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Relationships Among Deoxyribonucleic Acid, Ribonucleic Acid, and Specific Transfer Ribonucleic Acids in *Escherichia coli* 15T⁻ at Various Growth Rates[1]

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The levels of macromolecules in *Escherichia coli* 15T⁻ growing in broth, glucose, succinate, and acetate media were determined to compare relationships among deoxyribonucleic acid (DNA), ribosomal ribonucleic acid (rRNA), transfer RNA (tRNA), and protein in cells at different growth rates. DNA and protein increased in relative amounts with decreasing growth rate; relative amounts of rRNA and tRNA decreased, tRNA making up a slightly larger proportion of RNA. For several amino acid-specific tRNAs studied, acceptor capacities per unit of DNA increased with increasing growth rate. The syntheses of tRNA and rRNA are regulated by similar, yet different, mechanisms. Chromatographic examination on columns of benzoylated diethylaminoethyl-cellulose of isoaccepting tRNAs for arginine, leucine, lysine, methionine, phenylalanine, serine, and valine did not reveal differences in the isoaccepting profiles for rapidly (broth culture) and slowly growing (acetate culture) cells. Therefore, isoacceptors for individual amino acids appear to be regulated as a group. Lower efficiencies of ribosomal function in protein synthesis can be explained, in part, by a low ratio of tRNA to the number of ribosomes available and by a decreasing concentration of tRNA with decreasing growth rate. Data on the tRNAs specific for seven amino acids indicate that the decreasing concentration of tRNA is a general event rather than a severe limitation of any one tRNA or isoaccepting tRNA.

There is evidence that transfer ribonucleic acid (tRNA), in addition to functioning in the transfer of amino acids, may function in regulating cellular metabolism. The general role of tRNA in protein synthesis is fairly well understood; its role in regulatory processes, however, is not.

tRNA is implicated in regulating protein synthesis (40) at the levels of transcription and translation. Evidence indicates that tRNA is involved at the transcriptional or translational levels in regulating the bacterial synthesis of biosynthetic enzymes for histidine (7, 25, 35), isoleucine, and valine (15, 18, 28, 41) and can be involved in a regulatory role at the translational level as shown with a eukaryotic system in vitro (2, 3). During phage infection in *Escherichia coli*, an isoaccepting tRNA for leucine (presumably functioning in a critical role in host protein synthesis) is destroyed by a nuclease, possibly virus directed (39, 40).

An approach to understanding tRNA in regulatory involvement in cellular metabolism includes examining the levels of the general classes of macromolecules in the cell. Results of studies on the bacteria *Aerobacter aerogenes* (32, 34), *Salmonella typhimurium* (34, 38), and *E. coli* (34), made under various growth conditions, showed similar levels of deoxyribonucleic acid (DNA), RNA, and protein. The most significant observation was the greatly increased levels of cellular RNA at high growth rates relative to the cellular levels of DNA and protein.

Results of diverse experimental procedures to determine whether increased RNA reflects an

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equivalent increase in tRNA did not agree (22, 32, 34).

This paper concerns the assumption that tRNA is subject to regulation defined by the needs of the cell. In analyzing the cellular levels of tRNA (as a function of the growth rate) to determine general and specific macromolecular relationships within a single bacterial strain, we examined levels of DNA, RNA, and protein and determined levels of tRNA and of individual acceptor capacities for several tRNAs. Also, we attempted to determine whether the syntheses of isoaccepting species of tRNA are regulated coordinately during fast and slow growth.

MATERIALS AND METHODS

Materials. All radioactive amino acids and [2-^{14}C]-uracil (55 mCi/mmol) were obtained from Schwarz BioResearch. Amino acids used in determining acceptor capacities had specific activities of 50 mCi/mmol. Amino acids used to charge tRNA for chromatography had these specific activities: L-[^{3}H]arginine (1.10 Ci/mmol), L-[^{14}C]arginine (220 mCi/mmol), L-[^{3}H]leucine (2.0 Ci/mmol), L-[^{14}C]leucine (180 mCi/mmol), L-[^{3}H]lysine (4.0 Ci/mmol), L-[^{14}C]lysine (228 mCi/mmol), L-[^{3}H]serine (1.8 Ci/mmol), L-[^{14}C]serine (160 mCi/mmol), L-[^{3}H]phenylalanine (10.0 Ci/mmol), L-[^{14}C]phenylalanine (455 mCi/mmol), L-[^{3}H]valine (6.0 Ci/mmol), L-[^{14}C]valine (200 mCi/mmol). NCS solubilizer was obtained from Nuclear-Chicago; deoxyribonuclease I and lysozyme (crystallized two times) were from Worthington Biochemical Corp; and Whatman CF-11 cellulose was from Reeve Angel.

BD-cellulose (benzoylated diethylaminoethyl [DEAE]-cellulose) was prepared by the method of Gillam et al. (19) except that 6.0 mol of benzoyl chloride was used per mole of anhydroglucose. Benzoyl chloride (reagent grade) was obtained from Eastman Kodak Co.; DEAE-cellulose (0.80 meq/g) was from Schleicher and Schuell Co. Pyridine (reagent grade), obtained from Fisher Scientific Co., was used as soon as opened.

