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Transient Rates of Synthesis of Five Aminoacyl-Transfer Ribonucleic Acid Synthetases During a Shift-Up of *Escherichia coli*

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The steady-state levels of a number of aminoacyl-transfer ribonucleic acid synthetases are known to be positively correlated with growth rate in *Escherichia coli*. To describe the regulation of these enzymes during a nutritional shift-up, use was made of the recent identification of polypeptide chains of several synthetases in whole cell lysates resolved by the O'Farrell two-dimensional gel system. A culture growing in acetate minimal medium was shifted to glucose-rich medium and pulse labeled with [^{3}H]leucine and [^{3}H]isoleucine for 30- or 6-s intervals during the 20 min after the shift. After mixing with a uniformly [^{35}S]sulfate-labeled reference culture, the samples were subjected to two-dimensional gel electrophoresis. The ^{3}H/^{35}S ratio in the resolved synthetase polypeptides provided an accurate estimation of their transient rates of synthesis. Five aminoacyl-transfer ribonucleic acid synthetases (those for arginine, glycine, isoleucine, phenylalanine, and valine) exhibited an increase in formation within 30 to 90 s after the shift-up. The magnitude of the increases corresponded to the final steady-state values and were reached within 2 to 3 min. The addition of rifampin revealed that the increase in the differential rate of valyl-transfer ribonucleic acid synthetase formation was the result of increased messenger ribonucleic acid transcription and not of a relaxation of some translation restriction.

The interest in the growth rate-related variation of aminoacyl-transfer ribonucleic acid (tRNA) synthetases stems from the seeming paradox that such "metabolic regulation" (12) is opposite to the response of amino acid-restricted cultures, and from the easily grasped logic of having synthetases controlled with the rest of the translation apparatus. Metabolic regulation has now been described in *Escherichia coli* for arginyl-tRNA synthetase (5, 6, 11, 12), glutaminyl-tRNA synthetase (6), glycyl-tRNA synthetase (6), isoleucyl-tRNA synthetase (6), leucyl-tRNA synthetase (6), lysyl-tRNA synthetase (6), methionyl-tRNA synthetase (2), phenylalanyl-tRNA synthetase (6), seryl-tRNA synthetase (5), threonyl-tRNA synthetase (5, 6, 11, 12), and valyl-tRNA synthetase (ValRS). Fifteen aminoacyl-tRNA synthetases in yeast are reported to display a pronounced metabolic regulation (R.C. Johnson, P.R. Vanatta, and J.R. Fresco, manuscript in preparation), and in eukaryotic cells there is a general tendency for the synthetase level to be proportional to the rate of protein synthesis (8).

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To sharpen the relation between metabolic regulation and the amino acid-related responses shown by many synthetases, we have examined the transient rates of formation of five synthetases in the first 20 min after a shift-up from acetate minimal medium to glucose-rich medium. These measurements were made possible by the ability of the O'Farrell two-dimensional gel system to resolve synthetase polypeptide chains from complex protein mixtures (10). Pulse labeling revealed that these synthetases (arginyl-, glycyl-, isoleucyl-, phenylalanyl-tRNA synthetase; ValRS) undergo an accelerated rate of synthesis almost immediately upon the shift-up, and within 2 to 3 min most have attained their final definitive rate of synthesis.

MATERIALS AND METHODS

Bacterial strain. The *E. coli* B derivative AS19 is a prototrophic strain possessing enhanced permeability to various molecules, including rifampin.

Media. All media were totally defined, synthetic media based on potassium morpholinopropane sulfonate (MOPS) medium (7). Carbon sources were employed at 0.4% (wt/vol). Cells were shifted to a rich medium described previously, with leucine and iso-

leucine omitted. The generation time in acetate medium was 130 min.

Bacterial growth. Cultures were grown aerobically in Erlenmeyer flasks with rotary shaking at 37°C. Bacterial mass was monitored at 420 nm with a Zeiss PMQII spectrophotometer. All growth rates were determined in cultures having optical densities between 0.1 and 0.4 when measured at 420 nm. Growth rates were expressed in terms of the specific growth rate constant, k, as calculated from the expression k = ln 2/mass doubling time in hours).

