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Derepressed Levels of Glutamate Synthase and Glutamine Synthetase in *Escherichia coli* Mutants Altered in Glutamyl-Transfer Ribonucleic Acid Synthetase

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The levels of glutamate synthase and of glutamine synthetase are both derepressed 10-fold in strain JP1449 of *Escherichia coli* carrying a thermosensitive mutation in the glutamyl-transfer ribonucleic acid (tRNA) synthetase and growing exponentially but at a reduced rate at a partially restrictive temperature, compared with the levels in strain AB347 isogenic with strain JP1449 except for this thermosensitive mutation and the marker *aro*. These two enzymes catalyze one of the two pathways for glutamate biosynthesis in *E. coli*, the other being defined by the glutamate dehydrogenase. We observed a correlation between the percentage of charged $tRNA^{Glu}$ and the level of glutamate synthase in various mutants reported to have an altered glutamyl-tRNA synthetase activity. These results suggest that a glutamyl-tRNA might be involved in the repression of the biosynthesis of the glutamate synthase and of the glutamine synthetase and would couple the regulation of the biosynthesis of these two enzymes, which can work in tandem to synthesize glutamate when the ammonia concentration is low in *E. coli* but whose structural genes are quite distant from each other. No derepression of the level of the glutamate dehydrogenase was observed in mutant strain JP1449 under the conditions where the levels of the glutamine synthetase and of the glutamate synthase were derepressed. This result indicates that the two pathways for glutamate biosynthesis in *E. coli* are under different regulatory controls. The glutamate has been reported to be probably the key regulatory element of the biosynthesis of the glutamate dehydrogenase. Our results indicate that the cell has chosen the level of glutamyl-tRNA as a more sensitive probe to regulate the biosynthesis of the enzymes of the other pathway, which must be energized at a low ammonia concentration.

The biosynthesis of glutamate from ammonium and a carbon source is catalyzed in *Escherichia coli* through two distinct pathways (3) by three enzymes coded by unlinked genes (4). The first pathway (equation 1) catalyzed by the glutamate dehydrogenase is functional only at a relatively high ammonia concentration because of the high K_m (1.5 to 3 mM) of the glutamate dehydrogenase for NH_4^+ (20). The biosynthesis of this enzyme has been reported to be under the control of glutamate (4).

$$\text{2-oxoglutarate} + NH_3 + \text{NADPH} \rightleftarrows \text{glutamate} + \text{NADP} \quad (1)$$

The second pathway can synthesize glutamate even at low ammonia concentration because of the high affinity of the glutamine synthetase for NH_4^+ (20). This enzyme promotes the assimilation of ammonia into glutamine (equation 2), which then serves as a donor of an amino group for the synthesis of glutamate from 2-oxoglutarate, catalyzed by the glutamate synthase (equation 3).

$$\text{glutamate} + NH_3 + \text{ATP} \rightleftarrows \text{glutamine} + \text{ADP} + P_i \quad (2)$$

$$\text{2-oxoglutarate} + \text{glutamine} + \text{NADPH} \rightleftarrows 2\,\text{glutamate} + \text{NADP} \quad (3)$$

(Abbreviations: NADP, Nicotinamide adenine dinucleotide; NADPH, reduced NADP; ATP, adenosine triphosphate; ADP, adenosine diphosphate.)

The properties of these three enzymes responsible for glutamate biosynthesis together with the central position of glutamate in the biosynthesis of amino groups in *E. coli* (7) suggest that the second pathway (equations 2

and 3) has a central role for glutamate biosynthesis and ammonia assimilation in *E. coli* growing in a medium containing a low concentration of NH_4^+ (20). Such a role was demonstrated for this pathway in *Klebsiella aerogenes*, where a mutant defective in the glutamate synthase cannot grow in minimal medium when the ammonia, used as the only nitrogen source, is at a concentration lower than 1 mM (5).