Bacterial growth conditions. *E. coli* strain 15T$^-$ (555-7), obtained from K. G. Lark, was grown in M9 minimal medium (1), containing a carbon source, supplemented with $CaCl_2$ (15 μg/ml), thymine (8 μg/ml), and the required amino acids arginine (68 μg/ml), methionine (60 μg/ml), and tryptophan (28 μg/ml). Carbon sources were 0.5% acetate, 0.9% succinate, 0.4% glucose, or, in the case of nutrient broth, 0.4% glucose with 0.3% beef extract and 0.5% peptone. Cells were grown with vigorous aeration in the various media at 37 C. Succinate and glucose cultures, grown in 1-liter volumes, were harvested several generations subsequent to addition of a 10-ml overnight inoculum grown in succinate or glucose medium, respectively. Acetate cultures were prepared by inoculating 100 ml of medium with 1 ml of acetate culture. The 100-ml culture was diluted to 1 liter upon reaching an absorbance of 0.300 (450 nm, 1-cm path) and harvested when the culture was again at 0.300 absorbance. Broth cultures were grown in 600 to 800 ml of medium with a 0.1% inoculum. All cultures were harvested between 0.300 and 0.600 absorbance.

Growth determinations. Culture turbidity was determined with a Beckman DU-2 spectrophotometer at 450 nm. Determining broth cultures required using a correction curve, previously determined by diluting a standard culture.

Cell density was determined with a Coulter particle counter (with a 30-μm orifice) or by viable counts using the pour-plate method. Results obtained by the two methods were in agreement.

Determination of protein, RNA, and DNA. Pellets from three 25-ml portions of exponentially growing culture (100 ml for acetate cultures) were treated in triplicate with 4 ml of ice-cold 10% trichloroacetic acid and washed with 2 ml of cold 5% trichloroacetic acid. The cell precipitate then was suspended in 2 ml of 0.5 M $HClO_4$, and the suspension was stirred intermittently for 20 min at 70 C. After centrifuging, 1.6 ml of the supernatant solution was withdrawn, and to the remaining suspension 1.6 ml of 0.5 M $HClO_4$ was added with stirring at 70 C as before. After centrifuging, a second 1.6-ml sample of the supernatant solution was withdrawn and combined with the first. The volume of combined supernatant solutions was adjusted to 3.6 ml with 0.5 M $HClO_4$. The remaining pellet was dissolved in 1.0 ml of 0.5 M NaOH for determining protein by the method of Lowry et al. (26). The $HClO_4$ extracts were used to determine RNA (14) and DNA (8).

Preparation of RNA. Total RNA was extracted by a modified method of Morris and DeMoss (29). The cell pellet from 200 ml of culture was resuspended in 2 ml of buffer (40 mM potassium acetate, pH 4.8, and 4 mM magnesium acetate) containing deoxyribonuclease (30 μg/ml) and lysozyme (300 μg/ml). The suspension was quick-frozen twice at -70 C, followed by incubation for 1 min at 37 C upon final thawing. Then the lysate, adjusted to 1.25% sodium dodecyl sulfate and 0.1% bentonite, was subjected to sonic oscillation for 3 min in a 10 kc Raytheon sonic oscillator at 5 to 7 C. All subsequent steps were performed at 0 to 5 C unless otherwise indicated. After centrifugation, the supernatant solution was combined with 1 vol of phenol, stirred intermittently for 10 min, then centrifuged again. The aqueous phase was removed, and the phenol phase was washed once with 0.5 vol of buffer. After 0.1 vol of 20% potassium acetate (pH 4.8) was added, the RNA in the combined aqueous phases was precipitated by adding 2 vol of 95% ethanol; the recovered RNA was precipitated once again. The pellet was dissolved in 1.8 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 8.0), and the solution was incubated for 1 h at 37 C to remove attached amino acids (37). RNA was precipitated as described, reprecipitated, washed with ethanol, and redissolved in 1 ml of water. The concentration of RNA was determined by the absorbance at 260 nm, on the basis that 1 mg/ml equals 24 A_{260} units for a 1-cm path length.

Ribosomal RNA (rRNA) and 5*S* rRNA were prepared by the method of Comb and Zehavi-Willner (10) except that chromatography was omitted. The 5*S* rRNA preparation was examined for purity by poly-

acrylamide gel electrophoresis as described below.

Preparation of tRNA for resolution of isoacceptors. Total RNA was dissolved in 2 M LiCl and allowed to stand overnight at 4 C. After centrifugation, tRNA was precipitated from the supernatant solution and again from aqueous solution with 0.1 vol of potassium acetate and 3 vol of ethanol. The precipitate was washed with ethanol, dried in vacuo, and dissolved in water.

Preparation and chromatography of aminoacyl-tRNA. tRNA (40 to 100 μg) was aminoacylated in the presence of 19 unlabeled amino acids not including the labeled amino acid. After extraction of the reaction mixture with phenol, aminoacyl-tRNA was precipitated from the resultant aqueous phase on adding 200 to 400 μg of carrier tRNA and then was precipitated twice more from aqueous solution with 0.1 vol of potassium acetate and 2 vol of ethanol. The precipitate was washed with ethanol, dried in vacuo, and dissolved in 0.3 ml of the buffer used to start the elution. This solution, adjusted to contain a total of 2 mg of tRNA, was applied to a BD-cellulose column (0.9 by 115 cm) previously equilibrated with the starting buffer. After the column was washed briefly with starting buffer, a linear gradient elution was begun with monitoring at 254 nm. Buffers and gradients are indicated with each figure. Unless otherwise stated, elution volumes were 200 ml and fractions were 2 ml. Flow rates were 0.5 to 0.9 ml/min. Carrier RNA was added to each fraction (150 μg/ml), and the tRNA was precipitated in cold 10% trichloroacetic acid and collected by filtration on glass-fiber (GF/C) filters. The amount of radioactivity was determined in vials containing 5 ml of toluene-base scintillation fluid.

