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Chemical Measurement of Steady-State Levels of Ten Aminoacyl-Transfer Ribonucleic Acid Synthetases in *Escherichia coli*

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Polypeptide chains of 10 aminoacyl-transfer ribonucleic acid synthetases (those for arginine, glutamine, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, threonine, and valine) have been identified in lysates of *Escherichia coli* resolved by the O'Farrell two-dimensional gel system. By labeling cells uniformly with [^{14}C]glucose and by measuring the total amounts of these polypeptides by their radioactivity, estimations of the steady-state, molecular amounts of these enzymes were made and compared to the number of ribosomes and elongation factors in these cells. Portions of a reference culture grown on glucose and labeled with [^{14}C]leucine or [^{35}S]sulfate were mixed with four cultures grown in widely different media containing [^{3}H]leucine or [^{3}H]leucine plus [^{3}H]isoleucine. From the isotope ratios of the total protein and of the spots containing the synthetase chains, the chemical amount of each synthetase relative to that of the reference culture was determined. The results, where comparable, show reasonable agreement with enzyme activity measurements. In general, these synthetases each exhibit a positive correlation with growth rate in unrestricted media, indicating a strong tendency for the levels of transfer ribonucleic acid, synthetases, elongation factors, and ribosomes to remain approximately, though not exactly, in balance at different growth rates.

Studies of enzyme regulation have traditionally relied largely on enzyme activity assays to quantitate enzyme levels and rates of synthesis. Several problems are associated with such measurements. The presence of inhibitors or activators of enzyme activity can yield misleading results. Susceptibility of enzymes to inactivation and/or degradation in vivo can lead to underestimation of their rate of formation; changes in rates of inactivation may simulate changes in rates of synthesis. Mutant enzymes cannot easily be compared in amount with wild-type enzymes. Finally, for enzymes that vary in amount only two- to fourfold, measurements of transient changes are virtually impossible.

All of these difficulties have been encountered in work with aminoacyl-transfer ribonucleic acid (tRNA) synthetases (reviewed in reference 11). Efforts have been made to bypass some of these problems by using specific antisera to measure enzyme protein (e.g., references, 4, 13, 18), by density labeling with deuterium followed by resolution of heavy and light enzyme to detect inactivation of preformed enzyme (e.g., references 1, 20), and by chemical purification of synthetases following double isotope labeling to estimate transient rates of protein synthesis (14).

The idea of the present study was to apply the O'Farrell (12) two-dimensional gel system for resolving total cell protein to obtain hitherto unavailable data on aminoacyl-tRNA synthetases. The specific objectives have been (i) to measure the cellular content of synthetases in absolute chemical units, (ii) to compare molecular amounts of synthetases with each other and with interacting parts of the protein synthesizing machinery, and (iii) to examine these parameters at different growth rates. These objectives have been achieved for 10 enzymes whose subunits have been successfully located in the gels. Fortunately, they include four enzymes that have already been examined for growth rate-related regulation by other methods: arginyl-tRNA synthetase (ArgRS), glutamyl-tRNA synthetase (GluRS), leucyl-tRNA synthetase (LeuRS), and valyl-tRNA synthetase (ValRS). Three others, isoleucyl-tRNA synthetase (IleRS), phenylalanyl-tRNA synthetase (PheRS), and threonyl-tRNA synthetase (ThrRS) have previously been studied

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only for amino acid-related regulation. For the remaining three, lysyl-tRNA synthetase (LysRS), glutaminyl-tRNA synthetase (GLnRS), and glycyl-tRNA synthetase (GlyRS), no systematic studies of growth rate-related regulation have been published.

MATERIALS AND METHODS

Bacterial strain. The *Escherichia coli* B/r derivative NC3 is a prototrophic strain possessing several characteristics desired in a standard strain: rapid growth in minimal medium, synchronizability, smooth growth during biosynthetic or nutritional restrictions, and a defective B-type deoxyribonucleic acid (DNA) restriction system.

Media. All media were totally defined; synthetic media were produced by adding one or more supplements to morpholinopropane sulfonate (MOPS) buffered medium (10). Carbon sources were used at the following concentrations: 0.4% (wt/vol) potassium acetate; 0.4% (vol/vol) glycerol; and 0.4% (wt/vol) D-glucose. Rich medium was made by adding 20 L-amino acids, four nucleic acid bases, five vitamins, and D-glucose to obtain the following concentrations (W. G. McKeever and B. L. Wanner, personal communication): 0.1 mM cysteine (dissolved and added just before use) and tryptophan; 0.2 mM histidine, methionine, and tyrosine; 0.4 mM arginine, asparagine, aspartate, isoleucine, lysine, phenylalanine, proline, and threonine; 0.6 mM glutamine, glutamate, and valine; 0.8 mM alanine, glycine, and leucine; 10.0 mM serine; 0.2 mM adenine, guanine, cytosine, and uracil; 0.02 mM thiamine-hydrochloride, calcium-pantothenate, *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, and 2,3-dihydroxybenzoic acid; 0.4% (wt/vol) glucose. All components (except water) were filter sterilized; water was autoclaved in tightly capped bottles.

