JOURNAL OF BACTERIOLOGY, June 1977, p. 1098-1108
Vol. 130, No. 3
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Chloramphenicol-Induced Changes in the Synthesis of Ribosomal, Transfer, and Messenger Ribonucleic Acids in *Escherichia coli* B/r

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Received for publication 25 January 1977

The synthesis of ribosomal ribonucleic acid (rRNA), transfer RNA (tRNA) and messenger RNA (mRNA) was measured in *Escherichia coli* B/r after the addition of 100 μg of chloramphenicol (CAM) per ml to cultures growing either in one of three minimal media (succinate, glycerol, or glucose) or in one of the same three media supplemented with 20 amino acids. (i) During CAM treatment, rRNA and tRNA were synthesized in the same relative proportions (85:15) as during exponential growth. The faster accumulation of tRNA relative to rRNA in CAM was due to a decreased stability of rRNA that is synthesized in the presence of or immediately before the addition of CAM. (ii) CAM stimulated the synthesis of rRNA and tRNA two- to eightfold. The results fell into two groups; one group was from studies done in minimal media and the other was from amino acid-supplemented media. In each group the stimulation decreased with increasing growth rate of the culture during exponential growth before the addition of CAM; however, the stimulation in minimal media was lower than that in amino acid-supplemented media. (iii) CAM caused an increase in the proportion of rRNA and tRNA synthesis and a corresponding decrease in the proportion of mRNA synthesis. In minimal media, the residual proportion of mRNA synthesis after CAM treatment was 10 to 15% of total RNA synthesis; in amino acid-supplemented media this proportion was 0 to 10%. In either case, the residual proportion of mRNA synthesis was independent of the proportions observed during exponential growth in these media. (iv) The absolute rate of mRNA synthesis decreased severalfold with the addition of CAM; i.e., the rate of synthesis of rRNA and tRNA was increased at the expense of mRNA synthesis. (v) During exponential growth, the fraction of the instantaneous rate of total RNA synthesis that corresponds to mRNA is a function of both the growth rate and the presence or absence of amino acids in the growth medium: in the absence of amino acids, this fraction decreased with increasing growth rate; in the presence of amino acids, the fraction increased slightly with growth rate. These results are consistent with a regulation of rRNA and tRNA synthesis at the transcriptional level, e.g., with a CAM-induced increase in the affinity of RNA polymerase for the rRNA and tRNA promoters. The results also suggest the occurrence of a regulation of RNA polymerase enzyme activity, i.e., of an activation of RNA polymerase that is inactive during exponential growth. A distinction between these alternatives requires measurements of the rRNA chain growth rates during CAM treatment.

Addition of the protein synthesis inhibitor chloramphenicol (CAM) to a bacterial culture is a convenient means to induce an increased transcription of ribosomal ribonucleic acid (rRNA) and transfer RNA (tRNA) genes (16, 18, 24). However, for rRNA this induction is difficult to quantitate because rRNA synthesized in the absence of protein synthesis is unstable (18). In the experiments presented here, this difficulty was overcome as follows.

We have observed previously that 5*S* rRNA is relatively stable in the presence of rifampin, a drug that inhibits RNA synthesis and that renders 16*S* and 23*S* rRNA unstable (33). Since 5*S* rRNA is cotranscribed with 16 and 23*S* rRNA (10, 14, 29) near the 3′ end of the total rRNA precursor (6), a 16*S* and a 23*S* rRNA

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molecule must have been synthesized for every 5S rRNA molecule observed. Hence, even if the 16 and 23S portions of the rRNA precursor are rapidly degraded, one might still infer their synthesis from the accumulation of the more stable 5S rRNA. Although we attempted this approach in the current investigation, we found that 5S rRNA synthesized in the presence of CAM is not completely stable; rather, it shows a slow turnover. But it was found that 5S rRNA and tRNA are synthesized in constant proportions and that tRNA is completely stable. Hence, tRNA accumulation can also be used to monitor rRNA synthesis. Since the rate of tRNA synthesis is several times greater than the rate of 5S rRNA synthesis, the measurement of tRNA provides greater sensitivity and accuracy.

