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Regulation of biosynthesis of aminoacyl-transfer RNA synthetases and of transfer-RNA in *Escherichia coli*

Regulación de la biosíntesis de aminoacil-tRNA sintetasas y de RNA de transferencia en *Escherichia coli*

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We have isolated temperature resistant revertants from temperature sensitive *E. coli* strains containing either a thermolabile glutaminyl-tRNA synthetase or leucyl-tRNA synthetase.

Among the revertants which still contained the thermolabile leucyl-tRNA shynthetase we found two classes of regulatory mutants *(leuX* and *leuY)* which have elevated levels of this enzyme. The *leuX* mutation specifies an operator-promoter region adjacent to the structural gene *(leuS)* for the enzyme. The *leuY* gene maps away from the *leuS* gene and codes for a protein. Using these mutants we demonstrated that the levels of leucyl-tRNA are related to the derepression of the leucine and isoleucine-valine operons.

Among the revertants which still contained the thermolabile glutaminyl-tRNA synthetase we characterized three classes of mutants, *glnT, glnU,* and *glnR*. The *glnT* and *glnU* mutants contain elevated levels of $tRNA^{Gln}$, while the *glnR* mutant possesses elevated levels of glutaminyl-tRNA synthetase. The level of glutamine synthetase, the enzyme responsible for the formation of glutamine, is also derepressed in the *glnT* and *glnR* mutants.

Transfer RNA and aminoacyl-tRNA synthetases are two very important classes of macromolecules. Not only do they assume a central role in the mechanism of protein biosynthesis (Söll and Schimmel, 1974; Kisselev and Favorova, 1974), they (or their product,aminoacyl-tRNA) are also involved in many other processes, including the regulation of biosynthesis of some amino acids (Bertrand *et al.*, 1975; Brenner and Ames, 1971; Stephens *et al.*, 1975; Umbarger, 1971). Thus it is important for our knowledge of cellular physiology to understand the factors controlling the levels of tRNA and of aminocyl-tRNA synthetases. An answer to the question of how this regulation is achieved not only advances our knowledge of intracellular control, it may also show other reactions in which aminoacyl-tRNA synthetases or tRNA are involved.

Mutants altered in the regulation of aminoacyl-tRNA synthetase biosynthesis may provide answer to the following specific questions: (i) Is there coordinate control of all aminoacyl-tRNA synthetases or of a subset thereof? Alternatively, are some aminoacyl-tRNA synthetases regulated independently? (ii) Is there coordinate regulation of all tRNA species? Alternatively, is each isoacceptor family (e.g. all $tRNA^{Leu}$ species) or each tRNA species (e.g. $tRNA^{Leu}$) individually controlled? iii) Is the regulation of an aminoacyl-tRNA synthetase coupled to that of its cognate isoacceptor tRNA family? (iv) Is the regulation of amino acid biosynthesis linked to that of the cognate tRNA or aminoacyl-tRNA synthetase? A very brief summary of our present knowledge for *E. coli* is given here, since we have attempted to study this question in this organism.

The biosynthesis of tRNA is a complicated process (Smith, 1976) involving transcription of tRNA genes into precursor molecules larger than mature tRNA. The additional nucleotides are then removed by special nucleases. Concomitant with trimming process enzymatic nucleotide modifications occur which yield the many modified nucleosides found in tRNA. To date there is little knowledge about the regulation of tRNA biosynthesis. It is known that the synthesis of tRNA and ribosomal RNA in bacteria are dependent on the growth rate and are regulated by similar, yet different mechanisms (Dennis, 1972; Ikemura and Dahlberg, 1973; Rosset, Julien and Monier, 1966; Skjold, Juarez and Hedgcoth, 1973). Considering the complexity of tRNA biosynthesis, regulation at many points can be envisaged. Thus, one may expect a variety of regulatory mutants.

In order to study the role of the aminoacyl-tRNA synthetases, many mutations affecting the activity of these enzymes have been isolated and characterized. To date, mutations in the structural genes of seventeen aminoacyl-tRNA synthetases of *E. coli* and *S. typhinurium* have been described (Söll, 1976; Johnson *et al.*, 1977). The most frequent synthetase mutations are temperature sensitive mutations found in strains that harbor a thermolabile aminoacyl-tRNA synthetase (Söll, 1976). The bacterium itself is temperature sensitive because the particular thermolabile enzyme cannot produce enough aminoacyl-tRNA to allow growth at the non-permissive temperature. These mutation have afforded information on the map locations of the aminoacyl-tRNA synthetase genes; they are not clustered Altough there is much more limited information on the location of tRNA genes (Smith, 1972; Hill, 1975), it does not appear that there is any close association of aminocyl-tRNA synthetase genes with the genes for the cognate tRNA or for the biosynthetic enzymes of the cognate amino acid.

Aminoacyl-tRNA synthetases were once thought to be constitutive (Boman *et al.*, 1961), and their levels unaffected by amino acid supply or growth rate. However, the work of Neidhardt and Williams (for review see Neidhart *et al.*, 1975) has shown that aminoacyl-tRNA synthetase formation depends on the amino acid concentration of the medium and on the growth rate of the cells. Possibly, two controls may act on synthetase formation, one responsive to the demands of protein synthesis, the other to amino acid restriction (Neidhart *et al.*, 1975). However, not all aminoacyl-tRNA synthetases are regulated in a uniform fashion; only for some enzymes is a repression-derepression-like mechanism observed. Overall, no clear picture has emerged so far. Mutations affecting the levels of one or more aminoacyl-tRNA synthetases may give us a deeper insight into this problem. To date, few mutants are available. A few *E. coli* mutants were discovered in which the regulation of individual aminoacyl-tRNA synthetases was altered. Some strains produce elevated levels of seryl-tRNA synthetase as a result of a mutation in an operator-like locus (Clarke *et al.*, 1973; Pizer *et al.*, 1972). Similar mutants with increased amounts of threonyl-tRNA synthetase were isolated among strainge resistant to the antibiotic borrelidin, which inhibits the activity of this enzyme (Paetz and Nass, 1973). However, the latter mutants have not yet been characterized genetically. These findings suggested the existence of a genetic mechanism controlling the levels of aminoacyl-tRNA synthetases.