Since a few aminoacyl-transfer ribonucleic acids (tRNA's) have been implicated in the regulation of the biosynthesis of the enzymes responsible for the biosynthesis of the corresponding amino acid (16), we have studied and report in this paper our observations on the influence of the intracellular concentration of glutamyl-tRNA on the level of the enzymes of the two pathways for glutamate biosynthesis in *E. coli*. Moreover, in the context of the requirement of certain pseudouridine residues for the regulatory function of a few tRNA's (27), we speculate on the possible role of such a residue in the regulatory function proposed here for (a) glutamyl-tRNA.

MATERIALS AND METHODS

General. Uniformly labeled [^{14}C]- or [^{3}H]glutamate and histidine were purchased from New England Nuclear Corp. Imidazole and 2-oxoglutarate were obtained from Sigma Chemical Co. Tryptone and the yeast extract were Difco products. We measured protein concentrations according to Lowry et al. (18). The RPC-5 (24) was a gift from R. J. Cedergren.

Bacterial strains. *E. coli* strains JP1449, AB347, EM-111, and EM-120 were obtained from D. Söll and have been described previously (21, 26). The thermosensitive mutant strain JP1449 altered in the catalytic subunit of the glutamyl-tRNA synthetase (GluRS) (13) is isogenic with strain AB347 except for this ts mutation and for the marker *aro*. Wild-type strain LT2 and mutant *hisT* 1504 of *Salmonella typhimurium* (27) were obtained from B. N. Ames.

Growth of E. coli and preparation of extracts. The *E. coli* strains were grown in a medium containing 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter. The cells were rapidly cooled during exponential growth, harvested by centrifugation, and washed in 0.01 M $MgSO_4$. After centrifugation, they were suspended in 0.05 M imidazole, pH 7.3, and lysed by passage through a French press at 12,000 lb/in^2. After centrifugation of the extracts for 1 h at 80,000 $\times$ *g*, the supernatants were assayed within a few hours for enzyme activity.

Enzyme assays. The glutamate synthase and the glutamate dehydrogenase activities were measured by following the oxidation of NADPH at 340 nm in a medium containing 0.16 mM NADPH, 0.05 M imidazole-hydrochloride, pH 7.3, 0.01 M 2-oxoglutarate, 0.1 mg of ethylenediaminetetraacetic acid/ml, and either 0.01 M glutamine for the glutamate synthase or 0.1 M NH_4Cl for the glutamate dehydrogenase. The initial velocity of the reaction was measured at room temperature (20 C) for two concentrations of cell extract, and the rates of oxidation of NADPH observed before the addition of glutamine or NH_4Cl were subtracted. Under these conditions, the activities of these enzymes were proportional to the amount of cell extract added.

To determine the amount of glutamine synthetase present in the cell irrespective of its state of adenylation, we measured its transferase activity in the presence of 0.4 mM $MnCl_2$ according to the method described by Prusiner et al. (25).

Measurement of the extent of acylation of tRNA^{Glu}. To measure the extent of aminoacylation of tRNA^{Glu} in exponentially growing cells, we lysed them by a procedure (2) that prevented further aminoacylation of the tRNA in the cell extract and stabilized the glutamyl-tRNA. The various strains growing exponentially in 200 ml of tryptone-yeast extract medium were killed by rapid addition of 10 ml of a 100% (wt/vol) solution of trichloroacetic acid, followed by strong mixing during 10 min. The bacteria were rinsed in 5% trichloroacetic acid at 0 C and centrifuged. The pellets were suspended in 2 ml of 0.25 M sodium acetate, pH 6.5, 1 mM ethylenediaminetetraacetic acid, and 0.05% sodium dodecyl sulfate.

