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# Mutants of *Escherichia coli* with an Altered Tyrosyl-Transfer Ribonucleic Acid Synthetase

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We have isolated several mutants defective in the gene for tyrosyl-transfer ribonucleic acid (tRNA) synthetase (*tyrS*). One of these mutants is described in detail. It was isolated as a tyrosine auxotroph with defects both in the tyrosyl-tRNA synthetase and in the tyrosine biosynthetic enzyme, prephenate dehydrogenase. It also had derepressed levels of the tyrosine-specific 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthetase. The latter finding suggested that a wild-type *tyrS* gene was required for repression of the tyrosine biosynthetic enzymes. The following results demonstrated that this hypothesis was not correct. (i) When the defective *tyrS* gene was transferred to another strain, the tyrosine-specific DAHP synthetase in that strain was not derepressed, and (ii) two other mutants with defective tyrosyl-tRNA synthetases had repressed levels of the tyrosine biosynthetic enzymes. The *tyrS* gene was located near minute 32 on the *Escherichia coli* chromosome by interrupted mating experiments.

Amino acyl transfer ribonucleic acid (tRNA) synthetases direct the attachment of amino acids to specific tRNA molecules; their role in protein synthesis has been well established. These enzymes have also been implicated in bacterial regulatory processes. In particular, they are involved in regulating the levels of the biosynthetic enzymes for certain amino acids in *Escherichia coli* and *Salmonella typhimurium*. Mutants in which the tRNA synthetase for histidine (16, 19), valine (7), or isoleucine (24) is impaired have derepressed levels of the respective amino acid biosynthetic enzymes. Studies with analogues of histidine (20) and valine (8) indicated that inhibition of the attachment of the naturally occurring amino acid to tRNA caused derepression of the amino acid biosynthetic enzymes. It is apparently the amount of histidine bound to tRNA that affects the level of repression of the histidine enzymes (22), but an analogous role for tRNA has not been clearly demonstrated for the other amino acids mentioned. The levels of the biosynthetic enzymes for arginine (11), phenylalanine (17), and tryptophan (5) are not increased in cells containing a genetic defect in the appropriate tRNA synthetase. Investigations with analogues of tyrosine had suggested that tyrosyl-tRNA synthetase was not involved in repressing the tyrosine biosynthetic enzymes (18).

We recently isolated a tyrosine-requiring mutant of *E.* coli K-12 that contained an altered tyrosyl-tRNA synthetase. This mutant permitted us to determine whether the presence of an intact synthetase for tyrosine was required to repress the tyrosine biosynthetic enzymes. The original mutant was also defective in prephenate dehydrogenase—one of the enzymes in the biosynthetic pathway for tyrosine—but the levels of the tyrosine-specific 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthetase were derepressed. These observations suggested that there was a direct relationship between the defective synthetase and the high levels of the tyrosine biosynthetic enzymes. However, genetic studies with this mutant and the isolation of two other mutants with an altered tyrosyl-tRNA synthetase demonstrated that repression of the tyrosine biosynthetic enzymes may still occur in a strain containing a defective tyrosyl-tRNA synthetase.

## MATERIALS AND METHODS

**Bacterial strains.** Table 1 describes the origin and properties of the strains used in these experiments.

**Growth media.** The medium contained (grams per liter): $KH_2PO_4$, 5.32; $K_2HPO_4$, 10.6; and $(NH_4)_2SO_4$, 2.0. The *p*H was adjusted to 7 with KOH. $MgSO_4 \cdot 7H_2O$, glucose, and thiamine were added, after autoclaving, to final concentrations of 0.2, 2.0, and 0.005 mg/ml, respectively. The rich medium contained 10 g

TABLE 1. *Strains of bacteria used*

| Strain | Source | Properties |
|---|---|---|
| CW3747 | A. Torriani, Massachusetts Institute of Technology | $F_{13}'$, *met*$^-$, constitutive for alkaline phosphatase |
| W3747 | A. Torriani | $F_{13}'$, *met*$^-$, differs from CW3747 only in the R2 gene for alkaline phosphatase (6) |
| JC500 | J. Eigner, Washington Univ. (originally from J. Clark, Univ. of Calif.) | F$^-$ *arg*$^-$, *his*$^-$, *leu*$^-$, *met*$^-$, *thy*$^-$, *gal*$^-$, *mal*$^-$, *lac*$^-$, *xyl*$^-$, *mtol*$^-$, *Tl*$^r$, *str*$^r$ |
| tyr2-3 | R. Somerville, Purdue Univ. | *tyr*$^-$ |
| KL16 | J. Eigner | Hfr, *thy tyrA his trp* ← |
| HfrH | D. Silbert, Washington Univ. | Hfr, *tyrA his trp leu* → |
| N25 | G. Nester | *met*$^-$ *phe*$^-$ |

of nutrient broth and 10 g of Casamino Acids per liter. For the assay of prephenate dehydrogenase and DAHP synthetase, the cultures were grown in the glucose-salts medium of Spizizen (23).

**Isolation of tyr$^-$ mutants.** (i) Mutagenesis with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NG) was done as follows. A culture, grown overnight in minimal medium plus required growth factors was centrifuged, resuspended in the same medium lacking glucose, and incubated with 30 μg of the mutagen per ml for 30 min at 37 C with shaking. A 1-ml amount was diluted into 20 ml of fresh medium containing glucose, growth factors, and 0.1 mM tyrosine and was grown overnight. (ii) Mutagenesis with ethylmethanesulfonate (EMS) was done as follows. An exponentially growing culture was treated with the mutagen for 3 hr (0.02 ml of EMS per 3 ml of culture) before diluting 20-fold into tyrosine-containing medium. (iii) Pencillin selection was done by the procedure of Gorini and Kaufman (9). In some cases the cells were taken through two cycles of penicillin before plating (14).

