JOURNAL OF BACTERIOLOGY, Nov. 1971, p. 790-798

Vol. 108, No. 2
Printed in U.S.A.

Mutants of *Escherichia coli* Unable to Make Protein at 42 C[1]

R. R. B. RUSSELL[2] AND A. J. PITTARD[3]
School of Microbiology, University of Melbourne, Australia

Received for publication 4 August 1971

Members of a collection of mutants of *Escherichia coli* unable to form colonies on nutrient agar at 42 C have been characterized on the basis of their growth response to a shift from 32 to 42 C in liquid medium. Forty-four mutants, which show an abrupt, nonlethal cessation of growth when moved to the restrictive temperature, have been characterized with respect to the effect of the mutation responsible for temperature sensitivity on deoxyribonucleic acid, ribonucleic acid, and protein synthesis. In 12 mutants, the mutation causing temperature sensitivity of growth primarily affects protein synthesis, in each case through an altered aminoacyl-transfer ribonucleic acid synthetase. Mutants with temperature-sensitive glutamyl-, phenylalanyl-, and valyl-transfer ribonucleic acid synthetases have been obtained, and the genes specifying these enzymes have been mapped by conjugation and transduction. Another mutant has been shown to possess a temperature-sensitive tryptophanyl-transfer ribonucleic acid synthetase, but this is not responsible for inability to grow at 42 C on media containing tryptophan.

The study of conditionally expressed mutations has, in recent years, extended considerably the range of cellular processes amenable to both genetic and biochemical analyses. In the bacterium *Escherichia coli*, numerous mutants have been reported in which temperature sensitivity, defined as an inability to grow at temperatures not inhibitory to the wild type, has been caused by alterations to one of the enzymes involved in the biosynthesis of either deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein. Many of the *E. coli* mutants that are unable to make protein at the restrictive temperature have been shown to possess mutations affecting aminoacyl-transfer RNA (tRNA) synthetases, and strains have been described with thermolabile alanyl-, phenylalanyl-, and valyl-tRNA synthetases (11, 18, 43). In addition, mutants of a number of other aminoacyl-tRNA synthetases have been obtained and mapped, on the basis of criteria other than temperature sensitivity. Such mutants may exhibit resistance to amino acid analogues or amino acid auxotrophy. Thus mutants have been obtained for the aminoacyl-tRNA synthetases of arginine, glycine, histidine, isoleucine, serine, tryptophan, and tyrosine (7, 8, 13, 15, 16, 17, 26, 37, 39). Recently Murgola and Adelberg (24) reported a new method of obtaining conditional lethal mutants and found conditional streptomycin-lethal mutations of glutamyl-tRNA synthetase.

Our purpose is to describe the isolation of a number of temperature-sensitive (TS) mutants of *E. coli* and, in particular, to report the genetic and biochemical analysis of those strains in which protein synthesis is specifically inhibited at 42 C.

MATERIALS AND METHODS

Organisms. All strains used in this investigation were derivatives of *E. coli* K-12; their genotypes are described in Table 1 and 2.

Media and culture methods. The media and culture methods used were as described by Adelberg and Burns (1). Nutrient agar consisted of Oxoid Nutrient Broth no. 2, 0.3% yeast extract, and 1% agar.

Isolation of TS mutants. Mutations were obtained in two strains, AB3282 and KA56. In each case, after mutagenesis with nitrosoguanidine by the method of Adelberg et al. (2), portions were spread on nutrient agar plates to give up to 200 colonies per plate. The plates were incubated at 32 C until colonies became visible and then were moved to 42 C. Colonies that grew no larger during overnight incubation at 42 C were picked, purified, and retested for temperature sensitivity.

For the isolation of mutants from KA56, an enrichment step was interposed in which the culture was

[1] This report is taken from a thesis submitted by R.R.B.R. in partial fulfillment of the requirements for a Ph.D. degree.

[2] Present address: CSIRO Division of Food Research, Cannon Hill, Queensland, Australia.

[3] Present address: Pasteur Institute, Paris.

TABLE 1. *Description of strains of Escherichia coli*

Strain	Sex	Genotype[a]
AB3282	F⁻	*thi-1, leu-351, proA-2, trp-356, his-4, ilvC-7, argE-3, galK-2, lacY-1, mal-358, str-704, tsx-358*
AB347	Hfr0-12	*thi-1, thrA-1, leuA-1, aroC-4, str-723*
AB2826	F⁻	*aroB-351*
KA56	HfrH	*thi⁻, galE*-PL5, *rel-1*
K6-1	F⁺	*aroD-351, pps-351*

[a] Symbols used are those of Taylor (40). Numbers refer to allele numbers allotted to mutations in this laboratory.

treated with mutagen, washed, and suspended in minimal medium supplemented with Casamino Acids, with glycerol as carbon source. After several hours of growth at 32 C, the culture was shifted to 42 C, and 1 hr later galactose was added to a final concentration of 2%. Under these conditions the lack of uridine diphosphate galactose-4-epimerase in KA56 caused actively growing cells to lyse (14). On the other hand, the population of TS mutants whose growth was inhibited at 42 C was enriched by this step. Maximum killing was observed 5.5 hr after the addition of galactose. At this stage, the culture was diluted and spread on agar plates for screening as described above.

Growth studies. Cultures of the strains to be tested, grown overnight in nutrient broth at 32 C, were inoculated into duplicate flasks containing 10 ml of fresh broth to give a population of about 10^7 cells/ml. The flasks were equipped with side arms, and increases in optical density were followed in a Klett-Summerson photoelectric colorimeter with a no. 54 filter. One flask was incubated at 32 C, the other at 42 C. After 5 hr, the number of cells in the culture growing at 42 C that were still able to form colonies at 32 C was determined, and samples from cultures grown at both temperatures were examined under a microscope.