Cashel (5) and Lazzarini et al. (17) suggested that ppGpp is involved in the control of rRNA synthesis. This nucleotide may interact directly with RNA polymerase to change its affinity for the rRNA and tRNA promoters (31, 32). CAM inhibits the synthesis of ppGpp in vivo (5, 12). If the normal regulation of rRNA synthesis does not require continued protein synthesis, then one would expect that CAM stimulates the rate of rRNA synthesis because it inhibits the synthesis of ppGpp. Further, this stimulation might be expected to be growth rate dependent: in slow-growing bacteria only a small fraction of the functioning RNA polymerase is engaged in the synthesis of stable RNA (e.g., 1, 26); therefore, the stimulation in slow-growing bacteria is potentially greater than that in fast-growing bacteria. In fact, Midgeley and Gray (24) observed an increase in the rate of accumulation of RNA after CAM addition which decreased with increasing growth rate of the bacteria before CAM treatment.

Our results show that the synthesis rates of rRNA and tRNA are coordinately increased by CAM, whereas at the same time the synthesis of messenger RNA (mRNA) is decreased. This redistribution of transcriptional activities depends on the growth rate, as was expected (see above), but it also depends on the presence or absence of amino acids in the growth medium. This amino acid effect is not understood. A quantitative evaluation of the results suggests that CAM may activate a fraction RNA polymerase that is inactive during exponential growth; this effect is particularly strong in slow-growing bacteria.

(This work was carried out by V. Shen in partial fulfillment of the requirements for the degree of Ph.D. in Molecular Biology at the University of Texas at Dallas.)

MATERIALS AND METHODS

Bacterial strain and growth conditions. Cultures of *Escherichia coli* B/r (ATCC 12407) were grown at 37°C in minimal C medium (15) supplemented with either 0.8% succinate or 0.2% glycerol or glucose and with or without a mixture of 20 synthetic L-amino acids, each to a final concentration of 20 μg/ml. Experimental cultures were inoculated with a fresh stationary-phase culture in the same medium (at least a 400-fold dilution). Culture growth was followed by measuring the turbidity (absorbance at 460 nm [A_{460}]; 1-cm light path). The bacterial growth rates were 2.0, 1.67, and 1.09 doublings/h in glucose, glycerol, and succinate medium supplemented with 20 amino acids, respectively, and 1.33, 1.03, or 0.6 doublings/h in glucose, glycerol, or succinate minimal medium. When bacteria were grown in only 20 amino acids (each at 20 μg/ml), the growth rate was 0.95 and growth ceased at an A_{460} of about 0.2. Thus, we assumed that bacteria in succinate amino acid medium, which grow exponentially at 1.09 doublings/h to an A_{460} of 2.0, are utilizing succinate.

Determination of RNA. Samples (0.5 ml of radioactively labeled cultures or 5 ml of unlabeled cultures) were added to 2 ml of 1 or 3 M trichloroacetic acid at 0°C, and the bacteria were collected on glass-fiber filters (Reeve-Angel 984H). The filters were kept in 1 ml of 0.2 N NaOH at 25°C overnight to hydrolyze RNA. An equal volume of 0.5 M perchloric acid was then added to each hydrolysate, and alkaline-insoluble, acid-precipitable material (including deoxyribonucleic acid [DNA]) was removed by membrane filtration. For radioactive RNA, 0.5 ml of the filtrate was dissolved in 5 ml of a Biosolv-3-toluene mixture, and the radioactivity was counted in a liquid scintillation spectrophotometer. For non-radioactive bacteria, the absorbance of the filtrate was read at 260 nm.

Gel electrophoresis. One milliliter of radioactively labeled culture was added to 1 ml of ethanol-phenol "stopping solution" at 0°C (21). After centrifugation the bacteria were resuspended in 0.5 ml of 100°C electrophoresis sample solution [0.2× tris(hydroxymethyl)aminomethane-ethylenediaminetetraacetic acid-borate (TEB) buffer (30), 1% sodium dodecyl sulfate, 10% glycerol, and 0.001% bromophenol blue], boiled for 45 s, and then stored at room temperature. A small volume (50 or 100 μl) of this lysate was applied to the gel. A 2.8% polyacrylamide-bisacrylamide (19:1)-0.5% agarose slab (12 cm long, 1.5 mm thick) was used to separate large-molecular-weight RNA species; a 10% polyacrylamide slab was used to separate small-molecular-weight RNA species. They were subjected to electrophoresis at room temperature for 1.5 and 3.5 h, respectively, at 10 V/cm in 1× TEB buffer containing 0.1% sodium dodecyl sulfate. The slab gels were dried on Whatman no. 1 filter paper and autoradiographed for 3 or 5 days on a sheet of Kodak PR Royal X-matic film. After development, the film was scanned on a Joyce-Loebl densitometer to locate the RNA bands. The gel (with the filter paper) was then cut into 2-mm slices. The RNA in the slices was hydrolyzed in 0.5 ml of 0.2 N NaOH overnight, and

the radioactivity was counted after addition of 5 ml of a Biosolv-3-toluene mixture in a liquid scintillation spectrophotometer.