**Genetic procedures.** (i) The preparation of phage P1 and the methods for transduction were essentially those described by Apirion (1). Phage was ultraviolet irradiated for 40 sec before transduction (2). (ii) The general procedures for mating experiments were those described by Apirion (1). For interrupted mating, the F$^-$ culture was grown in minimal medium with the appropriate growth factors present. Hfr strains were grown in rich medium. Mating pairs were interrupted by vigorous agitation with a Vortex mixer for 1 min.

**Assay of tyrosyl-tRNA synthetase.** The preparation of extracts and the assay were adapted from the procedures described by Muench and Berg (15). Exponentially growing cultures were harvested by centrifugation, washed with saline, and suspended in 0.01 the original volume in 20 mM Tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 7), 10 mM $MgCl_2$, and 10% glycerol. Extracts were prepared by sonic disruption of the cells in a Bronwill Sonicator (Biosonik) for 1 min per ml of buffer. Cell debris and ribosomes were removed by centrifugation for 1 hr at 30,000 rev/min in a Spinco model L ultracentrifuge, and the extracts were dialyzed against the same buffer. To detect activity in transductants or recombinants, cells were inoculated into 25 ml of medium and harvested after overnight growth. A 1-ml amount of the buffer was added to the washed cells, and the extract was prepared as above. The extracts were centrifuged as above, but they were not dialyzed. The assay solution contained: 20 μmoles of Tris-hydrochloride buffer (*p*H 7.0); 2 μmoles of $MgCl_2$; 2 μmoles of KCl; 0.8 μmole of glutathione; 0.2 μmole of adenosine triphosphate; 0.04 mg of bovine serum albumin; 4.0 nmoles of $^{14}$C-tyrosine (50 μc/μmole); 40 nmoles of each of the other amino acids; and four $A_{260}$ units of *E. coli* B tRNA in a final volume of 0.2 ml. After the addition of the extract at 0 C, the tubes were placed in a 37 C water bath for 10 min.The reaction was terminated by cooling the tubes in an ice-water bath and adding 0.5 ml of 10% trichloroacetic acid. The contents of the tube were filtered onto glass fiber filters, washed with 5% trichloroacetic acid, dried, and counted in an end-window low-background Nuclear Chicago Counter. One unit of enzyme is equivalent to the formation of 1 nmole of tyrosyl-tRNA in 10 min at 37 C. This unit is the same as that defined by Muench and Berg (15).

**Assay of prephenate dehydrogenase.** Cells in the log phase were harvested by centrifugation and washed once in 0.1 M Tris-hydrochloride (*p*H 7.8), 1.0 mM $MgCl_2$, and 0.1 mM ethylenediaminetetraacetic acid. They were resuspended in 2 ml of this same buffer and disintegrated by sonic treatment in a Mullard MSE instrument (Measuring & Scientific Equipment, Ltd., London, England) employing maximum power for 1 min. The supernatant solution obtained after centrifugation at 44,000 × *g* for 30 min was used in the assay. Assays were always performed on the same day on which the extracts were made. The assay was that previously described (21). One unit of enzyme forms 1 nmole of product in 1 min.

**Assay of tyrosine-specific DAHP synthetase.** Extracts were prepared as described for prephenate dehydrogenase, except that the buffer employed was 0.05 M $KPO_4$, *p*H 6.8, and extracts were passed through a G25 Sephadex column. The specific activities of the tyrosine-specific and phenylalanine-specific DAHP synthetase were determined from the inhibition of activity by 0.5 mM tyrosine and phenylalanine in the reaction mixture. The assay was performed by the procedure described by Jensen and Nester (12). All assays were performed on the day extracts were prepared. One unit of enzyme forms 1 nmole of DAHP per min.

**Protein determination.** Protein was usually determined by the method of Groves, Davis, and Sells (10).

In some experiments, the method of Lowry et al. (13) was also used with essentially the same results.

**Chemicals.** DL-*m*-Fluorotyrosine was a generous gift from P. Berg. $^{14}$C-tyrosine was purchased from New England Nuclear Corp. The *E. coli* B tRNA was obtained from Schwarz BioResearch, Inc. NG was purchased from Aldrich Chemical Co., and the EMS came from Eastman Kodak Co. The antibody against the wild-type tyrosyl-tRNA synthetase was kindly provided by P. Berg. Its preparation was described by Calendar and Berg (3).

## RESULTS

**Isolation of a mutant defective in tyrosyl-tRNA synthetase.** A tyrosine auxotroph (designated SS-1) derived from strain CW3747 was isolated by penicillin selection after mutagenesis with NG—a procedure that has been used successfully for obtaining several amino acid auxotrophs from this strain. This tyrosine-requiring mutant had the unusual property of not incorporating radioactive leucine into protein when tyrosine in the medium was replaced by fluorotyrosine (Fig. 1). Fluorotyrosine is known to be incorporated into bacterial protein in place of tyrosine (R. L. Calendar, Ph.D. Thesis, Stanford Univ., Stanford, Calif.), and another tyrosine auxotroph did incorporate radioactive leucine into protein in the presence of fluorotyrosine.

Further investigation revealed that extracts of this mutant lacked tyrosyl-tRNA synthetase activity (Fig. 2). Tyrosyl-tRNA synthetase activity was detected in extracts from the parent strain (CW3747), and no inhibition of this activity was observed when extracts of the parent and mutant were mixed. The ability of the extract from strain SS-1 to attach other amino acids to tRNA was comparable to that of wild-type strain (Table 2).