Measurement of DNA, RNA, and protein synthesis. Cells were grown in nutrient broth at 32 C until the exponential phase of growth was reached. Portions then were inoculated into four flasks, each containing 10 ml of broth, to give a density of approximately 10^8 cells/ml. One flask served as a control. The other three flasks contained, respectively, 0.1 μCi of isotopically labeled leucine, thymidine, or uracil per ml. After 30-min of growth at 32 C, the flasks were transferred to 42 C, and at intervals thereafter growth and incorporation of the several radioisotopes into acid-precipitable material were determined. The incorporation of label was assayed by transferring 0.5-ml samples from each of the flasks into 0.5 ml of ice-cold 10% trichloroacetic acid. The trichloroacetic acid precipitates were left for at least 30 min before being collected on membrane filters (0.45-μm pore diameter; Millipore Corp., Bedford, Mass.) and washed with an additional 15 ml of cold trichloroacetic acid. The filters were dried at 60 C and placed in vials with 5 ml of scintillation fluid [toluene containing 5.0 g of 2,5-diphenyloxazole and 200 mg of 1,4-bis-2(5-phenyloxazolybenzene) per liter] and counted in an Ansitron liquid scintillation spectrometer.

Test of relaxed or stringent (RC) phenotype. A 1-ml amount of a mid-log phase culture of the strain to be tested, growing in minimal medium, was collected on a membrane filter (Millipore Corp.) and washed with buffer. The cells were suspended by shaking the filter in 10 ml of minimal medium that lacked one of the amino acids for which the strain was auxotrophic. The filter was removed, and ^{14}C-uracil was added. The culture was divided in two, and the required amino acid was added to one portion. After 1 hr of incubation, samples were taken into trichloroacetic acid for processing as described above.

Mapping experiments. The conditions under which broth crosses were carried out were as described previously (30), except that cultures were grown and mated at 32 C. When selection was made for recombinants able to grow at 42 C, plates were first incubated at 32 C for several hours to allow phenotypic expression before being transferred to the 42 C incubator.

Transduction was by bacteriophage P1*kc*.

Preparation of cell extracts. Cells were grown in broth at 32 C to mid-log phase, washed twice with 0.9% NaCl by centrifugation, and suspended in tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 7.3) containing 10% glycerol at a density of 10^{10} cells/ml. This suspension was treated sonically for 40 sec in a 500-watt ultrasonic oscillator (output setting 3; Measuring Scientific Equipment, Ltd., London). Extracts were stored at −20 C.

Assay of aminoacyl-tRNA synthetases. The adenosine triphosphate-pyrophosphate exchange assay was that described by Calender and Berg (5), with incubation for 15 min at 42 C. The tRNA-charging assay was that described by Muench and Berg (23), except that a Tris-hydrochloric acid buffer (*p*H 7.5) was used and

TABLE 2. *Description of temperature-sensitive (TS) strains*[a]

No.	Sex	*ts* Allele	Allele no. of gene affected by *ts* mutation	Other markers
JP1023	F⁻	*363*	*gltX-351*	As in AB3282
JP1112	F⁻	*352*	*pheS-353*	As in KA56
JP1116	Hfr	*353*	*pheS-354*	As in KA56
JP1135	Hfr	*354*	*valS-351*	As in KA56
JP1136	Hfr	*355*	*valS-352*	As in KA56
JP1159	F⁻	*356*	*valS-353*	As in KA56
JP1192	F⁻	*357*	*valS-354*	As in KA56
JP1199	Hfr	*358*	*valS-355*	As in KA56
JP1230	Hfr	*359*	*pheS-360*	As in KA56
JP1260	F⁻	*364*	?	As in KA56, *trpS-378*
JP1288	Hfr	*361*	*valS-356*	As in KA56
JP1330	Hfr	*362*	*valS-357*	As in KA56
JP1449	Hfr	*363*	*gltX-351*	*thr⁻, leu⁻, str-723*
JP1451	F⁻			*trpS-378*

[a] Mutations of TS mutants were originally given allele numbers with the prefix *ts*. When the affected genes were identified, the mutations were renamed and allocated allele numbers in a series with other alleles of the same gene; e.g., *ts-352* became *pheS-353*.

incubation was at 42 C. Precipitates were collected on membrane filters (Millipore Corp.) and counted in a scintillation counter. The tRNA was prepared from strain KA56 by the method of von Ehrenstein (10).

RESULTS

When cells of AB3282 were treated with mutagen, 21 of 4,000 colonies examined were unable to grow at 42 C (i.e., were TS). When KA56 cells were treated with mutagen and exposed to galactose killing at 42 C before plating, 289 colonies (of approximately 12,000 examined) were found to be temperature sensitive.

Of these 310 isolates, 97 were found to be capable of growth on nutrient agar at 42 C when large inocula were streaked; these were studied no further. To facilitate later studies of macromolecular syntheses in the remaining 213 mutants, a more quantitative assessment of growth inhibition was made by following the growth of aerated liquid broth cultures at 32 and 42 C. The growth rate for both parental strains was approximately 40% faster at 42 C than at 32 C. All mutants tested were able to grow exponentially at 32 C, although a majority had mean doubling times longer than that of the parent strain. Some also displayed an aberrant morphology, which was generally more exaggerated at 42 C. Taking into account all parameters measured, it was possible to assign all the mutants studied to one of five arbitrarily chosen groups (A to E). (Growth curves of the wild type and of representative members of each of the five groups are shown in Fig. 1).

Group A mutants showed no net increase in cell density or viable count and appeared morphologically normal at 42 C.

Group B mutants grew at 42 C for 1 to 5 hr before growth stopped completely, more or less abruptly.