DNA-RNA hybridization. *E. coli* DNA was purified by the procedure of Massie and Zimm (22), with slight modifications (H. Brunschede, unpublished data). Unlabeled "competitor" rRNA and [^{14}C]rRNA as internal standard were prepared from 30*S* and 50*S* ribosomal subunits after two sucrose gradient sedimentations, the first one at 10^{-2} M Mg^{2+} (isolation of 70*S* peak) and the second one after dialysis against 10^{-4} M Mg^{2+}.

Pulse-labeled RNA was obtained by labeling 1 ml of culture (A_{460} = 0.4), either growing exponentially or 5 min after CAM treatment, with [5-^{3}H]uridine (22 Ci/mmol; 50 μCi/ml, final concentration) for 1 min. At the end of the pulse, the culture was quickly poured into an equal volume of a boiling sodium dodecyl sulfate-lysis mixture (3); the lysate was kept at 100°C for 45 s and then stored at room temperature.

Hybridization according to Gillespie and Gillespie (13) was carried out in glass vials containing 0.5 ml of 50% formamide, 0.02 M ethylenediaminetetraacetic acid, 3× SSC (0.15 M NaCl plus 0.015 M sodium citrate), and 0.5% sodium dodecyl sulfate, 5 μl of pulse-labeled RNA lysate (containing 0.01 to 0.03 μg of RNA, which is mostly rRNA and tRNA), and 0.005 μg of [^{14}C]rRNA with or without 1.5 μg of non-radioactive competitor rRNA. They also contained two filters, one of which was a blank (filter without DNA; its radioactivity was subtracted from the sample radioactivity); the other filter was loaded with 15 μg of denatured *E. coli* DNA. After incubation at 37°C for 3 days, the filters were removed and thoroughly washed from both sides, and the radioactivity was counted in a liquid scintillation spectrophotometer.

DNA (15 μg/filter) was more than 500-fold in excess over RNA (excess of rDNA over rRNA was only one- to twofold, assuming six rRNA genes per genome and nonreplicating chromosomes in the stationary cells from which the DNA was prepared).

In the competition experiments, unlabeled rRNA was in more than a 50-fold excess over labeled RNA.

Chemicals. CAM, rifampin, and all radiochemicals were purchased from Schwarz/Mann (Orangeburg, N.Y.). Acrylamide and bisacrylamide were bought from Eastman Organic (Rochester, N.Y.). All other chemicals were purchased from Sargent-Welch (Skokie, Ill.).

RESULTS

Electrophoretic separation of RNA species. High-molecular-weight RNA species (>16*S*), which were radioactively labeled in the presence or absence of CAM, were separated by electrophoresis through soft gels (2.8% polyacrylamide, 0.5% agarose; Fig. 1a). Low-molecular-weight species (4*S*, 5*S*) were separated in harder gels (10% polyacrylamide; Fig. 1b). The accumulation of both radioactive high- and low-molecular-weight RNA is seen to be stimulated by CAM (area under the peaks). Further, the CAM-rRNA is defective in maturation (27). By plotting the sedimentation value versus movement (Fig. 1c), the precursors of 23*S* and 16*S* RNA were identified as 25*S* and 16.5*S* peaks, respectively. Also, a 26*S* peak was observed (Fig. 1a, c), which has not been reported previously.

Instability of RNA synthesized in the presence of CAM. When rifampin was added to bacteria labeled in the presence of CAM, a large fraction of labeled RNA become acid soluble (Fig. 2b), suggesting that not only mRNA but also rRNA was being degraded.

