linked multiple mutational events during chromosome replication (GUEROLA, INGRAHAM and CERDA-OLMEDO 1971). We have examined 14 mutants with an effect on protein synthesis from a group of temperature-sensitive strains isolated using this procedure.

Each of the mutants, as well as the control strain, was analyzed for its growth characteristics and viability at 30° and after a temperature shift to 44°. At 30° in tryptone broth, the control cells doubled in 36 min, and the different mutants

had generation times between 40 and 70 min. The control cells showed no change in their growth rate after the shift, whereas the mutants exhibited a decline in growth rate and a drop in cell viability at the restrictive temperature. Figure 1 illustrates this growth response for the control (SK901) and two mutant strains, SK1043 and SK1045. Thirteen of the mutants showed no increase in cell mass after one generation at 44°. The remainder demonstrated a reduced rate of growth after the temperature shift. With one exception, all of the mutant strains also showed a decline in cell viability upon incubation at 44°. Figure 2 shows the loss in viability for a typical mutant (SK1152) and the continued growth at this temperature of the control strain. SK1045 was not killed at 44°, but maintained a constant cell number for up to four hours. A measure of the conditional lethality for the different mutants is the time required for a 50% reduction in cell number at 44°. This occurred over a range of 0.75 to 4.0 hours for the different mutants examined (Table 1). The growth response of the mutants at the elevated temperature was very similar to that observed previously for strain SK911 (*rimE*) (KUSHNER, MAPLES and CHAMPNEY 1977).

The reversion frequencies for all of the mutant strains have been determined. For the mutants isolated after chromosomal mutagenesis, temperature-resistant revertants were isolated at frequencies between 3.3×10^{-6} and 1.0×10^{-9}. The six strains isolated by the phage P1 localized mutagenesis technique had rever-

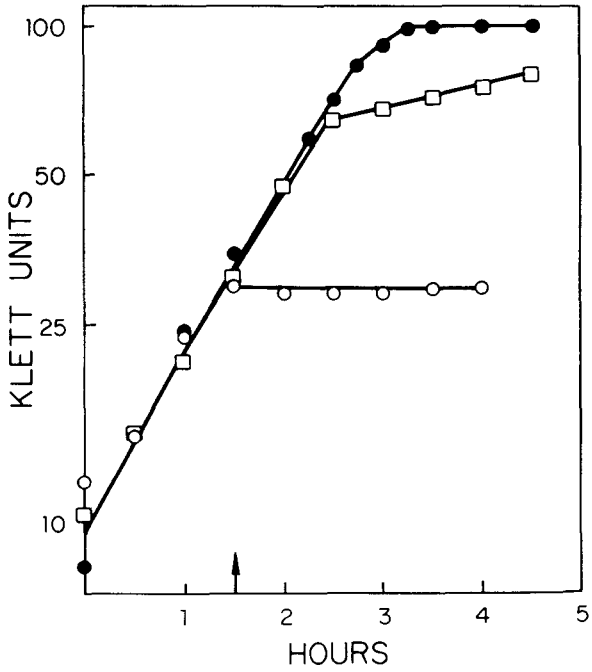

FIGURE 1.—Growth curves for two temperature-sensitive mutants and a control strain. The increase in cell density (Klett units) is shown for cells growing at 30° and shifted (arrow) to 44°. SK901 (control) (●); SK1043 (□); SK1045 (○).