CF-11 cellulose chromatography. Relative amounts of tRNA and rRNA were determined by fractionation on columns of CF-11 cellulose (5). tRNA passes directly through the column when the eluant is 85 mM NaCl in a solution of 95% ethanol-water (35:65). That minor adjustment of Barber conditions (5) removes some high-molecular-weight RNA which elutes with tRNA when the eluant is 65 mM NaCl in a solution of 95% ethanol-water (35:65). rRNA elutes with the water eluant. Column sizes for analytical purposes were 9 by 30 mm or 9 by 60 mm. tRNA was collected in 2-ml fractions and rRNA in 5-ml fractions, and the amounts were determined by measuring A_{260}. To determine acceptor capacities of RNA in the fractions, 1.92 mg of RNA from cells grown in glucose medium was fractionated on a column (15 by 80 mm) of CF-11 cellulose. tRNA was recovered by adjusting the eluate to 0.230 M NaCl in 95% ethanol-water (44:56), applying to a smaller CF-11 cellulose column (9 by 30 mm), eluting with water, and precipitating with ethanol. RNA in the water fraction from the first column was recovered by precipitation with ethanol.

Polyacrylamide gel electrophoresis. Total RNA preparations were fractionated on polyacrylamide gels by the method of Davis (11) with modifications. Unless otherwise indicated, the gel size was 0.5 by 5.0 cm and consisted of 4.5 cm of 10% acrylamide (5% *N,N'*-methylenebisacrylamide cross-linkage) and an upper layer of 0.5 cm of 3% acrylamide (with 5% cross-linkage). Davis large-pore spacer gel was eliminated, and the large-pore buffer was adjusted to 20% sucrose. Total RNA (200–400 μg), dissolved in the large-pore buffer, was subjected to electrophoresis for 50 min at 350 V and a current of 3 mA per gel. Gels were stained with acridine orange and were destained electrophoretically by the method of Richards et al. (33).

To determine tRNA, cultures of 10 to 20 ml were grown as described. RNA was labeled during logarithmic growth for 20 min with [2-^{14}C]uracil, followed by a chase period with unlabeled uridine; it was extracted as described. Approximately 10 to 20 μg of RNA, applied to a polyacrylamide gel consisting of a 2-cm layer of 3% gel on 3 cm of 10% gel, was subjected to electrophoresis. Unstained gel slices 1-mm thick were digested with 30% H_2O_2 (0.7 ml) at 60 to 70 C for several hours. To determine the amount of radioactivity, 0.6 ml of NCS solubilizer and 5 ml of toluene-base scintillation fluid were added to the solubilized gel slices.

Preparation of aminoacyl-tRNA synthetases. A pellet of cells grown to late log phase in 2 liters of M9 medium with glucose was suspended in 60 ml of 40 mM Tris-hydrochloride (pH 7.5), 12 mM 2-mercaptoethanol, and 4 mM $MgCl_2$ (Tris buffer). After sonic oscillation for 10 min at 5 to 7 C, the suspension was clarified by centrifuging for 15 min at 12,100 $\times$ *g*. To the supernatant solution, 6 ml of 10% streptomycin sulfate (in Tris buffer) was added dropwise with stirring. After 30 min, the precipitate was removed by centrifuging for 15 min at 12,100 $\times$ *g*; the supernatant solution was applied to a column of DEAE-cellulose (5 g) equilibrated with Tris buffer. Next, we added (sequentially) 25 ml of the above buffer, 50 ml of buffer containing 0.075 M KCl, and 50 ml of buffer containing 0.30 M KCl. The synthetases were eluted as a yellow band in the second 25-ml fraction of the last buffer applied. To the fraction containing synthetases, we added a mixture of 11.2 g of $(NH_4)_2SO_4$ and 0.243 g of $(NH_4)_2CO_3$. The precipitate, collected by centrifuging 15 min at 12,000 $\times$ *g*, was dissolved in 1.5 ml of Tris buffer. The solution then was applied to a Sephadex G-50 column (15 by 1.5 cm) equilibrated with Tris buffer. Fractions (2 ml) were taken, and those containing the colored band were pooled. The solution, adjusted to 1 mM in glutathione, was distributed into 0.2-ml portions which were stored at −20 C.

Amino acid acceptor capacities. Maximal acceptance was determined on approximately 150 μg of RNA in 0.125 M Tris-hydrochloride buffer (pH 7.5) containing 175 to 300 μg of protein from the enzyme preparation, 25 μM ^{14}C-amino acid, 2.5 mM sodium (Na_2) adenosine triphosphate, 25 mM magnesium acetate, and 10 mM reduced gluthathione (for arginine and serine assays) or 30 mM 2-mercaptoethanol (for leucine, lysine, methionine, phenylalanine, and valine assays). A total of 0.2 ml of reaction mixture was incubated for 30 min at 37 C and then was chilled at 0 C. A 100-μliter sample of the solution was transferred onto a Whatman 3MM disk (2.4 cm), which then was dropped into cold 10% trichloroacetic

acid. The disks were washed by the procedure of Barnett and Jacobson (6). Incubation for 30 min gave maximal acceptance.