In order to isolate regulatory mutants for tRNA or aminoacyl-tRNA synthetase biosynthesis we devised the following scheme. Temperature sensitive *E. coli* strains containing a thermolabile aminoacyl-tRNA synthetase do not grow at the non-permissive temperature presumably because the defective enzyme can-

not form enough aminoacyl--tRNA. However, temperature resistant revertants of such a strain may well include those with a stable aminoacyl-tRNA synthetase due to a back mutation to a second site reversion in the structural gene, or to missense suppression. More importantly, one may also expect temperature resistant strains which still contain the thermolabile enzyme. Among these strains would be included some which have increased amounts of the thermolabile enzyme, thus producing sufficient aminoacyl-tRNA for cell growth at elevated temperatures. tRNA overproducing strains may also be found among the temperature resistant revertants since it is well documented that enzymes are protected against heat inactivation by the interaction with their substrates (Yaniv and Gros, 1969; Mitra *et al.*, 1970; Lapointe and Söll, 1972). Because tRNA forms a very tight complex with aminoacyl-tRNA synthetases, one may expect an *in vivo* protection of the enzyme by increased amounts of the cognate tRNA. This has some support since it has been found that *E. coli* strains bearing a defective elongation factor T_u are viable when the intracellular pool of tRNA is increased (Kuwano *et al.*, 1973). A rather interesting class of mutants which would allow growth at the non-permissive temperature has been found by Böck and his colleagues (Ruffler *et al.*, (1974). They have isolated revertants in which the temperature sensitivity of an *alaS*⁻ bearing strain is found to be suppressed by mutations in genes coding for ribosomal proteins. Additional experiments indicate that the altered ribosomal proteins slow down the rate of protein synthesis thus allowing the defective aminoacyl-tRNA synthetase to produce sufficient aminoacyl-tRNA for reduced growth (Buckel *et al.*, 1976). Lastly, there could be many other types of changes that lead to the observed phenotype.

In this study, we have concentrated on the selection of temperature resistant revertants of a *leuS*⁻ strain and a *glnS*⁻ strain. For the *leuS*⁻ strain we have characterized revertants which contain increased levels of leucyl-tRNA synthetase. Overproduction of this enzyme may be caused by mutations in two different genetic loci. The *leuX* mutation defines a *cis*-dominant operator-promoter region adjacent to the structural gene for the enzyme. The *leuY* locus codes for a protein and maps away from the structural gene for leucyl-tRNA synthetase. For the *glnS*⁻ strain, we have described temperature resistant revertants with increased levels of $tRNA^{Gln}$, revertants containing elevated levels of $tRNA^{Gln}$ and $tRNA^{Met}$, and revertants with elevated levels of glutaminyl-tRNA synthetase.

Isolation and Characterization of Regulatory Mutants Affecting Leucyl-tRNA Synthetase Levels

Isolation of Revertants

The mutation responsible for the thermolabile leucyl-tRNA synthetase was originally isolated in *E. coli* strain KL231 (Low *et al.*, 1971). Its *leuS31* allele displayed three distinct phenotypes: temperature sensitivity (no growth above 40°C), resistance to the leucine analog azaleucine (at least 30 times over the lethal level for wild type), and retardation of growth when isoleucine-valine is included on solid minimal medium. Revertants of strain KL231 were selected on LB plates supplemented with thymine at 42°.

Revertants Make More Leucyl-tRNA Synthetase

The revertants were screened for the presence of the *leuS31* allele by checking their tolerance to azaleucine on minimal agar plates. As a confirmation the thermolability of the leucyil-tRNA synthetase was measured in cell-free extracts of these strains. In all cases the *in vitro* thermolability of leucyl-tRNA synthetase corresponded to *in vivo* azaleucine resistance. tRNA was prepared from the revertants, and strains KL231 and wild type strain D2. The amount of tRNA in all strains was the same (approximately $50A_{260}$ units/gram of cells). When the amount of leucine acceptor RNA was measured, we found that tRNA isolated from the revertant strains accepted 100-160 pmoles of leucine per A_{260} unit; while tRNA isolated from D2 accepted 150 pmoles per A_{260} unit and tRNA from KL231 accepted 120 pmoles per A_{260} unit. Thus, there is no gross alteration in either the *in vivo* level of total

tRNA or the *in vivo* concentration of leucine acceptor RNA.