Bacterial growth. Cultures were grown aerobically at 37°C in Erlenmeyer flasks with rotary shaking. Bacterial mass was monitored at 420 nm with a Zeiss PMQII spectrophotometer. All growth rates were determined in cultures between optical densities at 420 nm of 0.1 and 2.0 by suitably diluting the culture before optical density measurement. Growth rates are expressed in terms of the specific growth rate constant k, as calculated from the expression k = (ln 2)/(mass doubling time [hours]).

Radioactive labeling. Cultures to be labeled uniformly with D-[U-^{14}C]glucose were grown for at least six generations in glucose plus MOPS medium containing D-glucose (9.4 mM; 27 μCi/μmol). For uniform labeling with L-leucine in minimal media, cultures were grown with the designated carbon source (acetate, glycerol, or glucose) and a labeling mixture consisting of L-isoleucine (80 μM), L-valine (120 μM), and either L-[4,5-^{3}H]leucine (160 μM; 125 μCi/μmol), or L-[U-^{14}C]leucine (250 μM; 135 μCi/μmol). For the rich medium, the labeling mixture of branched-chain amino acids was used in place of the regular amounts of these amino acids.

In some experiments protein was labeled by growth of the cells in MOPS medium containing [^{35}S]K_2SO_4 and mixed with cultures labeled with a mixture of L-[4,5-^{3}H]leucine and L-[4,5(m)-^{3}H]isoleucine as described previously (2).

Two-dimensional gels. A sample of culture (80 to 1,000 μg of protein) was rapidly chilled. The cells were collected by centrifugation, washed with 1 volume of 10 mM tris(hydroxymethyl)aminomethane, 5 mM $MgCl_2$, pH 7.4, and then resuspended in 0.08 ml of the same buffer. The cells were lysed by sonic treatment for three 15-s pulses at a power setting of no. 3 using a microtip (Heat Systems Ultrasonics, Inc.). Throughout sonication the samples were chilled in an ice-water bath. The lysates were treated essentially as described by O'Farrell (12) with certain modifications (17). Gels had to be soaked overnight in the staining solution to obtain ideal staining in a reproducible manner. The gels were then dried under vacuum on filter paper, and autoradiograms were made.

Measurement of radioactivity in gels. Two methods were used to measure the amount of various isotopes in the two-dimensional polyacrylamide gels. In the first, used exclusively when ^{3}H and ^{14}C were to be determined, portions of interest in the gels were cut out with a punch made from a syringe needle (15 to 18 gauge) and oxidized in a Packard sample oxidizer. After resolution of the ^{3}H as water and the ^{14}C as CO_2, the samples were counted in Monophase 40 (for ^{3}H) and a mixture of Carbosorb II and Permafluor V (for ^{14}C) according to Packard instructions.

In the second method, used with ^{3}H and ^{35}S mixtures, the portions of interest in the gels were cut out and processed as previously described (17).

Identification of spots. Purified proteins received from the sources listed in Table 1 were used to determine the correspondence of a particular polypeptide with a particular protein spot on the O'Farrell gel system (Fig. 1). Identification was usually based on inspection of gels containing stainable quantities of marker protein and radioactive *E. coli* NC3 total protein and gels containing just the latter.

Chemicals and radiochemicals. All chemicals were the highest grade available from ordinary commercial suppliers. Radioactive glucose was purchased from Amersham-Searle Corp. (Arlington Heights, Ill.) and [^{35}S]sulfate was obtained from New England Nuclear Corp. (Boston, Mass.). Radioactive amino acids were purchased either from Amersham-Searle or Schwarz/Mann (Div. of Becton, Dickinson & Co., Orangeburg, N. Y.). The ampholine and other materials used in the polyacrylamide gels were obtained from the suppliers listed by O'Farrell (12).

RESULTS

Qualitative reliability of the gels. Separated protein spots in the O'Farrell two-dimensional gel system are expected on theoretical grounds to be homogeneous polypeptides (12). This assumption was examined by mixing cultures of *E. coli* strain NC3 grown in the same medium, but containing in one case [^{3}H]leucine and

TABLE 1. *Identification of polypeptides of aminoacyl-tRNA synthetases on two-dimensional gels*

Protein	Donor	Subunit			
		Structure	Name	Mol wt[a]	Spot identification[b]
ArgRS	W. Konigsberg	α	α	70,000	E58.0
GlnRS	H. Weissbach	α	α	69,000	G61.0
GluRS	D. Söll	$\alpha\beta$	α	56,000	
			β	46,000	F48.1
GlyRS	P. Berg	$\alpha_2\beta_2$	α	33,000	
			β	80,000	E77.5
IleRS	M. Yarus; W. Konigsberg	α	α	114,000	F107
LeuRS	H. Weissbach	α	α	104,000	D100
LysRS	I. Hirshfield	α_2	α	69,000	D58.5
PheRS	A. Böck	$\alpha_2\beta_2$	α	38,000	G36.0
			β	96,000	D94.0
ThrRS	H. Weissbach				G65.0
ValRS	D. Söll	α	α	110,000	E106

[a] Subunit structures and molecular weight estimations of these enzymes are taken from published accounts referenced in the review by Neidhardt et al. (11), except LysRS information is from a manuscript supplied by I. Hirshfield.