Members of group C died at 42 C, and many lysed at various times after shifting to 42 C. The viable count decreased (in some cases to as low as 10^3 cells/ml in 5 hr), and under the microscope many cells were observed to have lysed.

Group D mutants grew at a considerably reduced, but still apparently exponential, rate at 42 C.

Within the 7-hr growth period observed, the group E mutants displayed a continuing decrease in growth rate, but never ceased to grow entirely. A number of mutants in this group

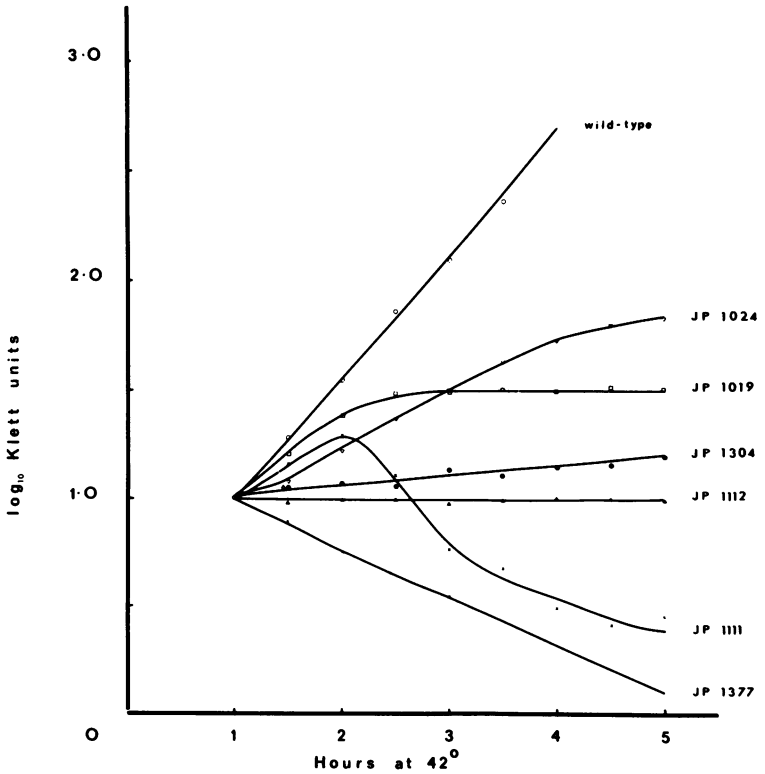

FIG. 1. *Growth of representative TS mutants in nutrient broth at 42 C. The various types of growth curve are shown as wild-type, KA56; A, JP1112; B, JP1019; C, JP1111 and JP1377; D, JP1304; E, JP1024.*

showed no increase in colony-forming units at 42 C, but under the microscope were seen to have formed long filaments.

Table 3 gives the numbers of mutants assigned to each group. The main purpose of the growth studies was to select those mutants in which temperature sensitivity was rapidly expressed at 42 C, as we considered such strains would be most amenable to the study of macromolecular synthesis. Consequently, the 45 strains displaying growth characteristics similar to those of JP1112 in Fig. 1 were chosen for further study.

Effect of ts mutations on synthesis of DNA, RNA, and protein. Figure 2 shows the rate at which isotopically labeled precursors of macromolecules were incorporated into the parental strain KA56. The incorporation of all three labels proceeded more or less in parallel, although the incorporation of thymidine dropped after a time presumably as a consequence of the action of thymidine phosphorylase (12). With each of the 45 mutant strains examined, the rate of incorporation of one or more of the precursors was drastically reduced. On the basis of these results, the TS mutants were grouped as shown in Table 4.

The classification in Table 4 is based on the effect of *ts* mutations on strains with "relaxed" control of RNA synthesis. The responses of "stringent" strains into which the *ts* mutations had been introduced by conjugation or transduction revealed that all mutations, apart from the ones causing temperature sensitivity of protein synthesis, resulted in the same precursor incorporation pattern as in "relaxed" backgrounds. The "TS-protein" mutations depressed the incorporation of ^{14}C-leucine in relaxed strains, but depressed incorporation of both ^{14}C-leucine and ^{14}C-uracil in stringent strains at 42 C. The incorporation of precursors into a relaxed mutant representative of the TS protein class is shown in Fig. 3.

Identification of genes affected by ts mutations. Twelve "TS-protein" mutations were mapped and identified and found to affect aminoacyl-tRNA synthetases. Mutants of valyl-, phenylalanyl-, and glutamyl-tRNA synthetase were found.

Valyl-tRNA synthetase mutants. Seven *ts* mutations were mapped near min 85 on the genetic map (40) by interrupted conjugation experiments and by demonstration of cotransduction with *pyrB*, at frequencies between 19 and 56% (average, 46%). Extracts of all seven lacked valyl-tRNA synthetase activity at 42 C (Table 5). While the activity of other aminoacyl-tRNA synthetases remained intact (Table 6). The mutated gene, *valS*, was mapped accurately by an inter-

TABLE 3. *Number of mutants assigned to each of the types of growth curve described in Fig. 1*

Parent	Growth curve type					Total
	A	B	C	D	E	
AB3282	1	4	0	2	5	12
KA56	44	41	42	26	48	201

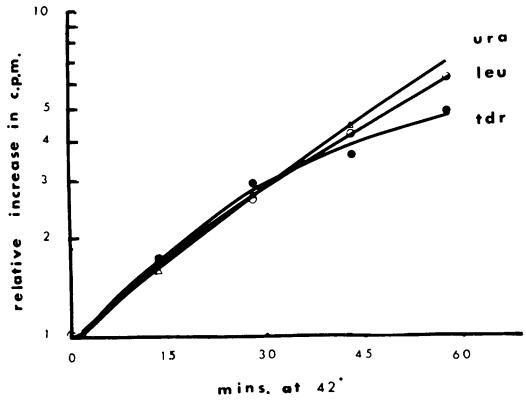

FIG. 2. *Incorporation of radioactive precursors of DNA, RNA, and protein by the wild-type strain KA56. ^{3}H-thymidine, tdr; ^{14}C-uracil, ura; ^{14}C-leucine, leu.*