The amount of leucyl-tRNA synthetase present in cell extracts of the revertant strains grown at 42° was determined by antiserum titration (Fig. 1). The strains were found to contain 0.5-7 times the amount of leucyl-tRNA synthetase present in strain KL231. The leucyl-tRNA synthetase levels of strains D2, KL231 and two enzyme overproducing strains,

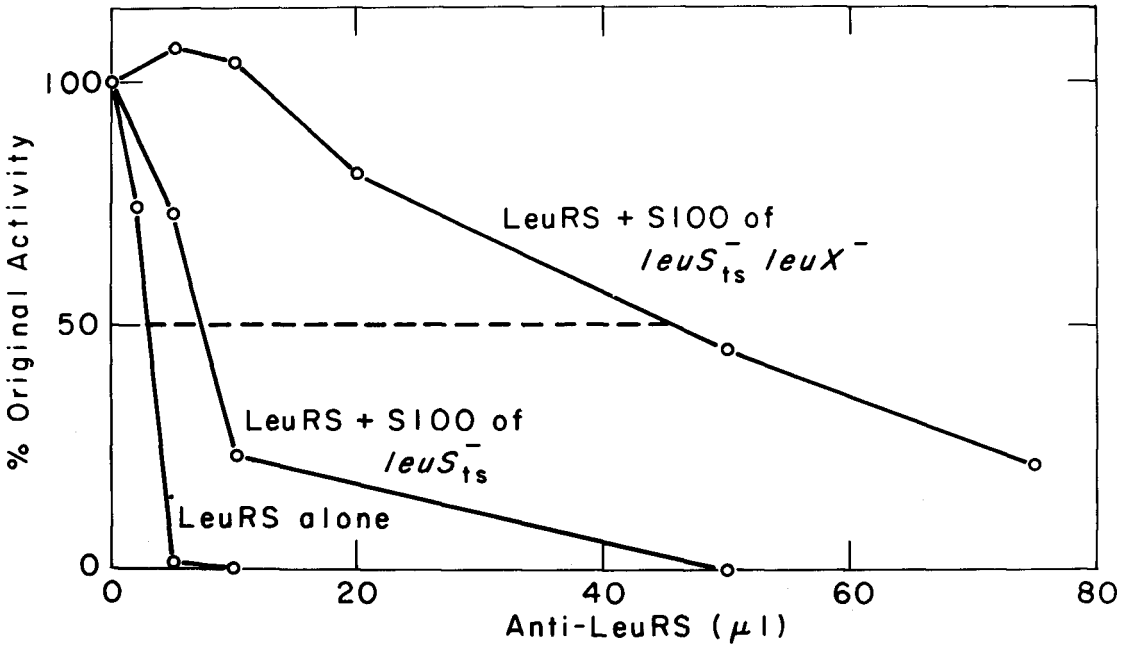

Fig. 1. Inhibition of leucyl-tRNA synthetase activity in cell extracts of various strains by antiserum. Pure leucyl-tRNA synthetase; leucyl-tRNA synthetase + cell extract of strain KL231 *(leuS⁻)* pure leucyl-tRNA synthetase + cell extract of strain GV10 *(leuS⁻ leuX⁻)*. The dashed line was used to calculate the amount of antiserum needed for half inactivation.

GV10 *(leuS⁻ leuX1)* and GV18 *(leuS⁻ leuY1)*, are listed in Table I. Depending on the growth of the cells and on the preparation of the cell extract 1.5. to 2-fold variations in the absolute level of enzyme were observed. These strains were used in the further characterization.

TABLE I

Some properties of *leuX⁻* and *leuY⁻* strains

Strain	*LeuRS Level*	*Growth Limit*
KL231 *($leuS^-_{ts}$)*	1.0	40°C
GV10 *($leuS^-_{ts}$ leuX1)*	6.9	45°C
GV18 *($leuS^-_{ts}$ leuY1)*	4.3	42°C

A possible explanation for the increased levels of leucyl-tRNA synthetase in cells grown at high temperature is the loss of a gene function at that temperature. Therefore we determined the amount of enzyme in cells grown at 30° and 42°C. As judged by antiserum titration strains D2, GV10, and GV18 contained the same amount of leucyl-tRNA synthetase when grown at the two temperatures. Thus, the enzyme over-production which suppresses the *leuS31* mutation in these strains is not caused by thermal interference with the expression of a regulatory gene.

Mapping Studies

In order to map the loci involved in the over-production of leucyl-tRNA synthetase, the stability of the mutations causing the temperature resistance of strains, GV10 and GV19 was investigated. No temperature sensitive isolates were detected from over 1200 single colonies of each strain. Thus the mutations are stable.

The growth limit, the temperature above which a strain will no longer grow (on LB plates) was a very good indication, that strains GV10 and GV19 contained different mutations resulting in temperature resistance (Table 1).

Transductions involving Plvir grown on strains GV10, GV18, and KL231 indicate that in GV10, a regulatory locus, which we designate *leuX*, causes the temperature resistance and is tightly linked to the gene for leucyl-tRNA synthetase, *leuS*, but *leuX* is separable from *leuS* with a low frequency of 1%. In contrast, the mutation causing temperature resistance in GV18 maps away from the structural gene between *lac* and *gal* and is cotransducible with *proC*. We have designated this regulatory locus *leuY*.

A dominance analysis of the $leuX^+$ and $leuX^-$, $leuY^+$ and $leuY^-$ alleles was carried out to further elucidate the nature of these loci. $LeuX^-$ was proven. to be *cis*-dominant and *trans*-recessive operator-promoter type mutation affecting the synthesis of leucyl-tRNA synthetase. The $leuY^+$ allele however is *trans*-dominant and appears to specify a diffusable compound whose presence regulates the *in vivo* level of leucyl-tRNA synthetase. In order to investigate the possibility that *leuY* specifies a protein, we checked to see if $leuY^-$ is a nonsense mutation. If so, its gene product is a protein. $leuY^-$ is indeed a nonsense mutation.

Biochemical Characterization of leuX and leuY Mutations

The two regulatory loci appear to affect only the synthesis of leucyl-tRNA synthetase, no effect on the levels of the other nineteen aminoacyl-tRNA synthetases or their cognate tRNA was found.