Electrophoretic separation of RNA species showed that tRNA labeled in the presence of CAM is stable after the addition of rifampin whereas 5*S* rRNA breaks down slowly (Fig. 3). Rifampin alone, without the addition of CAM, caused breakdown of 23*S* rRNA (Fig. 4, dashed line), but the combination of rifampin (rif) and CAM caused an acceleration of this breakdown (Fig. 4, solid line). In the first case (+rif, −CAM), incomplete 50*S* ribosomal particles are degraded (30). In the second case (+rif, +CAM), unprotected 23*S* rRNA, expected to be more susceptible to nucleases, was degraded. tRNA was stable under either condition (Fig. 4, dotted line).

Without rifampin, the stability of (unprotected) rRNA labeled after CAM (i.e., −rif, +CAM) cannot be determined in this manner because the breakdown of RNA would be masked by de novo synthesis of RNA.

Coordinate synthesis of rRNA and tRNA during CAM treatment. Since 5*S* rRNA and 4*S* tRNA were relatively stable in the presence of rifampin and CAM (Fig. 3 and 4), the rates of synthesis of these species in CAM could be assumed to be equal, or nearly equal, to their rates of accumulation. In the experiment of Fig. 5, RNA was labeled with [^{14}C]uracil beginning at 0, 10, or 20 min after the addition of CAM. The cells had been prelabeled during exponential growth (one to two generations before addition of CAM) with [^{3}H]uracil, such that the ^{14}C/^{3}H isotope ratio in each RNA species was a measure for the specific ^{14}C activity of that RNA species. The isotope ratios for 5*S* rRNA and tRNA are seen to increase almost linearly with the same slope during the first 30 min after CAM treatment (Fig. 5a). This means that during CAM treatment both species of RNA were synthesized in the same proportions in which they were synthesized during exponential growth and that these proportions do not change with time in CAM. Hence, the preferential accumulation of tRNA during CAM treatment (11, 16) results entirely from prefer-