The growth rate of strain SS-1 in the presence of tyrosine was not significantly different from that of the parent strain CW3747 either at 37 or

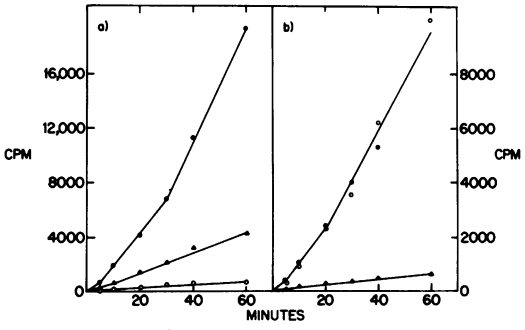


FIG. 1. *Effect of fluorotyrosine on protein synthesis in two tyrosine-requiring mutants of E. coli. Exponentially growing cells were filtered, washed, and resuspended to a cell concentration of 2 × 10$^8$ per ml in tyrosine-free medium and samples were added to three flasks which were incubated at 37 C for 5 min before the addition of 0.1 mM tyrosine (●), 0.1 mM fluorotyrosine (○), or neither (▲); $^{14}$C-leucine (2 μc/μmole, final concentration 0.02 mM) was added 2 min later. Samples (1 ml) removed at the indicated times were added to an equal volume of 10% trichloroacetic acid. The samples were heated at 80 to 90 C for 30 min before filtering. (a) strain SS-1, (b) strain tyr2-3.*

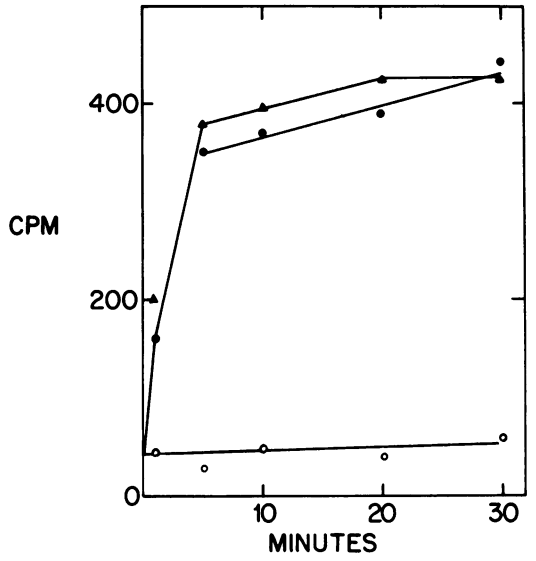


FIG. 2. *Attachment of tyrosine to tRNA by extracts of CW3747 and the tyrosine-requiring mutant (strain SS-1). The final protein concentration in the assay tube was 50 μg/ml for both strains CW3747 and SS-1. Strain CW3747, ●; strain SS-1, ○; mixed extracts, ▲.*

TABLE 2. *Attachment of amino acids to tRNA by extracts from strain SS-1 and CW3747*[a]

| Amino acid | Amino acid bound to tRNA (pmoles/$A_{260}$) | |
|---|---|---|
| | Strain CW3747 | Strain SS-1 |
| Arginine | 42.4 | 42.4 |
| Glutamate | 12.0 | 11.3 |
| Histidine | 13.2 | 13.2 |
| Leucine | 57 | 57 |
| Phenylalanine | 18.5 | 20.0 |
| Threonine | 23.6 | 23.6 |
| Tyrosine | 21.2 | 3.0 |

[a] Each amino acid was incubated in the usual assay mixture except that the reaction was run at several concentrations of tRNA (0.92, 1.82, or 3.64 $A_{260}$ units per 0.2 ml), and the time of incubation was 30 min. The tubes contained 10 μg of protein from the extract of either CW3747 or SS-1.

at 42 C. Overnight cultures of strain SS-1, however, always had a prolonged lag period before they began exponential growth.

**Properties of the mutationally altered tyrosyl-tRNA synthetase.** Although no tyrosyl-tRNA synthetase activity was found under the usual assay conditions, activity was detected with about six times more extract than had been added to the assay shown in Fig. 2. Furthermore, the activity was greatly enhanced by increasing the tyrosine concentration (Fig. 3). It appeared that the synthetase in strain SS-1 was defective in its ability to bind tyrosine, as extracts from strain CW3747 did not show these increases in activity (*see also* Table 7). Although fluorotyrosine was not tested as a substrate for the defective synthetase, the most likely explanation for its inability to be incorporated into protein in strain SS-1 (Fig. 1) was that it is an exceedingly poor substrate for the altered synthetase. The $K_m$ value (0.48 mM) for fluorotyrosine with wild-type tyrosyl-tRNA synthetase was reported to be 16 times higher than that for tyrosine (4).

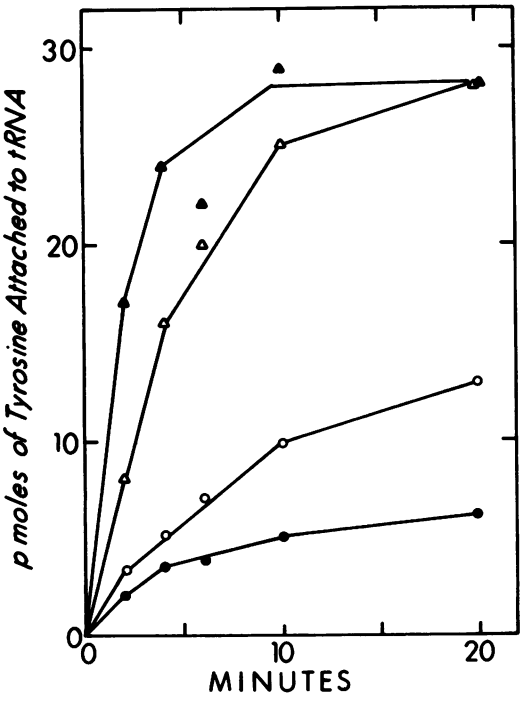


FIG. 3. *Effect of tyrosine concentration on the activity of tyrosyl-tRNA synthetase from strain SS-1. The activity of tyrosyl-tRNA synthetase was assayed as a function of time with varying concentrations of tyrosine. Symbols: 0.05 mM, ●; 0.1 mM, ○; 0.25 mM, △; 0.5 mM, ▲. The protein concentration was 0.37 mg/ml in the assay solution. Samples of 0.05 ml were removed at the indicated times and added to 0.5 ml of 10% trichloroacetic acid.*

Attempts to increase the activity of the synthetase from the mutant were unsuccessful. Preparation of the extract in the presence of tyrosine had no effect, and dialysis did not alter the activity.

