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# Protein and Nucleic Acid Synthesis in Two Mutants of *Escherichia coli* with Temperature-Sensitive Aminoacyl Ribonucleic Acid Synthetases[1]

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ABSTRACT

EIDLIC, LIA (Purdue University, Lafayette, Ind.), AND FREDERICK C. NEIDHARDT. Protein and nucleic acid synthesis in two mutants of *Escherichia coli* with temperature-sensitive aminoacyl ribonucleic acid synthetases. J. Bacteriol. 89:706-711. 1965.—Two temperature-sensitive mutants of *Escherichia coli* were isolated which grow almost normally at 30 C and fail to grow at 37 C. One (I-9) was derived from a strain with stringent amino acid control of ribonucleic acid (RNA) synthesis; the other (IV-4) was derived from a strain with relaxed amino acid control of RNA synthesis. When cultures of these mutants growing at 30 C were shifted to 37 C, IV-4 synthesized RNA preferentially to protein but I-9 did not. Cell-free extracts of both mutants and their parent strains were examined for their ability to catalyze adenosine triphosphate (ATP)-dependent attachment of amino acids to soluble RNA (sRNA). These measurements indicated that I-9 possesses a temperature-sensitive valyl sRNA synthetase, and that IV-4 possesses a temperature-sensitive phenylalanyl sRNA synthetase. The behavior of these mutants suggests that amino acids permit RNA synthesis in stringent strains only after activation or attachment to sRNA, that relaxed strains can overproduce RNA without a complete array of fully functioning aminoacyl sRNA synthetases, and that these enzymes are obligatory for the biosynthesis of proteins.

Activation of amino acids and their attachment to soluble ribonucleic acid (sRNA) are believed to be obligatory steps in protein synthesis (for a review of the evidence consult Simpson, 1962). Amino acids have also been implicated in the regulation of the overall rate of RNA synthesis in bacteria (the evidence was recently reviewed by Neidhardt, 1964). This regulatory function of amino acids has been visualized by two groups of investigators (Stent and Brenner, 1961; Kurland and Maaløe, 1962) as the neutralization by amino acids of an inhibitory effect of uncharged sRNA on RNA polymerase. Studies in vitro on this enzyme have supported the sRNA theory of regulation by showing that sRNA molecules are indeed less inhibitory to RNA polymerase when they are charged with amino acids (Tissiéres, Bourgeois, and Gros, 1963). Estimations of the degree of charging of cellular sRNA by measuring its resistance to periodate oxidation have provided additional support by showing some correlations between the degree of charging and the rate of RNA synthesis under a few special conditions (Ezekiel, 1963).

This paper reports the isolation and identification of two mutants of *Escherichia coli*, each of which appears to possess an aminoacyl sRNA synthetase which fails to operate at 37 C. By examining the behavior of these cells when they are shifted from 30 to 37 C, it has been possible to obtain direct evidence of the physiological importance of aminoacyl sRNA synthetases in protein and RNA synthesis.

## MATERIALS AND METHODS

*Organisms.* This study involved four strains of *E. coli:* KB, a prototrophic wild strain which exhibits stringent control of RNA synthesis by amino acids ($RC^{st}$); K10, a thiamine-requiring wild strain which exhibits relaxed control of RNA synthesis by amino acids ($RC^{rel}$); I-9, a temperature-sensitive mutant derived from KB; and IV-4, a temperature-sensitive mutant derived from K10.

[1] This paper is taken from a thesis to be submitted by L. Eidlic to the Faculty of the Graduate School of Purdue University in partial fulfillment of the requirements for the Ph.D. degree. A portion of this work was presented at the 64th Annual Meeting of the American Society for Microbiology, Washington, D.C., 3-7 May 1964.

The procedure by which the mutants were isolated is described below.