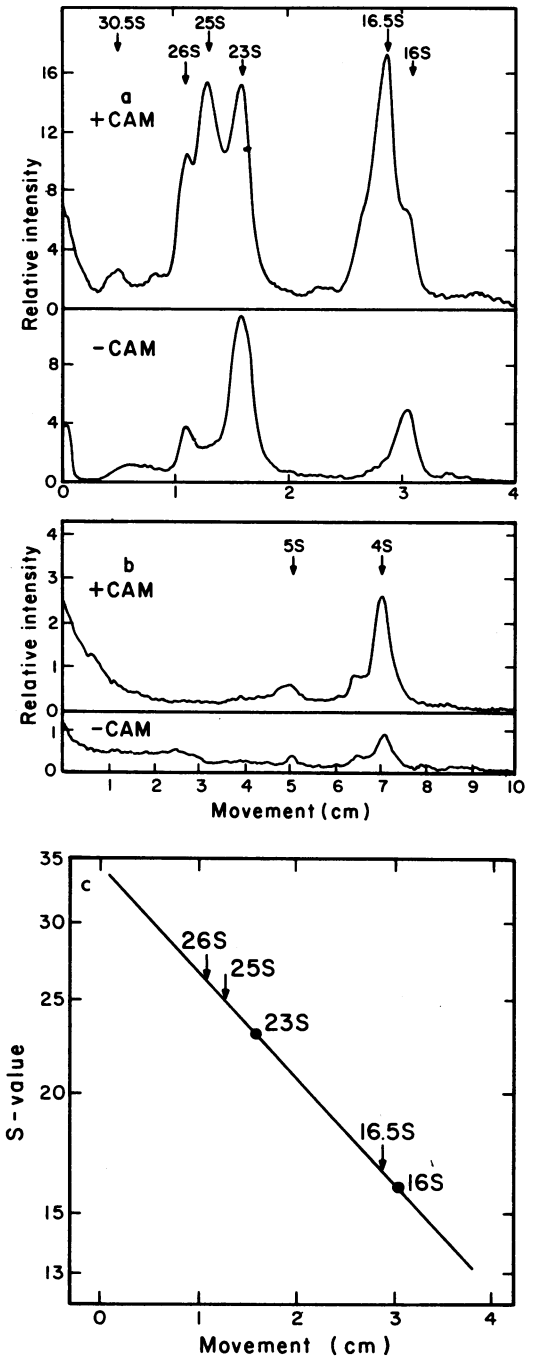

FIG. 1. *Separation of RNA species on polyacrylamide gels. An exponential culture (succinate plus amino acids medium) was labeled with [^{14}C]uracil (1.5 μCi/ml, 62 mCi/mmol) beginning at A_{460} = 0.32. After 15 min, CAM was added to one portion of the culture. After 20 min of further incubation, the cells of both CAM-treated and untreated culture were harvested, lysed, and analyzed by gel electrophoresis as described in the text. Densitometer tracings of the autoradiogram from the CAM-treated and untreated lysates are shown. (a) Separation of large-molecular-weight RNA species in a 2.8% polyacrylamide and 0.5% agarose composite gel. (b) Separation of small-molecular-weight RNA species in a 10% polyacrylamide gel. The relative intensity of the tracing (ordinate scale) was normalized to the same amount of sample lysate and the same length of film exposure time in (a) and (b). The sedimentation value of each band in (a) was obtained from Fig. 4c. (c) The matured 23S and 16S rRNA peaks from the minus CAM sample in (a) were used as standards (○) to define a straight line according to which the S-values of the other peaks (arrows) observed in (a) were estimated.*

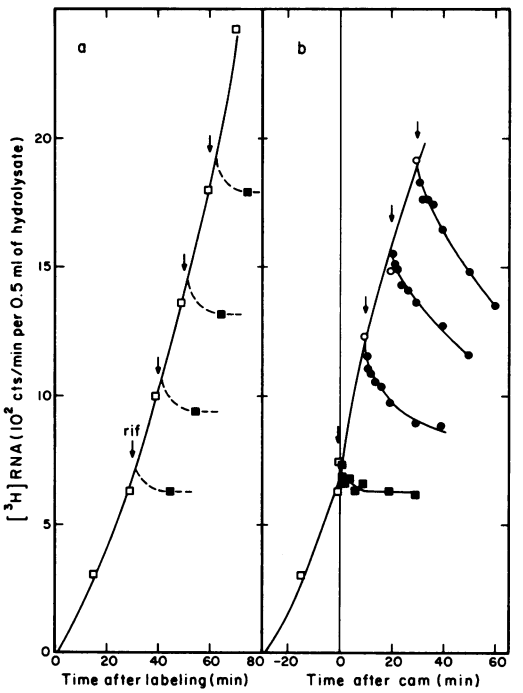

FIG. 2. *Stability of RNA synthesized during CAM treatment. (a) An exponentially growing culture (glucose plus amino acids medium) was labeled with [5-^{3}H]uracil (0.5 μCi/ml, 22 Ci/mmol). (b) After 30 min, a portion of the labeled culture was added to CAM (100 μg/ml). At the times indicated by the arrows, 5-ml portions of either CAM-treated or untreated culture were added to rifampin (rif) (200 μg/ml, final concentration). Samples (0.5 ml) were added to 2 ml of 1 M trichloroacetic acid, and the radioactivity in RNA (after removal of DNA) was determined as described in the text. Symbols: (□) −CAM, −rif; (■) −CAM, +rif; (○) + CAM, −rif; (●) +CAM, +rif. From the (−CAM, +rif) cultures, only one sample was taken at 15 min after rif, which indicates the final plateau (reference 3; see Fig. 5b, curve for rif at −1 min).*