Pulse labeling of cultures. Cultures were grown for at least 10 generations on acetate MOPS medium containing one-tenth the normal sulfate concentration (1). To measure the rates of protein synthesis, a portion (1.0 ml) of the culture was removed and added to a prewarmed flask containing 0.045 ml of a labeling mixture containing 15 μCi (29 Ci/mmol) of [^{3}H]isoleucine and 30 μCi (38 Ci/mmol) of [^{3}H]leucine. After either 30 or 60 s, incorporation was stopped by adding a 500-fold excess of unlabeled isoleucine and leucine. After a 40-s chase, the sample was harvested by pouring onto ice. It was mixed with a suitable amount of a reference culture grown on acetate and labeled with $^{35}S[SO_4]$ for three generations.

Two-dimensional gels. Gel electrophoresis of the samples, staining of the gels, and measurement of radioactivity in the protein spots was performed as described by O'Farrell (10) with modifications described previously (14).

Measurement of radioactivity in synthetase polypeptides. Spots identified as synthetase polypeptides (arginyl-tRNA synthetase, E58.0; glycyl-tRNA synthetase, E77.5; isoleucyl-tRNA synthetase, F107; phenylalanyl-tRNA synthetase [β subunit], D94.0; and ValRS, E106) have already been described (6). Centers of these spots were punched out, and their $^3H/^{35}S$ ratio was determined as previously described (6). Radioactivity levels corrected for cross counting and background (10 cpm) were 30 to 300 cpm for ^{35}S and 100 to 1,000 cpm for ^3H. The radioactivity of gel areas without autoradiographically visible spots was negligible (<1 cpm). This value for each spot, divided by the $^3H/^{35}S$ ratio of the total protein, provided a measure of the differential rate of synthesis of the polypeptide comprising that spot. These values, calculated after the nutritional shift-up, were expressed relative to the value in the acetate MOPS culture before the shift-up.

Chemicals and radiochemicals. All chemicals were the highest grade available from ordinary commercial suppliers. Radioactive amino acids were purchased from Amersham/Searle. [^{35}S]sulfate was purchased from New England Nuclear. The materials used in the polyacrylamide gels were obtained from the suppliers listed by O'Farrell (10).

RESULTS

Transient rates of synthesis during a shift-up. Figure 1 presents the results of an experiment in which a culture of *E. coli* strain AS19 growing on acetate MOPS was shifted to glucose-rich medium by the addition of appropriate supplements. The differential rates of synthesis of each of five synthetase polypeptides are presented relative to their differential rate of formation before the shift-up. Each showed an accelerated synthesis within 30 to 90 s after the shift, and most had reached the value characteristic of steady-state growth in rich medium within a few minutes. There is a tendency for an initial cycling in the rate of synthesis immediately after the shift; there appears to be a peak at 2 to 3 min and a trough at 4 to 6 min. In a second experiment in which the polypeptides of ValRS, isoleucyl-tRNA synthetase, and arginyl-tRNA synthetase were measured, a similar cycling was evident (results not shown).

Effect of rifampin on synthesis of ValRS. Two cultures of *E. coli* strain AS19 were prepared in acetate MOPS medium and labeled during exponential growth with [^{35}S]sulfate as in the previous experiment. Each culture then received rifampin (final concentration, 100 μg/ml); one of the two cultures was shifted within 12 s to glucose-rich medium by the addition of appropriate supplements, and the other received no nutritional supplements. The differential rate of synthesis of ValRS was measured for each culture at different periods during the ensuing 10 min. The results are presented in Fig. 2. In each culture there was a sharp decline in the differential rate of ValRS synthesis. There was no indication of accelerated synthesis in the culture shifted to glucose-rich medium.