RESULTS

Bacterial growth. Growth rates (generations per hour) and respective nutritive media for *E. coli* 15T⁻ are presented in Table 1. The various media were selected to elicit a range of bacterial growth rates. All nutrients were well above growth-limiting concentration; therefore, only the quality of nutrient limited the growth rates. We consistently harvested cell cultures at about the half-way point of exponential growth, a point in the growth curve where both mass and number of cells increased maximally. Cultures, checked periodically for turbidity and viability to determine growth rate, were maintained at the exponential rate for several generations before harvesting.

Macromolecular levels. Cellular levels of DNA, RNA, and protein (Fig. 1) agreed relatively well with published values (17, 32, 34, 38). The level of RNA with respect to DNA or protein increased with growth rate approximately fourfold, while a constant protein/DNA level was maintained. The synthesis of RNA is noncoordinate with the synthesis of DNA and protein and may be subject to a more intricate regulation (31).

tRNA levels. To learn whether the syntheses of the two stable classes of RNA are regulated in a similar way, we determined the fraction of tRNA in the total stable RNA by chromatography on CF-11 cellulose and by polyacrylamide gel electrophoresis. All leucine accepting activity elutes from columns of CF-11 cellulose exclusively in the ethanol fraction; 5*S* rRNA, which elutes in the water fraction (unpublished observations), also can be resolved by polyacrylamide gel electrophoresis, thus eliminating an error inherent in determining tRNA by chromatography on methylated albumin or by sedimentation in sucrose gradients. An additional advantage in the determination by polyacrylamide gel is that (because *E. coli* 15T⁻ has a strict thymine requirement) the use of ^{14}C-uracil reduces the error caused by DNA contamination (16).

As growth rate increased, the fraction of tRNA in total RNA decreased moderately with either method of determination (Fig. 2). Our results agreed relatively well with values obtained by some workers for *A. aerogenes* (34) and *E. coli* (13, 16, 17) but differed slightly from the results of other workers. Neidhardt and Magasanik (32) reported that in *A. aerogenes* the tRNA fraction (of total RNA) remained constant at two growth rates, and Rosset et al. (34) observed that in *E. coli* and *S. typhimurium* this fraction increased only at low growth rates. Major differences, however, were reported by Gray and Midgley (20) in values for *E. coli* (a decrease of tRNA from 52% at 0.34 generation per hour to 21% at 1.74 generations per hour) and by Kjeldgaard and Kurland (22) in values for *S. typhimurium* (a decrease of tRNA from 65% at 0.2 generation per hour to

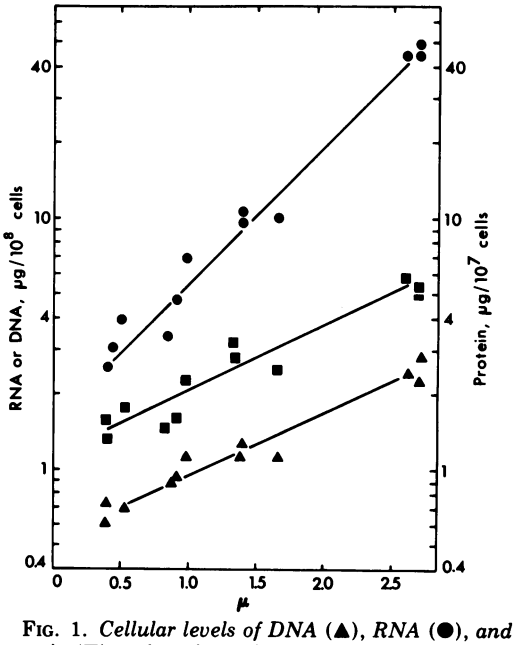

FIG. 1. *Cellular levels of DNA* (▲), *RNA* (●), *and protein* (■) *as functions of the growth rate* (μ).

TABLE 1. *Changes in RNA relative to DNA with varying growth rate*

Medium	Growth rate[a]	RNA/DNA	tRNA/DNA	pmol of tRNA/μg of DNA						
				Arg	Leu	Lys	Met	Phe	Ser	Val
Acetate	0.44	4.0	0.62	1.0	1.6	0.64	1.2	0.60	0.96	1.1
Succinate	0.91	5.3	0.79	1.1	2.0	0.95	1.1	0.95	1.1	1.4
Glucose	1.5	8.3	1.1	1.7	3.1	1.4	2.0	1.5	1.7	2.0
Nutrient broth	2.7	16.7	1.9	3.3	5.2	2.3	4.0	2.7	3.5	3.0

[a] Generations per hour.

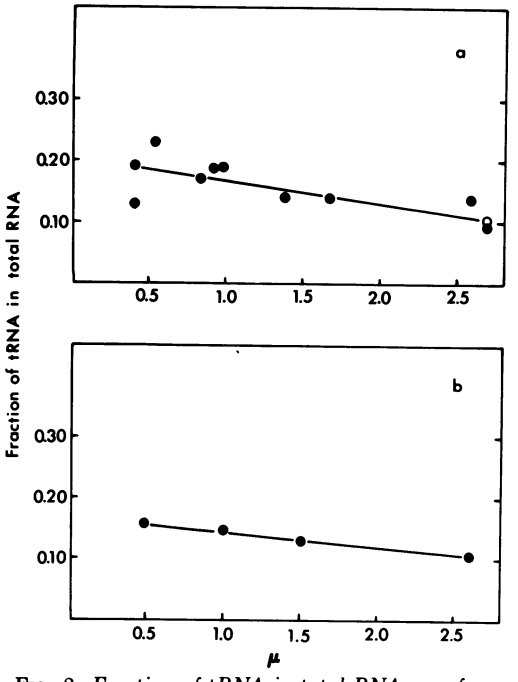

FIG. 2. *Fraction of tRNA in total RNA as a function of the growth rate. Values were determined by fractionation on (a) CF-11 cellulose or (b) by polyacrylamide gel electrophoresis.*

20% at 2.4 generations per hour). Those workers concluded that the ratio of tRNA to DNA was independent of growth rate, implying a coordinate regulation of the synthesis of tRNA and DNA. Those results are not in accord with data presented here nor with the data of Wong et al. (43).