The tRNA's were then extracted according to the method of Atherly and Suchanek (2) and dissolved in 0.05 M sodium *N*-2-hydroxyethyl piperazine-*N'*-2'-ethanesulfonic acid, pH 6.5, 0.005 M $MgCl_2$. One aliquot was incubated at 37 C during 2 h in 1.8 M tris-(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.0 (32), to deacylate the tRNA's, which were then ethanol precipitated twice. Since acylated tRNA^{Glu} of *E. coli* can be inactivated by periodate oxidation, presumably because of the presence of 5-methylamino-2-thiouridine at the 5'-terminal position of the anticodon of at least the major isoacceptor tRNA^{Glu} (22), the proportion of acylated tRNA^{Glu} could not be obtained by specific inactivation of unacylated tRNA with periodate. We thus measured the amount of unacylated tRNA^{Glu} in total tRNA by charging it with [^{14}C]glutamate in the presence of an excess of GluRS. The acceptor activities for glutamate of the deacylated tRNA and of nondeacylated tRNA's in the presence of partially purified GluRS were measured under conditions described previously (14) and allowed us to calculate the percentage of charged tRNA^{Glu}. The amount of GluRS added was sufficient to charge in 10 min of incubation at 30 C three times more tRNA^{Glu} than the amounts used for our measurements. Under these conditions, less than 10% of the glutamyl-tRNA was hydrolyzed.

Preparation of histidyl- and glutamyl-tRNA's from S. typhimurium. Strains LT2 and *hisT* 1504 of *S. typhimurium* were grown in minimal salt medium containing 0.5% glucose (6) and harvested in late exponential phase. We verified that the harvested cells of strain LT2 were sensitive to the action of 0.02 M aminotriazole and 0.5 mM triazolealanine, whereas the mutant *hisT* 1504 gave normal colonies on minimal salt medium in the presence of these inhibiting agents (8). Partially purified GluRS and histidyl-

tRNA synthetase were prepared by chromatography of a sonicated extract of strain LT2 on diethylaminoethyl (DEAE)-cellulose, followed by a fractionation of the pools of each enzyme on hydroxyl apatite as described previously for the GluRS (14). Total tRNA was isolated from each strain by the method of Zubay (32), where the isopropanol fractionation step was replaced by a chromatography on DEAE-cellulose (31). After deacylating them by incubation for 90 min at 37 C in 1.8 M Tris-hydrochloride, pH 8.0, the tRNA's from strains LT2 and *hisT* 1504 were charged with [^{14}C]- and [^{3}H]glutamate, respectively. The same procedure was followed for histidine. The proteins were then removed by chromatography on DEAE-cellulose.

Chromatography of glutamyl-tRNA on RPC-5. Unfractionated tRNA acylated with glutamate was dissolved in 0.01 M sodium acetate, pH 4.5, 0.01 M $MgCl_2$, 0.001 M 2-mercaptoethanol, and 0.45 M NaCl and applied at a rate of 15 ml/h on a column (0.9 by 33 cm) equilibrated with the same buffer. The temperature of the column was kept at 37 C. The tRNA was eluted with a gradient of 0.45 to 0.8 M NaCl in the same buffer at a rate of 40 ml/h. The conductivity of the elution was measured with a Radiometer conductivity meter type CDM2e. Aliquots of the fractions were counted in a 6:7 mixture of toluene and Triton X-100 containing 1 g of 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene and 10 g of 2,5-diphenyloxazole per liter.

RESULTS

Glutamate synthase and glutamate dehydrogenase levels in thermosensitive mutants with altered GluRS and in their parental strain, AB347. The specific activity of the glutamate synthase was measured in extracts of cells harvested during exponential growth at various temperatures. In the thermosensitive mutant strain JP1449 altered in the GluRS (13), the level of glutamate synthase was derepressed ninefold compared with the level found in its parental strain, AB347, when both strains were grown at 37 C (Table 1). At 32 C, this level was only twofold higher in the mutant. An intermediate derepression of the level of the glutamate synthase was found in a partial revertant strain, JP1449-18ts* (Table 1), which grows very slowly at 43 C and whose GluRS is thermolabile (13).