TABLE 4. *Macromolecular syntheses which are principally affected at 42 C in rel^{-} strains carrying ts mutations, which show growth of type A*

Macromolecular syntheses affected	No. of mutants
DNA	1
RNA	7
DNA and RNA	5
Protein	12
RNA and protein	16
DNA, RNA, and protein	4

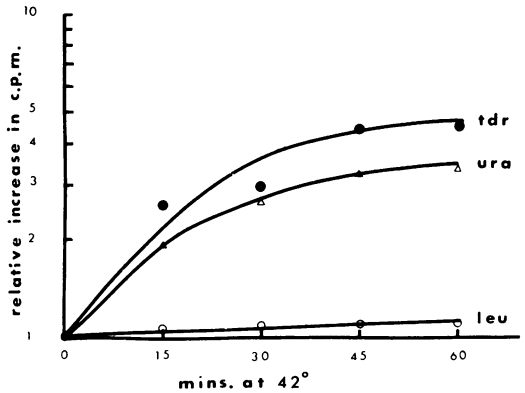

FIG. 3. *Incorporation of radioactive precursors of DNA, RNA, and protein by strain JP1112.*

TABLE 5. *Activity of KA56 and valS mutant extracts in the tRNA-charging assay*

Strain	*ts* Allele	Specific activity of valyl-tRNA synthetase[a]
KA56	+	67.0
JP1135	*354*	0.7
JP1136	*355*	0.5
JP1159	*356*	1.7
JP1192	*357*	0.02
JP1199	*358*	2.4
JP1288	*361*	0.02
JP1330	*362*	1.1

[a] Nanomoles of ^{14}C-valine attached to tRNA in 10 min at 42 C per milligram of protein.

rupted mating experiment in which the reference markers were *leu* (at min 1) and *rif*, which is located at min 77.5, on the far side of *argH* from *metB* (*unpublished data*). The results allow the calculation of the position of *valS* at min 84. Another experiment indicated that *valS* is on the clockwise side of *pyrB* (*unpublished data*). This position for *valS* is in agreement with that reported by Tingle and Neidhardt (41).

TABLE 6. *Percentage of wild-type activity in extracts of TS mutants in the tRNA-charging assay at 42 C*

Amino acid	JP1023 (*ts*-363)	JP1112 (*ts*-352)	JP1192 (*ts*-357)	JP1260 (*ts*-364)
Alanine	107	86	79	92
Arginine	132	104	110	78
Aspartic acid	109	106	108	114
Asparagine	122	144	94	78
Cysteine	62	141	91	138
Glutamic acid	11[a]	149	109	141
Glutamine	83	119	87	142
Glycine	123	104	100	84
Histidine	110	111	106	130
Isoleucine	109	106	94	83
Leucine	130	89	82	134
Lysine	99	98	103	79
Methionine	79	132	121	79
Phenylalanine	98	25[a]	80	71
Proline	73	127	112	64
Serine	110	108	112	130
Threonine	112	97	100	133
Tryptophan	122	87	93	20[a]
Tyrosine	114	90	96	77
Valine	105	130	8[a]	141

[a] For this rough screening, the "no-extract" and "no-tRNA" controls were omitted, so that blank values could not be subtracted from the results obtained. This accounts for the apparent residual activity of mutant enzymes; later, more detailed experiments showed that mutant extracts contained no detectable activity whatever.

Phenylalanyl-tRNA synthetase mutants. The three mutations, *ts-352*, *ts-353*, and *ts-359*, were mapped at min 33 by conjugation and were cotransduced with *aroD* at frequencies of 38%, 44%, and 43%, respectively. All three mutations affected phenylalanyl-tRNA synthetase (Table 7) and were presumed to be alleles of the *pheS* locus (4). One mutation was used to map the region in more detail, and a gene order of *aroD pps pheS* was found (Table 8). During the mapping of the *pheS-353* mutation, a new suppressor, which allows *pheS-353* strains to grow at 42 C, was found. This suppressor was recently discribed (36).

Glutamyl-tRNA synthetase mutant. A single *ts* mutation was found by conjugation to map close to min 45. This was *ts-363*, isolated in JP1023. It is cotransducible with *aroC* at a low frequency (3/528 transductants) and with *dsdA* at a frequency of 10%. As *aroC* and *dsdA* are 49% cotransducible (20), the *ts-363* mutation must lie on the far side of *dsdA* from *aroC*.

When aminoacyl-tRNA synthetases for all 20 common amino acids were assayed in extracts of JP1023, only the aminoacyl-tRNA synthetase for glutamic acid was absent (Table 6). It was not possible to demonstrate charging of tRNA with glutamic acid by extracts of JP1023 at 32 or 42 C or by extracts of strains into which the *ts-363* mutation had been introduced by transduction or conjugation, but TS$^+$ revertants and transductants did have activity. Other experiments, in which mutant and parent extracts were mixed, gave no indication of the existence of an inhibitory factor (Table 9). The mutation resulting in temperature sensitivity of glutamyl-tRNA synthetase was named *gltX-351*.

