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Growth Rate Modulation of Four Aminoacyl-Transfer Ribonucleic Acid Synthetases in Enteric Bacteria

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The specific activities of arginyl-, glutamyl-, seryl-, and valyl-transfer ribonucleic acid (tRNA) synthetases were measured in the wild-type and mutant strains of *Salmonella typhimurium* LT2 and *Escherichia coli* B/r. In media restricted only by carbon and energy source availability, the specific activities of all four enzymes were proportional to the growth rate, with the exception of seryl-tRNA synthetase in *S. typhimurium*, which remained essentially constant. Structural gene densities were calculated for these four enzymes and were found not to account for the variation of specific activity with growth rate. Various means of restricting the growth rate of wild-type and mutant cells revealed that synthetase levels are not as tightly coordinated with growth rate as is stable RNA. The effect of exogenous amino acids on synthetase variation with growth rate was investigated in a number of ways. In most instances no evidence was found that the exogenous supply of individual amino acids modified the growth rate-related regulation of synthetase levels. An exception was seryl-tRNA synthetase activity, which rose dramatically upon catabolic depletion of exogenous serine from synthetic rich media and which, in *E. coli*, showed a similar rise upon arginine limitation. The previously reported response of many synthetases to endogenous amino acid limitation and the response of many of them to the growth-supporting property of the medium seem to be only indirectly related; these separate responses may be different aspects of a single control system or may reflect the existence of separate, interacting control mechanisms.

Aminoacyl-transfer ribonucleic acid (tRNA) synthetases occupy an interesting position in cellular metabolism. Since many of them are involved in regulation of their cognate amino acid biosynthetic pathways, they can be viewed as the terminal enzymes of these pathways, and aminoacyl-tRNA's can be viewed as the true end products. Focus on the relationship of synthetases to biosynthetic pathways has led to an experimental approach in which cultures of bacteria are restricted for an amino acid (usually using auxotrophic or bradytrophic strains, or strains with a mutationally altered aminoacyl-tRNA synthetase) and the behaviors of individual synthetases are observed. Ten synthetases in *Escherichia coli* and *Salmonella typhimurium* studied in this manner have been shown to become either transiently or, in a few cases, permanently elevated under these circumstances (18).

The synthetases may also be regarded as an integral part of the cell's translation apparatus, and this view has led to a different approach to regulation of synthetases. Several elements of the bacterial cell's translation machinery vary in level with growth rate. These include ribosomal RNA (15), ribosomal protein (8), tRNA (23), and elongation factors T and G (10). The early literature on aminoacyl-tRNA synthetases contains conflicting information on possible growth effects (reviewed in 18), and therefore Parker and Neidhardt (21) initiated the first systematic study of the effect of growth rate on synthetase level. They observed a positive correlation for the arginyl- and valyl-tRNA synthetases in *S. typhimurium* LT2 and *E. coli* B/r and a similar, but lesser, variation of the leucine enzyme. Nutritional shifts revealed that the synthetases for arginine and valine behaved similarly to stable RNA and quite differently from biosynthetic enzymes under amino acid control (20, 21). Radioactive labeling and antibody neutralization both supported the idea that the specific activity measured in crude extracts was a true reflection of the differential rate of synthesis of these two enzymes

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(20). Cassio et al. (5) have reported that methionyl-tRNA synthetase also exhibits a variation with growth rate.

Here we report an extension of this study designed to determine whether other synthetases exhibit a similar "metabolic regulation" and to explore further the relationship of this phenomenon to amino acid-specific effects. Our results indicate: (i) that at least two other synthetases, those for glutamic acid and serine, exhibit metabolic regulation, (ii) that the levels of enzymes of this class are only loosely coupled to growth rate and can be uncoupled in a number of ways, and (iii) that for some synthetases the amino acid supply seems to be the dominant controlling signal, whereas for others this signal is irrelevant under physiological conditions.

(The results reported here are taken from a thesis presented by W.G.M. to Purdue University, Lafayette, Ind., in partial fulfillment of the requirements for the Ph.D. degree [1975]. A preliminary report was presented at the Annual Meeting of the American Society for Microbiology, 27 April to 2 May 1975.)

MATERIALS AND METHODS

Bacterial strains. Two prototrophic strains were used: *S. typhimurium* NTI is an LT2 strain described previously (16) and *E. coli* NC3 is a B/r derivative that lacks one or more of the usual host restriction enzymes. Four mutant derivatives of *E. coli* NC3 were used: strain NC32 *(valS)* is temperature sensitive for growth as the result of an altered valyl-tRNA synthetase; strain NC34 *(fus)* is temperature sensitive for growth as the result of an altered protein chain elongation factor G; strain NC36 *(leuA)* is temperature sensitive for growth as the result of an altered first enzyme in the leucine biosynthetic pathway; and strain NC39 *(cya)* is pleiotropically negative for growth on arabinose, galactose, and lactose as the result of a deficiency in adenyl cyclase activity. All strains were constructed by B. Wanner in this laboratory, using P1-mediated transduction to introduce the indicated lesions from mutants produced in this laboratory or, in the case of the G factor, obtained from D. Schlessinger.

Media. All media were totally defined, synthetic media produced by adding one or more supplements to MOPS medium (16). Carbon sources were employed at the following concentrations: 0.4% (wt/vol) potassium acetate; 0.4% (vol/vol) glycerol; and 0.4% (wt/vol) D-glucose. Rich medium contained 20 amino acids, 2 purines, 2 pyrimidines, and 5 vitamins as described previously (16). All directions for preparation of these media, including filter sterilization of all components, were presented previously (16).

Growth conditions. A single colony from a minimal medium MOPS plate was used to start a culture the night before an experiment by inoculation into a MOPS medium containing a carbon source set to be limiting at an optical density (OD) of 1 (3×10^8 cells/ml). A dilution was made on the limited culture to allow at least nine generations of growth before the culture was harvested at an OD of 1. The temperature-sensitive cells were all grown at temperatures that were limiting but that would permit steady-state growth. A glucose-limited overnight culture, grown at a nonrestricting temperature, was used to inoculate fresh minimal medium also at a nonrestricting temperature. The temperature was raised in small increments until the final restricting temperature was reached. A digital thermistor controller (model 71A, Yellow Springs Instruments Co., Yellow Springs, Ohio) made this procedure easy. Shifts from rich medium to rich medium lacking one or more amino acids were accomplished by filtering the culture (100 ml or less at an OD of 1) through a 142-mm membrane filter (pore size, 0.45 μm; Millipore Corp., Bedford, Mass.). Natural depletions were accomplished by adding a predetermined, low concentration of the appropriate amino acid to the fresh rich medium and allowing the cells to deplete the amino acid from the medium.

Growth was measured with a Zeiss PMQ II spectrophotometer, and its rate is expressed as a first-order rate constant, k, in units per hour (16).