Test for synthetase stability in acetate cultures. Cultures prepared on acetate MOPS medium were prelabeled with [^{35}S]sulfate as in the previous experiments, and then labeled for a 1-min pulse with the labeling mixture of [^{3}H]isoleucine and [^{3}H]leucine. The $^3H/^{35}S$ ratios of several synthetase polypeptides were then measured in the usual way. The results are expressed relative to the $^3H/^{35}S$ ratios of the unfractionated protein of the samples and are presented in Table 1. For comparison, separate cultures grown on acetate and labeled for long periods with either [^{35}S]sulfate or [^{3}H]isoleucine and [^{3}H]leucine were mixed. The same synthetase polypeptides were analyzed for ^3H and ^{35}S, and measured isotope ratios were expressed relative to the $^3H/^{35}S$ ratio of the unfractionated protein. The same mixing experiment was performed also with glucose as a carbon source. The values with the steady-state cultures labeled for long periods (Table 1) are measures of the amino acid composition of the individual synthetase polypeptides. They represent a comparison of the ratio of (leucine plus isoleucine)/(methionine plus cysteine) of the synthetase to the ratio present in *E. coli* bulk

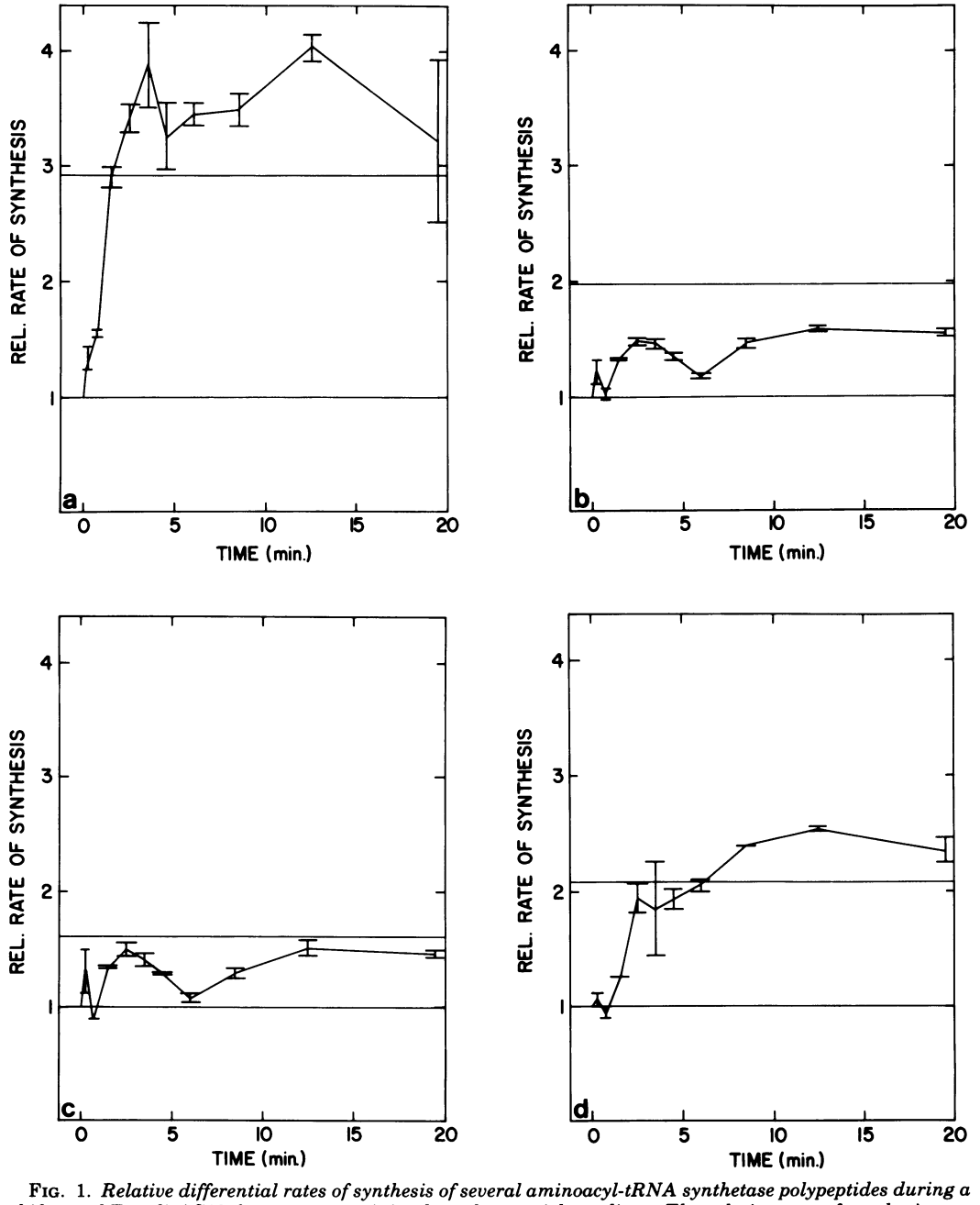

FIG. 1. *Relative differential rates of synthesis of several aminoacyl-tRNA synthetase polypeptides during a shift-up of E. coli AS19 from acetate minimal to glucose-rich medium. The relative rate of synthesis was calculated as the $^3H/^{35}S$ ratio of a synthetase divided by the $^3H/^{35}S$ ratio of the total extract protein and then normalized to the value of the preshift sample. Each horizontal datum bar covers the time interval of the ^3H pulse for that sample. For most times, duplicate results are