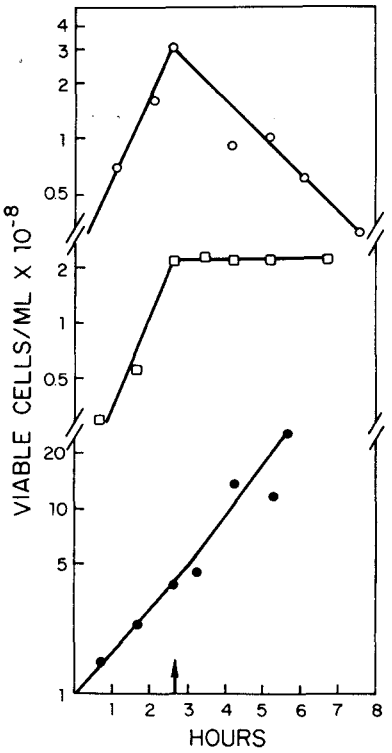

FIGURE 2.—Cell viability of two temperature-sensitive mutants and a control strain. The increase in cell number (viable cells per ml) is indicated for cells growing at 30° and shifted (arrow) to 44°. SK901 (control) (●); SK1152 (○); SK1045 (□).

sion frequencies between 3.0×10^{-6} and 3.5×10^{-8}. These frequencies are indicative of point mutations conferring temperature sensitivity on the different strains. A more detailed examination of the characteristics of the revertants is in progress.

The relative rates of RNA and protein synthesis for each strain at 44° were measured by determining the rate of incorporation of radioactive uracil and leucine. The kinetics of incorporation of uracil and leucine into the control strain and three mutants, representing the strains derived by both localized mutagenesis techniques, are indicated in Figure 3. The relative rates of synthesis for every mutant are listed in Table 2. The rates of both RNA and protein formation were reduced in all mutants in comparison with the control strain. Protein synthesis was more severely affected, with 18 strains showing a rate 50% or less of that of the control. Only seven mutants had an RNA synthesis rate lower than the corresponding protein synthesis rate.

The temperature-sensitivity of protein synthesis in the majority of these strains suggested a mutational effect on a component of translation. Therefore, all of the conditional mutants were tested for alterations in their sensitivity to four different antibiotic inhibitors of protein synthesis. We examined the growth response of each mutant and a control strain to increasing concentrations of three

TABLE 1

Characteristics of temperature-sensitive mutants

Strain	Isolation method*	$t_{1/2}$(44°)†	Antibiotic sensitivity‡	Assembly defect§
SK1024	NTG	60	—	50S (0.32)
SK1029	NTG	48	str^R	50S (0.23)
SK1043	NTG	90	spc^S	— (0.83)
SK1044	NTG	120	$spc^S ery^R$	— (0.87)
SK1045	NTG	—‖	$spc^S ery^S$	30S (2.00)
SK1046	NTG	78	—	— (0.74)
SK1047	NTG	200	—	— (0.78)
SK1048	NTG	90	—	— (1.00)
SK1049	NTG	60	str^S	30S (1.80)
SK1151	NTG	105	—	— (0.85)
SK1152	NTG	60	ery^R	— (0.88)
SK1154	NTG	60	$str^R spc^R ery^R$	— (0.86)
SK1155	NTG	114	$spc^S ery^S$	— (0.88)
SK1156	NTG	90	ery^R	— (0.75)
SK1218	Td	240	—	30S (1.24)
SK1219	Td	210	ery^S	— (0.85)
SK1220	Td	110	—	30S (1.34)
SK1221	Td	45	—	30S (1.74)
SK1224	Td	135	str^R	30S (1.45)
SK1226	Td	60	—	30S (1.25)

* Temperature-sensitive mutants were isolated after nitrosoguanidine (NTG) mutagenesis of the bacterial chromosome or by transduction (Td) with mutagenized bacteriophage P1 as described (MATERIALS AND METHODS).

† Indicates the time required for 50% loss of viability at 44°.

‡ Altered sensitivity (S) or resistance (R) to streptomycin (str), spectinomycin (spc) or erythromycin (ery). Mutants SK1218–SK1226 were isolated from a spc^R parent strain.

§ Indicates affected subunit and 50S/30S specific activity ratio ( ).

‖ Strain SK1045 remains viable at 44° (Figure 2).