Calendar and Berg (3) prepared antibody to purified tyrosyl-tRNA synthetase. Extracts of strain SS-1 containing the defective tyrosyl-tRNA synthetase were able to block the activity of the antibody when tested against the wild-type tyrosyl-tRNA synthetase (Table 3). This result indicated that the amount of synthetase-like protein formed in the mutant was similar to the amount of enzyme present in wild-type cells. The low activity of the mutant could be attributed to a decrease in the specific activity of the tyrosyl-tRNA synthetase rather than to the formation of less enzyme.

**Evidence for an additional mutation in strain SS-1.** Several spontaneous revertants of strain SS-1 able to grow in the absence of tyrosine were isolated. These revertants were still defective in tyrosyl-tRNA synthetase, and they had levels of prephenate dehydrogenase 10-fold higher than those found in strain CW3747. When strain SS-1 was tested for prephenate dehydrogenase, no activity was detectable (Table 4). The latter

TABLE 3. *Evidence for the presence of protein antigenically related to tyrosyl-tRNA synthetase in mutant extracts*[a]

| Components in first incubation[b] | Addition to second incubation[b] | Tyrosyl-tRNA synthetase activity remaining after reaction with antibody |
|---|---|---|
| | | % |
| None | CW3747 extract, 17 μg | 100 |
| Antibody, 2.1 μg | CW3747 extract, 17 μg | 82 |
| Antibody, 4.3 μg | CW3747 extract, 17 μg | 46 |
| Antibody, 4.3 μg + mutant extract, 8 μg | CW3747 extract, 17 μg | 77 |
| Mutant extract, 8 μg | None | 2 |

[a] The mutant extract was prepared from a *tyr*$^+$ transductant (Table 5).

[b] Both incubations were 10 to 15 min at 0 C. The tubes were supplemented with bovine serum albumin in the first incubation, resulting in 20 μg of protein in a final volume of 0.04 ml. The volume was increased to 0.08 ml in the second incubation by the addition of extract from CW3747. After the second incubation, 0.02 ml from each tube was added to 0.18 ml of the standard solution for assay of tyrosyl-tRNA synthetase.

observation suggested that the tyrosine requirement in strain SS-1 might have arisen as a result of mutations in more than one gene.

The genes for the tyrosine biosynthetic enzyme, prephenate dehydrogenase (*tyr*A), and for the phenylalanine biosynthetic enzyme, prephenate dehydratase (*phe*A), are closely linked on the *E. coli* chromosome (25). Phage P1 grown on the original *tyr*$^-$ mutant (SS-1) was used to transduce a phenylalanine auxotroph (N25 *phe*A$^-$) to prototrophy. The transductants were selected on plates containing tyrosine and were screened for growth in the presence and absence of tyrosine. After 24 hr, more than 90% (160 colonies) of the transductants isolated had grown only in the presence of tyrosine. After 48 hr, all of these colonies had grown also in the absence of tyrosine. Extracts were prepared from four of the transductants. Prephenate dehydrogenase was undetectable in these extracts (Table 4), whereas the activity of tyrosyl-tRNA synthetase was normal. The transfer of the *tyrA* gene from SS-1 to N25 demonstrated that the prephenate dehydrogenase of strain SS-1 is defective but permits slow growth in the absence of tyrosine in a cell that has normal tyrosyl-tRNA synthetase activity (*see also* Fig. 5).

**Transduction of strain SS-1 to tyr$^+$.** Strain SS-1 was transduced to tyrosine prototrophy by using phage P1 grown on the parent strain CW3747 (Table 5). For comparison, the frequency of *leu*$^+$ transductants was also determined in experiment 2. Four *tyr*$^+$ transductants from experiment 1 and 23 from experiment 2 were tested for tyrosyl-tRNA synthetase; none of them had regained activity. Several of the transductants were also assayed for prephenate dehydrogenase and were found to have a high level of activity similar to that reported for the *tyr*$^+$ revertant (Table 4). The high level of several tyrosine biosynthetic enzymes (DAHP synthetase was also derepressed) probably results in the synthesis of enough tyrosine to permit the defective tyrosyl-tRNA synthetase to function, allowing the cells to grow in the absence of tyrosine in the medium.

TABLE 4. *Levels of prephenate dehydrogenase*

| Strain | +Tyrosine[a] | −Tyrosine[a] |
|---|---|---|
| CW3747 | 6.8 | 11.9 |
| SS-1 | 0 | |
| N25 | Not determined | 21.2 |
| N25 *phe*$^+$*tyr*$^-$ transductants | 0 | |
| SS-1 *tyr*$^+$ revertant | Not determined | 169 |
| SS-1 *tyr*$^+$ transductant | 30[b] | 116 |

[a] Extracts were prepared either from cultures grown in a medium containing 30 μg of tyrosine per ml (+tyrosine) or from cultures grown in a medium that did not contain tyrosine (−tyrosine). Expressed as enzyme units per milligram of protein.

[b] Strain SS-1 *tyr*$^+$ transductant was grown with 100 μg of tyrosine per ml.