[b] The nomenclature of the spots will be described in a future note. Their location is given in Fig. 1. The position of polypeptides in the second (vertical) dimension of O'Farrell gels is chiefly a function of molecular weight (mol wt). In most cases the position of marker proteins is consistent with reported values for their molecular weight. Apparent exceptions are: spot E58.0 (reported mol wt: 70,000; apparent mol wt: 58,000), spot D58.5 (reported mol wt: 69,000; apparent mol wt: 58,500), and spot G61.0 (reported mol wt: 69,000; apparent mol wt: 61,000).

[^{3}H]isoleucine, and in the other, [^{35}S]sulfate. The ^{3}H/^{35}S ratio of each spot in gels made from these lysates was therefore a function of the (leucine plus isoleucine)-(cysteine plus methionine) content of the particular polypeptide. For any one gel, therefore, counting different parts of the same spot should give an invariant ratio if the spot is a single polypeptide. Also, the ^{3}H/^{35}S ratio of each spot in one gel, normalized to the ^{3}H/^{35}S content of the total protein should be the same as the corresponding spot in another gel made from cells grown in a different medium. Examples of this latter check are presented in Table 2. They support the notion that these resolved spots are indeed homogeneous.

When duplicate extracts, differently labeled, were mixed and run on gels, individual spots were found to differ in isotope ratio across their horizontal dimension. Apparently, migration of polypeptides in this system (and particularly in the isoelectric focusing dimension) is perceptibly affected by subtle changes in polypeptide structure after cell rupture. This problem was eliminated by mixing whole cells of the different cultures before sonication.

Synthetase amounts in reference culture. A culture of *E. coli* strain NC3 grown in glucose MOPS at 37°C with [^{14}C]glucose to label proteins uniformly was used to prepare a two-dimensional gel. The total radioactivity in each synthetase polypeptide and in the entire gel was measured. From these values the numbers of molecules of each synthetase per genome-equivalent of DNA could be calculated from the data of Dennis and Bremer (5) as described in Table 3. The molecular abundance of these 10 synthetases prove to be similar, varying only 2.7-fold between the most (IleRS) and least (LysRS) abundant.

Variation of synthetase protein with growth rate. In one set of experiments, portions of a reference culture (glucose MOPS) labeled with [^{14}C]leucine were mixed with cultures grown at different rates in other media labeled with [^{3}H]leucine. Gels were prepared from each cell mixture. In another set of experiments, the reference culture was labeled with [^{35}S]SO_4 and mixed with other cultures labeled with a mixture of [^{3}H]leucine plus [^{3}H]isoleucine. For each set of experiments, the ratio of isotopes (^{3}H/^{14}C or ^{3}H/^{35}S) in each resolved protein spot divided by the ratio of isotopes (^{3}H/^{14}C or ^{3}H/^{35}S, respectively) in the unfractionated protein is a measure of the relative amount of protein in the spot compared to that in the reference culture. The data showing the relative amounts of 10 aminoacyl-tRNA synthetases are plotted as a function of growth rate in Fig. 2. Note in the ^{3}H/^{35}S data that the values at all growth rates are mathematically normalized to the value for the glucose-grown cells. Hence, the value for the glucose-grown