30S ribosomal subunit inhibitors, kanamycin, spectinomycin and streptomycin, and one 50S subunit inhibitor, erythromycin (PESTKA 1971). Figure 4A shows the response of the control and two mutants (SK1154 and SK1155) to increasing concentrations of spectinomycin. Strains SK1043, SK1044 and SK1045, like SK1155, revealed an enhanced growth sensitivity to spectinomycin (Table 1), whereas strain SK1154 was resistant to inhibition by the drug (Figure 4A). (The spectinomycin resistance of strains SK1218 through SK1226 was present in the parental strain from which they were derived.)

Resistance to erythromycin was exhibited by mutants SK1154 (Figure 4B), SK1044, SK1152, and SK1156, while an enhanced sensitivity to this 50S inhibitor was shown by strains SK1045, SK1155 and SK1219 (Figure 4B). Finally, three mutants resistant to streptomycin were discovered among the temperature-

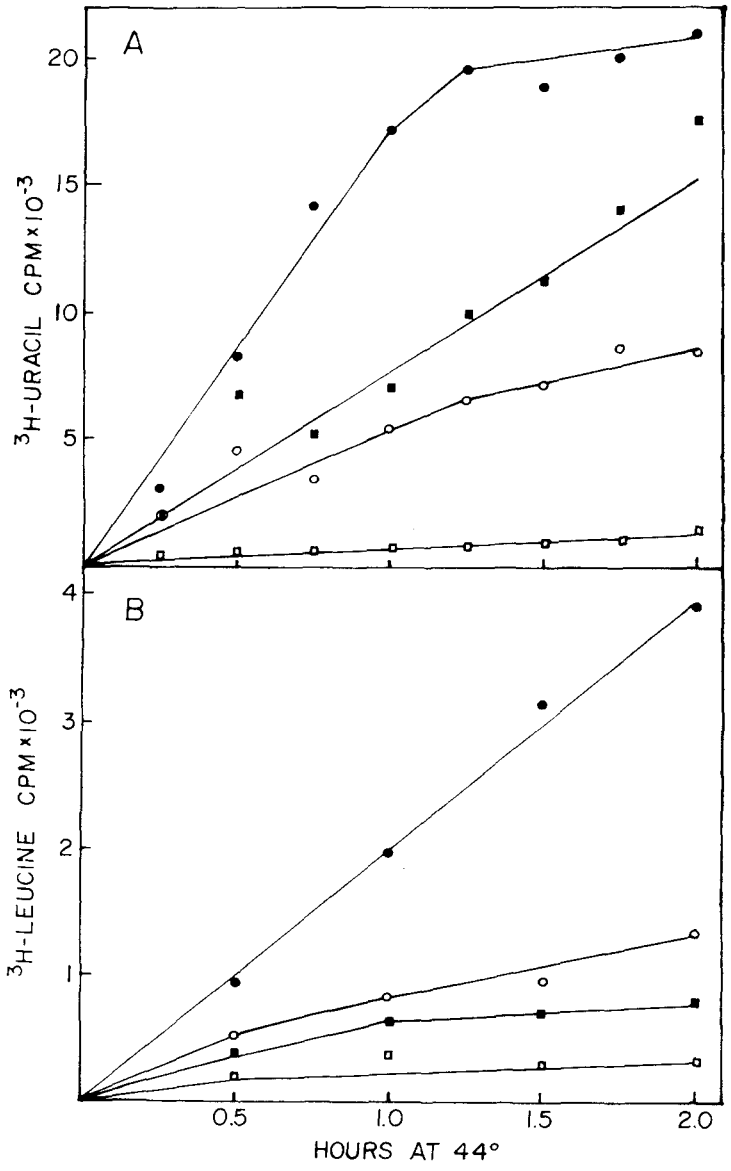

FIGURE 3.—Kinetics of RNA and protein synthesis at 44°. A. Incorporation of ^{3}H-uracil into TCA precipitable material from cells growing at 44°. B. Incorporation of ^{3}H-leucine into TCA precipitable material from cells growing at 44°. SK901 (control) (●); SK1045 (□); SK1156 (○); SK1220 (■).