TABLE 5. *Transduction of SS-1 to tyr*$^+$

| Recipient | Donor | *tyr*$^+$ transductants per $10^8$ cells | *leu*$^+$ transductants per $10^8$ cells |
|---|---|---|---|
| Experiment 1 | | | |
| SS-1, *str*$^r$ [a] | CW3747 | 790 | |
| SS-1, *str*$^r$ [a] | | 0 | |
| Experiment 2 | | | |
| SS-1, *leu*$^-$ *str*$^r$ [b] | CW3747 | 1,000 | 3,000 |
| SS-1, *leu*$^-$ *str*$^r$ [b] | SS-1 | 0 | 12,000 |
| SS-1, *leu*$^-$ *str*$^r$ [b] | | 0 | 0 |

[a] A spontaneous mutant resistant to streptomycin was isolated from SS-1.

[b] A *leu*$^-$ derivative of SS-1 *str*$^r$ was isolated after mutagenesis with NG and penicillin selection.

**Transfer of tyrA and tyrS genes to strain JC500.** CW3747 carries the $F'_{13}$ episome and transfers chromosomal markers at a low frequency. The *tyr*$^-$ mutant of strain CW3747 (SS-1) and the F$^-$ strain JC500 were incubated together for 2 hr and *thy*$^+$ recombinants were selected on plates containing tyrosine. About 25% of the 300 colonies that grew on plates that contained tyrosine were unable to grow in the absence of tyrosine. Eight tyrosine-requiring recombinants were assayed for tyrosyl-tRNA synthetase and were found to lack this activity. One of these recombinants was chosen for further study. Phage P1 grown on this *tyr*$^-$ derivative was used to transduce the *phe*$^-$ mutant (N25) to *phe*$^+$. Eighty-three per cent of the *phe*$^+$ transductants were also *tyr*$^-$. However, they grew in the absence of tyrosine after 48 hr, as had the *tyr*$^-$ transductants obtained with P1 grown on strain SS-1. This result, plus the inability to detect prephenate dehydrogenase activity in extracts of the *thy*$^+$ *tyr*$^-$ recombinant, led us to conclude that the two mutations present in strain SS-1 had been transferred to this JC500 recombinant during mating.

**Tyr$^+$ recombinants obtained by interrupted mating.** The strain JC500 *thy*$^+$ *tyr*$^-$ recombinant carrying defects in both prephenate dehydrogenase and tyrosyl-tRNA synthetase was used as the recipient in interrupted mating experiments (Fig. 4). *Tyr*$^+$ recombinants were selected by using two Hfr strains with different origins and different

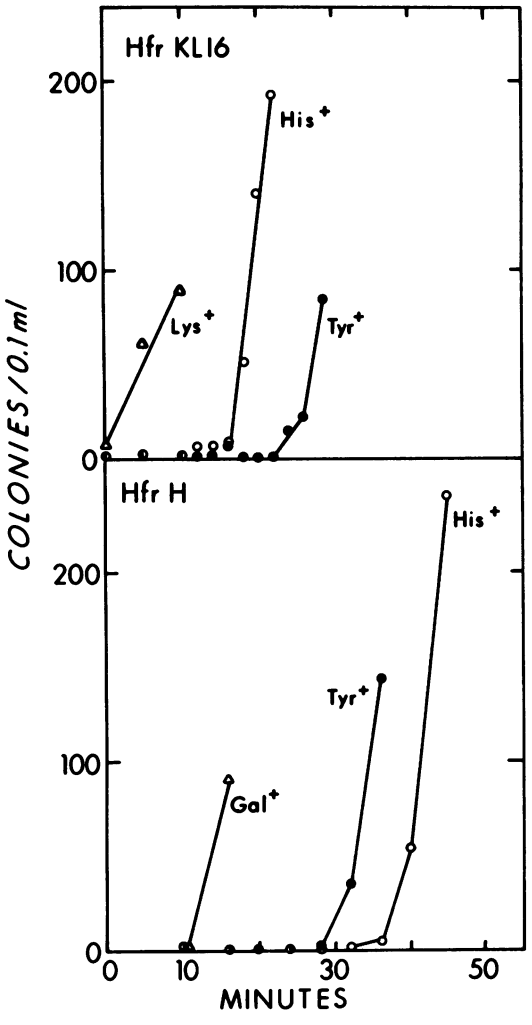


FIG. 4. *Time of entry of several markers in the tyr⁻ recombinant of strain JC500. The F⁻ strain JC500 was made thy⁺ tyr⁻ by recombination with strain SS-1 (see text) and lys⁻ by mutagenesis with EMS and penicillin selection. An exponentially growing culture at 2 × 10⁸ cells/ml was mixed with an Hfr strain at a ratio of 1:1. After 5 min at 37 C with gentle mixing, the culture was diluted 20-fold. The mixing was stopped and, at the indicated times, samples were removed, diluted 2-fold, and agitated to break apart the mating pairs. A 0.1-ml amount was plated immediately. The time scale refers to the time after dilution.*

directions of transfer (Table I). The gene which conferred tyrosine prototrophy entered the recipient at about 24 min with strain KL16 as donor and at 32 min with strain HfrH as donor. Recombinants, taken at the 24th min in the cross with strain KL16 and at the 32nd min in the cross with strain HfrH, had regained tyrosyl-tRNA synthetase activity (eight from each cross were assayed). None of the recombinants obtained from the mating with strain HfrH recovered prephenate dehydrogenase activity; two of eight from the cross with strain KL16 also recovered prephenate dehydrogenase activity. *TyrA* is located at minute 50 on the *E. coli* chromosome and should enter almost immediately when strain Hfr KL16 is used as the donor, but not until 50 min when strain HfrH is the donor (25). Therefore, it was not surprising to find that some of the recombinants with strain KL16 had regained both enzymatic activities.

