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# Close Linkage of the Genes *serC* (for Phosphohydroxy Pyruvate Transaminase) and *serS* (for Seryl-Transfer Ribonucleic Acid Synthetase) in *Escherichia coli* K-12

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*Escherichia coli* strain K28, isolated after nitrosoguanidine mutagenesis, was found to be auxotrophic for serine. It was also temperature sensitive for growth as a result of producing an altered seryl-transfer ribonucleic acid (tRNA) synthetase (EC 6.1.1.11, L-serine: tRNA ligase [AMP]). The auxotrophy was caused by a mutation in the structural gene for phosphohydroxy-pyruvate transaminase (*serC*), which was distinct from, but closely linked to, the structural gene for seryl-tRNA synthetase (*serS*). We conclude that the relevant genes are in the order *gal-serS-serC-aroA*.

A correlation has frequently been observed between the occurrence of amino acid auxotrophy and alteration of the corresponding aminoacyl-transfer ribonucleic acid (tRNA) synthetases (5, 9, 10, 16, 18). In most cases, auxotrophy could be related to a change in the kinetic parameters or to a greatly reduced level of enzyme in the mutant (6). However, the isolation and mapping of serine auxotrophs of *Escherichia coli* K-12, with presumably defective seryl-tRNA synthetases, presented an anomoly because the kinetic parameters and level of enzyme were closed to those of the wild type (8). With serine auxotrophs K19 and K28 (8), the only differences observed were that seryl-tRNA synthetase from K19 and K28 had slightly lower specific activity compared to the wild type, and synthetase from K28 was temperature sensitive in crude extracts and at every stage of purification.

In this paper we show that in strain K28 the lesion responsible for auxotrophy was in the structural gene for phosphohydroxypyruvate transaminase, the second enzyme in the biosynthesis of serine. This mutation was found to map close to the previously reported mutations in the structural gene of seryl-tRNA synthetase.

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## MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used in this study are described in Table 1. Figure 1 shows the genetic map of *E. coli* with the location of the pertinent markers. In the text only the relevant genes will be indicated after the strain number.

**Media and reagents.** LB medium was prepared as described (14). Minimal medium 56/2 (1) was used supplemented with any necessary growth factors such as L-amino acids (each 100 μg/ml), pyrimidines (40 μg/ml), thiamine (0.1 μg/ml), and glucose (0.2%). Streptomycin was used at a concentration of 100 μg/ml.

Uniformly labeled L-[$^{14}$C]amino acids were obtained commercially, and *E. coli* B tRNA was purchased from Schwarz/Mann. [3-$^{14}$C]phosphoglyceric acid was a gift of L. Pizer.

**Bacterial crosses.** Donor and recipient cells were grown in LB medium at 37 C to a concentration of approximately $2 \times 10^8$/ml. Cells were mixed together in a ratio of 1 donor to 10 recipient cells, gently aerated for 80 min at 37 C, and then plated onto minimal agar plates necessary for growth of the recombinants. The plates were incubated at 30 or 37 C depending on whether temperature-sensitive or -resistant recombinants were required.

Transductions were carried out using P1 *vir*. Lysates were prepared by adsorbing $10^7$ P1 plaque-forming units onto $5 \times 10^7$ freshly grown cells ($5 \times 10^8$/ml) in LB medium which contained 2.5 mM $Ca^{2+}$. After 20 min of adsorption at 37 C, 4 ml of 0.4% agar was added, and the mixture was poured onto a plate containing LB medium, 1% agar, 0.1% glucose, and