TABLE 2

*Relative rates of RNA and protein synthesis for different temperature-sensitive mutants**

Strain	RNA synthesis	Protein synthesis
SK1024	31	19
SK1029	36	11
SK1043	2	13
SK1044	7	15
SK1045	3	9
SK1046	4	75
SK1047	12	47
SK1048	19	8
SK1049	5	12
SK1151	3	3
SK1152	10	9
SK1154	43	18
SK1155	5	59
SK1156	25	25
SK1218	26	13
SK1219	62	41
SK1220	66	17
SK1221	26	17
SK1224	64	44
SK1226	57	19

* The rates were calculated as a percent of the control rate of ^{3}H-uracil or ^{3}H-leucine incorporation after temperature shift-up, as described in MATERIALS AND METHODS. For SK901 the absolute rates were 17,675 cpm per hour for ^{3}H-uracil and 1,875 cpm per hour for ^{3}H-leucine. Results are the average of two determinations. For comparison, all rates were determined between one and two hours following the temperature shift.

sensitive strains (SK1029, SK1154 and SK1224) and one mutant (SK1049) showed an enhanced streptomycin sensitivity. No cells were found with an altered response to kanamycin. Table 1 lists the antibiotic sensitivity of each mutant.

Temperature-sensitive protein synthesis in these cells could be caused by a defect in ribosomal subunit formation. To discover if ribosome assembly was impaired by the temperature-sensitive lesion, the cells were labeled with ^{3}H-uracil after a growth shift to 44°. The labeled cell extracts were sedimented through sucrose gradients to separate the ribosomal subunits. Figure 5C shows a control gradient profile, and Figures 5A and 5B depict the profiles of mutants SK1024 and SK1029. The gradient patterns indicate a clear deficiency in the formation of 50S subunits for both mutants. The specific activity (^{3}H-uracil cpm/A_{260}) for each subunit was calculated by summing the total radioactivity and absorbance for the two gradient peaks. The specific activity is a measure of the amount of

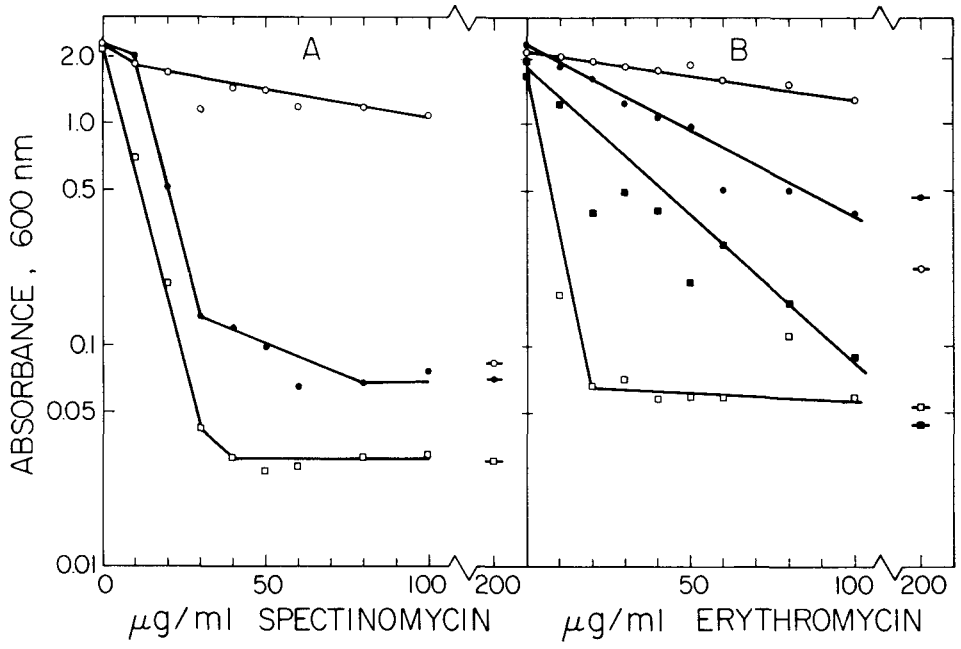

FIGURE 4.—Antibiotic sensitivity of temperature-sensitive mutants and control strain. The final cell density (absorbance at 600 nm) is indicated for each strain incubated for 18 hours at 37° in the presence of the indicated amount of (A) spectinomycin or (B) erythromycin. SK901 (control) (●); SK1154 (○); SK1155 (□); SK1219 (■).