*Growth of cultures.* Except in the mutant isolation procedures, which required a special medium described below, the following three media were used: (i) a minimal medium consisting of basal salts solution P (Fraenkel and Neidhardt, 1961) supplemented with 0.4% glucose and, when required, 5 $\mu$g/ml of thiamine; (ii) an enriched medium containing 10% Tryptone, 5% yeast extract, and 0.2% glucose; and (iii) a low phosphate-enriched medium containing basal solution TM (Fraenkel and Neidhardt, 1961) supplemented with 0.2% Tryptone, 0.2% glucose, and $10^{-3}$ M phosphate. Liquid cultures were grown aerobically on a rotary action shaker at 30 or 37 C. Growth was measured by optical density in a Zeiss spectrophotometer (model PMQ II) at 420 m$\mu$.

*Measurement of protein, RNA, and deoxyribonucleic acid (DNA).* Synthesis of these macromolecules was measured by either colorimetric or isotopic methods. Portions of cultures to be assayed colorimetrically were filtered through membrane filters (coarse porosity; Schleicher & Schuell Co., Keene, N.H.). The cells on the filter were washed with ice-cold solution P and then resuspended in a known volume of distilled water with the aid of a mechanical mixer (Vortex). Portions of the suspension were then assayed for protein by the phenol method (Lowry et al., 1951), for RNA by the orcinol method (Schneider, 1945), and for DNA by the diphenylamine method (Burton, 1956).

To estimate protein and nucleic acid synthesis by isotope incorporation, cultures were grown in the low phosphate enriched medium containing either sufficient $P^{32}$ as orthophosphate to yield a specific activity of ca. 13,000 counts per min per $\mu$mole, or sufficient $C^{14}$-labeled L-arginine to yield a specific activity of ca. 500 counts per min per $\mu$g. The cells from culture samples were collected on membrane filters and washed thoroughly with ice-cold solution P. Radioactivity was then measured in a Nuclear-Chicago thin end-window, gas-flow counter.

*Isolation of temperature-sensitive mutants.* The wild strain was grown overnight in a rich medium containing (per liter): 37 g of Brain Heart Infusion (Difco), 3 g of yeast extract, 1 g of Tryptone, and 20 ml of a separately sterilized solution containing (per 100 ml) 50 mg each of L-methionine, L-histidine, L-tyrosine, and L-asparagine, 5 mg of thymidine, and 500 mg of deoxyadenosine. Approximately 0.1 ml of ethyl methane sulfonate was added to 5 ml of such a culture in a 250-ml flask, and the mixture incubated at 30 C with shaking for 2 hr. Fresh medium (45 ml) was then added to the flask, and the incubation was continued for 5 hr. A subculture was made to obtain an exponential-phase culture, and when the cell density was ca. $10^9$ cells per milliliter the temperature was increased to 37 C. Penicillin (2,000 units per ml) was added 30 min later, and the incubation was continued at 37 C until there was evidence of cell lysis. Suitably diluted portions of the culture were then spread on agar plates containing the same rich medium and placed at 37 C. Colonies appearing within 15 hr were marked, and the plates were incubated at 30 C for 40 hr. New colonies appearing at the lower temperature were picked for further purification and study.

This procedure was followed several times to accumulate a large number of temperature-sensitive mutants. The rationale behind each step of the procedure was described elsewhere (Neidhardt, 1964).

*Assay of aminoacyl sRNA synthetases.* Cell-free extracts were prepared by sonic treatment of washed cells with a Branson Sonifier. The extracts were dialyzed overnight against phosphate buffer (0.006 M, *p*H 7.3) containing mercaptoethanol (0.006 M) at 5 C. All three of the conventional methods of measuring aminoacyl sRNA synthetases were used. (i) Adenosine triphosphate (ATP)-dependent formation of aminoacyl hydroxamate was measured by use of the $C^{14}$-labeled amino acid method of Loftfield and Eigner (1963), modified slightly by Nass and Neidhardt (*in preparation*). (ii) Amino acid-dependent exchange of $P^{32}$-labeled pyrophosphate with ATP was followed by the method described previously (Fangman and Neidhardt, 1964*a*). (iii) ATP-dependent attachment of $C^{14}$-labeled amino acid to sRNA was measured as described previously (Fangman and Neidhardt, 1964*a*). The amino acids used in this assay each had a specific activity of 20 $\mu$c/$\mu$mole, except L-histidine (0.6 $\mu$c/$\mu$mole).