Although the total amount of leucine-tRNA was unaltered in the $leuX^-$ and $leuY^-$ strains, it was possible that there was a change in the amount of the individual isoacceptors. Thus we examined the elution profiles or radioactive Leu-tRNA by reversed phase chromatography. As seen in Figure 2A and C the profiles of Leu-tRNA. from strains D2 ($leuS^+$), KL231 ($leuS^-$), and GV18 ($leuS^-$ $leuY^-$), were all similar. The difference between these profiles and that of Leu-tRNA from strain GV10 ($leuS^-$ $leuX^-$) was as increase of peak 2 (fraction 22-35) at the expense of peak 1 in the profile of GV10 (Fig. 2B). Additional chromatography on Sepharose 4B indicated that the tRNAS which give rise to the "new" peak in Fig. 2 are probably undermodified $tRNA^{Leu}$ species; this would also explain the decrease in the amount of fully modified $tRNA^{Leu}$ (peak 1 in Fig 2B). Thus no major

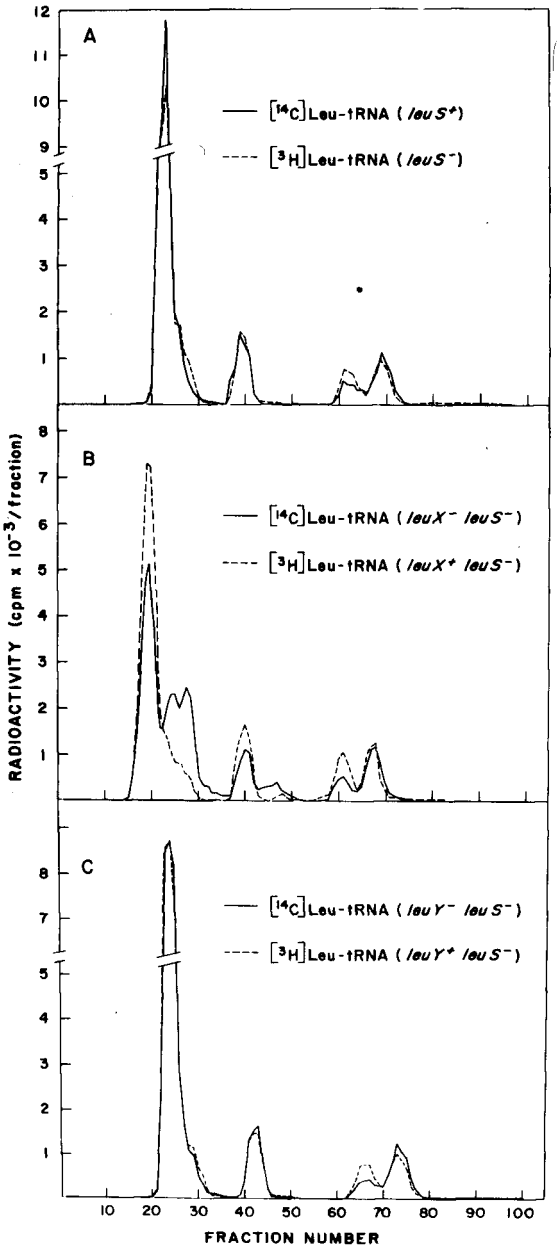

Fig. 2 RPC-5 chromatography of unfractionated tRNA from various strains: (A) D2 ($leuS^+$) and KL231 ($leuS^-$); (B) GV10 ($leuS^-$ $leuX^-$) and KL231 and (C) GV18 ($leuS^-$ $leuY^-$) and KL231.

changes in the isoacceptor distribution of $tRNA^{Leu}$ were observed, though differences in the modification of $tRNA^{Leu}$ are probable. This apparent undermodification in *leuX*$^-$ strains may be caused by the great excess of leucyl-tRNA synthetase preventing interaction of tRNA modifying enzymes with some of the $tRNA^{Leu}$ population.

Whether such a difference in the amounts of the leucine isoacceptors may play a role in the regulation of the *leu* or *ilv* operons is an interesting question. Hatfield and Burns (1970) have shown that leucyl-tRNA binds to the immature forms of threonine deaminase and later studies (Calhoun and Hatfied, 1973, 1975) indicate that only $tRNA_4^{Leu}$ is effective. Therefore it is important to determine whether the *in vivo* charged leucyl-tRNA has an isoacceptor distribution different from that found by *in vitro* charging. Such experiments are now feasible using borate-cellulose chromatography (LaRossa *et al.*, 1977a) in conjunction with reversed phase chromatography (Pearson *et al.*, 1971).

Expression of the Leu and Ilv Operons is Dependent on Leucyl-tRNA

Lewis and Ames (1972) showed an inverse relationship between the *in vivo* levels of histidyl-tRNA and the expression of the *his* operon in *S. typhimurium*. A similar role for leucyl-tRNA also has been suggested since the *leu* and *ilv* operons are derepressed in *leuS*$^-$ strains of *E. coli* (Low *et al.*, 1971) and *hisT*$^-$ strains of *S. typhimurium* (Rizzino *et al.*, 1974; Cortese *et al.*, 1974; Allaudeen *et al.*, 1972). Yet the study *of leuS*$^-$ strains did not differentiate between leucyl-tRNA and leucyl-tRNA synthetase as the active element in repression. Having determined the level of leucyl-tRNA in the *leuX* and *leuY* strains we investigated the extent of derepression of the *leu* operon and *ilv* gene cluster as a function of leucyl-tRNA levels. For this reason we measured the activities of acetohydroxyacid synthetase, the second enzyme in the isoleucine-valine biosynthetic pathway, and of isopropylmalate synthetase, an enzyme involved in leucine biosynthesis. An inverse relation between the levels of leucyl-tRNA and the activity of these enzymes was found (Table II). No derepression was seen for histidinol phosphate phosphatase, an unrelated enzyme. Thus leucyl-tRNA is the macromolecule responsible for derepression of the *leu* and *ilv* operons in strains KL231 (*leuS*$^-$). Since *hisT* mutants of *S. typhimurium* are also derepressed for the *his*, *ilv*, and *leu* operons (Rizzino *et al.*, 1974; Cortese *et al.*, 1974; Allaudeen *et al.*, 1972) these results imply a similar mechanism of control for the three operarons in both *E. coli* and *S. typhimurium*.