Crude extracts. Culture samples were processed as described previously (21), except that the buffer contained 10% (vol/vol) glycerol. For extracts to be assayed for threonine deaminase the procedure was modified by washing the cells twice, omitting dialysis, and employing the buffer of Burns (4) to wash the cells and suspend them for sonic treatment. All assays were performed within 12 h; all activities were stable for at least 2 days.

RNA and protein determination. For whole cells, a slight modification of the trichloroacetic acid-NaOH method of Neidhardt and Magasanik was used (17). Protein was determined colorimetrically by the method of Lowry et al. (14), with bovine serum albumin as a standard. RNA was determined colorimetrically by the orcinol method of Schneider (22), with yeast RNA type XI as a standard (Sigma Chemical Co., St. Louis, Mo.). The absorbance at 670 nm was measured.

Aminoacyl-tRNA synthetase assays. The synthetases were assayed by measuring the attachment of ^{14}C-labeled L-amino acid to tRNA at 37 C in a reaction mixture similar to that described previously (1). There were four changes in this procedure: (i) sodium cacodylate, pH 7.3, was substituted for tris(hydroxymethyl)aminomethane buffer; (ii) the dipotassium adenosine 5'-triphosphate stock was made to pH 6.8; (iii) the reduced glutathione stock was made to pH 7.0; and (iv) tRNA prepared from *E. coli* B was used. The final concentration of the radioactive amino acid in the assay tube was 0.1 mM at 5 μCi/μmol. The mixture was incubated for 5 min (for arginyl-tRNA synthetase) or 10 min (for the other three synthetases). Subsequent processing of the samples was accomplished as described by Parker et al. (20). For the synthetases measured in extracts of cells grown on glucose, this asssay system gave trichloroacetic acid-precipitable counts from 8- to 20-

fold above a background obtained by stopping the reaction immediately after the amino acid was added. Duplicate assays using two concentrations of protein were always run. At least 90% of the duplicates at the same protein concentration were within ±10% of the mean. The specific activity is expressed as units per milligram of protein, where 1 unit is the amount of enzyme that attaches 1 μmol of amino acid to tRNA per h under the conditions employed.

Ornithine transcarbamylase assays. Ornithine transcarbamylase activity was determined in cell extracts by measuring, colorimetrically, the production of citrulline at 37 C (12). The specific activity is expressed as micromoles of citrulline formed per hour per milligram of protein under the conditions employed.

Threonine deaminase assays. Threonine deaminase activity was determined in cell extracts by measuring, colorimetrically, the production of α-ketobutyrate essentially by the method of Burns (4) but with several modifications. The phosphate buffer of Datta (7) was used and the conditions used by Datta to stop the reaction were slightly modified. The reaction was stopped after 10 min at 37 C by adding 0.2 ml of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl to the entire 1.0 ml of reaction mixture. After 15 min at room temperature, 1.0 ml of 2.5 N NaOH was added. The color was allowed to develop for 10 min and was then read at 540 nm within 15 min. The specific activity is expressed as micromoles of α-ketobutyrate formed per hour per milligram of protein under the conditions employed.

Chemicals and radiochemicals. All chemicals were the highest grade available from ordinary commercial suppliers. Deacylated tRNA derived from *E. coli* B was purchased from General Biochemicals Co., Chagrin Falls, Ohio. Radioactive amino acids were purchased from Schwarz/Mann, Orangeburg, N.Y., and Amersham-Searle Corp., Arlington Heights, Ill.

RESULTS

Examination of synthetase levels in diverse media. Cultures of both *E. coli* NC3 and *S. typhimurium* NT1 were harvested during steady-state growth in media of various composition, and the activity of the four synthetases was measured. Figure 1 shows the results for *E. coli* NC3. The specific activities of all four synthetases increased as a function of increasing growth rate set by acetate, glycerol, glucose, and the synthetic rich medium. The increases were not coordinate but varied over this range of growth rate as follows: seryl-tRNA synthetase, 2.0-fold; valyl-tRNA synthetase, and glutamyl-tRNA synthetase, 2.3-fold; and arginyl-tRNA synthetase, 3.3-fold. Raising or lowering the exogenous cognate amino acid supply did not consistently affect the synthetase levels. Effects of omitting noncognate amino acids will be considered later.

The results with *S. typhimurium* NT1 were similar for three synthetases, which exhibited increased specific activities in cultures growing at increasingly rapid rates. Over the same range of growth rate (acetate, $k = 0.31$, compared with rich medium, $k = 1.90$), the synthetase levels increased as follows: glutamyl-tRNA synthetase, 1.8-fold; arginyl-tRNA synthetase, 2.0-fold; and valyl-tRNA synthetase, 2.3-fold (data not shown). Generally, the exogenous cognate amino acid supply did not affect synthetase levels. The level of seryl-tRNA synthetase remained constant from acetate to glucose media and even decreased slightly from glucose to the fully supplemented rich medium (Fig. 2).

Metabolic regulation and gene density. A potentially significant factor in the rate of synthesis of a particular protein at different cellular growth rates is the variation in numbers of copies of the structural gene for that protein. This variation is brought about by the fact that multiple growing forks occur in the deoxyribonucleic acid (DNA) of enteric bacteria dividing faster than once per hour. Hence, genes located near the origin of replication become amplified relative to those near the terminus. To examine the effect of this phenomenon on synthetase formation, we calculated the "gene density" of various synthetase structural genes at different growth rates. "Gene density" was defined as the number of gene copies per genome amount of DNA, and it was calculated by the aid of a Fortran program written by Robert Blumenthal. The assumptions in the calculation were: (i) that equation 5 of Cooper and Helmstetter (6) accurately describes chromosome replication as a function of growth rate, (ii) that replication is symmetrical and bidirectional for the chromosome of *E. coli* (origin near 74 min, terminus near 25 min [3]) and of *S. typhimurium* (origin between 109 and 112 min, terminus between 20 and 53 min [9]), (iii) that mutant cells growing at restricted rates have a pattern of DNA replication similar to that of wild-type cells growing at the same rate, (iv) that the protein/DNA ratio is virtually constant with growth rate, and (v) that synthetases not yet located on the *S. typhimurium* chromosome occupy analogous positions to their counterparts on the *E. coli* chromosome. The results for *E. coli* are shown in Table 1. They demonstrate clearly that differential gene amplification cannot account for the observed variation in synthetase level, either because the magnitude of amplification is too small (as for the *gltE* and *valS* genes) or because the gene density actually decreases with increasing growth rate (as for the *argS*, *gltM*, *gltX*, and *serS* genes). An interesting sidelight of this