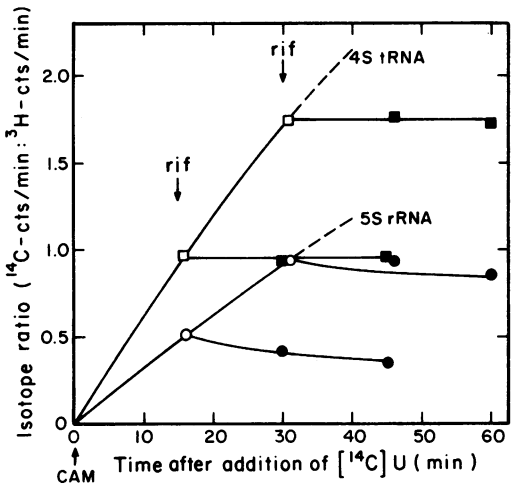

FIG. 3. *Stability of 4S tRNA and 5S rRNA synthesized during CAM treatment. An exponentially growing culture (glucose plus amino acids medium) was labeled with [^{3}H]uracil (0.5 μCi/ml, 22 Ci/mmol), beginning at A_{460} = 0.1. All ^{3}H label was exhausted from the medium and chased into stable nucleic acids within two generations. At A_{460} = 0.4 (zero time), [^{14}C]uracil (1.5 μCi/ml, 62 mCi/mmol) and CAM (100 μg/ml) were added. Portions of the labeled culture were added to rifampin (200 μg/ml, final concentration) either 15 or 30 min after addition of CAM and [^{14}C]uracil (↓). Cells (in 1 ml) were harvested, lysed, and analyzed on 10% polyacrylamide gels as described in the text. Symbols:* (□) *tRNA, +CAM −rif;* (■) *tRNA, +CAM +rif;* (○) *5S rRNA, +CAM −rif;* (●) *5S rRNA, +CAM +rif. The isotope ratios (^{14}C/^{3}H) are a measure for the specific radioactivity of the RNA synthesized during CAM treatment. The constant isotope ratio in tRNA after cessation of RNA synthesis means that [^{14}C]tRNA synthesized in the presence of CAM is as stable as [^{3}H]tRNA synthesized during exponential growth. The difference in the isotope ratios for 4S and 5S RNA observed here was unusual (cf. Fig. 9a and 10c) and is presumed to reflect a higher (than normal) background in the ^{3}H channel.*

ential breakdown of 16 and 23S rRNA rather than preferential (noncoordinate) synthesis of tRNA.

In Fig. 5a, the slopes of the labeling kinetics for 4S and 5S RNA are decreasing with time in CAM (about 30% per 20 min), but the slopes are independent of the time of the beginning of labeling (0, 10, or 20 min). This shows that in CAM the rates of synthesis of tRNA and rRNA slowly decrease (i.e., the decrease in the labeling rate is not due to pool effects or RNA turnover).

CAM-induced stimulation of rRNA and tRNA synthesis. According to the results in Fig. 5, the change in the rate of tRNA accumulation after the addition of CAM is equal to and can be used as a measure for the otherwise not observable change in the rate of rRNA synthesis. Using this approach, it was found that CAM produces always, in every growth medium tested, a significant (1.8- to 8-fold) stimulation of rRNA and tRNA synthesis (Fig. 6–8).

Any apparent stimulation in the accumulation of radioactive tRNA might also reflect an increase in the specific radioactivity rather than an increase in the synthesis rate of RNA.

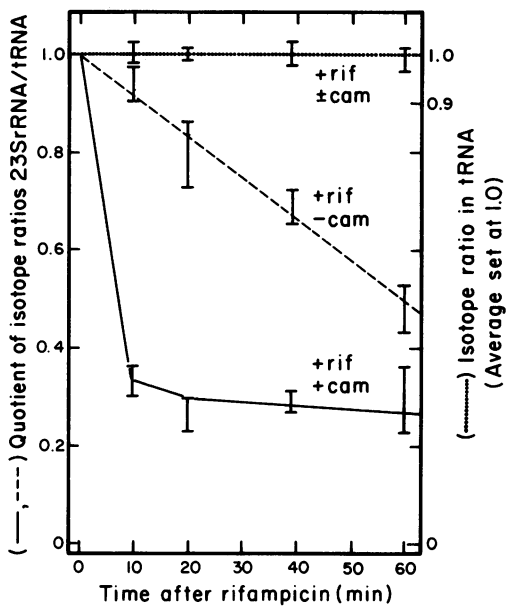

FIG. 4. *Stability of 23S rRNA and 4S tRNA synthesized during CAM treatment. Exponentially growing bacteria (glucose plus amino acids medium) were concentrated by centrifugation and treated for 2 min with ethylenediaminetetraacetic acid to increase their permeability for rifampin (rif) (33). The treatment was terminated by a 10-fold dilution to the original cell density (A_{460} = 0.4), and the culture was divided into two portions. After 3 min, 100 μg of CAM per ml was added to one portion. After a further 3 min, [^{14}C]uracil (0.5 μCi/ml, 62 mCi/mmol) and, again 2 min later (= zero time), 60 μg of rifampin per ml were added to both portions. Samples (1 ml) were mixed with an equal volume of exponentially growing bacteria whose stable nucleic acids were labeled with [^{3}H]uracil. Lysates from each sample were prepared for electrophoresis and analyzed as described in the text. The vertical bars represent the range from three different experiments. The average of the isotope ratios for 4S tRNA in each experiment was set at 1.0 (right ordinate). The isotope ratios of 23S rRNA were divided by the isotope ratios in tRNA observed in the same sample (left ordinate). For each isotope ratio determination, the summed ^{3}H and ^{14}C radioactivities in the whole peak were used (Fig. 1a). For further experimental details and rationale of analysis see Fig. 1 and 2 of reference 33.*