Isolation and Characterization of Mutants with Elevated Levels of $tRNA^{Gln}$ or Glutaminyl-tRNA Synthetase

Strain AB4143 is unable to grow above 41°C on LB agar and harbors a thermolabile glutaminyl-tRNA synthetase (Körner *et al.*, 1974). From this mutant, spontaneous temperature resistant revertants were isolated at 42°C. The revertants appeared at a frequency of 10^{-5}. These temperature resistant strains were checked for the thermolability and the amount of glutaminyl-tRNA synthetase, and for the concentration of $tRNA^{Gln}$. All the revertants examined exhibited identical biochemical and genetic

TABLE II

Some properties of *leuX*$^-$ and *leuY*$^-$ strains

Strain	*Temperature* °C	*In vivo charged Leu-tRNA (%)*	*Rate of Protein Synthesis*[a]	*Aceto hydroxyacid Synthetase*	*Isopropyl malate Synthetase*
D2 (*leuS*$^+$)	37	74	47	10	0.6
KL231 (*leuS*$^-$)	30	18	3	135	17.6
GV10 (*leuX*$^-$ *leuS*$^-$)	37	64	47	9	2.3
GV18 (*leuY*$^-$ *leuS*$^-$)	37	22	36	38	9.0

[a]Measured by β-Galactosidadse formation.

characteristics and still contained the thermolabile glutaminyl-tRNA synthetase. The amount of glutaminyl-tRNA synthetase in cell extracts of strains AB4143, the wild type strain AB3441, and the revertants was determined by antibody titration. The temperature resistant character of the revertants was not due to the overproduction of the thermolabile enzyme; each revertant strain contained twice as much $tRNA^{Gln}$. Unfractionated tRNA of strains AB3441 and AB4143 accepted 30 pmoles of [^{14}C]glutamine per A_{260} unit while tRNA from the revertants accepted 60 pmoles of [^{14}C]glutamine per A_{260} unit. Since Chase *et al.* (1974) had reported significant changes in the levels of tRNA isoacceptors in *E. coli* grown under various stress conditions (e.g. high and low temperatures and amino acid starvation) strains AB3441, AB4143, and the revertants were grown at 30°, 37°, and 41°C. The tRNA was extracted and assayed for the level of glutamine acceptance. At all growth temperatures the tRNA from the revertant strains contained twice as much $tRNA^{Gln}$ than the wild type or mutant strain. One of these revertants, strain KL361, was chosen for further analysis.

Since the selection of temperature resistant revertants from strain AB4143 resulted in strains with gene duplications (see below) we attempted another selection at 45°. Among those strains which still contained the thermolabile glutaminyl-tRNA synthetase we selected two for further characterization. Preliminary characterization of strain A57 showed it to possess elevated levels of $tRNA^{Gln}$, while strain A46 had elevated levels of the cognate aminoacyl-tRNA synthetase.

Genetic Characterization of the Revertant Strains

The high reversion frequency (10^{-5}) of strain KL361 suggested a gene duplication to be responsible for the temperature resistant phenotype. This was proven to be the case, since the reversion frequency was dependent on the *recA* allele. The duplicated locus *(glnT)* was mapped between minutes 75 and 81 on the *E. coli* chromosome. The temperature resistant character in strains A46 and A57 was stable. The $glnR^-$ locus is between minute 60 and minute 10 on the *E. coli* chromosome. Studies to map this locus better and also to define genetically the $glnU^-$ mutation are in progress.

Biochemical Characterization of Revertant Strains

The *glnR* mutation causes 5-fold increased levels of glutaminyl-tRNA synthetase in strain A46. The levels of the nineteen other aminoacyl-tRNA synthetases and the amino acid acceptor activities of tRNA were similar to those in strain AB3441 (Table III).

As was the case for strain KL361 the tRNA of strain A57 showed twice the glutamine acceptor activity of tRNA from wild type strain AB3441 (Table III). The unfractionated tRNA of strains KL361 and A57 were assayed to determine the levels of the remaining 19 ammo acid acceptor RNAs. Only $tRNA^{Gln}$ is elevated in strain KL361 when compared to the parental and the wild type strains. However, in strain A57 the methionine acceptance was also elevated from 70 to 110 pmoles/A_{260} unit. This indicates that the *glnU* mutation regulates

TABLE III

Glutaminyl-tRNA synthetase and glutamine tRNA levels
In $glnR^-$, $glnT^-$, and $glnU^-$ strains

Strain	*GlnRS Level*	*$tRNA^{Gln}$ Level*
AB3441 *(glnS$^+$)*	1	1 (30 pmoles/A_{260})
AB4143 *(glnS$^-$)*	1	1
A46 *(glnS$^-$ glnR$^-$)*	5	1
KL361 *(glnS$^-$ glnT$^-$)*	1	2
A57 *(glnS$^-$ glnU$^-$)*	1	2

the synthesis of two tRNA species, possibly they are transcribed together on a common precursor molecule. Indeed, Inokuchi and Ozeki (personal communication) have evidence for such a tRNA precursor. It is also important to note that there was no general overproduction of tRNA in the revertants: 75 A_{260} units of tRNA per gram wet weight can be extracted from cells of strains AB3441, AB4143, KL361, and A57.