*Genetic crosses.* The ability of the wild strains to grow at 37 C was symbolized as $ts^+$; the inability of the mutants, $ts^-$. Sensitivity to streptomycin was symbolized as $str^s$, resistance as $str^r$. Crosses were performed between strain K10 (Hfr, $str^s ts^+$), and strain I-91 ($F^+$, $str^r ts^-$), a streptomycin-resistant derivative of strain I-9. Separate cultures of K10 and I-91 were grown overnight on enriched medium at 30 C. The culture of I-91 was aerated for an additional 17 hr to convert these $F^+$ cells into $F^-$ phenocopies (Lederberg, Cavalli, and Lederberg, 1952). About $5 \times 10^9$ $F^-$ cells were then mixed with about $5 \times 10^8$ Hfr cells in a total volume of 10 ml of enriched medium. The mixture was held at 30 C without shaking for 1 hr, then for 3 hr with gentle shaking. To detect recombinants ($str^r$, $ts^+$), samples of the mating mixture were plated on enriched agar containing 200 $\mu$g/ml of streptomycin and were incubated at 37 C after a 2-hr period at 30 C. Samples of each of the parental cultures were plated similarly to detect the presence of any K10 cells that were $str^r$, and any I-91 cells that were $ts^+$. Recombinants were then picked and cultures were grown at 37 C to be tested for valyl sRNA synthetase activity by the attachment assay.

## RESULTS

In Table 1 are presented the growth-rate constants of the two temperature-sensitive

TABLE 1. *Steady-state growth rates of normal cells and of temperature-sensitive mutants*

| Organism | Medium* | Growth rate† | |
|---|---|---|---|
| | | 30 C | 37 C |
| KB (wild) | Minimal | 0.33 | 0.52 |
| | Enriched | 1.12 | 1.66 |
| I-9 (mutant) | Minimal | 0.31 | 0 |
| | Enriched | 0.93 | 0 |
| K10 (wild) | Minimal | 0.44 | 0.69 |
| | Enriched | 0.80 | 1.54 |
| IV-4 (mutant) | Minimal | 0.46 | 0 |
| | Enriched | 0.87 | 0 |

* The composition of the media is described in Materials and Methods.

† Expressed as the specific growth rate constant, $k$, calculated as $ln\ 2$/mean doubling time in hours.

mutants, I-9 and IV-4, and their respective parents, KB and K10. Each mutant grew almost as rapidly as its parent at 30 C in both minimal and enriched media. Both parents grew 1.5 times faster at 37 C than at 30 C, and neither mutant grew in any medium at 37 C.

The rich medium used during the isolation of these mutants had been chosen to lessen the probability of selecting a mutant with a temperature-sensitive step in monomer biosynthesis. To help pinpoint the reaction which had become altered in I-9 and IV-4, the ability of these cells to synthesize RNA, protein, and DNA at 37 C was examined. Cultures of mutant and of wild cells in balanced growth in rich medium at 30 C were shifted to 37 C, and the accumulation of RNA, protein, and DNA was measured colorimetrically (Table 2). Both wild strains exhibited a coordinately accelerated synthesis of all three macromolecules. Mutant I-9 for 2 hr showed unbalanced growth in which more DNA and protein accumulated than RNA. Mutant IV-4 also went into a phase of unbalanced growth, but one in which there was an overproduction of RNA relative to protein and DNA. Repetition of this experiment, but with minimal medium, yielded similar results (not shown). Measurements of macromolecule synthesis by isotope incorporation confirmed the colorimetric data (Table 3).