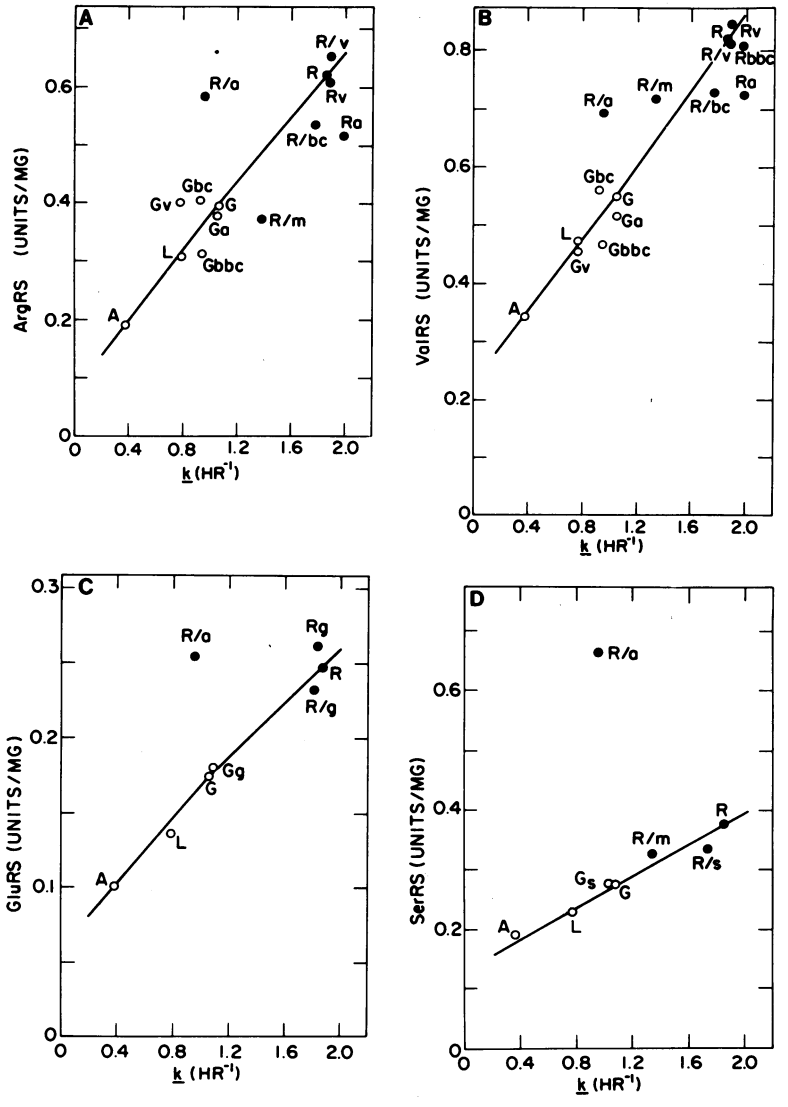

FIG. 1. *Levels of four aminoacyl-tRNA synthetases in diverse media in E. coli NC3. Each point represents the specific activity for a culture measured after growth in steady-state for at least nine generations. All values were normalized to the value of a glucose culture, usually grown, extracted, and assayed in parallel with the experimental culture. Symbols:* ○, *cultures grown in minimal medium;* ●, *cultures grown in rich media. Abbreviations: A, acetate; L, glycerol; G, glucose; Ga, glucose plus 6 mM arginine; Gs, glucose plus 15 mM serine; Gv, glucose plus 15 mM valine; Gbc, glucose plus branched-chain amino acids (0.6 mM valine, 0.4 mM isoleucine, 0.8 mM leucine); Gbbc, glucose plus balanced, branched-chain amino acids (1.2 mM valine, 0.6 mM isoleucine, 0.6 mM leucine); R, rich medium (completely synthetic); Ra, rich medium plus 6 mM arginine; Rv, rich medium plus 15 mM valine; R/v, rich medium lacking valine; R/bc, rich medium lacking branched-chain amino acids; R/a, rich medium lacking arginine; R/m, rich medium lacking methionine; R/S, rich medium lacking serine. (A) Arginyl-tRNA synthetase. (B) Valyl-tRNA synthetase. (C) Glutamyl-tRNA synthetase. (D) Seryl-tRNA synthetase.*

calculation is that coordination between the *gltX* and *gltE* genes must be provided in some manner since their gene products presumably appear in constant molar ratios in mature glutamyl-tRNA synthetase in cells at different growth rates.

RNA/protein ratios of *E. coli* NC3 and its mutant derivatives. Because some synthetases

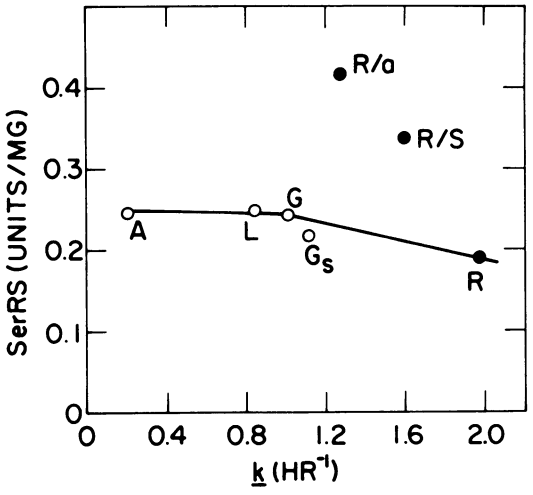

FIG. 2. *Levels of seryl-tRNA synthetase of S. typhimurium NTI in diverse media. The procedures, symbols, and abbreviations are as for Fig. 1.*

TABLE 1. *Gene densities of several genes in E. coli as a function of growth rate*[a]

Growth rate		Density					
k[b]	DT[c] (min)	*argS*[d]	*gltM*[e]	*gltX*[e]	*gltE*[e]	*serS*[f]	*valS*[g]
0.461	90	0.80	0.80	0.85	1.20	0.80	1.15
0.693	60	0.80	0.80	0.85	1.20	0.80	1.15
0.830	50	0.80	0.80	0.85	1.30	0.80	1.20
1.04	40	0.70	0.75	0.80	1.35	0.75	1.25
1.39	30	0.65	0.65	0.75	1.50	0.65	1.30
1.67	25	0.60	0.60	0.70	1.60	0.60	1.40
2.08	20	0.50	0.55	0.60	1.80	0.55	1.50

[a] The assumptions made and the method of calculation are given in the Results section. Values are rounded off to the nearest 0.05 gene copies per genome of DNA.

[b] First-order growth rate constant.

[c] DT, Doubling time in minutes.

[d] Arginyl-tRNA synthetase, 35 min on the *E. coli* K-12 genetic map. (All mapping references may be found in reference 18, except that for *gltX*, which is in reference 13.)

[e] Glutamyl-tRNA synthetase *gltM*, 38 min; *gltX*, 45 min; *gltE*, 72 min.

[f] Seryl-tRNA synthetase, 20 min.

[g] Valyl-tRNA synthetase, 85 min.

have been shown to be regulated in parallel with stable RNA in wild-type strains during steady-state growth and during carbon-energy mediated shifts-up and -down (21, 20), we compared the RNA/protein values with the synthetase levels under our growth conditions. The tight coupling of RNA/protein with growth rate is readily apparent in Fig. 3. Similar results were seen in *S. typhimurium* NT1 (data not shown). This close proportionality of RNA/protein with growth rate was independent both of medium composition and of the particular temperature-sensitive mutation used to restrict the cells. The only exception was found in *E. coli* NC34 (G-factor), which is known to have altered RNA control at restricted temperatures (2).