TABLE 1. *Description of bacterial strains*[a]

| Strain | Genotype | Source |
|---|---|---|
| S26R1e | Hfr (Cavalli), *phoA4, supD32, rel-1, tonA22*, T2$^r$ | J. R. Katze |
| K28 | Hfr (Cavalli), *serC13, serS14, phoA4, supD32, rel-1, tonA22*, T2$^r$ | J. R. Katze (NG treatment of S26R1e) |
| KL185 | F$^-$, *str-118, his-1, trp-1, pyrD34, thi-1, gal-6, xyl-7, mtl-2, malA1*, $\lambda^-$ | B. Low (*unpublished data*) |
| W3110 | F$^-$ | C. Hill |
| KL208 | Hfr, $\lambda^-$ | B. Low (*unpublished data*) |
| AB259 | HfrH, $\lambda^-$, *thi-1* | E. A. Adelberg |
| AB2829 | Hfr, *aroA354* | E. A. Adelberg |
| KL281 | Hfr (Cavalli), *serC13, serS14, serO17, phoA4, supD32, rel-1, tonA22*, T2$^r$ | TR revertant of K28 |
| KL282 | Hfr (Cavalli), *serC13, serS14, serS16, phoA4, supD32, rel-1, tonA22*, T2$^r$ | TR revertant of K28 |
| KL284 | Hfr (Cavalli), *serS14, phoA4, supD32, rel-1, tonA22*, T2$^r$ | Ser$^+$ revertant of K28 |
| KL285 | F$^-$, *str$^r$, serS14, serC13, his-1, trp-1* | Pyr$^+$ (Str$^+$) recombinant for mating of K28 and KL285 |
| KL286 | F$^-$, *str$^r$, serS14, his-1, trp-1* | Pyr$^+$ (Str$^+$) recombinant from mating of K28 and KL285 |
| KL290 | Hfr (Cavalli), *serC13, serS14, phoA4, supD32, rel-1, tonA22*, T2$^r$, *gal*$^-$ | NG treatment of K28 |
| KL291 | Hfr (Cavalli), *serC13, serS14, phoA4, supD32, rel-1, tonA22*, T2$^r$, *gal*$^-$, *nalA*$^-$ | Nalidixic acid-resistant derivative of KL290 |

[a] Genetic symbols are those described by Taylor (19). Abbreviations: *serC*, locus for phosphohydroxypyruvate transaminase structural gene; *serO*, locus of operator for seryl-tRNA synthetase (see following paper); TR, temperature resistant; NG, nitrosoguanidine.

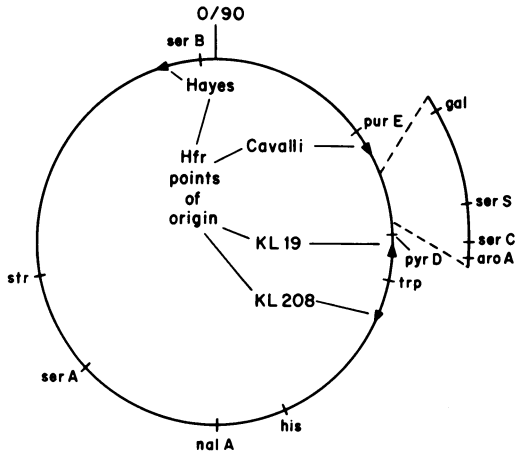


FIG. 1. *Genetic map of E. coli showing relative positions of genetic loci used in this study. Genetic symbols are those described by Taylor (19).*

2.5 mM $Ca^{2+}$. After overnight incubation at 37 C, the top agar was scraped into a centrifuge tube and stirred with 0.5 ml of chloroform. The mixture was centrifuged, and the supernatant fluid which contained the phage was retained. Transductions were begun by adsorbing phage and bacteria in a 1:5 ratio in LB medium containing 2.5 mM $Ca^{2+}$ as above. The cells were washed twice by centrifugation and plated out onto media selective for recombinants.

**Preparation of cell extracts.** Bacteria for cell extracts were grown to stationary phase in either LB or minimal media in a rotary shaker at either 30 or 37 C. The cells were collected by centrifugation, washed and suspended in standard buffer (0.02 M tris(hydroxymethyl)aminomethane, pH 7.4, 0.01 M magnesium acetate, 0.0005 M ethylenediaminetetraacetic acid, 0.02 M mercaptoethanol, and 10% glycerol [vol/vol]). The cells were broken by sonic treatment, and the solution was clarified by centrifugation at 70,000 $\times$ *g* for 30 min. The extracts at this stage normally contained between 1 and 3 mg of protein per ml as determined by the method of Lowry et al. (13) using crystalline bovine plasma albumin as standard.