The decreased protein synthesis relative to RNA and DNA by strain IV-4 tentatively implicates some step in protein synthesis as the temperature-sensitive reaction. The behavior of strain I-9, however, leads to an ambiguous conclusion. The preferential inhibition of RNA synthesis could be caused by a primary difficulty in the synthesis of this polymer, or it might be an indirect result of the inhibition of any of a number of cellular processes, because RNA synthesis is known to be highly "irritable" (Schaechter, 1961). When chloramphenicol was added at high concentrations (100 $\mu$g/ml) to a culture of I-9 which had been exposed to 37 C for 35 min, RNA synthesis did not proceed (Table 4), even though this concentration of chloramphenicol normally uncouples RNA synthesis from protein synthesis (Kurland and Maaløe, 1962). I-9, therefore, has a temperature-induced lesion (i) in the formation of some phosphorylated precursor of RNA which cannot be supplied exogenously to the cell, (ii) in the polymerization of RNA, or (iii) in some other reaction which must proceed even in the presence of chloramphenicol for RNA to be synthesized.

Cell-free extracts were prepared from cultures of all four organisms and examined for their content of 18 aminoacyl sRNA synthetases (Table 5). The synthetase levels of each mutant differed from those of its parent by less than a factor of two for 17 of the 18 amino acids tested, and these small differences were not considered significant. Valyl sRNA synthetase, however, was undetectable in the I-9 extract, and phenylalanyl sRNA synthetase was undetectable in the IV-4 extract.

An extract from each mutant was mixed with an equal portion (on a total protein basis) of an extract from the respective wild strain. No significant inhibition or stimulation of either the

TABLE 2. *Accumulation of RNA, protein, and DNA by normal cells and by temperature-sensitive mutants after a shift to 37 C*

| Organism | Time after shift to 37 C | RNA* | Protein* | DNA* | RNA/protein* |
|---|---|---|---|---|---|
| | *min* | | | | |
| KB (wild) | 0 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 30 | 2.11 | 2.00 | 2.09 | 1.05 |
| | 60 | 4.90 | 5.10 | 4.37 | 0.96 |
| I-9 (mutant) | 0 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 30 | 1.27 | 1.82 | 1.46 | 0.70 |
| | 120 | 1.08 | 1.73 | 1.85 | 0.63 |
| K10 (wild) | 0 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 30 | 2.15 | 1.91 | 1.71 | 1.15 |
| | 70 | 3.40 | 3.18 | 3.20 | 1.07 |
| IV-4 (mutant) | 0 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 30 | 2.82 | 1.24 | 2.22 | 2.26 |
| | 85 | 3.95 | 1.62 | 2.11 | 2.44 |
| | 125 | 5.08 | 1.95 | 3.34 | 2.60 |

* All values are normalized to the value at zero time. Colorimetric assays were used.

valyl sRNA synthetase of the KB extract or of the phenylalanyl sRNA synthetase of the K10 extract was found (Table 6); the drop in specific activity was almost precisely what would be expected by simple dilution of the wild extracts with inert protein.

Cultures of KB, I-9, K10, and IV-4 were grown in rich medium at 30 C and cell-free extracts were prepared. Other cultures of the four strains were grown at 30 C but shifted to 37 C 2 hr before

TABLE 3. *Incorporation of $P^{32}$-labeled phosphate and $C^{14}$-labeled arginine by normal cells and by temperature-sensitive mutants after a shift to 37 C*

| Organism | Time after shift to 37 C | Isotope incorporated* | | |
|---|---|---|---|---|
| | | $P^{32}$ (as $PO_4$) | $C^{14}$ (as L-arginine) | $P^{32}/C^{14}$ |
| | *min* | | | |
| KB (wild) | 0 | 1.00 | 1.00 | 1.00 |
| | 30 | 1.50 | 1.35 | 1.13 |
| | 60 | 2.09 | 1.84 | 1.15 |
| I-9 (mutant) | 0 | 1.00 | 1.00 | 1.00 |
| | 30 | 1.16 | 1.20 | 0.97 |
| | 60 | 1.25 | 1.14 | 1.11 |
| | 90 | 1.31 | 1.20 | 1.09 |
| K10 (wild) | 0 | 1.00 | 1.00 | 1.00 |
| | 30 | 1.59 | 1.82 | 0.88 |
| | 60 | 2.86 | 3.58 | 0.80 |
| IV-4 (mutant) | 0 | 1.00 | 1.00 | 1.00 |
| | 30 | 1.51 | 0.90 | 1.66 |
| | 60 | 1.77 | 0.96 | 1.84 |
| | 90 | 2.09 | 0.96 | 2.18 |