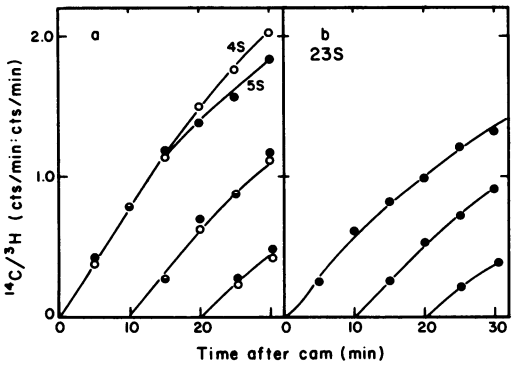

FIG. 5. *Comparison of tRNA and rRNA synthesis during CAM treatment. Exponentially growing bacteria (glucose plus amino acids medium) were prelabeled with [^{3}H]uracil (0.5 μCi/ml, 22 Ci/mmol), beginning at A_{460} = 0.1. During two generations, all label was exhausted from the medium and "chased" into stable nucleic acids. At A_{460} = 0.4, CAM (100 μg/ml) was added (zero time). At 0, 10, and 20 min [^{14}C]uracil (1.5 μCi/ml; 62 Ci/mol) was added to portions of the CAM-treated culture. Samples (1 ml) were analyzed by electrophoresis as described in the text. (a) Isotope ratios in 5S rRNA and tRNA. The ^{3}H radioactivities per sample are 1,400 and 3,700 cpm, respectively. (b) Isotope ratio in 23S rRNA. The ^{3}H radioactivity was 16,000 cpm. The equal slope of the curves for 4S and 5S RNA indicates that rRNA and tRNA are synthesized in CAM (^{14}C label) in the same proportions as during exponential growth before CAM (^{3}H label).*

The specific activity might increase due to reduced endogenous synthesis of pyrimidines or due to decreased recycling of non-radioactive pyrimidine nucleotides liberated from RNA degradation during the labeling. These two effects were minimized by using a high concentration of uracil, which represses most of the endogenous pyrimidine synthesis, and by beginning the labeling before the addition of CAM such that the RNA precursor pool and mRNA were equilibrated with label when the experiment began.

Figures 6 and 7 illustrate two examples in which [^{14}C]uracil was added to exponentially growing bacteria (prelabeled with [^{3}H]uracil) either at the time of CAM addition (Fig. 6) or 15 min earlier (Fig. 7). In Fig. 6c, the labeling of 4S and 5S rRNA is seen to be equally stimulated (twofold), but the labeling of 5S rRNA (in CAM) soon lags behind the labeling of 4S RNA, presumably due to a slow turnover of (unprotected) 5S rRNA. If the ^{14}C labeling is begun 15 min before CAM, the labeling of 4S tRNA is stimulated again about twofold, but 5S rRNA is now labeled more slowly (only 1.6-fold stimulation, Fig. 7c). A qualitatively similar difference was obtained for the labeling of 23S rRNA (cf. Fig. 6d and 7d).

The effect of the labeling program on the labeling of 5S and 23S RNA (relative to 4S