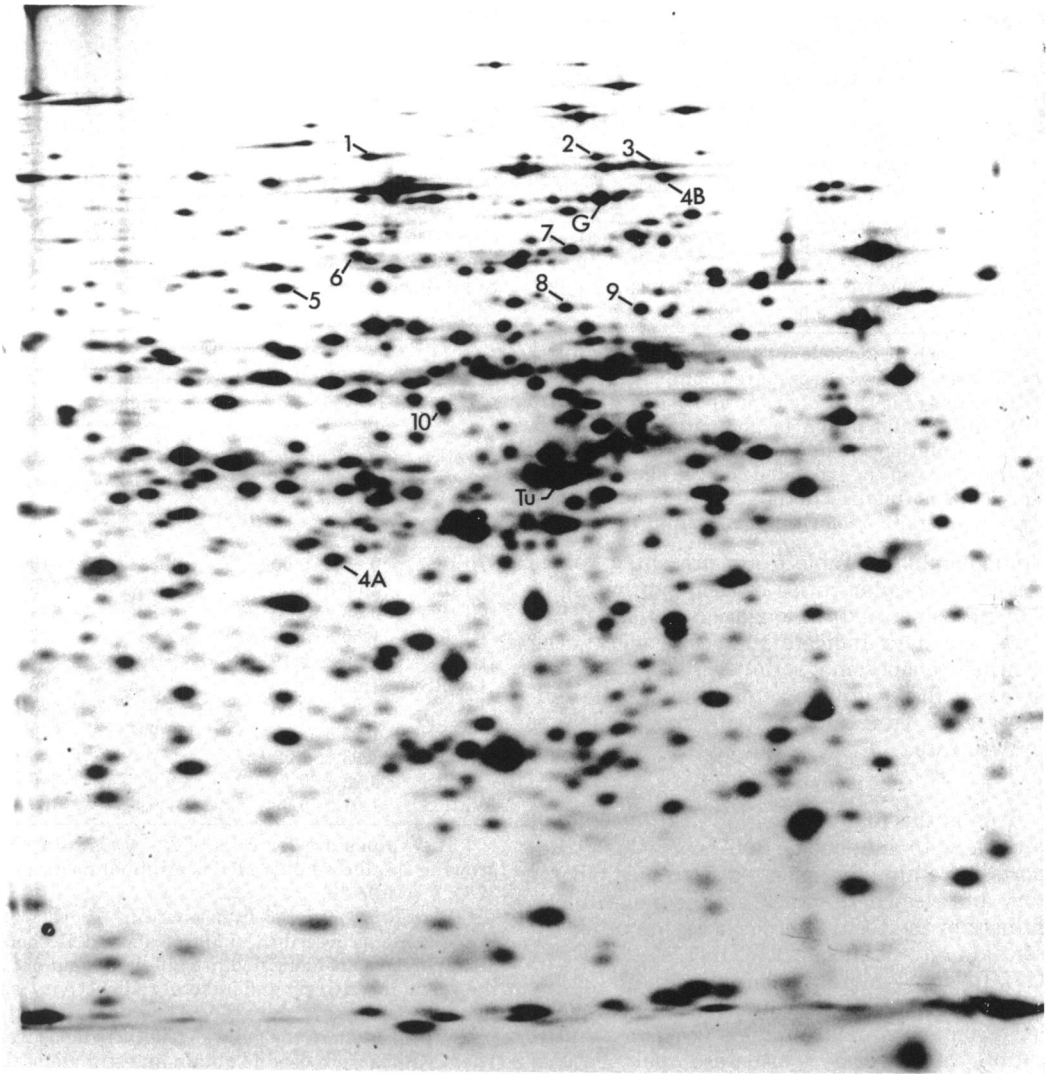

FIG. 1. *Autoradiogram of an O'Farrell gel of E. coli reference strain NC3 grown on glucose MOPS and labeled with [^{14}C]glucose, showing 10 identified synthetases, EF-G, and EF-Tu. The proteins are labeled as follows: 1, IleRS (F107); 2, ValRS (E106); 3, LeuRS (D100); 4a, PheRS (G36.0) α subunit; 4b, PheRS (D94.0) β subunit; 5, GlnRS (G61.0); 6, ThrRS (G65.0); 7, GlyRS (E77.5); 8, ArgRS (E58.0); 9, LysRS (D58.5); 10, GluRS (F48.1); G, EF-G (D84.0); Tu, EF-Tu (E42.0).*

cells is 1.0 and there is no variation between duplicates. In the ^{3}H/^{14}C data the empirical values of the glucose-grown cells must be 1.0 because leucine was used for both ^{3}H and ^{14}C labeling. Therefore the values need not be normalized and for the glucose-grown cells deviation from 1.0 is due to experimental error. The amounts of these 10 synthetases each display a positive correlation with growth rate, confirming for four of them the results obtained from enzyme assays and other measures. Also of interest is the complete coordinacy between the α and the β chains of PheRS at the various growth rates.

The ^{3}H/^{14}C data in Fig. 2 relating the amount of each synthetase polypeptide to the amount in the reference culture were combined with the absolute values for the reference culture given in Table 3 and the amount of total protein per genome (5) to calculate the number of molecules of each synthetase per genome at the various growth rates. These are presented in Table 4.

Molecular ratios of synthetases to other

TABLE 2. *Homogeneity of synthetase polypeptide spots*[a]

Synthetase	Spot no.	$^3H/^{35}S$ of spot/$^3H/^{35}S$ of total protein in medium: Acetate	Glycerol	Glucose	Rich less methionine
GlyRS	E77.5	1.57	1.70	1.44	1.98
IleRS	F107	0.86	0.92	0.81	0.97
PheRS	D94.0	1.27	1.31	1.41	1.33
ValRS	E106	0.92	0.99	0.80	

[a] For each of the four media shown, two cultures of *E. coli* NC3 were prepared, one labeled with [^{3}H]leucine plus [^{3}H]isoleucine and one labeled with [^{35}S]sulfate, and mixed in appropriate proportion. After resolution on the two-dimensional gels, the radioactivity of the indicated polypeptides was measured and normalized to that of total protein, which varied less than 5% for the four media.

components. In Table 5 are presented values for the molecular ratios of each synthetase to 70S ribosomes and to elongation factor Tu (EF-Tu). The data indicate that, in general, the synthetase/ribosome ratio decreases progressively at faster growth rates, whereas the synthetase/EF-Tu ratio is virtually independent of growth rate.

DISCUSSION

This is the first application of the O'Farrell technique to aminoacyl-tRNA synthetases. Reports have already appeared from this laboratory and elsewhere indicating the general usefulness of the technique for studying other essential proteins such as EF-Tu (7, 16, 17), EF-G (17), RNA polymerase subunits α and β (2, 17), the transcription termination factor, ρ, and at least some ribosomal proteins (2). The identification of several synthetase polypeptides proved to be fairly easy; 11 are reported here. Still, the polypeptide chains of half of the 20 synthetases remain to be identified, and for at least a few of them it may prove necessary to change the standard ampholine mixture to permit satisfactory resolution.

Molecular amounts of synthetases. The determination of absolute numbers of individual synthetase molecules per genome (Table 3) relies on several assumptions. First, we have assumed that all of the measured polypeptides are present in the cell as mature holoenzyme, with subunit stoichiometry as described for purified synthetases in the literature (11). Second, we have assumed complete recovery of each measured synthetase subunit. This assumption requires further comment.