In contrast, the level of glutamate dehydrogenase behaved in a very similar way in the wild-type strain and in these two thermosensitive mutants altered in the GluRS. Its specific activity doubled when the temperature increased from 32 to 37 C and then decreases at 43 C (Table 1).

As a control, to find whether the variations in growth rate were responsible for the derepression of the level of glutamate synthase, we measured the specific activity of this enzyme in extracts of the thermosensitive mutant strain KL231 altered in the leucyl-tRNA synthetase (17) after growth of this strain at the permissive temperature of 32 C and at 37 C, at which temperature the growth rate is reduced presumably by the restrictive amount of leucyl-tRNA. The reduction in growth rate from 32 to 37 C did not trigger a derepression of the level of the glutamate synthase (Table 1).

TABLE 1. *Specific activities of the glutamate synthase and of the glutamate dehydrogenase in thermosensitive mutants of the GluRS and in their parental strain*

E. coli strain	Growth temp (C)	Generation time (min)	Sp Act: Glutamate synthase (mU/mg of protein)[a]	Sp Act: Glutamate dehydrogenase (mU/mg of protein)
AB347	32	40	5.5	7.8
	37	20	6.3	15
	43	20	4.0	5.7
JP1449 (ts)	32	50	11	11
	37	90	53	20
JP1449-18ts*	32	50	6.3	6.8
	37	50	8.0	12
	43	150	24	8.7
KL231	32	45	10.4	8.2
	37	68	10.6	7.8

[a] One milliunit oxidizes 10^{-3} μmol of NADPH/min at 20 C.

The specific activities of the glutamate synthase and of the glutamate dehydrogenase (Table 1) were low compared with those measured by Prusiner et al. (25). Two factors might explain these differences. Their measurements were made at 30 C, compared with 20 C for ours. Moreover, their cells were grown in minimal medium containing 40 mM NH_4Cl, whereas our growth medium contained tryptone and yeast extract without added NH_4Cl.

Percentage of tRNA^{Glu} aminoacylated and level of glutamate synthase in various mutants with altered GluRS. The approach we used here (see Materials and Methods) to measure the percentage of tRNA^{Glu} acylated in vivo gave a value of 71% in wild-type strain AB347 growing exponentially at 37 C, in good agreement with the value of 69% obtained by Kaplan et al. (10), who used a different method. Two streptomycin-dependent mutants reported to have an altered GluRS (21) were also studied. We took the streptomycin-dependent mutant strain AB2847, known to map in the ribosomal cluster (J. Davies, personal communication), as

a control. These three strains were grown in the same tryptone-yeast extract medium in the presence of 0.2 mg of streptomycin per ml. The results (Fig. 1) show a correlation between the repression of the level of glutamate synthase and the percentage of aminoacylated $tRNA^{Glu_1}$. There was no significant derepression of the level of the glutamate synthase in strain AB2847 (Fig. 1).

Glutamine synthetase levels in strains JP1449 and AB347 at 37 C. We measured the transferase activity of the glutamine synthetase in extracts of the thermosensitive mutant strain JP1449 and of strain AB347 grown at 37 C. The activity increased linearly with the amount of cell extract added in the range of our measurements. The specific transferase activity of the glutamine synthetase, independent of the state of adenylation of this enzyme (28), was found to be 10-fold higher in mutant strain JP1449 than in strain AB347 during growth at 37 C (Table 2).