The position of *gltX* is very close to that reported by Eggertsson (9) for the *ochre*-suppressor *supN*. To study the linkage of *gltX* and *supN*,

TABLE 7. *Phenylalanyl-tRNA synthetase activity in pheS mutant extracts*

Strain carrying mutation *ts*$^-$	Percentage of wild-type specific activity of phenylalanyl-tRNA synthetase	
	PPi-exchange[a]	tRNA-charging[b]
352	26	0
353	99	1
359	23	0

[a] Wild-type specific activity: 3.9 μmoles of ^{32}P incorporated into adenosine triphosphate in 15 min at 42 C per mg of protein.

[b] Wild-type specific activity: 18 nm of ^{14}C-phenylalanine attached to tRNA in 10 min at 42 C per mg of protein.

TABLE 8. *Mapping of aroD, pps, and pheS by transduction*

Relevant markers: donor, JP1112 *ts-352*; recipient, K6-1 *aroD⁻ pps⁻*				
Class of recombinants				
aroD	*pps*	*ts-352*	No.	
+	−	+	112	
+	+	−	112	
+	+	+	51	
+	−	−	10	
	Possible gene orders			
Strain	*aroD*	*pps*	*ts*	
JP1112	+	+	−	(A)
K6-1	−	−	+	
	ts	*aroD*	*pps*	
JP1112	−	+	+	(B)
K6-1	+	−	−	
	aroD	*ts*	*pps*	
JP1112	+	−	+	(C)
K6-1	−	+	−	

[a] To produce the class of recombinants which is *aroD⁺pps⁻ts⁻*, alternative A requires four cross-over events, whereas B and C require only two. As this class is rare, alternative A is the more likely to be correct.

bacteriophage P1 was grown on a strain containing both *supN-23* and the *ochre* mutation *his-4*. This P1 lysate was used to transduce either *gltX⁺* (by selecting for ability to grow at 42 C) or *supN*-23 (by selecting for histidine-independence) into JP1023, which carries *his-4*. More than 100 of each class of transductants were tested, and in each case 98% cotransduction of *gltX* and *supN* was found.

Temperature-sensitive *trpS* mutation. Several attempts to map the mutation rendering JP1260 temperature sensitive were unsuccessful; as another approach to the identification of the TS lesion, the aminoacyl-tRNA synthetases of this strain were examined. The ability to attach tryptophan to tRNA at 42 C was greatly altered in extracts of JP1260 (Table 6).

Mutations of the gene specifying tryptophanyl-tRNA synthetase, *trpS*, have been studied by several workers (8, 16, 17) and also by J. Camakaris in this laboratory. All had located the *trpS* gene between *str* and *malA*, and Camakaris (*unpublished data*) had further shown that the gene order or the region was *str pabA trpS aroB malA*. P1 grown on JP1260 was therefore used to transduce *aroB⁺* into strain AB2826, and it was found that half of the transductants were unable to grow at 42 C. One of these Aro⁺ TS transductants was numbered JP1451. Enzyme assays confirmed that JP1451 contained a defective tryptophanyl-tRNA synthetase, and the mutation has accordingly been named *trpS-378*. However, although JP1451 was unable to grow on minimal medium, unlike strain JP1260, it was still able to grow on nutrient agar plates at 42 C. This suggested that, at 42 C, strain JP1451 had a requirement for some factor present in nutrient agar but absent from the minimal medium. By testing a range of growth factors for their ability to permit growth of JP1451 in minimal medium at 42 C, it was found that tryptophan stimulated growth.

It therefore appears that the *trpS-378* mutation introduces a temperature-conditional tryptophan auxotrophy, and furthermore it was found that the addition of tryptophan to the medium could restore the growth of JP1451 at 42 C to wild-type levels. In agreement with the results of Ito et al. (16), it was shown that the amount of tryptophan in the medium affected the growth *rate*, rather than the final cell *yield*.

When a culture of JP1451, growing in minimal medium at 32 C (at which temperature the growth rate in the absence of tryptophan is 80% of that in its presence) is shifted to 42 C, growth ceases and the synthesis of DNA, RNA, and protein is arrested, giving a pattern of isotope

TABLE 9. *Glutamyl-tRNA synthetase assays (tRNA-charging reaction) of ts-363 and wild-type strains*[a]

Extract	*ts* Allele	Assay temp (C)	Specific activity[b]
AB3282	+	32	2.8
AB3282	+	42	3.5
JP1023	363	32	<0.02
JP1023	363	42	<0.02
JP1023 TS⁺ revertant	+	42	2.9
AB3282 + JP1023 (1:1)[c]	+/363	42	2.93
KA56	+	42	3.94
KA56 + JP1023 (1:1)	+/363	42	3.12
AB347	+	42	3.9
JP1449[d]	363	42	<0.02

[a] All cultures were grown at 32 C.

[b] Nanomoles of ^{14}C-glutamic acid attached to tRNA in 10 min by 1 mg of protein from an active extract in assay.

[c] An extract of the TS mutant was mixed with the usual amount of wild-type extract in the standard assay.

[d] JP1449 is an *aroC⁺ gltX-351* transductant of AB347.

incorporation just like that of other strains with TS aminoacyl-RNA synthetases. On the subsequent addition of tryptophan, growth and isotope incorporation recommence.

When extracts of *trpS-378* strains were assayed at 32 or 42 C, it was not possible to show either attachment of tryptophan to tRNA, or catalysis of the tryptophan-dependent exchange of pyrophosphate (even when the concentration of tryptophan in the assay was increased 10-fold, to 20 mM). However, tryptophanyl-tRNA synthetase seems to be present in the cell and for normal functioning needs only the addition of exogenous tryptophan. This restoration of the enzyme's function in vivo at 42 C is indicated by the experiment described in Fig. 4. Even after several hours of exposure to the restrictive temperature, JP1451 was still able to resume growth (assayed in this case by protein synthesis) as soon as tryptophan was supplied.