* All radioactivities are normalized to the value of zero time. $P^{32}$ incorporation measures RNA and DNA accumulation, and also any polyphosphate that might be formed.

TABLE 4. *Failure of chloramphenicol to restore RNA synthesis in a temperature-sensitive mutant*

| Organism | Time after shift to 37 C | RNA* | Protein* | RNA/protein* |
|---|---|---|---|---|
| | *min* | | | |
| KB (wild) | 0 | 1.00 | 1.00 | 1.00 |
| | 30 | 1.86 | 1.77 | 1.05 |
| | 35† | — | — | — |
| | 80 | 4.00 | 2.59 | 1.54 |
| | 110 | 4.46 | 2.70 | 1.65 |
| I-9 (mutant) | 0 | 1.00 | 1.00 | 1.00 |
| | 30 | 1.12 | 1.43 | 0.75 |
| | 45 | 1.02 | 1.36 | 0.75 |
| | 50† | — | — | — |
| | 80 | 1.15 | 1.45 | 0.79 |
| | 125 | 1.19 | 1.63 | 0.73 |

* All values are normalized to the value at zero time. Colorimetric assays were used.

† This symbol indicates the time at which chloramphenicol (100 μg/ml) was added.

TABLE 5. *Aminoacyl sRNA synthetase activities of extracts from normal cells and from temperature-sensitive mutants*

| $C^{14}$-labeled L-amino acid | Specific activity* | | | |
|---|---|---|---|---|
| | KB (wild) | I-9 (mutant) | K10 (wild) | IV-4 (mutant) |
| Alanine | 0.0016 | 0.0010 | 0.0015 | 0.0028 |
| Arginine | 0.038 | 0.027 | 0.068 | 0.085 |
| Aspartic acid | 0.029 | 0.023 | 0.063 | 0.062 |
| Glutamic acid | 0.0081 | 0.0062 | 0.015 | 0.011 |
| Glutamine | 0.0076 | 0.0109 | 0.0051 | 0.0101 |
| Glycine | 0.0011 | 0.0009 | 0.020 | 0.021 |
| Histidine | 0.0102 | 0.0090 | 0.0083 | 0.0180 |
| Isoleucine | 0.0041 | 0.0047 | 0.0096 | 0.0052 |
| Leucine | 0.124 | 0.053 | 0.16 | 0.18 |
| Lysine | 0.047 | 0.033 | 0.045 | 0.037 |
| Methionine | 0.027 | 0.018 | 0.030 | 0.027 |
| Phenylalanine | 0.021 | 0.012 | 0.021 | <0.002† |
| Proline | 0.0004 | 0.0009 | 0.0017 | 0.0019 |
| Serine | 0.037 | 0.021 | 0.027 | 0.011 |
| Threonine | 0.0011 | 0.0028 | 0.0011 | 0.0006 |
| Tryptophan | 0.017 | 0.018 | 0.022 | 0.022 |
| Tyrosine | 0.014 | 0.015 | 0.015 | 0.023 |
| Valine | 0.134 | <0.001† | 0.159 | 0.137 |

* Expressed as micromoles of amino acid attached to sRNA per hour per milligram of protein. The assays were performed at 37 C. The cells had been grown at 30 C and then exposed to 37 C for 1 hr. All values are corrected for zero time controls. The sRNA had been prepared from strain KB.

† No activity was detected.