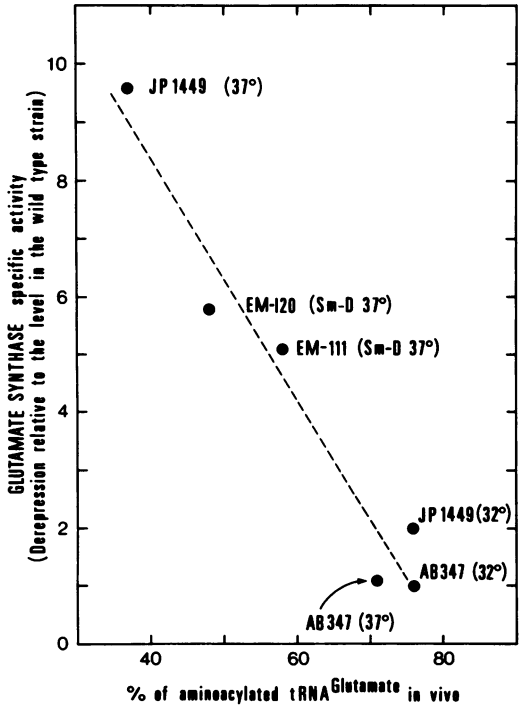

FIG. 1. *Correlation between the specific activity of the glutamate synthase and the percentage of charged $tRNA^{Glu}$ in mutant strains of Escherichia coli with altered GluRS and in strain AB347 whose GluRS is wild type. The growth temperature of the cultures is indicated in parentheses. The specific activity of the glutamate synthase in extracts from strain AB347 was 5.5 × 10^{-3} units/mg of protein (Table 1) when measured at 20 C and was given the relative value 1 on the ordinate. A value of 10 × 10^{-3} units per mg of protein (1.8-fold the wild-type value) was found in extracts of the streptomycin-dependent strain AB2847 grown at 32 C.*

Comparison of the glutamyl-tRNA species in wild-type strain LT2 and in mutant strain hisT 1504 or S. typhimurium. The few aminoacyl-tRNA species known to be involved in the regulation of the biosynthesis of an amino acid have been studied in *S. typhimurium*. To play their regulatory function, these tRNA's must have pseudouridine residues in the anticodon region. Indeed, in mutants of *S. typhimurium* altered in the locus *hisT*, the operons normally controlled by these aminoacyl-tRNA's are depressed, and these tRNA's, devoid of pseudouridine in the anticodon region, can be separated from the corresponding wild-type tRNA's on RPC-5 (27). To see whether the regulatory function of glutamyl-tRNA in *E. coli* indicated by our results had its counterpart in *S. typhimurium* and whether it was also dependent on the formation of pseudouridine residues synthesized by the enzyme coded by the *hisT* locus, we analyzed by chromatography on a column of RPC-5 a mixture of [^{14}C]glutamyl-tRNA from LT2 and of [^{3}H]glutamyl-tRNA from *hisT* 1504. The results (Fig. 2) show that the two glutamyl-tRNA's of *hisT* 1504 were eluted by a linear salt gradient at the same time as the corresponding tRNA's from LT2, suggesting (27) that the enzyme coded by the gene *hisT* modifies no $tRNA^{Glu}$ isoacceptor in *S. typhimurium*. We had previously verified that under our experimental conditions of chromatography on RPC-5, [^{14}C]histidyl-tRNA from strain LT2 and [^{3}H]histidyl-tRNA from the mutant strain *hisT* 1504 were not eluted together, as expected (27).

DISCUSSION

Two components are primarily altered in the thermosensitive mutant strain JP1449 defective in the catalytic subunit of the glutamyl-tRNA

TABLE 2. *Glutamine synthetase "transferase" activity measured at 37 C in extracts prepared from cells growing exponentially at 32 or 37 C in tryptone-yeast extract medium*

E. coli strain	Growth temp (C)	Specific transferase activity[a]
AB347	32	0.14
	37	0.17
JP1449 (ts)	32	0.03
	37	1.52

[a] Expressed as micromoles of glutamate hydroxamate formed per minute per milligram of protein.