TrpS-378 has been shown, when introduced into any of several strains, to cause temperature sensitivity of growth on minimal medium but not on nutrient agar. In broth, *trpS-378* slightly depresses the growth rate at 42 C, but this is remedied by the addition of tryptophan. These results suggested that *trpS-378* was not in fact responsible for the TS character of the strain in which it was originally isolated, JP1260. This possibility was investigated by transducing Str^r into JP1260 from a *trps*$^+$ strain. (One would predict that some 10% of Str^r transductants would receive *trpS*$^+$, but of 50 transductants tested, none were TS$^+$.) It thus appears that JP1260 contains a mutation, additional to *trpS-378*, which causes temperature sensitivity of growth and of protein synthesis. *TrpS-378*, on the other hand, does not on its own cause a noticeable depression of protein synthesis in broth at 42 C. This second mutation, *ts-364*, does not seem to affect the stability of an aminoacyl-tRNA synthetase (Table 6).

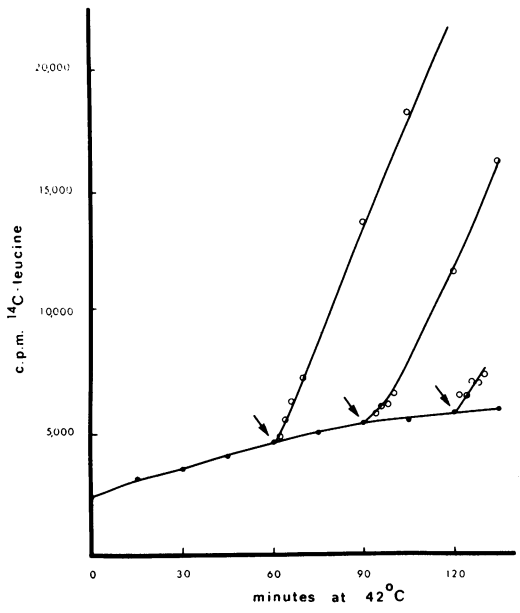

FIG. 4. *Incorporation of ^{14}C-leucine by JP1451 growing in minimal medium at 42 C. At the points indicated by arrows,* L-*tryptophan (to give 10^{-4}* M*) was added to portions of the culture.*

DISCUSSION

The collection of mutants described here provides a further instance (noted by several other groups 18, 28, 42, 43) in which *valS*, and to a lesser extent *pheS*, mutations are detected at a high frequency in collections of temperature-sensitive mutants. The *valS* mutants closely resemble ones already described by others in greater detail (28, 44). Two of the *pheS* mutants are of particular interest: one because it is suppressible (36), the other because it appears to retain its capacity for phenylalanine-dependent adenosine triphosphate-pyrophosphate exchange while being unable to charge tRNA with phenylalanine (Table 7). In this respect, it is like the *alaS* mutant of Böck (3). The other aminoacyl-tRNA synthetase mutants reported here are altered in both the amino acid activation and the tRNA-charging steps.

The aminoacyl-tRNA synthetase genes which have been mapped to date are scattered around the chromosome, separated from each other and from the genes specifying the biosynthesis of their particular amino acid (28, 40). One possible exception to this rule is the proximity of *pheS* to *aroD* (4), but the data in Table 8 show that *pheS* is separated from the *aroD* gene, which is concerned in the "common pathway" of aromatic amino acid biosynthesis. The gene for tyrosyl-tRNA synthetase has recently been reported to be near the known position of *pheS* (37), but experiments in this laboratory using a mutant with a detective tyrosyl-tRNA synthetase (obtained as a fluorotyrosine-resistant mutant) have failed to demonstrate cotransduction of *tyrS* and *aroD*, so it appears that *pheS* and *tyrS* are not closely linked (*unpublished data* of R.R.B.R.).

Recently, Murgola and Adelberg (24, 25) described mutants with altered glutamyl-tRNA synthetases, and they mapped two genes, *gltE* and *gltM*, responsible for the altered activities. Each of the mutant synthetases displays a higher K_m for glutamate than does the parental enzyme, but each (even the two presumed to be alleles of the same locus) shows a completely different effect on the incorporation of radioactive precur-

sors into macromolecules. The genetic data presented by Murgola and Adelberg, placing *gltE* close to *xyl* and *gltM* near *his*, indicate that their mutations are quite distinct from the *gltX* locus.

The *gltX-351* mutation results in loss of all detectable activity in the tRNA-charging assay, although approximately half of the wild-type capacity for glutamate-dependent pyrophosphate exchange is retained by crude extracts (*unpublished data*). By contrast, Murgola and Adelberg's (25) mutants have altered K_m values for glutamate and altered specificity for charging of $tRNA_{glu}$ subspecies.

Murgola and Adelberg suggested several possible explanations for the multiplicity of genetic loci affecting glutamyl-tRNA synthetase. One of the possibilities raised was that the enzyme consisted of non-identical subunits, as do bovine tryptophanyl-tRNA synthetase and *E. coli* glycyl-tRNA synthetase, which have been reported to be oligomers containing dissimilar subunits (29, 31). Several aminoacyl-tRNA synthetases can be dissociated into subunits, but the smallest subunits observed all have molecular weights between 33,000 and 45,000. As glutamyl-tRNA synthetase of *E. coli* was estimated by Lazzarini and Mehler (19) to have a molecular weight of 50,000, it seems probable that it exists as a monomer. On the other hand, Nass and Stoffler (27) calculated it to have a molecular weight of 75,000, like the arginyl- and prolyl-tRNA synthetases, both of which do have subunits.