Synthetase levels in *E. coli* NC3 derivatives with temperature-sensitive lesions related to protein synthesis. In contrast to the RNA/protein behavior, synthetase levels in cells growing at restricted rates were, in some instances,

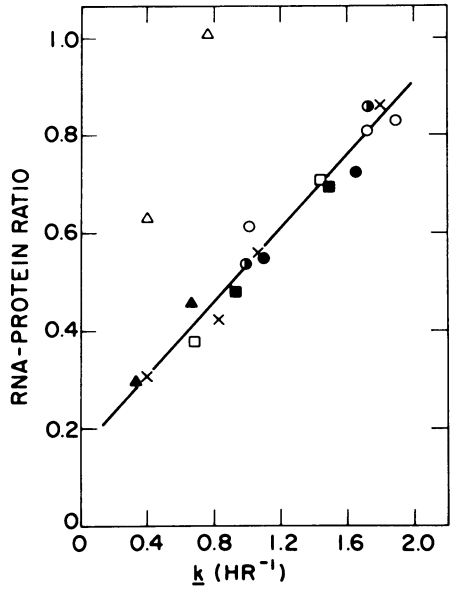

FIG. 3. *Ratios of RNA to protein in E. coli NC3 wild-type and mutant derivatives growing in a wide range of growth conditions. The cells were harvested from steady-state growth, and the chemical assays were performed as described in the text. When not specified, the growth temperature was 37 C. A glucose-growth culture was included as a control for most of the determinations.* ×, *Wild-type NC3 on four media: k ≃ 0.4, acetate; k ≃ 0.8, glycerol; k ≃ 1.0, glucose; k ≃ 1.8, rich.* ○, *Wild type on rich media lacking one or more amino acids: k = 1.1, arginine, k = 1.7, methionine; k = 1.9, branched-chain amino acids.* ●, *Wild type on rich media with a poor carbon source: k = 1.1, acetate, k = 1.7, glycerol.* □, *NC36 (temperature-sensitive leucine auxotroph) at 40 C (glucose) or 36 C (rich medium lacking branched-chain amino acids).* ■, *NC32 (temperature-sensitive valyl-tRNA synthetase mutant) at 38 C (glucose) or 35 C (rich).* △, *NC34 (temperature-sensitive G-factor mutant) at 37 C (glucose) or 35 C (rich).* ▲, *NC39 (deficient adenyl cyclase mutant) plus 5 mM cAMP. For all of the mutant derivatives, the symbol at the faster growth rate represents cells grown in a rich medium, and the symbol at the slower growth rate represents cells grown in glucose medium.*

quite different from the levels in cells growing unrestricted (in different media) at the same growth rate. The data in Fig. 4A illustrate, for example, that cells restricted in valyl-tRNA formation adjusted their arginyl-tRNA synthetase levels approximately normally for the particular growth rate, whereas cells restricted in leucine biosynthesis and in G-factor function failed to make this adjustment very precisely at low growth rates. The behaviors of glutamyl-tRNA synthetase and of valyl-tRNA synthetase were similar to that of the arginine enzyme and are not shown. The behavior of seryl-tRNA synthetase (Fig. 4B) indicated that the nature of the restricting lesion was more important in determining enzyme level than was the growth rate. One consistent pattern was observed with all synthetases in all mutants except that bearing the mutant *fus* allele (G factor): the level of enzyme in restricted cultures in rich medium was higher than in more slowly growing restricted cultures in minimal medium.

Effect of catabolite repression and cAMP on synthetase levels in *E. coli*. Strain NC39, a derivative of *E. coli* NC3 with a deficient adenyl cyclase activity, grows at only one-third the normal rate in glucose minimal or rich media. The data in Fig. 5A and B indicate that these cells have elevated levels of arginyl- and valyl-tRNA synthetases and that addition of cyclic adenosine 3',5'-monophosphate (cAMP; 5 mM) partially overcomes the growth restriction but does not permit a fine adjustment of synthetase level to growth rate. Data (not shown) for glutamyl-tRNA synthetase and seryl-tRNA synthetase were similar.

Wild-type cells (strain NC3) grew more slowly in rich medium when acetate or glycerol was used in place of glucose, and their synthetase levels (Fig. 5A and B) were only roughly near the value predicted by the growth rate. Major deviations included a high level of glutamyl-tRNA synthetase (not shown) in enriched acetate medium and a low level of seryl-tRNA synthetase (not shown) and valyl-tRNA synthetase in enriched glycerol medium.

Effects of individual amino acids on synthetase levels. Attention was given to the effect of exogenously supplied amino acids because of the implication of endogenous amino acids in the regulation of some synthetases. Included in Fig. 1 and 2 are the responses of the four synthetases to the inclusion or omission of both cognate and noncognate amino acids. Several results are noteworthy. First, in *E. coli* (Fig. 1), the omission of arginine from the rich medium reduced the growth rate to that found in glucose minimal medium and yet did not lead to a proportional reduction in synthetase level. Three of the synthetases remained at, or close to, the level found during unrestricted growth in rich medium, and the fourth one, seryl-tRNA synthetase, actually was elevated to nearly double that level. Except for this result with arginine omission, no other dramatic effects on the synthetase level were brought about by adding or omitting individual amino acids (or families, in the case of the branched-

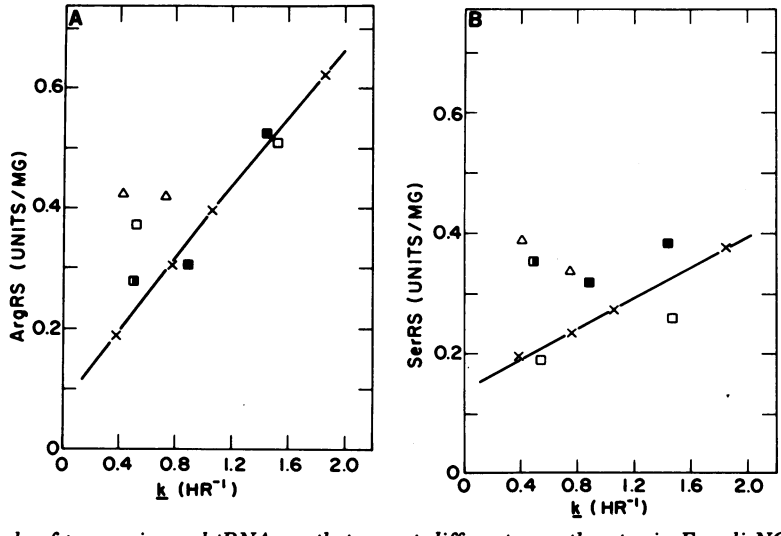

FIG. 4. *Levels of two aminoacyl-tRNA synthetases at different growth rates in E. coli NC3 and mutant derivatives. The points represent specific activities measured in cultures growing in steady state. The symbols and conventions are the same as for Fig. 3, with one addition: ◨, NC32 (temperature-sensitive valyl-tRNA synthetase mutant) at 39 C (glucose). (A) Arginyl-tRNA synthetase. (B) Seryl-tRNA synthetase.*