The recombinants with a defective prephenate dehydrogenase grew slowly in the absence of tyrosine even though they had regained tyrosyl-tRNA synthetase activity (Fig. 5). In this regard they were similar to the strain N25 transductants that had received the defective *tyrA* gene from strain SS-1. The original strain JC500 (and the recombinants from the mating with strain KL16 that had also regained prephenate dehydrogenase activity) had the same growth rate whether

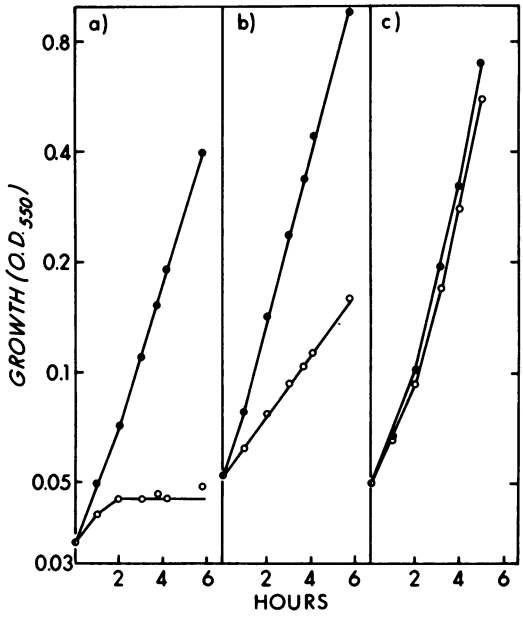


FIG. 5. *Growth of strain JC500 and recombinants. Each culture growing exponentially in the presence of 0.1 mM tyrosine was centrifuged, washed, and resuspended in the absence of tyrosine. The culture was divided into two flasks, only one of which contained tyrosine. The flasks were incubated at 37 C with shaking; samples were removed at the indicated times to measure turbidity. Samples: 0.1 mM tyrosine, ●; no tyrosine, ○. (a) Strain JC500 containing the tyrS and tyrA genes from strain SS-1. (b) Strain JC500 tyr⁺ recombinant from mating with HfrH. (c) Strain JC500 original thy⁻ mutant.*

tyrosine was present in the medium or not. The results obtained in the interrupted mating experiment indicate that the gene for tyrosyl tRNA synthetase—the *tyrS* gene—maps at approximately minute 32 on the *E. coli* chromosome.

**Tyr$^+$ recombinants obtained by transduction of strain JC500 tyr$^-$.** The ability to recover the the tyrosyl-tRNA synthetase activity by recombination in strain JC500 *tyr*$^-$ was not peculiar to the introduction of genes by mating. When this strain was transduced to *tyr*$^+$ by phage P1 grown on the original strain JC500, the transductants regained synthetase but not prephenate dehydrogenase activity. The growth of these transductants was similar to that of the *tyr*$^+$ recombinant of strain JC500 containing a defective prephenate dehydrogenase shown in Fig. 5.

**Levels of the tyrosine biosynthetic enzymes in strain SS-1 and strain JC500 tyr$^-$.** Strain SS-1 *tyr*$^+$ transductants have high levels of prephenate dehydrogenase (Table 4). The tyrosine-specific DAHP synthetase is also derepressed both in the transductants and in the original tyrosine-requiring mutant (SS-1). However, in the strain JC500 that had received both the defective tyrosyl-tRNA synthetase and prephanate dehydrogenase from strain SS-1, the tyrosine-specific DAHP synthetase is not derepressed (Table 6). To establish that this enzyme can be derepressed in this strain, a culture was grown in a chemostat with 5 $\mu$g of tyrosine per ml and a doubling time of approximately 180 min. Under this condition, derepression of the DAHP synthetase occurred.

**Other mutants defective in tyrosyl-tRNA synthetase.** The mutagenesis and selection procedure that led to the isolation of strain SS-1 was used to obtain a tyrosine auxotroph from strain W3747 and one from strain W3747 *his*$^-$. These two mutants were also defective in tyrosyl tRNA synthetase. The activity of the synthetase in crude dialyzed extracts of these mutants was similar to that found in strain SS-1 (Table 7). These enzymes were also defective in binding tyrosine; an increase in tyrosine concentration from 0.02 to 0.08 mM increased the activity of the mutant enzymes fourfold. This increase in tyrosine concentration caused only a 20 to 30% increase in the activity of the wild-type tyrosyl-tRNA synthetase.

The two mutants differed from strain SS-1 in several properties. Both mutants had a very high reversion rate to *tyr*$^+$, and neither of them formed colonies when plated on rich medium. The most significant difference was that both mutants had *repressed* levels of prephenate dehydrogenase and *repressed* levels of DAHP synthetase (Table 8).

The reversion to *tyr*$^+$ was so high in the strain W3747 *tyr*$^-$ mutant that it was not possible to do any genetic experiments, but the tyrosine auxotroph derived from strain W3747 *his*$^-$ could be transduced to tyrosine prototrophy with P1

TABLE 6. *Levels of tyrosine-specific DAHP synthetase*[a]

| Strain | Enzyme units/mg of protein |
|---|---|
| CW3747 | 52.9 |
| SS-1[b] | 136.2 |
| SS-1 *tyr*$^+$ transductant | 265 |
| JC500 | 58.8 |
| JC500 *tyr*$^-$[b] | 20.1 |
| JC500 *tyr*$^-$ grown in chemostat | 668 |

[a] The specific activity was calculated from the total DAHP synthetase times per cent of tyrosine specific activity. The phenylalanine-specific DAHP synthetase was also determined. The sum of both activities accounted for virtually all of the enzyme activity measured.