given. A data line has been drawn connecting the average rates at the midpoints of the labeling periods. The reference rate of synthesis in the preshift acetate medium is indicated by the horizontal line through 1.0. The steady-state rate characteristic of rich medium is indicated by the upper horizontal line, and is taken from reference 6. (a) Arginyl-tRNA synthetase, (b) glycyl-tRNA synthetase; (c) isoleucyl-tRNA synthetase; (d) phenylalanyl-tRNA synthetase; (e) valyl-tRNA synthetase.*

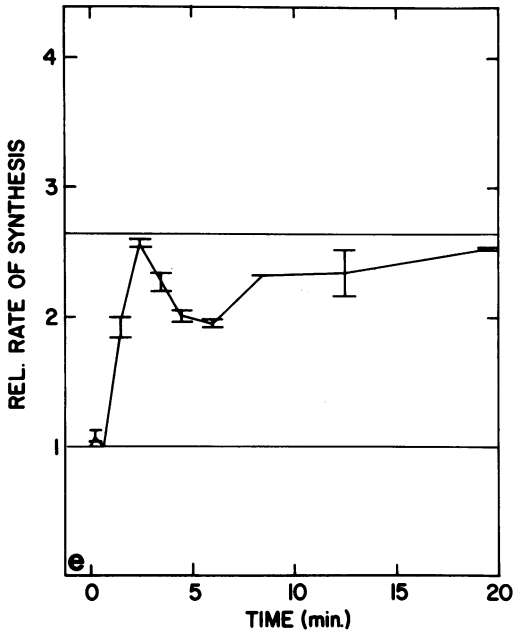

FIG. 1e.

protein. As expected, the values for each synthetase are fairly independent of the media used to grow the cells (acetate or glucose). Significant turnover of synthetase proteins during growth of the cells on acetate would lead to higher incorporation of ^{3}H-labeled amino acids during the 1-min pulse than would be predicted from the results with uniformly labeled cultures. The data shown in Table 1 show no convincing indication of such turnover.

DISCUSSION

These results make it clear that a prominent feature in the adjustment of *E. coli* cells to grow faster upon nutritional supplementation is a rapid acceleration in the rate of formation of several aminoacyl-tRNA synthetases. These data confirm the earlier conclusions that the positive correlation of synthetase activity with growth rate involves a variation in amount of synthetase protein and that this variation reflects a control on the rate of synthetase protein production rather than on its degradation (11).

The result with rifampin shows that the increased production of ValRS synthetase protein after the shift-up requires new transcription initiations and argues against a control mechanism operating by restricting the translation of synthetase messenger RNA.