E. coli contains two $tRNA^{Gln}$ species which differ in only seven nucleotides (Yaniv and Folk, 1975). The most significant difference is in the 5'-position of the anticodon, where $tRNA_1^{Gln}$ has 2-thiouridine derivative while $tRNA_2^{Gnl}$ has a cytidine. We then proceeded to determine if both isoacceptor species were overproduced in strain KL361. Reversed phase chromatography of unfractioned [^{14}C] Gln-tRNA of strain AB3441, resolved the two species (Figure 3). In a double label experiment we then compared

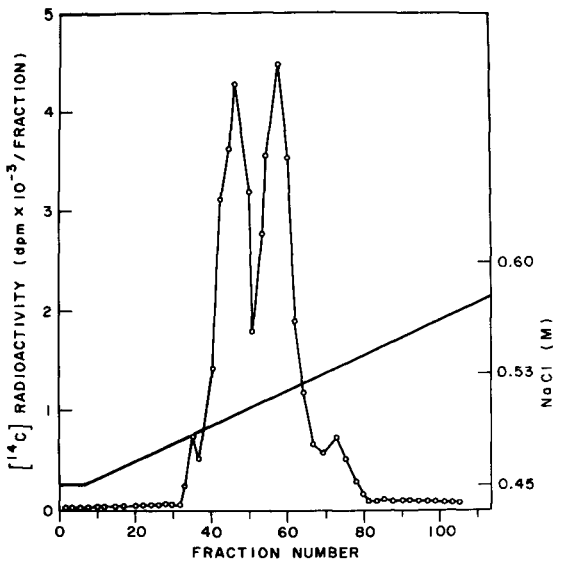

Fig. 3. RPC-5 chromatography of unfractionated tRNA extracted from strain AB3441. 10 A_{260} units of unfractionated tRNA from AB3441 were applied to the column.

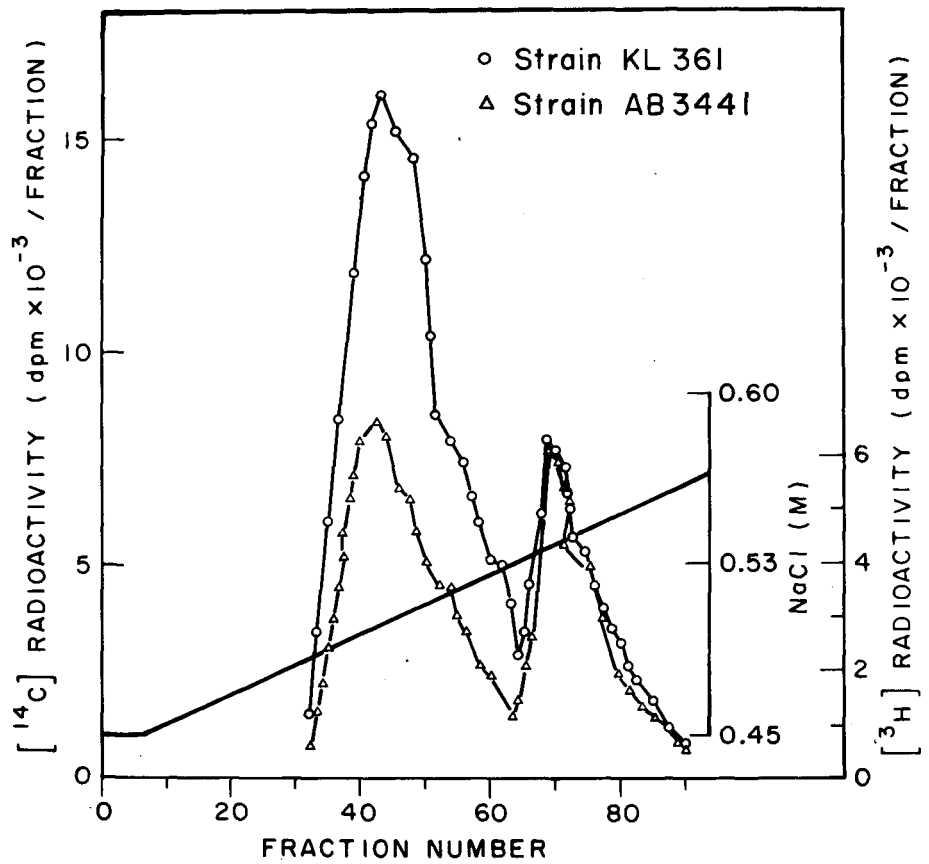

Fig. 4. RPC-5 chromatography of unfractionated [^{3}H] Gln-tRNA from strain AB3441 and [^{14}C] Gln-tRNA from strain KL361. 25 A_{260} units of unfractionated tRNA from each strain were applied to the column.

[^{14}C] Gln-tRNA of strain KL361 with [^{3}H] Gln-tRNA of strain AB3441. As can be seen from the elution profile (Figure 4) only one of the two tRNA^{Gln} species was elevated in strain KL361. We used chemical modification of the tRNA to identify which tRNA^{Gln} species was elevated in strain KL361. It is known that the 2-thiouridine in tRNA^{Gln} can be readily alkylated by cyanogen bromide; the resulting modified tRNA species cannot easily be aminoacylated (Seno, Agris and Söll, 1974). Therefore tRNA from strain KL361 was treated with cyanogen bromide and aminoacylated with [^{14}C] glutamine. As a control, untreated tRNA from strain AB3441 was charged with [^{3}H] glutamine. The aminoacylated tRNAs were cochromatographed on RPC-5. The elution profile (Figure 5) showed that the first peak of Gln-tRNA was absent in the cyanogen bromide treated [^{14}C] Gln-tRNA sample. Thus the first peak corresponds to tRNA$_1^{Gln}$ and the second peak to tRNA$_2^{Gln}$. It is important to note that cyanogen bromide treatment of the tRNA does not alter its elution profile upon RPC-5 chromatography (Katze, 1975). Therefore tRNA$_1^{Gln}$ is the isoacceptor present in 3-fold increased concentration in strain KL361.