[b] Extracts were prepared from cultures grown in a medium containing 30 $\mu$g of tyrosine per ml.

TABLE 7. *Activity of tyrosyl-tRNA synthetase in wild-type and tyrS mutants assayed at two concentrations of tyrosine*

| Strain | Tyrosyl-tRNA synthetase (enzyme units/mg of protein)[a] | |
|---|---|---|
| | 0.02 mM tyrosine | 0.58 mM tyrosine |
| CW3747 | 27 | 35 |
| W3747 | 30 | 45 |
| SS-1 | 0.14 | 0.54 |
| W3747 *his*$^-$ *tyrS2* | 0.07 | 0.26 |
| W3747 *tyrS3* | 0.09 | 0.39 |

[a] Each extract was assayed at several different protein concentrations to determine the specific activity. The activity of the synthetase from the *tyrS* mutants varied with different preparations of extracts, but the same stimulation with increased tyrosine concentration was always observed.

TABLE 8. *Levels of tyrosine biosynthetic enzymes in tyrosine auxotrophs*[a]

| Strain | Prephenate dehydrogenase[b] | Tyrosine-specific DAHP synthetase[b] |
|---|---|---|
| W3747 *his*$^-$*tyrS2* | 3.8 | 39.8 |
| W3747 *tyrS3* | 6.0 | 42.0 |

[a] Extracts were prepared from cultures grown in a medium containing 30 $\mu$g of tyrosine per ml.

[b] Expressed as enzyme units per milligram of protein.

phage grown on strain W3747. Transductants from three separate transduction experiments recovered tyrosyl-tRNA synthetase activity (17 were assayed). They grew equally well in the presence or absence of tyrosine in the medium and in rich medium.

The *tyr*$^+$ revertants obtained from strain W3747 *his*$^-$ *tyr*S2 and W3747 *tyr*S3 were similar to those obtained from strain SS-1. They were still defective in tyrosyl-tRNA synthetase activity, but they had derepressed levels of the tyrosine biosynthetic enzymes. Revertants also grew in rich medium.

**Isolation of other tyrosine auxotrophs.** Several other tyrosine auxotrophs have been isolated from strains CW3747 and W3747. With EMS as a mutagen and penicillin selection, two tyrosine auxotrophs were isolated—one from an exponential culture and one from an overnight culture in stationary phase. Both had normal levels of tyrosyl-tRNA synthetase. In two cases, NG was used as a mutagen with exponentially growing cultures; the tyrosine auxotrophs isolated (one in the absence of penicillin selection) were not defective in the tyrosyl-tRNA synthetase.

Our results suggest that both NG and nongrowing cells favor mutations in the *tyrS* gene, but not enough mutants have been isolated to draw any conclusions. We did not detect amino acyl tRNA synthetase mutants when auxotrophs for histidine, arginine, tryptophan, and proline were isolated by the same procedure.

## DISCUSSION

On the basis of biochemical and genetic data, the tyrosine auxotroph, strain SS-1, had mutations both in the *tyrS* gene, the gene that specifies tyrosyl-tRNA synthetase, and in the *tyrA* gene, the gene that codes for prephenate dehydrogenase. In this mutant the defect in the synthetase by itself did not lead to a tyrosine requirement. *Tyr*$^+$ transductants still had the mutated *tyrS* gene and grew well in the absence of tyrosine. These transductants had derepressed levels of prephenate dehydrogenase and the tyrosine-specific DAHP synthetase. The latter enzyme was also derepressed in the original *tyr*$^-$ mutant. The high levels of these enzymes in the transductants apparently provided sufficient tyrosine to permit the altered synthetase to function. The fact that the tyrosine biosynthetic enzymes were derepressed suggested that the wild-type *tyrS* gene was required for repression. Our results with other *tyrS* mutants from strain W3747 and with a *tyr*$^-$ recombinant of strain JC500 did not support this hypothesis.

Two other *tyrS* mutants had normal repressed levels of prephenate dehydrogenase and the tyrosine-specific DAHP synthetase. In these mutants the tyrosine requirement could be attributed to the defect in tyrosyl-tRNA synthetase because *tyr*$^+$ transductants recovered synthetase activity. These *tyrS* mutants have not been studied extensively, and it is conceivable that some mutations in the *tyrS* gene would not cause derepression of the tyrosine biosynthetic enzymes. We consider this possibility unlikely because when the *tyrS* mutation from strain SS-1 was transferred to another strain (JC500), the tyrosine biosynthetic enzymes were still repressed. The most reasonable explanation for our results is that strain SS-1 carries a third mutation in a gene that controls the level of the tyrosine biosynthetic enzymes.

There does not seem to be an obvious selective advantage to a cell to possess these three mutations. Two mutants that were defective in the *tyrS* gene but appear to have normal repressed levels of the tyrosine biosynthetic enzymes have been isolated by the same procedure as that used to obtain strain SS-1. It is known, however, that the frequency of multiple mutations with NG as a mutagen is high. A cell that had a "leaky" mutation *either* in the *tyrS* gene *or* in the *tyrA* gene and had derepressed levels of the tyrosine biosynthetic enzymes would probably not have been isolated as a tyrosine auxotroph after penicillin selection. However, a cell such as strain SS-1 which is defective in *both* the *tyrS* gene and the *tyrA* gene would still be selected as a tyrosine-requiring mutant even though the tyrosine biosynthetic enzymes were derepressed.

The mutation causing derepression of the tyrosine biosynthetic enzymes was not cotransducible with the *tyrA* gene from strain SS-1. When the *tyrA* gene from a strain SS-1 *tyr*$^+$ transductant (*tyr*A$^+$ and derepressed, *see* Table 4) was introduced into another strain by transduction, the prephenate dehydrogenase activity in the transductant was still repressed.