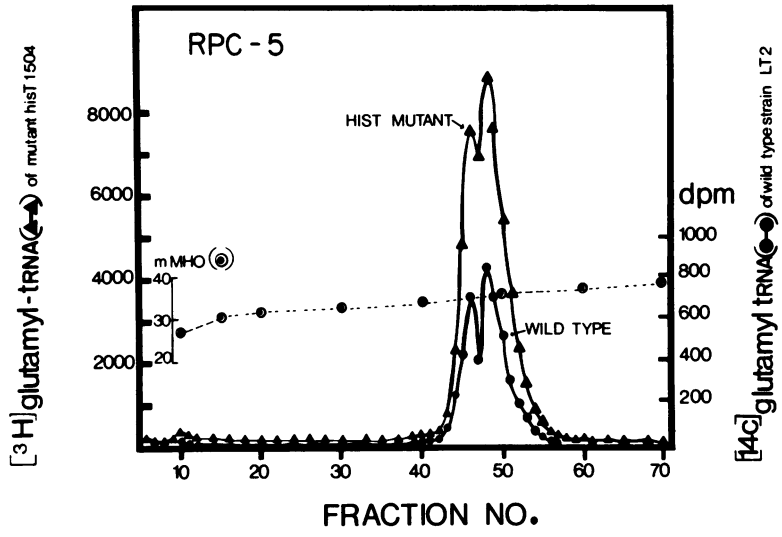

FIG. 2. *Chromatography of a mixture of [^{14}C]glutamyl-tRNA from strain LT2 and [^{3}H]glutamyl-tRNA from mutant strain hisT 1504 of Salmonella typhimurium on a column (1 by 30 cm) of RPC-5 at 37 C.*

synthetase: the GluRS itself and the level of glutamyl-tRNA. Consequently, the 10-fold derepression in the levels of glutamate synthase (Table 1) and of glutamine synthetase (Table 2) observed under these conditions indicates that GluRS or glutamyl-tRNA^{Glu} or both are involved in the regulation of the biosynthesis of these two enzymes, which catalyze the second biosynthetic pathway for glutamate in *E. coli*. On the other hand, the glutamate dehydrogenase, which defines the other pathway for glutamate biosynthesis, is present at the same level in mutant JP1449 as in the parental strain, AB347, at 38 C. Thus, its synthesis does not appear to be regulated by either GluRS or glutamyl-tRNA. This result agrees with the observation of Varrichio (30), who reported that the synthesis of this enzyme in *E. coli* is probably under the control of glutamate. It also indicates that glutamate itself is not acting as co-repressor for the biosynthesis of the enzymes of the glutamate cycle (second pathway).

The correlation observed between the level of glutamate synthase and the percentage of charged tRNA^{Glu} (Fig. 1) suggests that glutamyl-tRNA, whether or not in association with GluRS, is a good candidate for the role of co-repressor of the biosynthesis of the glutamate synthase. The fact that both glutamate synthase and glutamine synthetase are derepressed to approximately the same extent at 39 C in mutant strain JP1449 (Table 2) indicates that the biosynthesis of these two enzymes shares a common repressor or co-repressor, which our results suggest to be a glutamyl-tRNA. This model permits linking the control of the biosynthesis of these two enzymes, which work in tandem and are essential for glutamate biosynthesis and ammonia incorporation at low ammonia concentrations (20) and whose structural genes have been reported to map in quite separate and nonadjacent loci on the *E. coli* chromosome (3, 4). This model is also supported by the fact that glutamate plays a central role in ammonia assimilation in *E. coli* (7).

It is very unlikely that these derepressions are mediated by changes in the level of ammonia concentration in this thermosensitive mutant since they were observed during exponential growth in rich medium. Moreover, the following results reported by Tempest et al. (29) seem to rule out a direct repression of the biosynthesis of the glutamate synthase by ammonia in another prokaryote. Indeed, they observed a repressed level of this enzyme in *Aerobacter aerogenes* growing in minimal medium with glutamate as sole source of nitrogen, even though the pools of ammonia and glutamate were low. Our model is consistent with this result since under these growth conditions, the availability of glutamate for the aminoacylation of tRNA^{Glu} is certainly not the growth-limiting factor. The concentration of charged tRNA^{Glu} will thus be high, repressing the synthesis of glutamate synthase.

