

## Gene for Heat-Inducible Lysyl-tRNA Synthetase (*lysU*) Maps near *cadA* in *Escherichia coli*

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A hybrid ColE1 plasmid from the Clarke-Carbon colony bank with a 7-kilobase insertion was found to encode the inducible lysyl-tRNA synthetase along with the catabolic enzyme lysine decarboxylase. The gene for the inducible synthetase, *lysU*, must lie within 0.3 min of the lysine decarboxylase gene, *cadA*, at 92 min on the *Escherichia coli* genetic map.

Under ordinary circumstances lysyl-tRNA synthetase appears to be regulated in *Escherichia coli* like other aminoacyl-tRNA synthetases (6). The lysine enzyme, however, appears to be unique in several respects. There are multiple molecular species of this enzyme that are resolvable on two-dimensional gels (10). Two of these species, form I and form III, have an apparent molecular weight of 58,500 and differ slightly in isoelectric point. These isoenzymes are thought to be the products of the *lysS* gene, form III being a minor species arising from some modification of the major form, form I. These two species are the only forms made under most growth conditions (5).

Another pair of lysyl-tRNA synthetase species, forms II and IV, are evident in cells that are grown in the presence of alanine, leucine, fructose, or glycyl-leucine (5) and in certain mutants (5, 6). This pair of isoenzymes resemble the more common pair in several respects; however, forms II and IV are slightly larger (molecular weight, 60,500). Form II has the same isoelectric point as form I and also is the major member of its pair; like form III, form IV is the less abundant species, and these two minor forms also have the same isoelectric point (5). This resemblance suggests that the inducible pair are the products of a single gene, but no direct information concerning this is available. Interest in the inducible pair has increased due to the recent discovery that these forms are part of the heat shock response of *E. coli* (5, 8). Form II in particular is induced transiently during the 10 min immediately after any shift-up in growth temperature, and during steady-state growth at 46°C this form is the major species produced. As in the case of most other heat-inducible proteins in *E. coli*, form II is under the control of the regulatory gene (*htpR*) of the high-temperature production (HTP) regulon (8).

Mutants of *E. coli* with reduced lysyl-tRNA synthetase activity are readily isolated (7). In several of these, forms I and III have altered isoelectric points. From such a strain (strain IH2017) Hirshfield isolated a spontaneous secondary mutant (strain IH2018) which had near-normal lysyl-tRNA synthetase activity, but was temperature sensitive for growth. (These strains were isolated by I. N. Hirshfield and kindly supplied to us by him.) In this pseudorevertant, forms I and III retained their altered isoelectric points, but forms II and IV were produced in larger amounts. This mutant was used to screen the Clarke-Carbon colony bank of hybrid plasmids (3). One plasmid, pLC4-5, yielded a transconjugant that was temperature resistant.

The proteins encoded by pLC4-5 were revealed first by the chloramphenicol recovery method (9) and then by the use of minicells (12). Three proteins were produced by the former method; these were forms II and IV of lysyl-tRNA synthetase and a protein previously identified on two-dimensional gels as lysine decarboxylase (11). The same three proteins plus an unidentified protein were produced from pLC4-5 in minicells (Fig. 1).

Verification that the third protein was lysine decarboxylase was obtained by mixing a labeled minicell extract with purified lysine decarboxylase and developing the mixture on a two-dimensional gel. The results demonstrated comigration of the pLC4-5-encoded product with the authentic sample (Fig. 2). Similarly, the spots thought to be forms II and IV of lysyl-tRNA synthetase coincided with the appropriate spots in a total cell extract prepared from induced cells (data not shown). Also, transconjugant IH2018(pLC4-5) produced greater amounts of forms II and IV of the synthetase than the parent, strain IH2018 (data not shown).