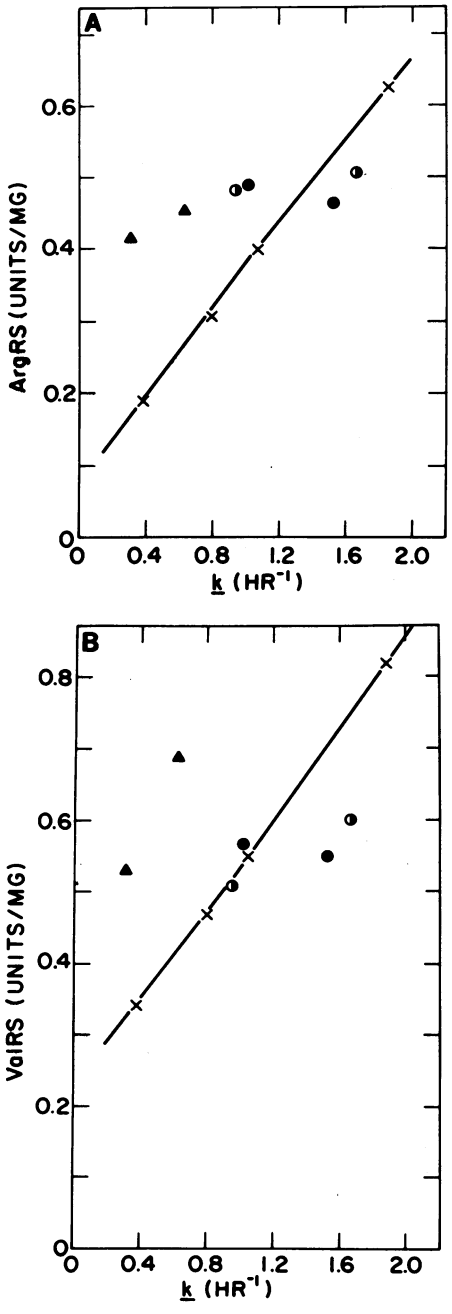

FIG. 5. *Levels of two aminoacyl-tRNA synthetases, under several growth conditions, in E. coli NC3 and adenyl cyclase mutants. The points represent specific activities measured in cultures growing in steady state. The basic media values (×) and the line are redrawn from Fig. 1A and B. ×, Wild-type NC3 on four media: k ≃ 0.4, acetate; k ≃ 0.8, glycerol; k ≃ 1.1, glucose; k ≃ 1.8, rich. ●, Wild type on rich media with poor carbon sources: k = 1.0, acetate, k = 1.5, glycerol. ▲, NC39 (deficient adenyl cyclase mutant). ◑, NC39 plus 5 mM cAMP. The symbol at the faster growth rate represents cells grown in a rich medium, and the symbol at the slower growth rate represents cells grown in glucose minimal medium. (A) Arginyl-tRNA synthetase. (B) Valyl-tRNA synthetase.*

chain amino acids), whether cognate or noncognate for a particular synthetase.

Second, in *S. typhimurium* the omission of arginine from rich medium had the same effect on growth rate and on the level of these four synthetases as in *E. coli* (data shown only for the serine enzyme in Fig. 2). Here, again, the seryl-tRNA synthetase displayed the greatest elevation during arginine restriction. In *S. typhimurium*, however, there was also an elevation of this enzyme when serine was omitted from rich medium (Fig. 2). No other effects of individual amino acids were observed.

Involvement of serine in regulation of seryl-tRNA synthetase in *S. typhimurium*. The lack of variation of seryl-tRNA synthetase with the growth rate in *S. typhimurium*, coupled with the fact that either arginine or serine omission from rich medium brought about a marked elevation of this enzyme activity (Fig. 2), led us to explore the regulation of this enzyme further.

A chance observation had earlier been made that the initial concentration of L-serine in the rich medium had to be 10 mM (about 10-times higher than that of other amino acids) or else diauxic growth occurred. Cells grown in rich medium with a low, normal concentration of serine (0.4 mM) and harvested at an OD of 1.0 after the diauxic lag had occurred were found to have an elevated level of seryl-tRNA synthetase (Fig. 6). Cells grown in rich medium with sufficient L-serine (10 mM) to support rapid growth without a diauxic shift had a low level of seryl-tRNA synthetase (Fig. 2 and 6). A reasonable explanation of the fact that a level of serine theoretically capable of supporting growth to an OD of about 10 was evidently depleted early in growth is that this amino acid was being catabolized rapidly by L-serine deaminase. This explanation was strongly supported by an observation (data not shown) that the omission of leucine and glycine or of leucine, glycine, and threonine virtually abolished the diauxic characteristic of growth on rich medium with a low concentration of serine (0.4 mM) and simultaneously lowered the level of seryl-tRNA synthetase; these combinations of amino acids are inducers of L-serine deaminase, at least in *E. coli* (11, 19).

Also reported in Fig. 6 are results with a glucose medium enriched only with amino acids (not purines, pyrimidines, or vitamins)

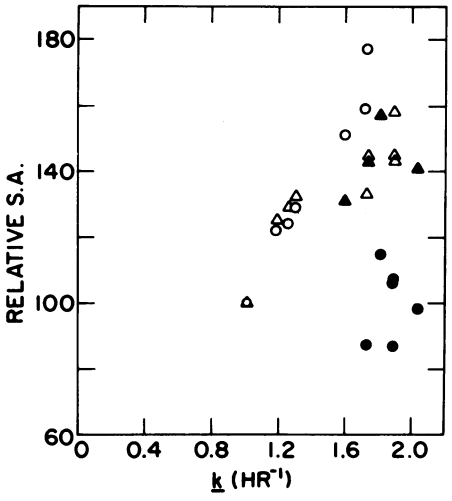

FIG. 6. *Levels of two aminoacyl-tRNA synthetases as a function of growth rate and cognate amino acid concentration in S. typhimurium NTI. The points represent specific activities measured in cultures growing in steady state on synthetic media with various amounts of amino acids. Except for glucose cultures ($k \simeq 1$), the media contained the 20 amino acids in the concentrations indicated in reference 16, except that serine was only 0.4 mM unless otherwise indicated. The cultures growing at a k of 1.6 or higher also contained the other rich medium supplements (bases and vitamins) described in reference 16. All of the values were normalized to a relative specific activity of 100 as the value of the respective enzyme in cells grown on glucose. Circles, Seryl-tRNA synthetase; the serine concentration was varied. Triangles, Valyl-tRNA synthetase; the valine concentration was varied. The filled symbols represent cultures containing greater than 4 mM cognate amino acid. The open symbols represent cultures containing either 0.4 mM serine or 0.6 mM valine.*

and with serine at a low concentration. This medium supported growth at a rate midway between glucose minimal and glucose-rich medium and yielded a level of seryl-tRNA synthetase likewise midway between that in cells grown on glucose minimal and glucose-rich medium with low serine. The implication, therefore, is that, as the rate of utilization of seryl-tRNA increases, the concentration of serine or some serine derivative falls, leading to derepression of seryl-tRNA synthetase. Saturating the cell with exogenous serine eliminates both the deficiency of serine and the elevation of the synthetase. This situation, which superficially leads to the same result as the other growth rate-related variations, seems to be restricted to this enzyme of those studied. Data are shown in Fig. 6 for valine and, as already presented in Fig. 1 for the other synthetases, indicating no involvement of the cognate amino acid in the variation of synthetase level with growth rate.