The mutation in strain SS-1 responsible for the derepressed levels of the tyrosine biosynthetic enzymes may be similar to the one recently described by Wallace and Pittard (26). They isolated mutants with high levels of the tyrosine biosynthetic enzymes, and the gene affecting regulation, designated as *tyrR*, maps near minute 34 on the *E. coli* chromosome. Our data indicate that the *tyrS* gene maps very close to this region. Until both genes can be studied by transduction analysis, it will be difficult to assess the relationship between them. We have not yet been able to cotransduce *tyrS* with other markers.

Strain SS-1, and in particular the *tyr*$^+$ transductants of strain SS-1, may resemble certain

mutants affected in the regulation of tryptophan biosynthesis (5). The latter mutants were defective in tryptophanyl-tRNA synthetase and also had high levels of the tryptophan biosynthetic enzymes. Doolittle and Yanofsky (5) examined a number of mutants defective in tryptophanyl-tRNA synthetase, and all of the mutants had repressed levels of the tryptophan biosynthetic enzymes. The original *trpS* mutants with high levels of the tryptophan biosynthetic enzymes are now believed to result from multiple mutational events in a single cell (5). Our results with tyrosine emphasize that the relationship between a mutationally altered amino acyl-tRNA synthetase and derepression of the amino acid biosynthetic enzymes requires careful scrutiny before a definite correlation between the two can be established.

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## LITERATURE CITED

1. Apirion, D. 1966. Altered ribosomes in a suppressor strain of *Escherichia coli*. J. Mol. Biol. **16**:285–301.
2. Apirion, D. 1967. Three genes that affect *Escherichia coli* ribosomes. J. Mol. Biol. **30**:255–275.
3. Calendar, R., and P. Berg. 1966. Purification and physical characterization of tyrosyl ribonucleic acid synthetases from *Escherichia coli* and *Bacillus subtilis*. Biochemistry **5**:1681–1690.
4. Calendar, R., and P. Berg. 1966. The catalytic properties of tyrosyl ribonucleic acid synthetases from *Escherichia coli* and *Bacillus subtilis*. Biochemistry **5**:1690–1695.
5. Doolittle, W. F., and C. Yanofsky. 1968. Mutants of *Escherichia coli* with an altered tryptophanyl-transfer ribonucleic acid synthetase. J. Bacteriol. **95**:1283–1294.
6. Echols, H., A. Garen, S. Garen, and A. Torriani. 1961. Genetic control of repression of alkaline phosphatase in *E. coli*. J. Mol. Biol. **3**:425–438.
7. Eidlic, L., and F. C. Neidhardt. 1965. Role of valyl-sRNA synthetase in enzyme repression. Proc. Nat. Acad. Sci. U.S.A. **53**:539–543.
8. Freundlich, M. 1967. Valyl-transfer RNA: role in repression of the isoleucine-valine enzymes in *Escherichia coli*. Science **157**:823–824.
9. Gorini, L., and H. Kaufman. 1959. Selecting bacterial mutants by the penicillin method. Science **131**:604–605.
10. Groves, W. E., F. C. Davis, Jr., and B. Sells. 1968. Spectrophotometric determination of microgram quantities of protein without nucleic acid interference. Anal. Biochem. **22**:195–210.
11. Hirshfield, I. N., R. DeDeken, P. C. Horn, D. A. Hopwood, and W. K. Maas. 1968. Studies on the mechanism of repression of arginine biosynthesis in *Escherichia coli*. III. Repression of enzymes of arginine biosynthesis in arginyl-tRNA synthetase mutants. J. Mol. Biol. **35**:83–93.
12. Jensen, R. A., and E. W. Nester. 1965. The regulatory significance of intermediary metabolites. Control of aromatic amino acid biosynthesis by feedback inhibition in *Bacillus subtilis*. J. Mol. Biol. **12**:468–481.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193** 265–275.
14. Lubin, M. 1962. Enrichment of auxotrophic mutant populations by recycling. J. Bacteriol. **83**: 696–697.
15. 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. Davies (ed.), Procedures in nucleic acid research. Harper and Row, New York.
16. 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.
17. Neidhardt, F. C. 1966. Roles of amino acid activating enzymes in cellular physiology. Bacteriol. Rev. **30**:701–719.
18. Ravel, J. M., M. N. White, and W. Shive. 1965. Activation of tyrosine analogs in relation to enzyme repression. Biochem. Biophys. Res. Commun. **20**:352–359.
19. Roth, J. R., and B. N. Ames. 1966. Histidine regulatory mutants in Salmonella typhimurium. II. Histidine regulatory mutants having altered histidyl-tRNA synthetase. J. Mol. Biol. **22**:325–334.
20. Schlesinger, S., and B. Magasanik. 1964. Effect of $\alpha$-methylhistidine on the control of histidine synthesis. J. Mol. Biol. **9**:670–682.
21. Schwink, I., and E. Adams. 1959. Aromatic biosynthesis. XVI. Aromatization of prephenic acid to *p*-hydroxyphenylpyruvic acid, a step in tyrosine biosynthesis in *Escherichia coli*. Biochim. Biophys. Acta **36**:102–117.
22. Silbert, D. F., G. R. Fink, and B. N. Ames. 1966. Histidine regulatory mutants in *Salmonella typhimurium*. III. A class of regulatory mutants deficient in tRNA for histidine. J. Mol. Biol. **22**:335–347.
23. Spizizen, J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Nat. Acad. Sci. U.S.A. **44**:1072–1078.
24. 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.
25. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. **31**:332–353.
26. Wallace, B. J., and J. Pittard. 1969. Regulator gene controlling enzymes concerned in tyrosine biosynthesis in *Escherichia coli*. J. Bacteriol. **97**:1234–1241.