An alternative possibility is that one of the genes is the structural gene for glutamyl-tRNA synthetase and that the others specify modifiers of the enzyme. Modifying factors have been reported for seryl- and methionyl-tRNA synthetases (6, 35); recently Rouget and Chappeville (34) described two interchangeable forms of *E. coli* B leucyl-tRNA synthetase. The exchange is stimulated by a factor in the supernatant fraction of the cell. Both forms of the enzyme have the same molecular weight, but one can activate only leucine, whereas the other can both activate it and attach it to tRNA.

It is worth noting the proximity of *gltX* to *supN*. The frequency of cotransduction of these two markers (98%) suggests that they are adjacent or allelic. *SupN* is an ochre suppressor, but it is not known which amino acid it inserts. Suppression at the level of translation can in theory be due to altered tRNA molecules, ribosomes, or aminoacyl-tRNA synthetases. No case of suppression due to mischarging of tRNA by a synthetase has yet been described in *E. coli* (21), but there is one probable example in *Neurospora* (32). A mutation which resulted in impaired fidelity of charging might well cause temperature sensitivity, and this could be due to either the attachment of glutamic acid to the wrong tRNA or the attachment of the wrong amino acid to $tRNA_{glu}$. However, the former case could be difficult to recognize, as it might have no effect on the activity observed in assays in which unfractionated tRNA is used. If an altered glutamyl-tRNA synthetase were to be responsible for suppression of an ochre mutation, there would have to be an ochre codon-recognizing transfer RNA molecule for it to charge. There is no evidence for the existence of such a tRNA in normal cells (38).

It is known that, for glytamyl- and arginyl tRNA synthetases, the activation step is greatly enhanced by the presence of tRNA (22, 33). For the arginine-specific enzyme, it has further been shown by Mitra et al. (22) that it is arginine-accepting tRNA which is required and that this tRNA also stabilizes the enzyme to H and heat. Although this information may give rise to speculation as to how a mutation altering $tRNA_{glu}$ could result in the observed phenotype of *gltX*-351 mutants, it should be noted that all assays of glutamyl-tRNA synthetase reported here were performed on crude extracts of cells which had been grown at 32 C. Both the quantity and quality of $tRNA_{glu}$ in the extracts should therefore have been normal, and the presence of excess tRNA in the charging assay should have been sufficient to fill the needs of the enzyme.

Further experiments are needed to purify the glutamyl-tRNA synthetases of wild-type and mutant strains, to investigate their interaction and ability to activate glutamic acid and to charge the several $tRNA_{glu}$ species, and to study the roles of *gltE*, *gltM*, and *supN*, in order to resolve the present problems.

ACKNOWLEDGMENTS

We thank H. L. Kornberg for strain K6-1 and E. McFall for a *dsdA* mutant. A. Stott and D. Legg are thanked for excellent technical assistance.

This work was supported by a grant from the Australian Research Grants Committee. During the course of this investigation, one of us (R.R.B.R.) was the recipient of a Commonwealth Postgraduate Award.

LITERATURE CITED

1. Adelberg, E. A., and S. N. Burns. 1960. Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. **79**:321–330.
2. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
3. Böck, A. 1969. Mutation affecting the charging reaction of alanyl-tRNA synthetase from *Escherichia coli* K10. Arch. Microbiol. **68**:165–178.
4. Böck, A., and F. C. Neidhardt. 1967. Genetic mapping of phenylalanyl-sRNA synthetase in *Escherichia coli*. Science **157**:78–79.
5. Calender, R., and P. Berg. 1966. Purfication and physical