Levels of biosynthetic enzymes. The results presented in the last section illustrate how a growth rate related variation in synthetase level might be brought about simply by subtle limitations of endogenous amino acid supply. On many grounds (discussed later) it is difficult to imagine that such a mechanism is generally responsible for the effects we have observed, but direct evidence is always useful. We have examined the levels of two biosynthetic enzymes with the notion that these levels could tell us something of the internal supply of amino acids under different growth conditions: ornithine transcarbamylase (for arginine) and threonine deaminase (for branched-chain amino acids). The results for threonine deaminase are shown in Table 2. They indicate that there is a systematic rise of threonine deaminase with rising growth rate only in *S. typhimurium*, not in *E. coli*, and only over the growth rate range presented by cultures growing on carbon sources poorer than glucose. Assuming that the level of threonine deaminase would reflect endogenous valine supply, there appears to be no limitation in cells growing in rich, complete medium. Furthermore, omitting the branched-chain amino acids (or simply valine) from rich medium elevates the threonine deaminase (Table 2) without affecting the valyl-tRNA synthetase level (Fig. 1B). Since arginine biosynthetic pathway control is modified

TABLE 2. *Specific activities of threonine deaminase from steady-state growing cultures of E. coli NC3 and S. typhimurium NT1*

Medium	*E. coli*		*S. typhimurium*	
	Sp act[a]	k[b]	Sp act[a]	k[b]
Acetate	34.7	0.416	13.1	0.297
Glycerol	39.1	0.770	24.6	0.866
Glucose	38.8	1.07	29.0	1.06
G + BBC[c]	22.0	0.945	—[d]	—
Rich[e]	8.51	1.89	2.02	2.03
Rich + BBC[f]	8.64	1.98	—	—
Rich − Val[g]	15.8	1.89	4.66	1.89
Rich − Arg[h]	27.2	1.04	3.57	1.30

[a] Micromoles of α-ketobutyrate formed per hour per milligram of protein.

[b] First-order growth rate constant.

[c] Glucose plus balanced, branched-chain amino acids (1.2 mM valine, 0.6 mM isoleucine, 0.6 mM leucine).

[d] Not measured.

[e] Synthetic rich medium.

[f] Rich medium plus balanced, branched-chain amino acids (see footnote c).

[g] Rich medium lacking valine.

[h] Rich medium lacking arginine.

in our strain of *E. coli*, the ornithine transcarbamylase measurements were performed only in *S. typhimurium*. The results (not shown) were that this enzyme was present at a near constant level in acetate-, glycerol-, and glucose-grown cells, was present at a very low level in rich medium, and was derepressed fourfold over the minimal medium level in cells grown in rich medium lacking arginine. The arginyl-tRNA synthetase in this strain, it may be recalled, varies monotonically with growth rate except for a great elevation in arginine-free, rich medium.

Altogether, these results argue against the endogenous level of arginine or valine being responsible for growth rate modulation of arginyl- and valyl-tRNA synthesis.

Effects of amino acid deprivations on synthetase level. The experience with serine depletion from rich medium suggested not only that this and similar depletions are likely to be frequently encountered by prototrophic enteric organisms, but also that such deprivations should be examined to learn how cells cope with such a stress. Specifically, we were interested in whether the cells respond to depletion of a single amino acid according to the amino acid-linked control of synthetases (which would predict a derepression) or according to metabolic regulation (which would predict either no change or, in some cases, a slight decrease in synthetase accumulation). In *S. typhimurium* NT1, as noted, exhaustion of serine from the rich medium leads to a diauxic lag followed by growth at a somewhat reduced rate. In *E. coli* NC3, exhaustion of arginine, methionine, or serine was marked by diauxic lags of characteristic duration. No lag was discernible when cells ran out of valine, but abrupt transfer of cells to a valine-free, rich medium did induce a growth lag. No lag was detected either upon exhaustion of glutamate (and glutamine) from the medium or by transfer of cells to a rich medium lacking these amino acids.

The differential rate of accumulation of seryl-tRNA synthetase in *S. typhimurium* cells during a serine down-shift is shown in Fig. 7; there was an initial 11-fold increase followed by a permanent 2.2-fold increase in accumulation of this enzyme, while no change occurred in the rate of accumulation of valyl-tRNA synthetase. In *E. coli*, serine removal brought about a two-fold rise in seryl-tRNA synthetase formation, but removal of valine or glutamate did not change the rate of accumulation of either their cognate synthetases or that for arginine (data not shown). Figure 8 shows the growth response of *E. coli* NC3 to exhaustion of arginine from rich medium, followed by readdition of

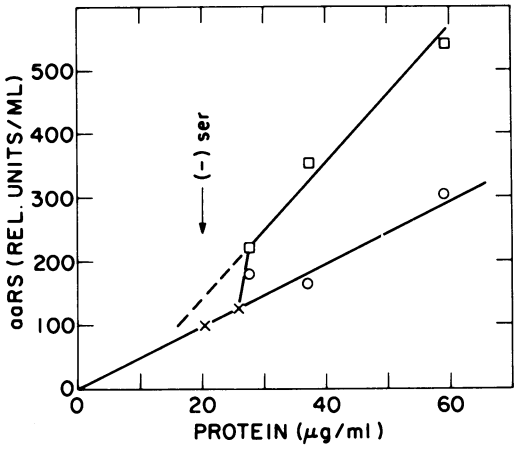

FIG. 7. *Differential plot of the rates of accumulation of seryl- and valyl-tRNA synthetases of S. typhimurium NTI during a serine exhaustion from rich medium. The growth rates were: preshift, k = 2.08; postshift, k = 1.89. The initial concentration of serine was 0.8 mM. The values are normalized to 100 as the value of the first sample. Symbols: ×, both synthetases; ○. valyl-tRNA synthetase; □, seryl-tRNA synthetase.*