This method of measuring the rate of formation of a protein in relation to the rate of total protein synthesis is particularly useful for examining transient changes, even of a small magnitude, since the rate of differential labeling is independent of any labeling delays caused by restricted entry of precursors or pool equilibration. It has been possible, therefore, to

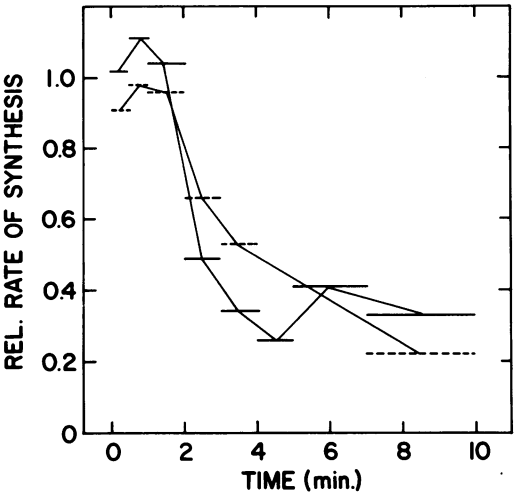

FIG. 2. *Effect of rifampin on the relative differential rates of synthesis of ValRS during a shift-up of E. coli AS19 from acetate minimal to glucose-rich medium. Two cultures growing on acetate received rifampin (final concentration, 100 μg/ml) at −12 s, and then one was shifted up at zero time. The relative rate of synthesis of ValRS was measured at intervals by the same procedures as for Fig. 1. The horizontal data bars cover the time intervals of the ^{3}H pulses for samples from the control (——) and from the culture shifted up (- - -).*

TABLE 1. *Test for turnover of synthetase polypeptides during growth of E. coli on acetate*

Protein[a]	(^{3}H/^{35}S of synthetase)/(^{3}H/^{35}S of total protein)		
	Pulse-labeled acetate culture[b]	Long-term-labeled cultures[c]	
		Acetate	Glucose
ArgRS	0.85, 0.95, 0.92	0.92	0.85
GlyRS	1.03, 0.88	1.27	1.41
IleRS	1.00, 0.86	0.86	0.81
PheRS	0.87, 0.74	–	0.73
ValRS	1.40	1.57	1.44

[a] Abbreviations: ArgRS, arginyl-tRNA synthetase; GlyRS, glycyl-tRNA synthetase; IleRS, isoleucyl-tRNA synthetase; PheRS, phenylalanyl-tRNA synthetase; and ValRS, valyl-tRNA synthetase.

[b] The pulse labeling with ^{3}H was performed as described in Materials and Methods for the shift-up experiment.

[c] The long-term labeling with ^{35}S was performed as described previously (6).

reveal that the acceleration of synthetase protein formation occurs within a minute of the nutritional supplementation of the medium and that the final elevated steady-state values are achieved within 2 to 3 min.

This result rules out certain explanations of synthetase variation with growth rate. For example, models in which the rate of synthetase formation is regulated by the total amount of other translation elements in the cell, such as tRNA, elongation factors, or ribosomal components, cannot explain the observed results. Explanations based on gene dosage, cell volume, or any other growth rate-related, structural property of the cell are also ruled out. Instead, some regulatory signal(s) must be generated extremely rapidly, within seconds, as the result of the nutritional supplementation of the medium. The nature of such a signal can only be speculated upon at this time, and there are many possible candidates for either positive or negative effectors (e.g., the charging level of tRNA, the conformation of one or another protein synthesis factor or of the synthetases themselves, etc.). Although acceleration of synthetase protein formation occurs as rapidly as that of ribosomal RNA (3, 9, 13) and ribosomal proteins (4, 15), it is not likely that identical molecular mechanisms are operative in controlling these different elements of the translation apparatus. Synthetase levels are not as tightly coordinated with growth rate as is stable RNA (5), and although some exhibit a mild stringent response, others do not (R. M. Blumenthal, P. G. Lemaux, F. C. Neidhardt, and P. P. Dennis, Mol. Gen. Genet., in press; S. Reeh, S. Pederson, and J. D. Friesen, Mol. Gen. Genet., in press). Further experiments are being performed to help identify the nature of the signals that effect these rapid changes in synthetase gene expression.

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