subunit synthesis at 44° and the equivalent formation of the two subunits should give a ratio of specific activities of 1.0. The ratio of 50S to 30S subunits for the control strain was 0.90. For the two mutants depicted, the ratios were 0.32 and 0.23, respectively (Table 1). These strains were the only ones to show defects in 50S assembly. Seven mutants had specific activity ratios of 1.24 or greater, suggesting an effect on 30S subunit formation (Table 1). The remaining mutants had gradient profiles and ratios similar to the control strain. Preliminary studies have indicated the presence of precursor 16S RNA in lysates of strains SK1024, SK1029, SK1218, SK1220, SK1221 and SK1224 grown at 44° and examined by polyacrylamide gel electrophoresis (unpublished observations).

Precise mapping of the genes conferring temperature sensitivity on these strains is in progress. Three-factor transductional crosses with phage P1 have indicated a close linkage of the temperature-sensitive character with *aroE* or streptomycin resistance (*rpsL*, CHAMPNEY and KUSHNER 1976) in six of the strains isolated by chromosomal mutagenesis. Temperature sensitivity in the six mutants derived by P1 localized mutagenesis must also be linked to *aroE*, by the nature of the selection method for these strains (KUSHNER, MAPLES and CHAMPNEY 1977). The mapping data for these mutants will be presented in detail after more strains have been examined.