Acceptor capacities of tRNA. Specific amino acid tRNAs are known to exist as multiple species and to recognize multiple codons (42). The tRNAs studied represent 27 of the possible 64 codons. Although recognizing only a single codon, $tRNA^{Met}$ was included because of its role in initiating protein synthesis. Thus, the particular tRNA species were selected on the premise that if regulatory processes involve modifying certain amino acid specific tRNAs, then those species are likely candidates to exhibit differences under altered growth conditions. Alternatively, if the synthesis of tRNA is regulated by the same mechanism as the synthesis of rRNA, then constant acceptor capacities, as a function of RNA, would be expected for the species surveyed.

The acceptor capacities for the tRNAs, as a function of DNA, are presented in Table 1 with tRNA/DNA ratios. Although the amount of DNA per cell increases with growth rate, there is a relatively greater increase in the amount of tRNA per cell with a subsequent increase in the tRNA/DNA ratio with increasing growth rate. Acceptor capacities for each of the tRNAs also increase relative to DNA with increasing growth rate, and the extent of the increase is about the same as that of the tRNA/DNA ratio. However, the increase is not uniform for the specific tRNAs studied, and the results may indicate possible differences in the regulation of synthesis or in the rates of synthesis of tRNAs. Data in Table 1 also show that acceptor capacities of tRNA as a function of RNA vary independently of total RNA (or ribosomal RNA) and confirm the observations of Fig. 2.

Analysis of isoaccepting tRNA from broth and acetate cultures. Cochromatography on columns of BD-cellulose of tRNA from cells grown in nutrient broth or acetate media provided a comparison of isoaccepting species of tRNA for a given amino acid. The results for $tRNA^{Leu}$ (Fig. 3), indicating no detectable differences for any of the four isoaccepting species, suggest that the biosyntheses of isoaccepting tRNAs are coordinately regulated. Thus, the increasing proportion of $tRNA^{Leu}$ in total RNA with decreasing growth rate reflects equivalent increases in the four isoaccepting species. To rule out the possibility that these results do not reflect accurately the in vivo use of isoacceptors, we treated preparations of unstripped tRNA from broth and acetate cultures with periodate prior to charging them with labeled amino acid so that we could compare them chromatographically with the respective tRNA not treated with periodate. Only the tRNA charged with amino acid is spared during periodation. Thus, a reasonably physiological representation of the distribution of charged species in vivo is achieved. However, chromatography revealed no difference in charged isoaccepting tRNAs in the in vivo situation.

There were no significant changes in the chromatographic profiles of $tRNA^{Arg}$ (Fig. 4) and $tRNA^{Phe}$ (Fig. 5) for either growth condition, and significant isoaccepting species were not observed. Isoaccepting profiles were obtained for $tRNA^{Lys}$, $tRNA^{Met}$, $tRNA^{Ser}$, and $tRNA^{Val}$ (Fig. 6-9), and no differences were observed between the two growth conditions. For $tRNA^{Met}$, the first peak eluting from the column is the formyl-accepting species and is the species involved in initiating protein synthesis (36). Clearly, the relative proportions of the two species of $tRNA^{Met}$ are the same for slow and fast growth.

RNA efficiency in protein synthesis. Efficiencies of protein synthesis for each nutrient

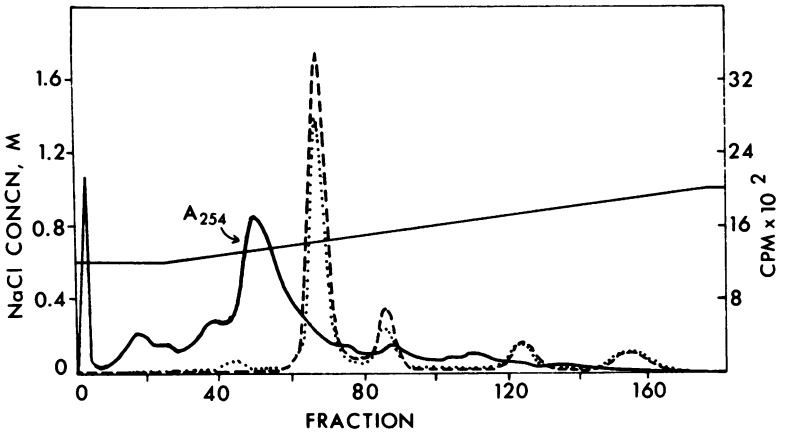

FIG. 3. *BD-cellulose chromatographic profile of leucyl-tRNA from cells grown in nutrient broth and acetate media. Elution was performed with a 500-ml gradient of sodium chloride containing 0.01 M magnesium chloride, 0.05 M sodium acetate (pH 5.0), and 2-methoxyethanol (0 to 10%, vol/vol). Fractions were 5 ml. Acetate ^{3}H-leucyl-tRNA (·····); broth ^{14}C-leucyl-tRNA (- - - - -).*