TABLE 6. *Aminoacyl sRNA synthetase activities of mixtures of extracts*

| Extract tested | Amino acid used | Specific activity* |
|---|---|---|
| KB (wild) | L-Valine | 0.061 |
| I-9 (mutant) | L-Valine | <0.001 |
| KB + I-9 (1:1) | L-Valine | 0.031 |
| K10 (wild) | L-Phenylalanine | 0.037 |
| IV-4 (mutant) | L-Phenylalanine | <0.002 |
| K10 + IV-4 (1:1) | L-Phenylalanine | 0.015 |

* Expressed as micromoles of $C^{14}$-labeled amino acid attached to sRNA per hour per milligram of total protein. The assays were carried out at 37 C with sRNA isolated from strain KB.

TABLE 7. *Aminoacyl sRNA synthetase activities measured by different procedures on extracts of cultures prepared at different temperatures*

| Organism | Growth temp* | Amino acid tested | Specific activity† | | |
|---|---|---|---|---|---|
| | | | sRNA attachment | Pyrophosphate exchange | Hydroxamate formation |
| | C | | | | |
| KB | 30 | L-Valine | 0.063 | 3.51 | 0.048 |
| (wild) | 37 | L-Valine | 0.075 | 3.58 | 0.047 |
| I-9 | 30 | L-Valine | 0.00018 | 1.38 | 0.006 |
| (mutant) | 37 | L-Valine | 0.00008 | 1.56 | 0.005 |
| K10 | 30 | L-Phenylalanine | 0.030 | 1.61 | 0.040 |
| (wild) | 37 | L-Phenylalanine | 0.035 | 2.05 | 0.035 |
| IV-4 | 30 | L-Phenylalanine | 0.0001 | 0.90 | 0.0021 |
| (mutant) | 37 | L-Phenylalanine | 0.0001 | 0.83 | 0.0016 |

* The temperature-sensitive mutants, where indicated, were exposed to 37 C for 2 hr before harvesting.

† The specific activities are expressed as micromoles of aminoacyl sRNA formed, pyrophosphate exchanged, or aminoacyl hydroxamate formed per hour per milligram of protein. The attachment and exchange assays were carried out at 30 C, the hydroxamate assay at 37 C. In other experiments, it was seen that from 30 to 37 C the temperature used in the assays had little effect on the results obtained by any of the assay procedures.

the cells were harvested to prepare extracts. All eight extracts were assayed by each of the three conventional methods for amino acid-activating enzymes (Table 7). By the sRNA attachment assay, both of the temperature-sensitive mutants had much less than 1% of the enzymatic activity of their parents, no matter what temperature had been used to prepare the cultures. The pyrophosphate-exchange assay indicated that the mutant extracts still retained 30 to 50% of their ability to react the amino acid in question with ATP. This "activating" activity could be ascribed either to the known ability of other aminoacyl sRNA synthetases to catalyze the activation step with these amino acids (e.g., Bergman, Berg, and Dieckmann 1961), or to some residual activation activity of the mutated enzymes. The hydroxamate assay measures the same enzymatic step as the pyrophosphate-exchange assay, but under different conditions (lower substrate concentrations and the presence of hydroxylamine). The mutant extracts showed only 5 to 10% of the hydroxamate-forming activity of the parents, indicating that even the ability to activate these amino acids has been altered in the mutants. Whether the reaction with ATP can be catalyzed by the mutant enzymes in vivo is not known.

The data in Table 7 failed to explain how the temperature-sensitive mutants could grow at 30 C, and raised the question of whether the inability to grow at 37 C was directly and solely caused by the alteration in the two aminoacyl sRNA synthetases. To answer this question, a preliminary test of the genetic identity of the temperature-sensitivity character and the altered enzyme character was made by crossing a temperature-sensitive mutant with a wild strain and examining the valyl sRNA synthetase activity of recombinants selected for growth at 37 C.