**Enzyme assays.** For the aminoacyl-tRNA synthetase activities, 25-$\mu$liter fractions of cell extract (5- to 100-fold diluted) were assayed in a 125-$\mu$liter reaction mixture under conditions previously described (11). Incubation was at 37 C for 10 min. All enzyme assays were performed within 12 hr after cell breakage.

Phosphohydroxypyruvate transaminase activity was measured as described by Pizer (17). Inorganic pyrophosphatase was assayed by the method of Bloemers et al. (2).

**Heat inactivation studies.** Cell extracts were diluted in standard buffer containing 1 mg of bovine plasma albumin per ml and heated in a water bath at 57 C for 3 min and then chilled to 0 C. The activity remaining was assayed as described above.

**Nitrosoguanidine mutation, selection of naladixic acid-resistant strains, and interrupted matings.** These methods were carried out as described by Miller (15).

## RESULTS

**Segregation of auxotrophy and synthetase mutations.** The lack of correlation between the phenotype of K28 and the properties of its seryl-tRNA synthetase previously reported (8) prompted us to study its genetic structure in more detail.

We used K28 to construct other strains, and some recombinants were observed which had either a serine requirement or a temperature-sensitive synthetase, but not both. A mating was performed between K28 (Hfr, $Str^s$, $Ser^-$) and KL185 ($F^-$, $Str^r$, $His^-$, $Trp^-$, $Ura^-$), and $Ura^+$, $Str^r$ recombinants were selected. Among the recombinants were the strains KL285 ($Trp^-$, $His^-$, $Ser^-$, $Str^r$) and KL286 ($Trp^-$, $His^-$, $Str^r$) both of which had temperature-sensitive seryl-tRNA synthetase as measured in crude cell extracts (Table 2). The presence of the temperature-sensitive synthetase could be correlated in every case with inability of the strain to grow at 45 C on LB plates. Strain KL286 is of particular interest since it was found to have the $Ser^+$ phenotype but retained the temperature-sensitive seryl-tRNA synthetase characteristic of the strain K28. To confirm the implication that $Ser^-$ phenotype and the temperature-sensitive seryl-tRNA synthetase were independent, strains K28 and KL285 were transduced with P1 phage grown on the wild-type strain W3110, and $Ser^+$ transductants were selected. Tests for growth at 45 C showed that approximately 30% of the $Ser^+$ transductants were temperature sensitive for growth, indicating that the gene responsible for auxotrophy (which we denote as *serC*) and the structural gene for seryl-tRNA synthetase, *serS*, were separate but 70% linked by P1 transduction.

If the strain K28 is a double mutant and the lesion causing auxotrophy and the structural gene, *serS*, are independent of each other, then reversion of one of the mutations should not affect the other. Spontaneous $Ser^+$ revertants of K28 and KL285 were therefore sought by concentrating overnight cultures (10-fold) and then plating them out on minimal media without serine. Reversion to $Ser^+$ was found to occur in approximately 1 in $10^8$ cells in K28 and 1 in $10^9$ for KL285. Five $Ser^+$ revertants were chosen at random for further study. They all had a temperature-sensitive seryl-tRNA synthetase, and all failed to grow normally at 45 C on LB plates.

Similarly, reversion to produce cells with normal synthetase should give rise to strains which would still be serine auxotrophs. Spontaneous revertants of K28 were isolated by plating cells onto LB plates and incubating at 45 C, the nonpermissive growth temperature. Nine colonies were obtained, all of which had the $Ser^-$ phenotype. The reversion frequency in this case was approximately 1 in $10^9$. Enzyme assays indicated two groups of revertants, one with seryl-tRNA synthetase with wild-type thermal stability (e.g., strain KL282 [Table 2]), and the other with a high level of activity of temperature-sensitive seryl-tRNA synthetase (e.g., strain KL281 [see following paper]). Thus, KL282 has a seryl-tRNA synthetase with a wild-type thermal inactivation rate at 57 C and still retains the gene defect causing auxotrophy. However, the low specific activity for seryl-tRNA synthetase in extracts of KL282 suggests that it is not a true revertant. (In some cases sonic treatment of K28 and its derivatives resulted in an almost clear solution which gave a very small pellet on centrifugation to bring down the cell debris. This would result in an increase in solubilized protein and a consequent decrease in specific activity of the enzyme assayed.)