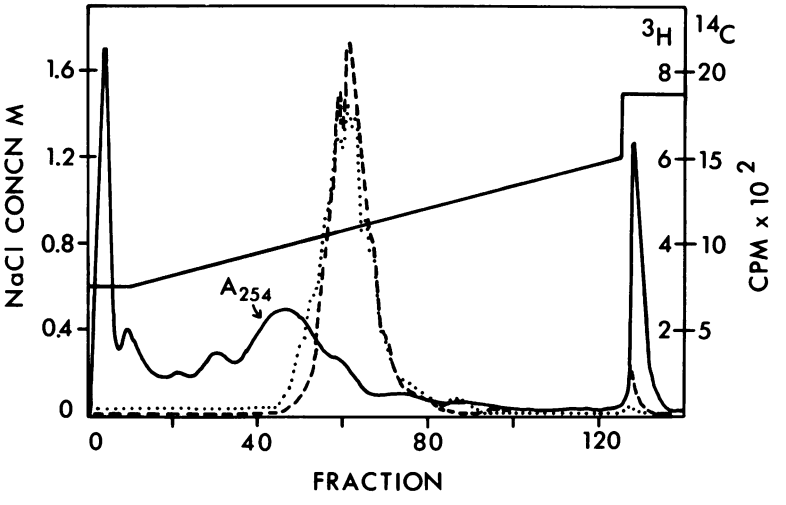

FIG. 4. *BD-cellulose chromatographic profile of arginyl-tRNA from cells grown in nutrient broth and acetate media. Elution was performed with a gradient of sodium chloride, followed by 1.5 M sodium chloride containing 15% (vol/vol) 2-methoxyethanol. All solutions also contained 0.01 M magnesium chloride and 0.01 M sodium acetate (pH 5.0). Acetate ^{3}H-arginyl-tRNA (·····); broth ^{14}C-arginyl-tRNA (- - - - -).*

condition are presented in Fig. 10 as the rate of cellular protein synthesis per unit mass of RNA (22). From 0.4 to 1.5 generations per hour efficiency for both tRNA and rRNA increased linearly with increasing growth rate. Beyond that point, efficiencies for rRNA decreased, an observation not in accord with results for *E. coli* and *S. typhimurium* (34) (indicating that efficiencies increase regularly with growth rate) or for *S. typhimurium* (22) (indicating that efficiencies remain essentially constant at all growth rates).

Number of tRNA molecules per ribosome. Levels of tRNA/ribosome (Table 2) agreed with corresponding values reported by Forchhammer and Lindahl (17) and with data of Mueller and Bremer (30) for cultures in glucose-minimal medium. Values for specific tRNAs averaged less than 1 for every two ribosomes ($tRNA^{Arg}$, $tRNA^{Ser}$) and rarely approached 1 for each ribosome ($tRNA^{Leu}$).

DISCUSSION

The synthesis of RNA is not regulated coordinately with the syntheses of DNA and protein. We found that tRNA and rRNA are regulated

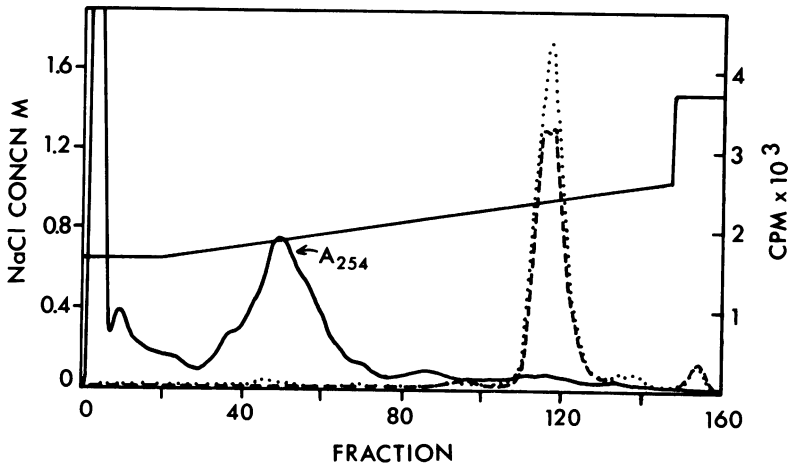

FIG. 5. *BD-cellulose chromatographic profile of phenylalanyl-tRNA from cells grown in nutrient broth and acetate media. Elution was performed with a gradient of sodium chloride containing 2-methoxyethanol (0 to 12%, vol/vol), followed by 1.5 M sodium chloride containing 15% (vol/vol) 2-methoxyethanol. All solutions also contained 0.01 M magnesium chloride and 0.01 M sodium acetate. Acetate ^{3}H-phenylalanyl-tRNA (·····); broth ^{14}C-phenylalanyl-tRNA (- - - - -).*