Our confidence in the qualitative recovery of synthetase polypeptides from cell extracts resolved by the O'Farrell system rests essentially on three observations. First, we have found that, exclusive of ribosomal proteins, the major amount of total *E. coli* protein in the lysis mixture actually enters the gels and appears as resolved spots. Second, for almost all of the polypeptides we have measured, the total amount of protein in each spot relative to that of other spots remains fairly constant from one gel to another. (An exception is the β chain of PheRS, to be discussed later.) And finally, the absolute

TABLE 3. *Quantities of synthetases in E. coli NC3 growing on glucose*[a]

Synthetase	Polypeptide: Wt fraction of total protein[b] ($\times 10^3$)	Holoenzyme: Wt fraction of total protein[c] ($\times 10^3$)	Holoenzyme: No. of molecules per genome[d]
ArgRS	0.81 ± 0.03	0.81	510
GlnRS	1.06 ± 0.02	1.06	676
GluRS	0.68 ± 0.06 (α)	1.25	539
GlyRS	1.50 ± 0.08 (β)	2.11	412
IleRS	2.29 ± 0.19	2.29	885
LeuRS	1.41 ± 0.18	1.41	597
LysRS	1.05 ± 0.05	1.05	333
PheRS	1.12 ± 0.02 (α) 1.93 ± 0.03 (β)	3.94	649
ThrRS	0.92 ± 0.14	0.92	346
ValRS	1.01 ± 0.09	1.01	425

[a] The values are for cells of *E. coli* strain NC3 growing in glucose plus MOPS minimal medium at 37°C, $k = 1.03^{-1}$.

[b] A gel was prepared from a culture grown with [^{14}C]glucose as described in Materials and Methods. Each polypeptide identified in Table 1 was cut out of the gel quantitatively and its total radioactivity was counted after oxidation to CO_2. The total radioactivity of the protein in the gel was similarly measured. These values were used to obtain an estimate of the weight fraction of the total protein which each polypeptide represented. The standard error of the mean is shown.

[c] The values for weight fraction given in column 1 for the polypeptides were corrected to include expected amounts of the other subunits of the same proteins, assuming the stoichiometry present in purified holoenzyme. In the case of PheRS, both subunits were measured, but the values for the β subunit were highly variable and therefore implied incomplete recovery. Only the values for the α chain were used to calculate the holoenzyme weight fraction.

[d] These values were obtained by multiplying the amount of protein per genome-equivalent of DNA (4×10^8 amino acid residues $\times$ 110 daltons per amino acid = 4.4×10^{10} daltons; reference 5) by the weight fraction characteristic of a particular enzyme (column 2), and then converting this value to number of molecules per genome by dividing by the holoenzyme molecular weight shown in Table 1.

amount of EF-G recovered in the gel has been found to be 94% of the theoretical amount calculated on the basis of a single EF-G per ribosome.

These observations, however, cannot establish rigorously that the recovery of any individual polypeptide chain is always equal to the recovery of total protein or to the recovery of a reference spot such as EF-G. Therefore, the values for individual synthetases must be taken as minimum values. In the case of the β subunit of PheRS, recoveries relative to that of EF-G ranged over fivefold in different gels; the figure given in Table 3 is an average of the three highest values obtained. If stoichiometry with the α subunit is assumed (and this is borne out by the coordinate variation of α and β displayed in Fig. 2), then the value given in Table 3 represents 68% recovery of the β subunit.

If the 10 synthetases remaining to be measured have the same average molecular abundance as the 10 studied here, then the combined total of synthetase molecules in the cell closely approximates the number of ribosomes.

Variation with growth rate. It should be emphasized that the relative changes in synthetase molecules per genome with growth rate presented in Table 4, and the relative changes in ratios of synthetases to ribosomes and to EF-