Similar studies have not yet been completed with strain A57.

In order to determine the consequences of the increased tRNA^{Gln} concentration in strain KL361 on other processes a number of descriptive experiments were done comparing this strain with strain AB3441 and strain AB 4143. The rate of synthesis of a specific protein, β-galactosidase, was determined in strains AB3441, AB4143, and KL361 (Table IV). This enzyme was chosen because its synthesis can be easily induced by isopropyl-thiogalactoside and its activity is easily measured. Strain AB 3441 synthesizes the enzyme more rapidly at 30° and 39°C than either strain AB4143 or KL361. As expected, enzyme synthesis is faster at 39°C than at 30°C. This is quite different from strain AB4143. When grown at 39°C, strain AB4143 synthesizes the enzyme twelve times slower than when grown at 30°C. However, the KL361 strain which also has a thermolabile glutaminyl-tRNA synthetase is able to synthesize protein at 39°C about ten times faster than AB4143. Clearly, the thermolability of the aminoacyl-tRNA synthetase does

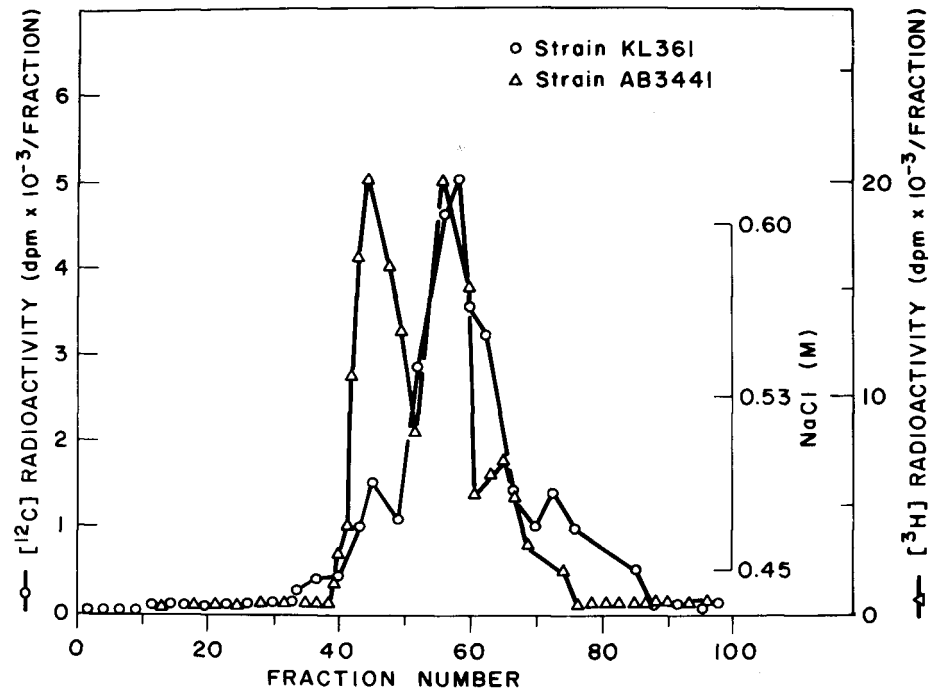

Fig. 5. RPC-5 chromatography of unfractionated [^{3}H] Gln-tRNA from strain AB3441 and [^{14}C] Gln-tRNA from strain KL361. The tRNA from strain KL36 was treated with BrCN before aminoacylation.

not restrict significantly the role of protein synthesis in strain KL361.

In order to correlate the rates of protein synthesis with the levels of aminoacylated tRNA, the *in vivo* levels of Gln-tRNA were determined in strains AB3441, AB4143, and KL361 (Table IV). The levels of Ser-tRNA served as a control. Since the 2-thiouridine in $tRNA^{Gln}$ is easily oxidized and the resulting modified tRNA can no longer be charged, we had to use a new method (LaRossa *et al.*, 1977a) *al.*, 1973). Recently, Lapointe *et al.*, (1975) have suggested that glutamyl-tRNA may repress not only glutamate synthetase, but also glutamine synthetase. Because other aminoacyl-tRNAS have been implicated as well in the regulation of the biosynthesis of their cognate amino acids (Brenchley and Williams, 1975), the levels of glutamine synthetase were determined in strains AB3441, AB4143, and KL361 in the transfer reaction which measures the activity of both the adenylated and unadenylated biosynthetic enzyme. The actual biosynthetic activity of the enzyme was determined by the phosphate release assay. Histidinol phosphate phosphatase, an enzyme in the histidine biosynthetic pathway, was also assayed to determine the general level of repression-derepression in the three strains. The strains were grown at 39°C where strain based on borate cellulose chromatography for the determination of the amount of *in vivo* charged tRNA. The amount of acylated tRNA increases slightly as the temperature rises and the rate of protein synthesis increases. However in strain AB4143 only 14% of the $tRNA^{Gln}$ are acylated at 39°C (near to non-permissive temperature). It should be noted that in strain KL361 a high level of Gln-tRNA is found despite the fact that the absolute concentrations of $tRNA^{Gln}$ is doubled when compared to the other two strains.