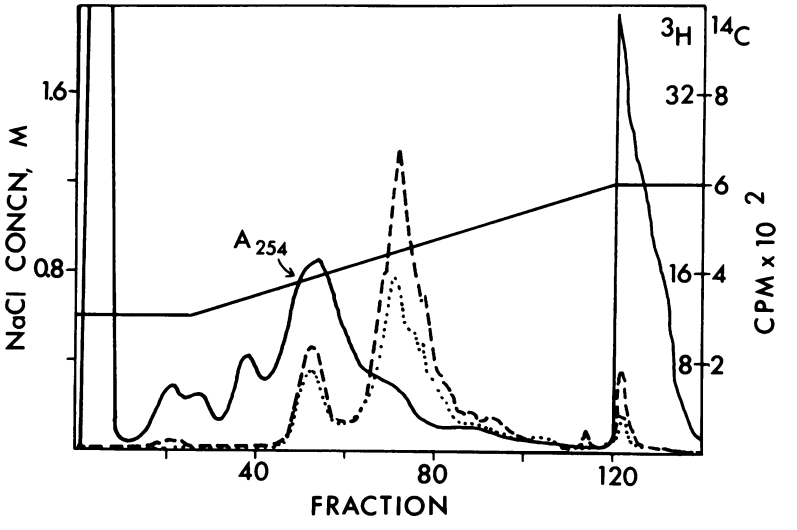

FIG. 6. *BD-cellulose chromatographic profile of lysyl-tRNA from cells grown in nutrient broth and acetate media. Elution was performed with a gradient of sodium chloride containing 0.01 M magnesium chloride and 0.05 M sodium acetate (pH 5.0), followed by 1.2 M sodium chloride containing 10% (vol/vol) 2-methoxyethanol. Acetate ^{3}H-lysyl-tRNA (·····); broth ^{14}C-lysyl-tRNA (- - - - -).*

by similar (yet differing) mechanisms, which is what Dennis (12) concluded. Even a set of isoaccepting tRNAs may be regulated independently of other tRNAs to a limited extent, although rates of synthesis or degradation could account for slight differences. The syntheses of isoaccepting tRNAs for the same amino acid appear to be regulated in the same manner under the extreme growth conditions. Because the tRNA/DNA ratio, for bulk tRNA and specific tRNAs, changes with growth rate, the amount of tRNA per cell must be determined by a regulatory mechanism and not by gene dosage (43).

Our results indicate that the limitation of protein synthesis is not determined in all instances by ribosomes (34) and that limitation by the supply of ribosomes might occur only, if

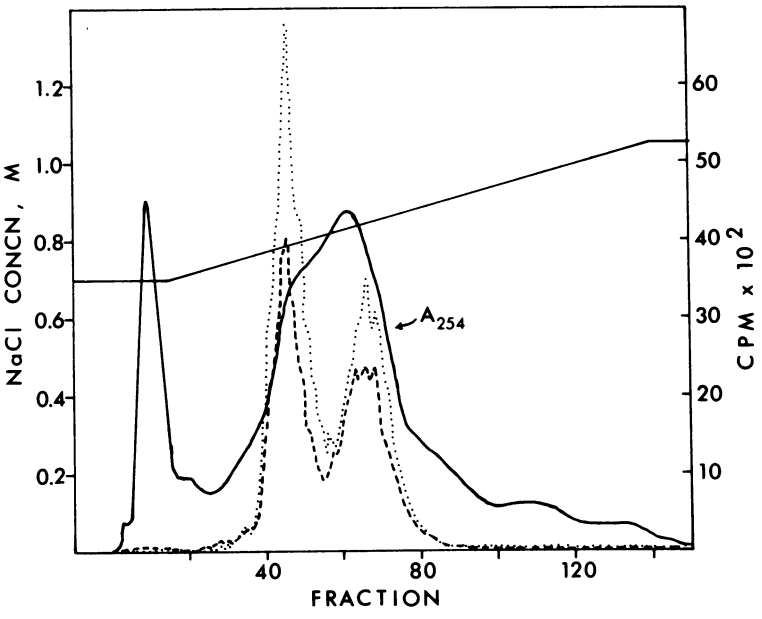

FIG. 7. *BD-cellulose chromatographic profile of methionyl-tRNA from cells grown in nutrient broth and acetate media. Elution was performed with a gradient of sodium chloride containing 0.01 M magnesium chloride, 0.01 M sodium acetate (pH 5), and 2-methoxyethanol (0 to 12%, vol/vol). Acetate ^{3}H-methionyl-tRNA (- - - - -); broth ^{14}C-methionyl-tRNA (· · · · ·).*

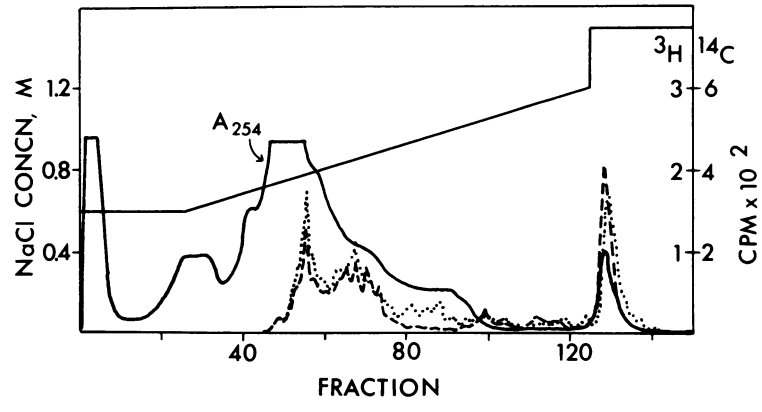

FIG. 8. *BD-cellulose chromatographic profile of seryl-tRNA from cells grown in nutrient broth and acetate media. Elution was performed with a gradient of sodium chloride containing 0.01 M magnesium chloride, 0.05 M sodium acetate (pH 5.0), and 2-methoxyethanol (0 to 10%, vol/vol), followed by 1.5 M sodium chloride containing 15% (vol/vol) 2-methoxyethanol. Acetate ^{3}H-seryl-tRNA (· · · · ·); broth ^{14}C-seryl-tRNA (-----).*

then, under conditions of glucose-minimal growth. Several reports present data pointing out that excess ribosomes are available for protein synthesis or that the efficiency of using rRNA in protein synthesis varies with different growth conditions (21, 23, 24, 34); yet, the RNA is immediately available for protein synthesis in shift-up experiments (21, 24).