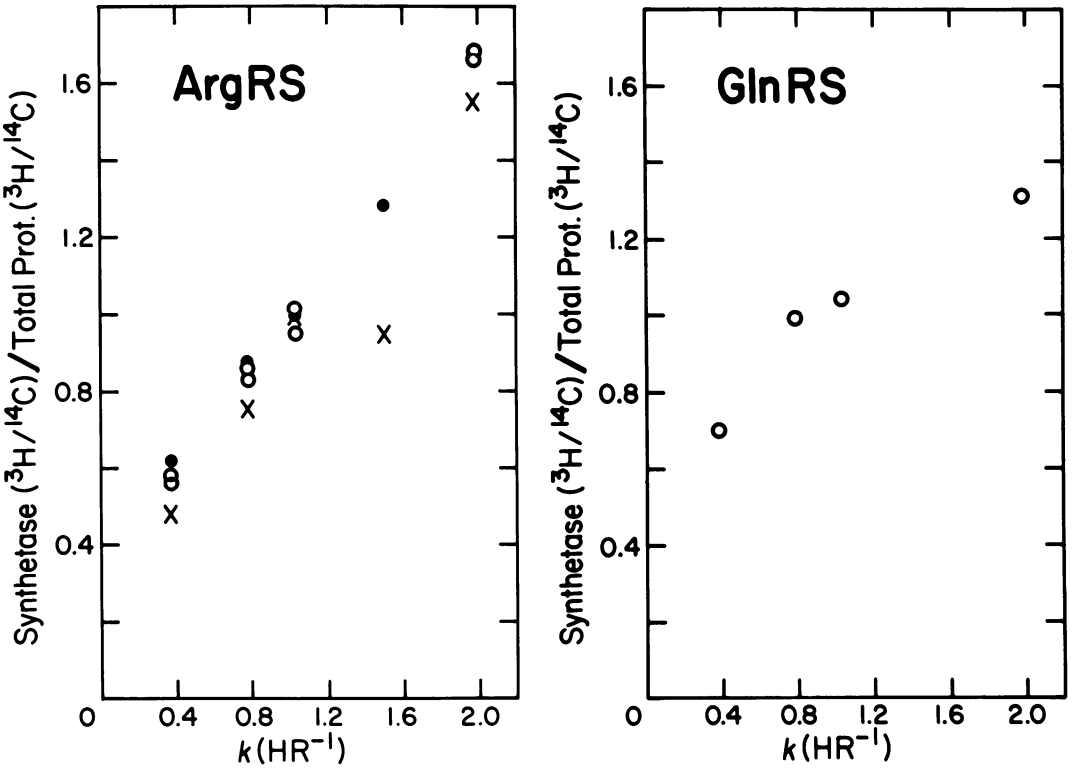

FIG. 2. *Levels of 10 aminoacyl-tRNA synthetase polypeptides as a function of growth rate in E. coli NC3. Five cultures of this strain were prepared as described in Materials and Methods; one each was grown on acetate (k = 0.38), glycerol (k = 0.77), glucose (k = 1.03), and rich (k = 1.98) medium containing [^{3}H]leucine labeling mixture, while a fifth was grown on glucose medium containing [^{14}C]leucine labeling mixture. Portions of the ^{14}C-labeled culture were mixed with each of the four ^{3}H-labeled cultures and O'Farrell gels were prepared. The spots identified as synthetase polypeptides were assayed for ^{3}H and ^{14}C content, and the ratio of these isotopes was divided by the $^3H/^{14}C$ ratio of the total protein. The resulting values (○), which are a measure of the amount of each protein relative to its amount in glucose-grown cells, are plotted as a function of the growth rate of the ^{3}H-labeled cultures (1). In experiments where ^{3}H-labeled culture was mixed with glucose-grown cells labeled with $^{35}SO_4$, rich medium minus methionine (k = 1.50) was used rather than rich medium, and a mixture of [^{3}H]leucine and [^{3}H]isoleucine was used rather than [^{3}H]leucine alone. The calculations are analogous to the ones made for the $^3H/^{14}C$ experiment. The level of synthetases is expressed relative to its amount in glucose-grown cells (●). In the case of PheRS, data are shown for both the α (△) and β (○) subunits. For ArgRS, GluRS, LeuRS, and ValRS enzyme activities (×) relative to the glucose culture are shown from the measurements of McKeever and Neidhardt (5) and Parker and Neidhardt (15).*