Since the high level of Gln-tRNA may increase the demand on glutamine in strain KL361 we determined the activity of glutamine synthetase, the enzyme which catalyzes glutamine formation. This enzyme is elaborately controlled both structurally and genetically (Ginsberg and Stadtman, 1973; Wolheuter *et al.*, 1973). Recently, Lapointe *et al.* (1975) have suggested that glutamyl-tRNA may repress not only glutamate synthetase, but also glutamine synthetase. Because other aminoacyl-tRNAs have been implicated as well in the regulation of the biosynthesis of their cognate amino acids (Brenchley and Williams, 1975), the levels of glutamine synthetase were determined in strains AB3441, AB4143, and KL361 in the transfer reaction which measures the activity of both the adenylated and unadenylated biosynthetic enzyme. The actual biosynthetic activity of the enzyme was determined by the phosphate release assay. Histidinol phosphate phosphatase, an enzyme in the histidine biosynthetic pathway, was also assayed to determine the general level of repression-derepression in the three strains. The strains were grown at 39°C where strain AB4143 has much lower levels of Gln-tRNA and a much slower growth rate than strains KL361, A57, A46, and AB3441. The amount of glutamine synthetase is elevated in strains

TABLE IV

Some properties of *glnR*$^-$, *glnT*$^-$ and *glnU*$^-$ strains

Strain	*In vivo charged Gln-tRNA (%)*	*Rate of β-Galactosidase Synthesis*	*Glutamine Synthetase*[a]		*Glutamine Pool*[a]
			Amount	*Spec. Activity*	
AB3441 (*glnS*$^+$)	74	94	1	1	1
AB143 (*glnS*$^-$)	14	3	1	1	20
A46 (*glnS*$^-$ *glnR*$^-$)	nd[b]	nd[b]	3.5	1	1
KL361 (*glnS*$^-$ *glnT*$^-$)	63	38	8	1	1
A57 (*glnS*$^-$ *glnU*$^-$)	nd[b]	nd[b]	1	1	1

[a] Relative to strain AB3441.
[b] Not determined.

KL361 and A46. However the biosynthetic activity of the enzyme and the activity of histidinol phosphate phosphatase is the same in all three strains. It is too early to say whether this phenomenon is expressed through the *in vivo* level of Gln-tRNA, or is a direct effect on this enzyme, since the determination of the activities of glutamate synthase and of glutamate dehydrogenase and of the concentration of some metabolites crucial for their regulation has not been completed.

DISCUSSION

In order to obtain mutants affecting the regulation of tRNA or aminoacyl-tRNA synthetase biosynthesis, we screened temperature resistant revertants of temperature sensitive strains possessing a thermolabile aminoacyl-tRNA synthetase. The rationale for this approach was outlined in the Introduction. Our expectations were fulfilled. Apart from the regulatory mutants described in this report strains which reproduce seryl-or valyl-tRNA synthetase have been isolated by this procedure (G. Theall and M. Baer, unpublished results) starting with temperature sensitive strains harboring these thermolabile aminoacyl-tRNA synthetases.

It may be appropriate at this point to discuss briefly the various possible regulatory mutations affecting tRNA biosynthesis. An overproduction of all tRNA species may be found in mutants of tRNA polymerase which increase its affinity for the promoters of tRNA genes. Possibly rRNA may also be overproduced in such strains since the synthesis of both classes of macromolecules is somehow correlated (Roseet, Julien and Monier, 1966). Since the synthesis of tRNAs is under stringent control (Ikemura and Dahlberg, 1973) one may expect mutations in those control elements to affect all tRNAs. Another type of mutant could lead to the increased transcription of specific tRNA genes. Depending on whether a monomeric tRNA gene arrangement or a multimeric transcription unit with different tRNAs is affected, the levels of one or more tRNA species would be elevated. The A57 revertant may fall into this category. Many tRNAs could be affected in mutants which alter the rate of precursor tRNA processing and thus eventually may alter the concentrations of various tRNA species.

How are all the changes seen in the *leuX*$^-$, *leuY*$^-$, *glnR*$^-$ *glnT*$^-$, and *glnU*$^-$ mutants correlated to the increased aminoacyl-tRNA synthetase or tRNA levels? Although we measured the levels of aminoacyl-tRNA, of the cognate free amino acids and of the magic spot compounds (p)ppGpp (Stephens *et al.*, 1975; Cashel and Gallant, 1974), we do not know yet the nature of the signal triggering these changes. However, these studies yielded an interesting result (data not shown) which suggest that the ratio of charged to uncharged tRNA controls the amount of magic spot formed rather than the absolute concentration of either.

It would be desirable to have novel schemes for mutant selection which would allow an easier biochemical determination of the aminoacyl-tRNA synthetase levels. In principle the same selection scheme could be envisaged starting from strains auxotrophic for an amino acid as a consequence of an aminoacyl-tRNA synthetase mutation with low affinity for the cognate amino acid. Such *E. coli* strains are available (Doolittle and Yanofsky, 1968; Kano *et al.*, 1969; Folk and Berg, 1970; Iaccarino and Berg, 1971; Buonocore *et al.*, 1972; Armstrong and Fairfield, 1975). Another approach may be the selection of mutants resistant to antibiotics which inhibit a particular aminoacyl-tRNA synthetase. In this way mutants have been obtained which overproduce wild type threonyl-tRNA synthetase (Paetz and Nass, 1973).

All the regulatory mutants known so far affect only a single aminoacyl-tRNA synthetase, suggesting that all of them are individually controlled. In a way this is not surprising since the structural genes of aminoacyl-tRNA synthetases are scattered throughout the *E. coli* genome (Söll, 1976). Possibly one may find mutants which display a coordinate control of several or of all aminoacyl-tRNA synthetases among revertants of strains containing two different thermolabile aminoacyl-tRNA synthetases or by analysis of a large number of revertants of a strain containing a single defective aminoacyl-tRNA synthesis. Such revertats may also contain strains with elevated levels of different tRNA species.

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