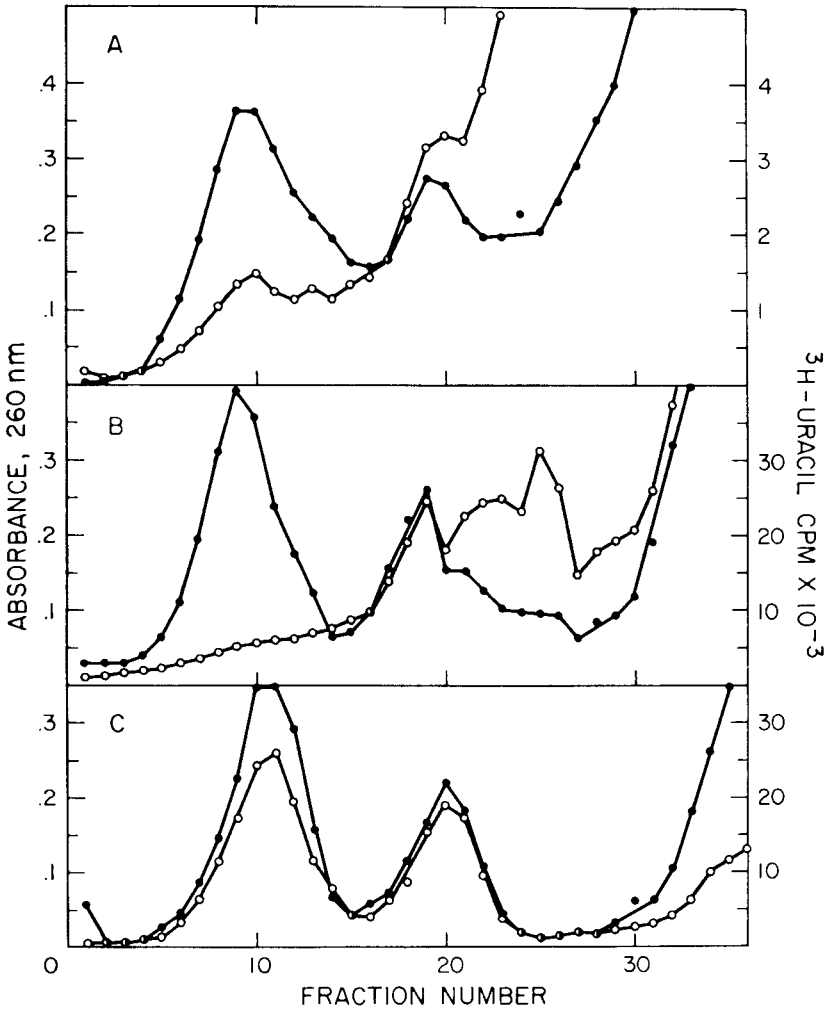

FIGURE 5.—Sucrose density gradient profiles of cell lysates from temperature-sensitive mutants and control strain. Lysates from cells labeled with ^{3}H-uracil at 44° were sedimented through linear sucrose gradients to separate the ribosomal subunits. The absorbance at 260 nm (●) and the CPM of ^{3}H-uracil (○) were measured for each fraction as described in MATERIALS AND METHODS. (A) SK1024; (B) SK1029; (C) SK901 (control).

DISCUSSION

Directed mutagenesis of a specific region of the *E. coli* chromosome has been useful in isolating a collection of temperature-sensitive, conditionally lethal mutants with apparent defects in protein synthesis. The clustering of many genes for the ribosomal proteins, protein synthesis factors and other translocational components at 72 min substantially increases the probability of isolating strains with defects in the translation process by this technique. Transduction of the *aroE* marker at 72 min with mutagenized P1 transducing particles should give

linked mutations within about 2 min of this gene on the bacterial chromosome (MURGOLA and YANOFSKY 1974). Alternatively, selection of revertants of a chromosomal marker at 73 min (*trpS*) after nitrosoguanidine mutagenesis should also be expected to give linked mutations in many strains as a consequence of the multiple mutational activity of this mutagen during chromosome replication (GUEROLA, INGRAHAM and CERDA-OLMEDO 1971). The ribosomal gene cluster is between *trpS* and *aroE* and would be expected to be enriched in mutated genes after this treatment. This localized mutagenesis approach has been recently used by others for the isolation of conditional mutants in ribosomal proteins and RNA polymerase (BERGER *et al.* 1975; CLAEYS *et al.* 1976).

A comparison of the types of mutants isolated after the two methods of localized mutagenesis is informative. Of the 14 mutants derived by nitrosoguanidine mutagenesis, nine were altered in their response to one or more of the antibiotics. Only four of the 14 showed ribosomal assembly defects. By contrast, only two of the six mutants from P1 mutagenesis showed antibiotic alterations, but five of the six revealed impaired ribosomal assembly. These differences may be related to the different activities of the two mutagens and to the replicational *versus* recombinational origin of the chromosomal mutations (GUEROLA, INGRAHAM and CERDO-OLMEDO 1971; HONG and AMES 1971).

The reduction in the rate of *in vivo* protein synthesis observed in all of these strains, together with the observed alterations in antibiotic sensitivity or ribosome assembly found in many of the mutants, suggests that genes for translational macromolecules have been mutated in many cases. The reduction in RNA synthesis seen in these mutants may reflect the stringent control of RNA synthesis observed during protein synthesis inhibition (CASHEL and GALLANT 1974), since both parental strains were *rel*$^+$. The few strains showing rates of RNA synthesis lower than the protein synthesis rates may also be affected in a transcriptional component, since the gene for the α subunit of RNA polymerase has been shown to map in this same chromosomal region (JASKUNAS, BURGESS and NOMURA 1975).

The alterations in antibiotic sensitivity seen in these mutants may be the consequence of additional mutational events, particularly in cells treated with nitrosoguanidine. Alternatively, they may reflect the interaction of an altered ribosomal component with other parts of this macromolecular complex. Several examples of pleiotrophic effects on ribosomal antibiotic sensitivity and function have been described for other antibiotic-resistant mutants of *E. coli* (APIRION and SCHLESSINGER 1969; SALTZMAN, BROWN and APIRION 1974; SALTZMAN and APIRION 1976).