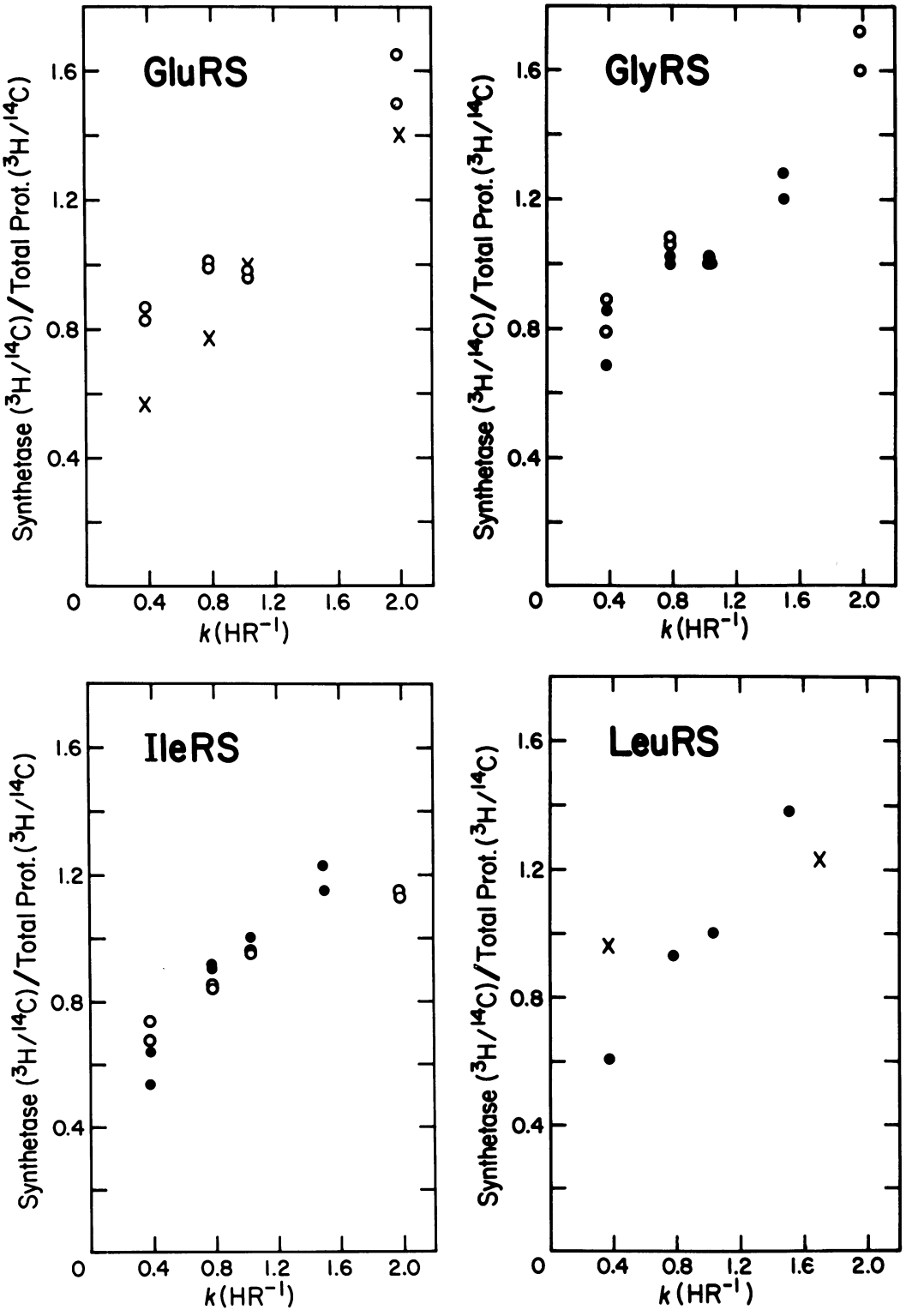

FIG. 2—*Continued*

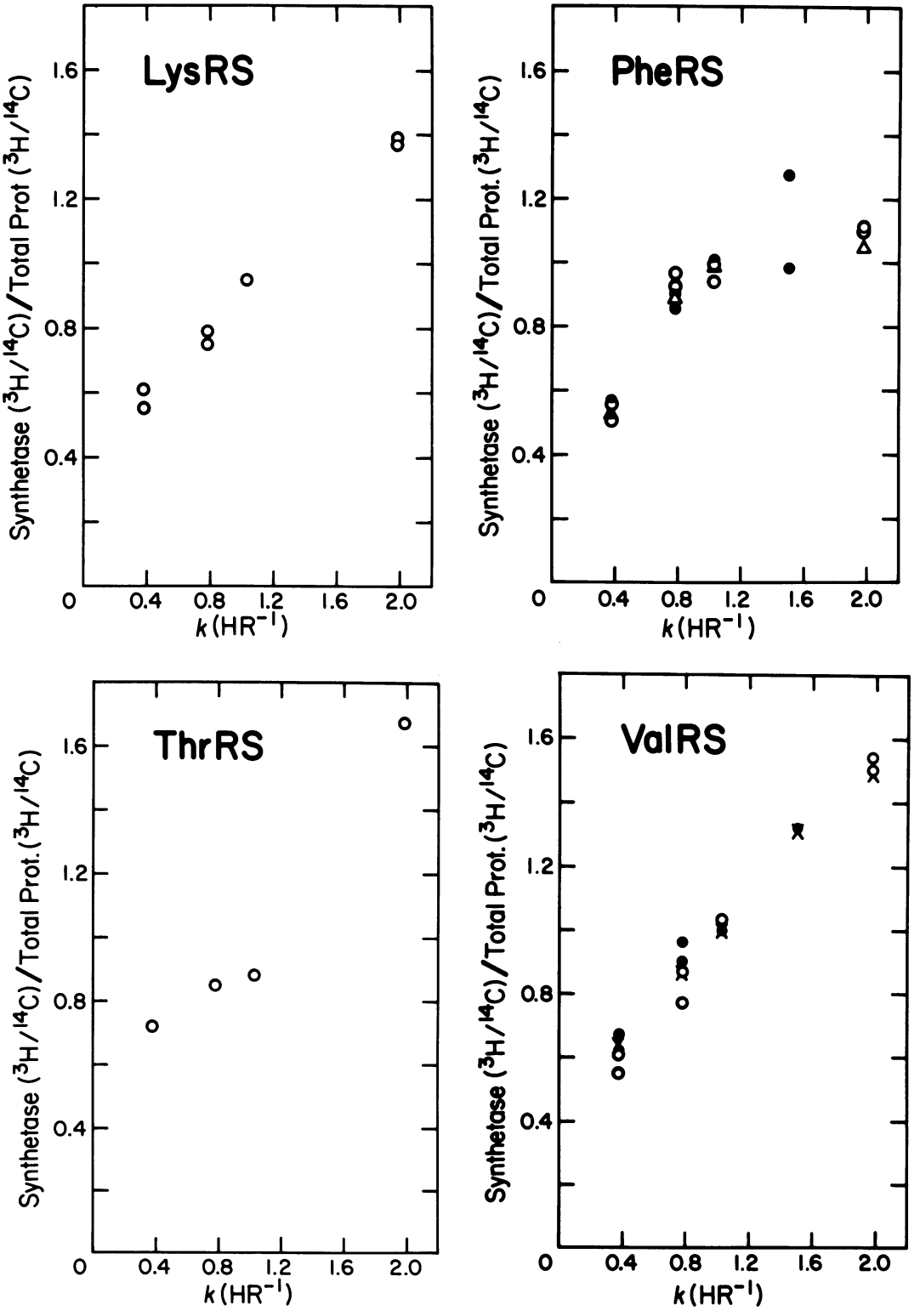

FIG. 2—*Continued*

Tu presented in Table 5, depend only on the data of Fig. 2. Since the results in Fig. 2 are obtained from ratios of ^{3}H-and ^{14}C-labeled protein mixtures rather than quantitation of entire spots, they are independent of the absolute recovery of polypeptides. Therefore, the important conclusions from Tables 4 and 5 do not rely on the absolute quantities reported in Table 3.