The lysine decarboxylase gene, *cadA*, has

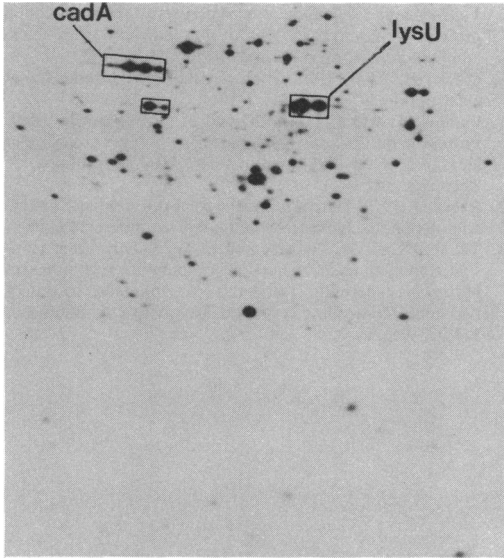


FIG. 1. Synthesis of protein from pLC4-5. An extract of [ $^{35}\text{S}$ ]methionine (1,193.0 Ci/mmol; 50  $\mu\text{Ci/ml}$ )-labeled minicells containing pLC4-5 was mixed with a whole-cell extract of strain W3110 labeled with [ $^3\text{H}$ ]leucine (145 Ci/mmol; 160  $\mu\text{Ci/ml}$ ). Two-dimensional gels were run, prepared for fluorography by impregnating with PPO (2,5-diphenyloxazole) (2), and exposed to Kodak XAR-5 film at  $-70^\circ\text{C}$ . (By exposing the gel to NS-5T film, the [ $^3\text{H}$ ]leucine was screened out and [ $^{35}\text{S}$ ]methionine-labeled proteins could be identified [data not shown].) On this fluorogram both  $^3\text{H}$ - and  $^{35}\text{S}$ -labeled proteins are visible. The plasmid proteins are enclosed in boxes; the *lysU* and *cadA* proteins are indicated (form II of the *lysU* proteins is on the left), together with one other unidentified protein made by the plasmid. Lysine decarboxylase and the unknown protein are present in more than one isoelectric species. This is common for plasmid proteins labeled in minicells, maxicells, or chloramphenicol-treated cells (unpublished data).

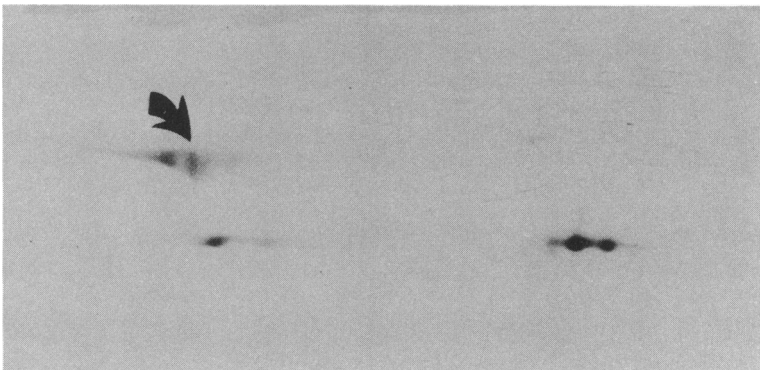


FIG. 2. Identification of a protein spot as lysine decarboxylase. A 1- $\mu\text{g}$  portion of purified lysine decarboxylase (obtained from E. Boeker) was mixed with 10  $\mu\text{l}$  of the [ $^{35}\text{S}$ ]methionine-labeled minicell extract described in the legend to Fig. 1. Comigration of the labeled protein with unlabeled purified protein, as described by Bloch et al. (1), is demonstrated by the spreading out of the lysine decarboxylase spot (arrow) and consequent dilution of the label.

been mapped at 92 min on the *E. coli* linkage map (13). By using purification and agarose gel electrophoresis (4), plasmid pLC4-5 was found to contain a chromosomal portion approximately 7.5 kilobases long. Therefore, the gene(s) for the inducible synthetase must lie within 0.3 min of *cadA*. Until there is evidence to the contrary, we will assume that the inducible synthetase forms are the products of a single gene, which we designate *lysU*.

The results presented here provide further evidence for the existence of at least two lysyl-tRNA synthetase structural genes, *lysS* and *lysU*. The former appears to map about 50 min away from the latter (I. N. Hirshfield, personal communication). Plasmid pLC4-5 should be useful for subcloning *lysU* to determine its structure and to examine the mechanism of its control by the heat induction regulator, *htpR*.

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