properties of tyrosyl ribonucleic acid synthetases from *Escherichia coli* and *Bacillus subtilis*.Biochemistry **5**: 1681-1690.
6. Cassio, D., and J. P. Waller. 1968. Etude de la méthionyl-tRNA synthétase d'*Escherichia coli*. 3. Dissociation en sousunités actives par action d'un facteur extrinsèque. Eur. J. Biochem. **5**:33-41.
7. Cooper, P. H., I. N. Hirschifield, and W. K. Maas. 1969. Map location of arginyl-tRNA synthetase mutations in *Escherichia coli* K-12. Mol. Gen. Genet. **104**:383-390.
8. Doolittle, W. F., and C. Yanofsky. 1968. Mutants of *Escherichia coli* with an altered tryptophanyl-transfer ribonucleic acid synthetase. J. Bacteriol. **95**:1283-1294.
9. Eggertsson, G. 1968. Mapping of ochre suppressors in *Escherichia coli*. Genet. Res. **11**:15-20.
10. von Ehrenstein, G. Isolation of sRNA from intact *Escherichia coli* cells, p. 588. *In* S. P. Colowick and N. A. Kaphan (ed.), Methods in enzymology, vol. 12A. Academic Press Inc., New York.
11. Eidlic, L., and F. C. Neidhardt. 1965. Protein and nucleic acid synthesis in two mutants of *Escherichia coli* with temperature-sensitive aminoacyl ribonucleic acid synthetases. J. Bacteriol. **89**:706-711.
12. Fangman, W. L., and A. Novick. 1966. Mutant bacteria showing efficient utilization of thymidine. J. Bacteriol. **91**:2390-2391.
13. Folk, W. R., and P. Berg. 1970. Isolation and partial characterization of *Escherichia coli* mutants with altered glycyl-transfer ribonucleic acid synthetases. J. Bacteriol. **102**:193-203.
14. Fukasawa, T., and H. Nikaido. 1959. Galactose-sensitive mutants of *Salmonella*.Nature (London) **184**:1168-1169.
15. Hofmann, E. P., R. C. Wilhelm, W. Konigsberg, and J. R. Katze. 1970. A structural gene for seryl-tRNA synthetase in *Escherichia coli* K-12. J. Mol. Biol. **47**:619-625.
16. Ito, K., S. Hiraga, and T. Yura. 1968. Tryptophanyl transfer RNA synthetase and expression of the tryptophan operon in the *trpS* mutants of *Escherichia coli*. Genetics **61**:521-538.
17. Kano, Y., A. Matsushiro, and Y. Shimura. 1968. Isolation of novel regulatory mutants of the tryptophan biosynthetic system in *Escherichia coli*. Mol. Gen. Genet. **102**: 15-26.
18. Kaplan, S., and D. Anderson. 1968. Selection of temperature-sensitive activating enzyme mutants in *Escherichia coli*. J. Bacteriol. **95**:991-997.
19. Lazzarini, R. A., and A. H. Mehler. 1964. Separation of specific glutamate- and glutamine-activating enzymes from *Escherichia coli*. Biochemistry **3**:1445-1449.
20. McFall, E. 1967. Mapping of the D-serine deaminase region in *Escherichia coli* K-12. Genetics **55**:91-99.
21. Menninger, J. R. 1966. Amber suppression and activating enzymes. J. Mol. Biol. **16**:556-561.
22. Mitra, S. K., K. Chakraburtty, and A. H. Mehler. 1970. Binding of transfer RNA and arginine to the arginine transfer RNA synthetase of *Escherichia coli*. J. Mol. Biol. **49**:139-156.
23. Muench, K. H., and P. Berg. 1966. Preparation of aminoacyl ribonucleic acid synthetases from *Escherichia coli*, p. 375-383. *In* G. L. Cantoni and D. R. Davis (ed.), Procedures in nucleic acid research. Harper and Row, New York.
24. Murgola, E. J., and E. A. Adelberg. 1970. Streptomycin-suppressible lethal mutations in *Escherichia coli*. J. Bacteriol. **103**:20-26.
25. Murgola, E. J., and E. A. Adelberg. 1970. Mutants of *Escherichia coli* K-12 with an altered glutamyl-transfer ribonucleic acid synthetase. J. Bacteriol. **103**:178-183.
26. Nass, G. 1967. Regulation of histidine biosynthetic enzymes in a mutant of *Escherichia coli* with an altered histidyl-tRNA synthetase. Mol. Gen. Genet. **100**:216-224.
27. Nass, G., and G. Stoffler. 1967. Molecular weight distribution of the aminoacyl-tRNA-synthetases of *Escherichia coli* by gel filtration. Mol. Gen. Genet. **100**:378-382.
28. Neidhardt, F. C. 1966. Roles of amino acid activating enzymes in cellular physiology. Bacteriol. Rev. **30**:701-719.
29. Ostrem, D. L., and P. Berg. 1970. Glycyl-tRNA synthetase: an oligomeric protein containing dissimilar subunits. Proc. Nat. Acad. Sci. U.S.A. **67**:1967-1974.
30. Pittard, J., and B. J. Wallace. 1966. Distribution and function of genes concerned with aromatic biosynthesis in *Escherichia coli*. J. Bacteriol. **91**:1494-1508.
31. Preddie, E. C. 1969. Tryptophanyl transfer ribonucleic acid synthetase from bovine pancreas. II. The chemically different subunits. J. Biol. Chem. **244**:3958-3968.
32. Printz, D. B., and S. R. Gross. 1967. An apparent relationship between mistranslation and an altered leucyl-tRNA synthetase in a conditional lethal mutant of *Neurospora crassa*. Genetics **55**:451-467.
33. Ravel, J. M., S. F. Wang, C. Heinemeyer, and W. Shive. 1965. Glutamyl and glutaminyl ribonucleic acid synthetases of *Escherichia coli* W. J. Biol. Chem. **240**:432-438.
34. Rouget, P., and F. Chappeville. 1970. Leucyl-tRNA synthetase. Purification of two interconvertible forms and evidence for an interconversion factor. Eur. J. Biochem. **14**:498-508.
35. Roy, K. L., and D. Söll. 1970. Purification of five serine transfer ribonucleic acid species from *Escherichia coli* and their acylation by homologous and heterologous seryl transfer ribonucleic acid synthetases. J. Biol. Chem. **245**:1394-1400.
36. Russell, R. R. B., and A. J. Pittard. 1971. New suppressor in *Escherichia coli*. J. Bacteriol. **107**:736-740.
37. Schlesinger, S., and E. W. Nester. 1969. Mutants of *Escherichia coli* with an altered tyrosyl-transfer ribonucleic acid synthetase. J. Bacteriol. **100**:167-175.
38. Söll, D. 1968. Studies of polynucleotides. LXXXV. Partial purification of an amber suppressor tRNA and studies on *in vitro* suppression. J. Mol. Biol. **34**:175-187.
39. Szentirmai, A., M. Szentirmai, and H. E. Umbarger. 1968. Isoleucine and valine metabolism of *Escherichia coli*. XV. Biochemical properties of mutants resistant to thiaisoleucine. J. Bacteriol. **95**:1672-1679.
40. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
41. Tingle, M. A., and F. C. Neidhardt. 1969. Mapping of a structural gene for valyl-transfer ribonucleic acid synthetase in *Escherichia coli* by transduction. J. Bacteriol. **98**: 837-839.
42. Tocchini-Valentini, G. P., L. Felicetti, and G. M. Rinaldi. 1969. Mutants of *Escherichia coli* blocked in protein synthesis: mutants with an altered G factor. Cold Spring Harbor Symp. Quant. Biol. **34**:463-468.
43. Yaniv, M., and Gros, F. 1967. Studies of temperature conditional mutants of *E. coli* with a modified aminoacyl RNA synthetase, p. 157-178. *In* D. Shugar (ed.), Genetic elements. Academic Press Inc., New York.
44. Yaniv, N., F. Jacob, and F. Gros. 1965. Mutations thermosensibles des systèmes activant la valine chez *E. coli*. Bull. Soc. Chim. Biol. **47**:1609-1626.