Although we found that ribosomal efficiencies for generations per hour greater than 1.5 are not constant, the efficiencies generally agreed with those of Forchhammer and Lindahl (17). The greatest change in ribosomal efficiency was a 100% increase between 0.5 and 1.7 generations per hour.

A similar comparison of "tRNA efficiencies" in protein synthesis reveals an increase of almost 200% for the same range of growth rates.

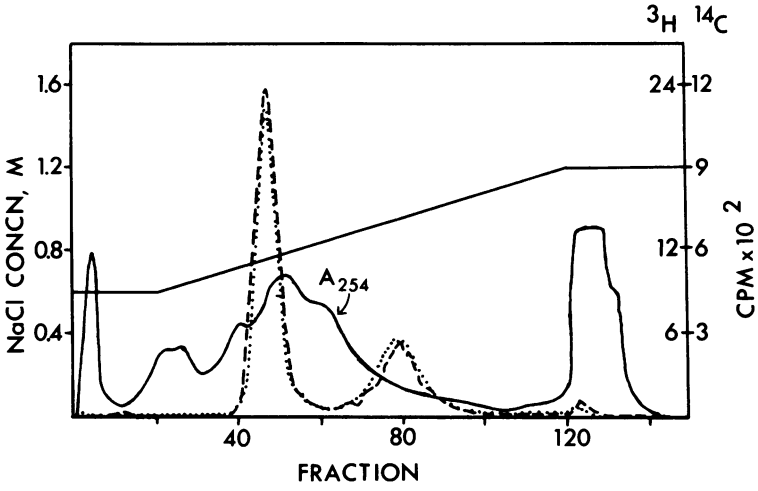

FIG. 9. *BD-cellulose chromatographic profile of valyl-tRNA from cells grown in nutrient broth and acetate media. Elution was performed with a gradient of sodium chloride containing 0.01 M magnesium chloride and 0.01 M sodium acetate (pH 4.0), followed by 1.2 M sodium chloride containing 10% (vol/vol) 2-methoxyethanol. Acetate ^3H-valyl-tRNA (·····); broth ^{14}C-valyl-tRNA (- - - - -).*

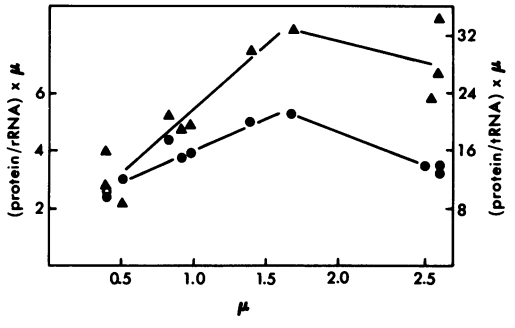

FIG. 10. *Efficiencies of rRNA (●) and tRNA (▲) in protein synthesis as functions of the growth rate.*

TABLE 2. *Number of tRNA molecules per ribosome*[a]

Medium	No. of tRNA molecules per ribosome			
	tRNA	$tRNA^{Arg}$	$tRNA^{Leu}$	$tRNA^{Ser}$
Acetate	18	0.56	0.91	0.54
Succinate	16	0.45	0.87	0.44
Glucose	12	0.42	0.74	0.38
Nutrient broth	9.0	0.42	0.63	0.43

[a] Based on estimated average molecular weights of 2.5×10^4 for tRNA and 1.8×10^6 for the ribosomal equivalent of rRNA.

Therefore, tRNA would appear to be a more reasonable limiting agent in protein synthesis than would ribosomes. Forchhammer and Lindahl (17) mentioned a possibly meaningful correlation between decreasing growth rate and a reduction in the cellular concentration of aminoacyl-tRNA. Their data and ours clearly indicate a greater availability of tRNA per ribosome at lower growth rates, but calculations based on differences in mass and size (9, 27) between rapidly and slowly growing cells reveal that the concentration of tRNA in the slowly growing cell (0.5 generation per hour) may be only 25% of the concentration in rapidly growing cells. The advantage gained by increasing the ratio of tRNA per ribosome may not be sufficient to offset the lower concentration of tRNA.

In *E. coli* strains MRE 600 (20) and 15 TAU-bar (16,17), messenger RNA (mRNA)/rRNA apparently is constant at various growth rates. Those observations alone do not indicate the efficiency with which ribosomes function in protein synthesis, but for different chain growth rates such data may indicate the limitation or regulation of protein synthesis by molecules or a mechanism independent of the availability of mRNA on the ribosome.

Acceptor capacities of tRNA for several amino acids did not show a change which could account for an inefficiency in protein synthesis resulting from a specific tRNA.

Because synthesis of a typical protein probably requires in excess of 20 different amino acid specific tRNAs to allow for codon degeneracy, the observed tRNA/ribosome ratios appear to be too low for the efficient synthesis of proteins. Our data support the idea that a portion of the

ribosomes are idle (23), although conceivably ribosomes share tRNA if diffusional limitations are not serious. In either case, it is apparent that the limited availability of tRNA, when compared with the number of ribosomes available, could be limiting protein synthesis in the cell.

If any of the tRNAs studied are involved in some way in regulating general protein synthesis, the role is more likely a quantitative rather than a qualitative contribution.

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