Another factor that can also be ruled out as a possible explanation for the derepression of the level of the glutamate synthase in strain JP1449 growing at limiting temperature is the reduction in growth rate, since this enzyme was found in larger amounts in *E. coli* during the exponential

growth than during the stationary phase (20). This conclusion is also supported by the absence of derepression of the level of glutamate synthase in the ts mutant strain KL231 growing at a reduced rate at the partially restrictive temperature of 37 C (Table 1) because of the suboptimal amount of leucyl-tRNA.

Even though we propose a common element in the regulation of the biosynthesis of the glutamate synthase and the glutamine synthetase, our model by no means implies that the biosynthesis of each of these two enzymes is regulated by identical mechanisms. They are working in tandem only under certain conditions, for instance when the ammonia level is very low in certain microorganisms (20). Under other conditions, the glutamate dehydrogenase is able to supply enough glutamate for optimal growth, and the cell probably does not need the glutamate synthase but still requires the glutamine synthetase. The regulation of the biosynthesis of the glutamine synthetase appears to be different and may be more complex than that of the glutamate synthase. Indeed, Prusiner et al. (25) observed that the addition of cyclic adenosine 3′,5′-monophosphate to the growth medium of *E. coli* causes an increase in the level of the glutamine synthetase but a decrease in the level of the glutamate synthase. Their result suggests that the transcription of the structural gene for glutamine synthetase is under the control of catabolite repression in *E. coli*. The unexpected but minor repression of the glutamate synthase level under the same stimulus indicates that biosynthesis of this enzyme is not under the direct control of this catabolite repression. Magasanik and his co-workers (19) found that in *K. aerogenes* the adenylated form of the glutamine synthetase represses its own biosynthesis, and that the nonadenylated form of this enzyme represses the biosynthesis of the glutamate dehydrogenase. If the glutamine synthetase has similar roles in *E. coli* and in *K. aerogenes*, our results suggest that a complex of the adenylated glutamine synthetase with a glutamyl-tRNA might be the repressor controlling the biosynthesis of this enzyme. The results shown in Tables 1 and 2 cannot be used to draw any conclusion on the role of the glutamine synthetase in the control of biosynthesis of the glutamate dehydrogenase in *E. coli* since the state of adenylation of the glutamine synthetase was not measured.

The main implication of our model is thus that one or all glutamyl-tRNA ^{lu} isoacceptors are involved, alone or in complex with other molecules, in the regulation of the biosynthesis of the glutamate synthase and of the glutamine synthetase. At least two tRNA^{Glu} isoacceptors have been resolved in *E. coli* (23). The sequence of only the major isoacceptor has been reported so far (22). A comparison of this sequence with that of the few tRNA's known to act as corepressors in *S. typhimurium* shows that it does not have in the anticodon region the pseudouridine residues reported to be essential for the regulatory role of histidyl-tRNA and of leucyl-tRNA (1, 27) in *S. typhimurium*. The similar behavior of [^{14}C]glutamyl-tRNA isolated from wild-type strain LT2 of *S. typhimurium* and of [^{3}H]glutamyl-tRNA from the mutant *hisT* during their co-chromatography on RPC-5 (Fig. 2) indicates that no tRNA^{Glu} isoacceptor is modified by the pseudouridine-forming enzyme coded by the *hisT* locus of *S. typhimurium*. An interesting feature of the main tRNA^{Glu} isoacceptor of *E. coli* (22) is the presence of a pseudouridine at the 13th position from the 5′ end of this molecule, adjacent to the adenosine residue present at the 5′ end of the so-called "dihydrouridine loop" in nearly all the tRNA's whose sequences are known (11). It is noticeable that among the tRNA's of known sequences (9; R. J. Cedergren, personal communication), only a few have a pseudouridine at this position, among them a tRNA^{Glu} from yeast (12). In the light of the results obtained by Singer et al. on the function of some pseudouridine residues in tRNA^{His} (27), it is tempting to speculate that this pseudouridine in tRNA^{Glu} might be important for the regulatory role proposed here for this macromolecule. A mutant of *E. coli* lacking this pseudouridine in tRNA^{Glu} would allow us to test this possibility. Such a mutant could be found among mutant strains of *E. coli* resistant to an analogue of glutamate.