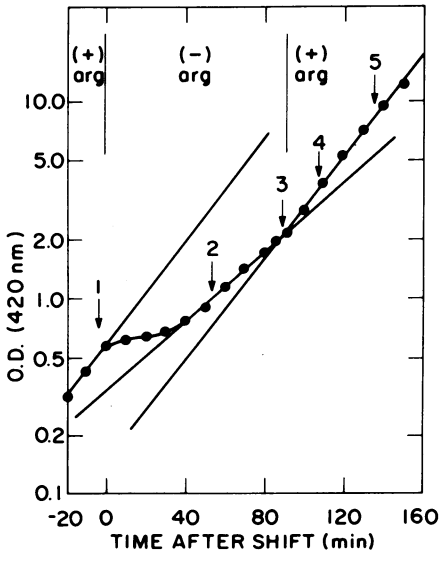

FIG. 8. *Growth response of E. coli NC3 to arginine deprivation and readdition in rich medium. The growth rates were: (+) arginine, k = 1.98; (−) arginine, k = 1.26; (+) arginine, k = 1.81. The initial concentration of arginine was 0.03 mM. The down-shift occurred when the cells exhausted the arginine from the medium. The arginine addition was done by diluting the cells fourfold into fresh medium containing arginine to give a final concentration of 0.4 mM, the normal concentration in rich medium. The OD values have been corrected for this dilution. At the arrows, samples were taken for enzyme measurement (see Fig. 9).*

arginine. The differential rates of accumulation of three synthetases are shown in Fig. 9. Neither the cognate enzyme, arginyl-tRNA synthetase nor valyl-tRNA synthetase showed any perturbations during these shifts; seryl-tRNA synthetase, however, derepressed nearly threefold upon the removal of arginine and returned to a normal rate of formation after the readdition of arginine. Subsequent experiments showed that the presence of ornithine (6 mM) or putrescine (0.1 mM) in the rich medium lacking arginine changed neither the growth rate nor the level of seryl-tRNA synthetase. Also, restriction for other amino acids, such as methionine, did not elevate the serine enzyme. In summary, only seryl-tRNA synthetase of the enzymes studied exhibited a response to depletion of its cognate amino acid from rich medium, and this enzyme also responded to arginine depletion.

DISCUSSION

The data in this study consist of measurements of enzyme activity in crude extracts, and their validity is therefore subject to question. Previous work from this laboratory (20, 21), however, has shown that such measurements by the procedures we have specified are true reflections of the rate of synthesis of arginyl-tRNA synthetase and valyl-tRNA synthetase in *S. typhimurium*. We assume that this validation likely holds for *E. coli* as well. Less evidence exists for the other two enzymes studied here, but preliminary measurements with antiserum prepared against seryl-tRNA synthetase (and kindly provided by W. Konigsberg) verified the results with enzyme assays in the few cases tested. Other, more indirect evidence, is provided by the fact that synthetase inactivation, when it occurs, is usually brought on by amino acid restriction (18), and we are reporting here a series of results in which the exogenous presence of individual amino acids has either no effect or a repressing effect on synthetase level. Also, *E. coli* NC3 cells growing on rich medium lacking arginine resemble cells growing on glucose minimal medium with respect to growth rate, RNA/protein ratio, and ease of sonic disruption; yet the levels of all four synthetases we studied more closely resemble those in cells growing in unrestricted, rich medium, eliminating these differences as possible sources of some systematic error.

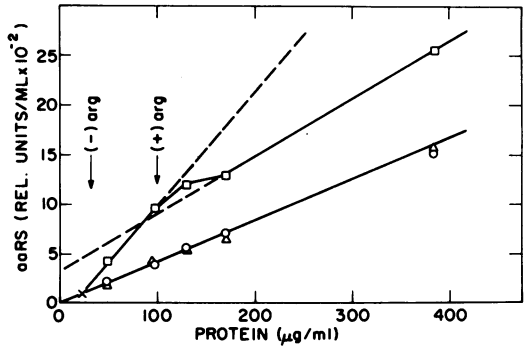

FIG. 9. *Differential plot of the rates of accumulation of arginyl-, seryl-, and valyl-tRNA synthetases of E. coli NC3 during arginine deprivation and readdition in rich medium. The growth response of the culture is shown in Fig. 8. The samples were taken at the growth intervals indicated by the arrows in Fig. 8. All the values are normalized to 100 as the value of the first sample. Symbols: ×, all three synthetases; △, arginyl-tRNA synthetase; ○, valyl-tRNA synthetase; □, seryl-tRNA synthetase.*

Generality of growth rate-dependent regulation. Evidence now exists for specific amino acid involvement (repression?) in the regulation of the aminoacyl-tRNA synthetases for arginine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, and valine (18). In one or another strain of *E. coli* or *S. typhimurium* these synthetases display either a transient or a long-lasting elevation in level during cell growth restricted by the supply of the specific amino acid. For the remaining 10 synthetases, there have been no reports of results with amino acid restriction. The results presented here bring the number of synthetases reported to display growth rate-related variation (metabolic regulation) to five: those for arginine, glutamic acid, methionine (5), serine (in *E. coli* only), and valine. Preliminary results not reported here extend this behavior also to the synthetases for glycine, isoleucine, threonine, and phenylalanine. Leucyl-tRNA synthetase (21) seems to show a marginal but definite degree of metabolic regulation, somewhat like seryl-tRNA synthetase in *E. coli*. The conclusion seems to be that a large number of synthetases display growth rate-related regulation, although the quantitative aspects of the phenomenon vary widely. Furthermore, there may be other synthetases like the seryl-tRNA synthetase of *S. typhimurium* reported here, which show no tendency to increase with increasing growth rate. Further systematic evaluation of synthetase levels is necessary before one can assess the meaning of this heterogeneous behavior. Interestingly, in yeast there is now evidence that at least 15 synthetases display a marked metabolic regulation, varying 3- to 20-fold in proportion to growth rate (R. C. Johnson, P. R. Vanatta, and J. R. Fresco, personal communication). The general tendency in eukaryotic systems for syn-

thetase level to be proportional to the rate of protein synthesis has been reviewed recently (18).

Regulation of synthetases and stable RNA. Earlier work has shown a similarity between the regulation of synthetases and of stable RNA. The synthetases for arginine, valine, and glutamic acid increase in level as much as does stable RNA with increasing growth rate (20, 21; results presented here). Furthermore, the behavior of the arginine enzyme during a nutritional shift-up (21) or shift-down (20) strongly resembles the behavior of ribosomal (or transfer) RNA. What we have shown here, however, is that the regulation of synthetases is not identical to that of stable RNA. The data in Fig. 3 through 5 show that the apparent coupling between synthetases and stable RNA is quite loose; growth of mutants under different restrictions can easily result in noncoordinate changes in steady-state levels of synthetases and stable RNA. It is possible that some of this non-coordination occurs as the result of restriction for a particular amino acid and, therefore, represents an override of the growth rate-related control by a control geared to the level of the individual amino acid. Not enough is known of the metabolism of individual amino acids during growth under various conditions to evaluate this possibility in all cases, but it seems reasonable, for example, to expect changes in amino acid catabolism as the result of changes in cAMP level or during growth in amino acid-containing media with poor carbon sources.