TABLE 4. *Variation of synthetases per genome in different media*

Synthetase	No. of synthetase molecules per genome[a] in medium			
	Acetate (k = 0.38)	Glycerol (k = 0.77)	Glucose (k = 1.03)	Rich (k = 1.98)
ArgRS	192	384	510	867
GlnRS	297	563	676	853
GluRS	308	485	539	873
GlyRS	222	379	412	676
IleRS	425	681	885	1053
LeuRS	237	486	597	(1,015)[b]
LysRS	132	236	333	483
PheRS	232	551	649	746
ThrRS	184	293	346	654
ValRS	155	298	425	629

[a] The values for cells grown in glucose medium were taken from column 3 of Table 3. For the other media, these values were corrected for the appropriate amount of protein per genome equivalent of DNA (acetate: 2.86×10^{10} daltons; glycerol: 3.85×10^{10} daltons; glucose 4.4×10^{10} daltons; rich: 4.4×10^{10} daltons; reference 5) and for the variation in synthetase amount per amount of total protein (Fig. 2, $^{3}H/^{14}C$ data for all synthetases except $^{3}H/^{35}S$ for LeuRS).

[b] Since the ^{35}S labeling protocol used a rich medium lacking methionine, the LeuRS data were extrapolated to estimate a value for k = 1.98.

The first important conclusion is that polypeptides of 10 aminoacyl-tRNA synthetases have been shown to vary with growth rate. For three, ArgRS, GluRS and ValRS, this finding confirms earlier work, and indicates a close correspondence under steady-state growth conditions of enzyme activity with chemical assays (6, 8, 14, 15). (The correspondence for LeuRS is, for unknown reasons, not close [15].) For the first time, growth rate-related regulation is established for GluRS, GlyRS, IleRS, LysRS, PheRS, and ThrRS. Since MetRS (3) and SerRS (8) have been shown to vary with growth rate in at least some strains of *E. coli*, the number of synthetases exhibiting this mode of regulation is now brought to 12. In passing, one may note the close coordinacy between the level of the α and β subunits of PheRS at different growth rates. The structural genes for these components lie close to each other, and could be contiguous according to recent information (M. M. Comer, and A. Böck, manuscript submitted for publication). The possibility is open, therefore, that they are part of a single operon.

The second finding of interest is that the synthetases studied here maintain a surprisingly constant ratio to EF-Tu. The individual synthetases deviate no more than 25% from

TABLE 5. *Molecular ratios of synthetases to ribosomes and to EF-Tu in different media*

Synthetases	No. of synthetase molecules in medium per:							
	70S ribosome[a] ($\times 10^{2}$)				EF-Tu[b] ($\times 10^{3}$)			
	Acetate	Glycerol	Glucose	Rich	Acetate	Glycerol	Glucose	Rich
ArgRS	5.9	5.9	5.8	5.1	8.3	9.0	9.8	11.2
GlnRS	9.2	8.6	7.7	5.1	12.8	13.2	13.0	11.1
GluRS	9.5	7.4	6.1	5.2	13.2	11.4	10.3	11.4
GlyRS	6.8	5.8	4.6	4.0	9.5	8.9	7.9	8.8
IleRS	13.1	10.4	10.0	6.2	18.2	16.0	17.0	13.7
LeuRS	7.3	7.5	6.8	6.0	10.2	11.4	11.5	13.2
LysRS	4.0	3.6	3.7	2.9	5.7	5.5	6.4	6.3
PheRS	7.0	8.4	7.4	4.4	10.0	12.9	12.5	9.7
ThrRS	5.7	4.5	3.9	3.9	7.9	6.9	6.6	8.5
ValRS	4.7	4.6	4.8	3.7	6.7	7.0	8.2	8.2
Total	73.2	66.7	60.8	46.5	102.5	102.2	103.2	102.1

[a] The number of synthetase molecules per genome in each medium (Table 3) was divided by the number of 70S ribosomes per genome (acetate, 3,230; glycerol, 6,520; glucose, 8,815; rich, 16,800) at the appropriate growth rates as calculated from the data of Dennis and Bremer (5).

[b] The number of synthetase molecules per genome in each medium (Table 3) was divided by the number of EF-Tu molecules per genome (acetate, 23,300; glycerol, 42,600; glucose, 52,100; rich, 76,800). The weight fraction of EF-Tu to total protein (0.0521) was determined in glucose-grown cells in the same manner as that for synthetases (see Table 3). Similarly, the molecular weight (44,000) was used to calculate the number of molecules per genome (52,100). From the gels described in Fig. 2 the variation in amount of EF-Tu in the various media was measured.

maintaining a constant ratio to EF-Tu molecules under these growth conditions, and the total of the 10 synthetases vary by only 1%. We have found, as has Furano (6), that the number of EF-Tu molecules per ribosome increases with decreasing growth rate. Some reports (19) indicate that the tRNA/ribosome ratio increases with decreasing growth rate. If this is so, then the major components providing charged tRNA for the ribosomal A site – synthetases, tRNA and EF-Tu – would all exhibit the same behavior and would remain balanced with respect to each other.

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