TABLE 2. *Enzyme activities*

| Strain | Seryl-tRNA synthetase[a] | $C_3/C_0$[b] | Transaminase[c] |
|---|---|---|---|
| S26R1e | 47 | 0.88 | 0.41 |
| K28 | 19 | 0.30 | <0.01 |
| KL185 | 56 | 0.96 | |
| KL281 | 99 | 0.32 | <0.01 |
| KL282 | 23 | 0.91 | <0.01 |
| KL284 | 21 | 0.22 | 0.47 |
| KL285 | 19 | 0.26 | |
| KL286 | 15 | 0.34 | 0.67 |

[a] Specific activity in units per milligram of protein from a typical experiment. Errors of ±20% were typical in these determinations. One unit of activity is defined as the amount of enzyme which catalyzes the incorporation of 1 nmole of serine into seryl-tRNA in 10 min.

[b] Ratio of seryl-tRNA synthetase activity after and before heating at 57 C for 3 min.

[c] Yield of *o*-phosphoserine-$^{14}C$ in micromoles per milligram of protein.

**Biochemical basis for auxotrophy.** Since the gene responsible for serine auxotrophy was found to map at a locus distinct from *serS*, an alternate explanation for the serine auxotrophy was sought. The biosynthesis of serine involves three steps (Fig. 2) (17). The structural genes *serA* and *serB* for the first and third of these enzymes (3-phosphoglyceric acid dehydrogenase and phosphoserine phosphatase) have

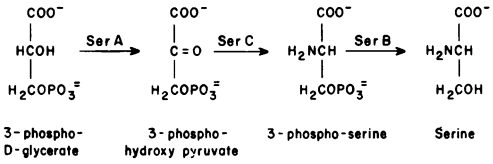


FIG. 2. *Steps in the biosynthesis of serine (17).*

been located at 57 and 89 min, respectively, on the Taylor map of *E. coli*. The mutation responsible for the auxotrophy of strain K28 has been reported to map between 17 and 19 min (8). No map position has been reported for the structural gene *serC* which specified the second enzyme (phosphohydroxypyruvate transaminase) in the serine biosynthetic pathway, although mutants defective in this enzyme have previously been isolated in *E. coli* B (4). The activities of the transaminase in the relevant strains were measured, and the yields of *o*-phosphoserine-$^{14}$C are given in Table 2. The assay for this enzyme involves a coupled system (17) which requires that the gene product of *serA* is functional since this converts the 3-phosphoglyceric acid to phosphohydroxypyruvate, the substrate for the transaminase.

Since previous work (8) has indicated that the mutation responsible for serine auxotrophy in K28 maps in the 17- to 19-min region we can assume that the *serA* gene in K28 is normal. Cell extracts from the serine auxotrophs were unable to catalyze the formation of *o*-phosphoserine from 3-phosphoglyceric acid. They are therefore deficient in the transaminase, the product of gene *serC*.

**Detailed mapping of serC and serS.** Although *serC* and *serS* were found to map close to each other (70% by P1 contransduction), their orientation relative to other genes on the chromosome was still unknown. Linkage to a third gene of known position is required, and *gal* was chosen since it maps close to *serS* (19).

K28 (*serS*$^-$, *serC*$^-$) was mutagenized with nitrosoguanidine (15), and a *gal*$^-$ derivative KL290 (*gal*$^-$, *serS*$^-$, *serC*$^-$) was isolated. Transduction of KL290 with P1 phage grown on wild-type strain W3110 failed to produce *gal*$^+$ *serC*$^+$ or *gal*$^+$ *serS*$^+$ contransductants, suggesting that both *serS* and *serC* are located more than 1 min from *gal*.