Synthetase regulation and cAMP. The results presented in Fig. 5 indicate that severe alterations in the cAMP metabolism level in a cell can disrupt the simple coordination between growth rate and the level of a particular synthetase. Nevertheless, the adenyl cyclase mutant still exhibits higher synthetase levels during fast growth in rich medium than during slow growth in minimal medium. Also, conditions that impose an enormous difference in the degree of catabolite repression in a cell (growth of the cyclase mutants with and without cAMP, for example) produce small changes (of no particular pattern) in synthetase level. It seems, then, that the formation of aminoacyl-tRNA synthetases is not under a straight-forward control by a cAMP-related signal.

Synthetase regulation and amino acid supply. Since many synthetases become derepressed, at least transiently, during endogenous limitation of their amino acid substrate, we wondered whether the growth rate-related variation (metabolic regulation) could somehow be explained on the basis of a cryptic amino acid limitation. Three lines of evidence indicate that such an explanation is unlikely.

First, manipulation of exogenous amino acid supply and endogenous demand for amino acid has failed in all cases but one to disrupt metabolic regulation. Only for the seryl-tRNA synthetase of *S. typhimurium* was it possible to eliminate growth rate-related variation by high exogenous levels of the cognate amino acid (Fig. 1 and 6). Second, using biosynthetic enzyme levels as a monitor of endogenous amino acid sufficiency has not revealed some unsuspected "hidden hunger" for arginine or valine to correlate with metabolic regulation of their synthetases (Table 2). Finally, the deliberate imposition of conditions that should exacerbate a subtle endogenous hunger for an amino acid (namely, depletion of that amino acid from a rich medium) failed to elevate the synthetases for arginine, valine, or glutamic acid in *E. coli* (Fig. 9). Only the enzyme for serine in this cell responded by derepressing, and it did so upon arginine deprivation as well. In *S. typhimurium* the picture was similar, although some experiments hinted that the arginine enzyme might respond to arginine depletion (results not shown).

Mechanism of growth rate-related regulation. At the present time two apparently different modes of synthetase regulation have been uncovered. Possibly these modes could result from two different, but interacting, control systems, one using some fairly general signal which measures metabolic (or growth) rate and one using some specific form of the cognate amino acid. It would not be surprising to discover that synthetase operons are controlled by multiple elements, given the multiplicity of signals that govern individual catabolic and biosynthetic operons. Still, evidence for multiple elements controlling synthetases has not been obtained, and it is possible to envision a single control mechanism which would accomplish both a general coupling to the ribosomal translation apparatus and a specific response to amino acid limitation. Such a mechanism has been presented elsewhere (18), but there is insufficient evidence by which to chose among many alternative models.

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LITERATURE CITED

1. **Anderson, J. J., and F. C. Neidhardt.** 1972. Unusual valyl-transfer ribonucleic acid synthetase mutant of *Escherichia coli*. J. Bacteriol. 109:307-314.
2. **Atherly, A.** 1973. Temperature-sensitive relaxed phenotype in a stringent strain of *Escherichia coli*. J. Bacteriol. 113:178-182.
3. **Bird, R. E., J. Louarn, J. Martuscelli, and L. Caro.** 1972. Origin and sequence of chromosome replication in *Escherichia coli*. J. Mol. Biol. 70:549-566.
4. **Burns, R. O.** 1971. L-Threonine deaminase-biosynthetic *(Salmonella typhimurium)*, p. 555-560. *In* H. Taylor (ed.), Methods in enzymology, vol. 17B. Academic Press Inc., New York.
5. **Cassio, D., Y. Mathien, and J. P. Waller.** 1975. Enhanced level and metabolic regulation of methionyl-transfer ribonucleic acid synthetase in different strains of *Escherichia coli* K-12. J. Bacteriol. 123:580-588.
6. **Cooper, S., and C. E. Helmstetter.** 1968. Chromosome replication and the division cycle of *Escherichia coli* B/r. J. Mol. Biol. 31:519-540.
7. **Datta, P.** 1966. Purification and feedback control of threonine deaminase activity of *Rhodopseudomonas spheroides*. J. Biol. Chem. 241:5836-5844.
8. **Dennis, P. P., and H. Bremer.** 1974. Macromolecular composition during steady-state growth of *Escherichia coli* B/r. J. Mol. Biol. 84:407-422.
9. **Fujisawa, T., and A. Eisenstark.** 1973. Bi-directional chromosomal replication in *Salmonella typhimurium*. J. Bacteriol. 115:168-176.
10. **Gordon, J.** 1970. Regulation of the *in vivo* synthesis of the polypeptide chain elongation factors in *Escherichia coli*. Biochemistry 9:912-917.
11. **Isenberg, S., and E. B. Newman.** 1974. Studies on L-serine deaminase in *Escherichia coli* K-12. J. Bacteriol. 118:53-58.
12. **Jones, M. E., L. Spector, and F. Lipmann.** 1955. Carbamyl phosphate, the carbamyl donor in enzymatic citrulline synthesis. J. Am. Chem. Soc. 77:819-820.
13. **Lapointe, J., and G. Delcuve.** 1975. Thermosensitive mutants of *Escherichia coli* K-12 altered in the catalytic subunit and in a regulatory factor of the glutamyl-transfer ribonucleic acid synthetase. J. Bacteriol. 122:352-358.
14. **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.
15. **Maaløe, O., and N. O. Kjeldgaard.** 1966. Control of macromolecular synthesis. W. A. Benjamin, New York.
16. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. 119:736-747.
17. **Neidhardt, F. C. and B. Magasanik.** 1960. Studies on the role of ribonucleic acid in the growth of bacteria. Biochem. Biophys. Acta 42:99-116.
18. **Neidhardt, F. C., J. Parker, and W. G. McKeever.** 1975. Function and regulation of aminoacyl-tRNA synthetases in prokaryotic and eukaryotic cells. Annu. Rev. Microbiol. 29:215-250.
19. **Pardee, A. B., and L. S. Prestidge.** 1955. Induced formation of serine and threonine deaminases by *Escherichia coli*. J. Bacteriol. 70:667-674.
20. **Parker, J., M. Flashner, W. G. McKeever, and F. C. Neidhardt.** 1974. Metabolic regulation of the arginyl and valyl transfer ribonucleic acid synthetases in bacteria. J. Biol. Chem. 249:1044-1053.
21. **Parker, J., and F. C. Neidhardt.** 1972. Metabolic regulation of aminoacyl-tRNA synthetase formation in bacteria. Biochem. Biophys. Res. Commun. 49:495-501.
22. **Schneider, W. C.** 1945. Phosphorous compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pertose nucleic acid. J. Biol. Chem. 161:293-303.
23. **Skjold, A. C., H. Juarez, and C. Hedgcoth.** 1973. Relationships among deoxyribonucleic acid, ribonucleic acid, and specific transfer ribonucleic acids in *Escherichia coli* 15T at various growth rates. J. Bacteriol. 115:177-187.