Another implication of our results is that the *E. coli* cell considers as distinct variables and has developed different sensors for glutamate and glutamyl-tRNA to control the biosynthesis of glutamate. The derepression of the glutamate synthase even in presence of a relatively high intracellular pool of glutamate in the case described by Tempest et al. (29) for *A. aerogenes* supports this view. Our results can be correlated with the existence of a regulatory factor that changes the affinity of the glutamyl-tRNA synthetase for glutamate and ATP in *E. coli* K-12 (15). In view of the central role of glutamate in the metabolism of *E. coli* and of many other prokaryotes and eukaryotes, and also of the regulatory function proposed here for glutamyl-tRNA, it is conceivably advantageous for a cell to be able to vary the quantitative relationship between the intracellular concentrations of free glutamate and of glutamyl-

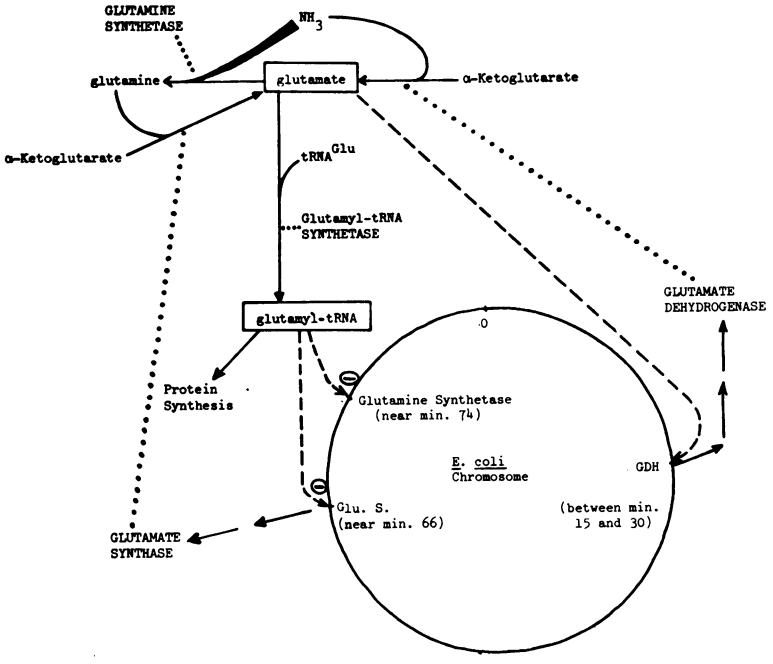

FIG. 3. *Regulation of glutamate biosynthesis in Escherichia coli. The enzymes involved in the biosynthesis of glutamate and the position of their structural genes on the chromosome are linked by series of arrows. The dotted lines indicate which reaction is catalyzed by a given enzyme. Discontinuous lines are drawn between structural genes and the molecules reported to repress their expression.*

tRNA.

Our conclusions are summarized in Fig. 3. The biosynthesis of the two enzymes that catalyze the pathway responsible for glutamate biosynthesis and ammonia assimilation at low ammonia concentrations in *E. coli*, the glutamine synthetase and the glutamate synthase, is under the repressive control of glutamyl-tRNA. Even though the structural genes for these two enzymes are not adjacent (3, 4; Fig. 3), the fact that they share a regulatory element makes them behave as one operon, at least under certain physiological conditions. Our results indicate that the biosynthesis of the glutamate dehydrogenase, which catalyzes the other main pathway for glutamate biosynthesis, active only at a relatively high ammonia concentration, is not controlled by glutamyl-tRNA, in agreement with a previous report (30) suggesting that the biosynthesis of this enzyme is repressed by glutamate (Fig. 3).

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