Interrupted matings between KL208 and KL291 (a nalidixic acid resistant derivative of KL290) were performed, and Ser$^+$ Nal$^r$ recombinants were selected. These recombinants were gridded, and the percentages of Gal$^+$ temperature-resistant colonies were determined by replica plating (Fig. 3). A low frequency of Ser$^+$ revertants was observed with KL291. The entry time of *serC*$^+$ was approximately 15 min after mating was commenced, and the genetic analysis shows that the *serS*$^+$ allele entered several minutes later, followed by *gal*$^+$. Thus *serS* is between *serC* and *gal* and closer to *serC* than to *gal*. In this experiment no *serC*$^+$, *serS*$^-$, *gal*$^+$ recombinants were obtained. This is consistent with the map order *serC-serS-gal* which would make *serC*$^+$ *serS*$^-$ *gal*$^+$ recombinants the rare, double crossover class. A Hayes Hfr strain, AB259, was also mated with KL291, and Ser$^+$ Nal$^r$ recombinants were selected. Out of 220 such recombinants, 192 were *serS*$^+$ and 118 were *gal*$^+$, indicating again that *serS* is more closely linked to *serC* than to *gal*.

Whereas no cotransduction was obtained between *serS* or *serC* and *gal*, the *serS* and *serC* genes were both found to cotransduce with another nearby marker, *aroA*. P1 phage grown on strain K28 (*aroA*$^+$, *serS*$^-$, *serC*$^-$) was used to infect strain AB2829 (*aroA*$^-$), and 70 *aro*$^+$ transductants were obtained. Ninety-three percent of these were found to be *serC*$^-$ whereas 60% were *serS*$^-$. All *serS*$^-$ colonies (42) were found to be *serC*$^-$ except for 1, thereby indicating the gene order *aroA-serC-serS*.

Confirmation of the linkage between *serS* and *serC* was obtained when an independently isolated strain with a temperature-sensitive seryl-tRNA synthetase was used as a donor of *serC*$^+$ in P1 transductions with strain KL282 (12). In this experiment, 64% of the transductants were found to be temperature sensitive.

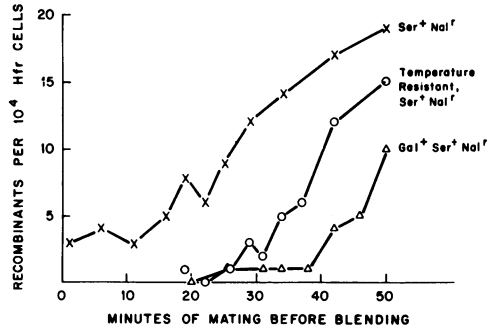


FIG. 3. *Results of an interrupted mating experiment using KL208 (Ser*$^+$*, TR, Gal*$^+$*, Nal*$^s$*) and KL291 (Ser*$^-$*, TS, Gal*$^-$*, Nal*$^r$*). Samples for blending were taken at approximately 2-min intervals, and Ser*$^+$ *Nal*$^r$ *recombinants were selected. These were then analyzed by replica plating (15) to determine the number which were temperature resistant for growth (TR) or Gal*$^+$.

## DISCUSSION

K28, which is auxotrophic for serine and also produces an altered seryl-tRNA synthetase, was isolated after mutagenesis with nitrosoguanidine. This mutagen has recently been shown to cause a significant frequency of pairs of mutations in closely linked positions (7), and our first finding was that K28 was indeed a double mutant. The genes *serC* (for phosphohydroxypyruvate transaminase) and *serS* (for seryl-tRNA synthetase) were both found to be affected. The two mutations were 70% cotransducible and very close to *aroA* on the Taylor map (19), *serS* being counterclockwise from *serC*. The *serS* mutation in K28 thus results only in the synthesis of a temperature-sensitive seryl-tRNA synthetase and is not the cause of auxotrophy in this strain. It now seems likely that the other serine auxotroph, K19, which gave a specific activity for seryl-tRNA synthetase close to that of the wild-type parent, is a *serC* mutant and not a *serS* mutant.

In the following paper (3) we report studies in which we make use of the close linkage of the *serS* and *serC* in characterizing strains producing high levels of seryl-tRNA synthetase.

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