The mating was performed between K10 (Hfr, $str^s ts^+$) and I-91 ($F^+$, $str^r ts^-$), and recombinants ($str^r$, $ts^+$) were selected by the described procedure. Two separate crosses of the same type were made (Table 8). The frequency of recombination was, in both experiments, far above the background mutational frequency. A total of 106 recombinants were grown in separate liquid cultures and tested for valyl sRNA synthetase activity by the attachment assay. Without exception, these recombinants appeared to possess the normal enzyme activity of the $ts^+$ parent.

A similar genetic analysis of strain IV-4 has not yet been possible because of its high rate of secondary mutation.

## DISCUSSION

The enzyme assays indicate strongly that I-9 possesses an altered valyl sRNA synthetase, and IV-4 an altered phenylalanyl sRNA synthetase. There is no evidence from the experiments in which mixtures of wild and mutant extracts were assayed that the mutants lack some stimulating

TABLE 8. *Genetic correspondence between temperature sensitivity and valyl sRNA synthetase activity*

| Hfr parent | $F^-$ parent | Frequency of $str^r ts^+$ recombinants per Hfr parent | Frequency of $str^r$ mutants in the Hfr culture | Frequency of $ts^+$ mutants in the $F^-$ culture | No. of recombinants with normal enzyme/no. tested |
|---|---|---|---|---|---|
| | | % | | | |
| K10 ($str^s ts^+$) | I-91 ($str^r ts^-$) | 1.3 | $<1.5 \times 10^{-7}$ | $<5 \times 10^{-9}$ | 14/14 |
| K10 ($str^s ts^+$) | I-91 ($str^r ts^-$) | 0.12 | $<3 \times 10^{-8}$ | $<3 \times 10^{-9}$ | 92/92 |

factor(s) or contain some factor(s) inhibitory to the attachment reaction. Unfortunately, even when assayed at 30 C, extracts prepared from mutant cells grown at 30 C fail to exhibit anywhere near normal levels of these enzyme activities. Thus, it has not been possible to correlate the growth inhibition at 37 C with a demonstration in vitro of the thermal inactivation of these enzymes. Fortunately, the preliminary genetic analysis of strain I-9 reported here demonstrates the great likelihood that the temperature-sensitive growth behavior and the altered valyl sRNA synthetase have a common genetic basis, and therefore that the growth behavior is indeed caused by the altered enzyme. Apparently, the altered enzyme is active in vivo at 30 C but not at 37 C, and is inactivated at any temperature in cell-free extracts. Further genetic work is being done to obtain similar information about strain IV-4, and to locate these lesions on the coli linkage map.

The failure of strain I-9 to produce RNA after protein synthesis had been halted by raising the temperature to 37 C in the presence and in the absence of chloramphenicol indicates that a complete array of amino acids (and nucleotide precursors) is not sufficient to permit RNA synthesis in a stringent strain if the cells lack a single aminoacyl sRNA synthetase. In other words, to permit RNA synthesis in stringent cells, amino acids must be at least activated or even attached to some kind of sRNA. This conclusion is strong support for the notion that amino acids "induce" RNA synthesis by combining with their cognate sRNA molecules, neutralizing the latter's inhibition of RNA polymerase (Stent and Brenner, 1961; Kurland and Maaløe, 1962). Since strain IV-4 is relaxed, its ability to overproduce RNA when phenylalanyl sRNA synthetase becomes growth rate-limiting would indicate that relaxed strains are either insensitive to uncharged sRNA or that they possess another way of "neutralizing" these molecules.

The obligatory role of aminoacyl sRNA synthetases in protein synthesis and in the regulation of RNA synthesis has been recently supported also by studies on the physiological behavior of a mutant of *E. coli* which had lost the ability to activate and attach *p*-fluorophenylalanine to sRNA (Fangman and Neidhardt, 1964*b*).

It should be emphasized, however, that, although our findings provide strong biological evidence for the involvement of aminoacyl sRNA synthetases in these processes, they do not provide proof of the involvement of sRNA.

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