The most direct indication of an affect on a translational component in these mutants was the observation that ribosomal assembly was affected at the restrictive temperature in nine mutants. The use of pre-existing ribosomes (formed at the permissive temperature) would allow only continued linear growth at 44° if no new ribosomes could be formed. This is consistent with the growth properties observed for many of the mutants. Ribosomal subunit assembly defects have been observed in other high and low temperature-sensitive strains, with prop-

erties similar to those described here (GUTHRIE, NASHIMOTO and NOMURA 1969; ROSSET *et al.* 1971; BRYANT and SYPHERD 1974).

The temperature-sensitive protein synthesis activity observed in these strains needs to be investigated in more detail. Assays are being conducted to examine specific steps in the process of protein synthesis, in order to better define the nature of the temperature-sensitive defect. Preliminary experiments indicate that ribosomal subunits from some of these strains cannot reassociate at 44° and that others are impaired in mRNA binding or tRNA binding (unpublished observations). The availability of these protein synthesis mutants will permit studies that should give a much clearer understanding of the relationships between structure and function for the macromolecules involved in the process of translation.

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LITERATURE CITED

APIRION, D., S. L. PHILLIPS and D. SCHLESSINGER, 1969 Approaches to the genetics of *Escherichia coli* ribosomes. Cold Spring Harbor Symp. Quant. Biol. **34**: 117–128.

APIRION, D. and D. SCHLESSINGER, 1969 Mutations in ribosome genes of *Escherichia coli*: pleiotropy and interactions in an organelle. Japan J. Genet. **44**: (Suppl. 1) 1–10.

BACHMANN, B., K. B. LOW and A. L. TAYLOR, 1976 Recalibrated linkage map of *Escherichia coli* K12. Bacteriol. Rev. **40**: 116–167.

BERGER, I., A. GEYL, A. BOCK, G. STOFFLER and H. G. WITTMANN, 1975 Localized mutagenesis of the *aroE-strA* section of the *Escherichia coli* chromosome coding for ribosomal proteins. Molec. Gen. Genet. **141**: 207–211.

BRYANT, R. E. and P. S. SYPHERD, 1974 Genetic analysis of cold-sensitive ribosome maturation mutants of *Escherichia coli*. J. Bacteriol. **117**: 1082–1092.

CHAMPNEY, W. S. and S. R. KUSHNER, 1976 A proposal for a uniform nomenclature for the genetics of bacterial protein synthesis. Molec. Gen. Genet. **147**: 145–151.

CASHEL, M. and J. GALLANT, 1974 Cellular regulation of guanosine tetraphosphate and guanosine pentaphosphate. pp. 733–745. In: *Ribosomes*. Edited by M. NOMURA, A. TISSIERES and P. LENGYEL. Cold Spring Harbor Laboratory, New York.

CLAEYS, I. V., J. H. MILLER, J. B. KIRSCHBAUM, S. NASI, B. MOLHOLT, G. GROSS, D. A. FIELDS and E. K. F. BAUTZ, 1976 Altered RNA polymerases resulting from temperature sensitive mutations in the *rif* region of the *E. coli* chromosome. pp. 56–65. In: *Control of Ribosome Synthesis*, Alfred Benzon Symposium IX, Munksggaard, Copenhagen.

DOOLITTLE, W. F. and C. YANOFSKY, 1968 Mutants of *Escherichia coli* with an altered tryptophanyl-transfer ribonucleic acid synthetase. J. Bacteriol. **95**: 1283–1294.

GUEROLA, N., J. L. INGRAHAM and E. CERDA-OLMEDO, 1971 Induction of closely linked multiple mutations by nitrosoguanidine. Nature (Lond.) New Biol. **230**: 122–125.

GUTHRIE, C., H. NASHIMOTO and M. NOMURA, 1969 Structure and function of *Escherichia coli* ribosomes. VIII. Cold-sensitive mutants defective in ribosome assembly. Proc. Natl. Acad. Sci. U.S. **63**: 384–391.

HONG, J. and B. N. AMES, 1971 Localized mutagenesis of any specific small region of the bacterial chromosome. Proc. Natl. Acad. Sci. U.S. **68**: 3158–3162.

ISONO, K., J. KRAUSS and Y. HIROTA, 1976 Isolation and characterization of temperature-sensitive mutants of *Escherichia coli* with altered ribosomal proteins. Molec. Gen. Genet. **149:** 297–302.

ISONO, K., A. G. CUMBERLIDGE, S. ISONO and Y. HIROTA, 1977 Further temperature-sensitive mutants of *Escherichia coli* with altered ribosomal proteins. Molec. Gen. Genet. **152:** 239–243.

JASKUNAS, S. R., M. NOMURA and J. DAVIES, 1974 Genetics of bacterial ribosomes. pp. 333–368. In: *Ribosomes.* Edited by M. NOMURA, A. TISSIERES and P. LENGYEL. Cold Spring Harbor Laboratory, New York.

JASKUNAS, S. R., R. R. BURGESS and M. NOMURA, 1975 Identification of gene for the α-subunit of RNA polymerase at the *str-spc* region of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. U.S. **72:** 5036–5040.

JASKUNAS, S. R., L. LINDAHL and M. NOMURA, 1975 Specialized transducing phages for ribosomal protein genes of *E. coli.* Proc. Natl. Acad. Sci. U.S. **72:** 6–10.

JASKUNAS, S. R., L. LINDAHL, M. NOMURA and R. R. BURGESS, 1975 Identification of two copies of the gene for the elongation factor EF-Tu in *E. coli.* Nature **257:** 458–462.

KURLAND, C. G., 1977 Structure and function of the bacterial ribosome. Ann. Rev. Biochem. **46:** 172–200.

KUSHNER, S. R., V. F. MAPLES and W. S. CHAMPNEY, 1977 Conditionally lethal ribosomal protein mutants: Characterization of a locus required for modification of 50S subunit proteins. Proc. Natl. Acad. Sci. U.S. **74:** 467–471.

LENGYEL, P., 1974 The process of translation; a bird's eye view. pp. 13–52. In: *Ribosomes,* edited by M. NOMURA, A. TISSIERES and P. LENGYEL. Cold Spring Harbor Laboratory, New York.

MURGOLA, E. J. and C. YANOFSKY, 1974 Structural interactions between amino acid residues at positions 22 and 211 in the tryptophan synthetase alpha chain of *Escherichia coli.* J. Bacteriol. **117:** 444–448.

NOMURA, M., 1973 Assembly of bacterial ribosomes. Science **179:** 864–873.

PESTKA, S., 1971 Inhibitors of ribosome functions. Ann. Rev. Microbiol. **25:** 487–562.

PITTARD, J. and B. J. WALLACE, 1966 Distribution and function of genes concerned with aromatic biosynthesis in *Escherichia coli.* J. Bacteriol. **91:** 1494–1508.

ROSSET, R., C. VOLA, J. FEUNTEUN and R. MONIER, 1971 A thermosensitive mutant defective in ribosomal 30S subunit assembly. FEBS Letters **18:** 127–129.

SALTZMAN, L. A., M. BROWN and D. APIRION, 1974 Functional interdependence among ribosomal elements as revealed by genetic analysis. Molec. Gen. Genet. **133:** 201–207.

SALTZMAN, L. and D. APIRION, 1976 Binding of erythromycin to the 50S ribosomal subunit is affected by alterations in the 30S ribosomal subunit. Molec. Gen. Genet. **143:** 301–306.

SCHLESSINGER, D., 1974 Genetic and antibiotic modification of protein synthesis. Ann. Rev. Genetics **8:** 135–154.

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