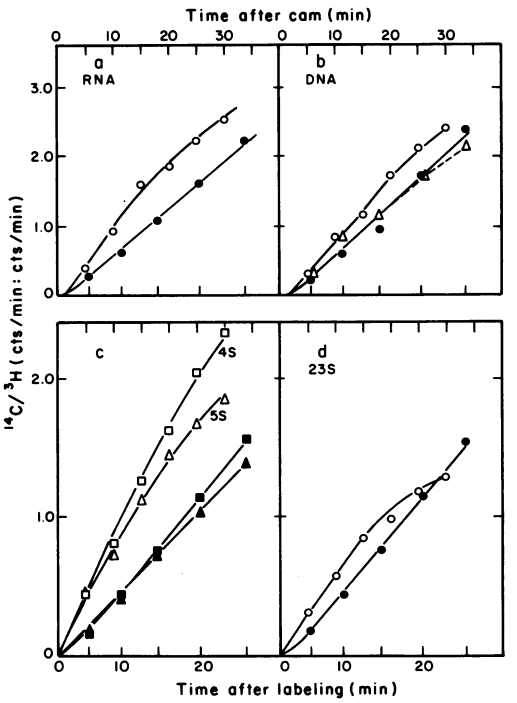

FIG. 6. *Changes of labeling rates of nucleic acids due to CAM. An exponentially growing culture (glucose plus amino acids medium) was prelabled with [^{3}H]uracil (0.5 μCi/ml, 22 Ci/mmol), beginning at A_{460} = 0.1. All ^{3}H label was chased into stable nucleic acids during two generations. At A_{460} = 0.4 the culture was divided into two portions. To one portion [^{14}C]uracil (1.5 μCi/ml, 62 mCi/mmol) and CAM (100 μg/ml) were added (zero time); to the other portion only [^{14}C]uracil was added. Samples were harvested, lysed, and subjected to electrophoresis as described in the text. Symbols: open, CAM-treated culture; closed, untreated culture. Bottom abscissa = expanded time scale for untreated culture (reference 20; expansion factor proportional to growth increment). Top abscissa = linear time scale for CAM-treated culture. (a) RNA = total acid precipitable radioactivity (in 20 μl of bacterial lysate) minus DNA. (b) DNA = alkali-stable material (1 h in 0.2 M NaOH; later the duration of this treatment was found to be insufficient; therefore, the DNA may be slightly contaminated [~20%] by RNA fragments). Symbols: (○) CAM, linear time scale; (△) CAM, expanded time scale; (●) no CAM, expanded time scale. (c) tRNA and 5S rRNA. (d) 23S rRNA. The absolute ^{3}H radioactivities were 45,000, 6,500, 4,200, 1,600, and 16,000 cpm (all within ±10%) for RNA, DNA, tRNA, 5S rRNA, and 23S rRNA, respectively.*

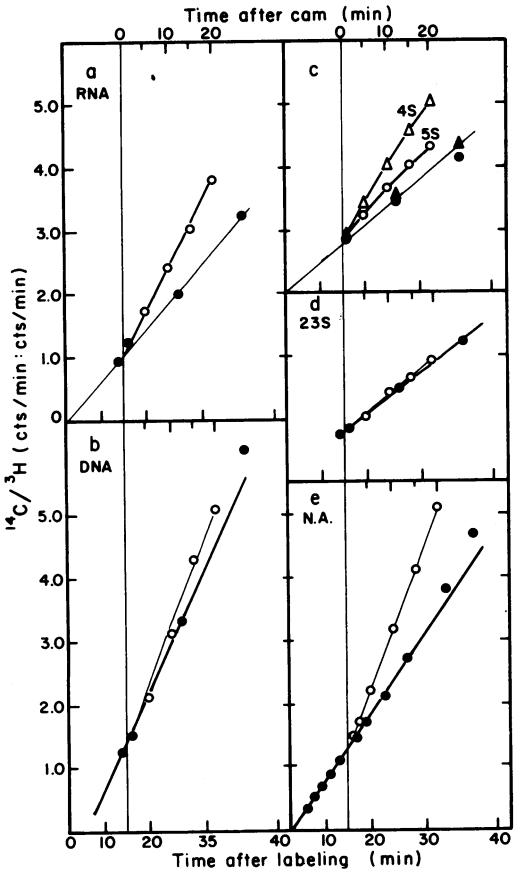

FIG. 7. *CAM-induced changes of the rates of labeling of nucleic acids after saturation of the nucleic acid precursor pools with label. Experimental procedures, symbols, and radioactivities are the same as in Fig. 6 except that [^{14}C]uracil was added 15 min before CAM in order to saturate the intracellular pool with ^{14}C-labeled nucleotides. (a) RNA; (b) DNA; (c) 5S rRNA, 4S tRNA; (d) 23S rRNA; (e) total nucleic acids.*

tRNA) indicates that 5*S* and 23*S* rRNA molecules are unstable when they are synthesized either during or immediately (5 to 10 min) before CAM treatment, i.e., when the assembly of 50*S* ribosomal particles is still incomplete. When label was added together with CAM (Fig. 6), then this breakdown was not immediately apparent because initially most of the degraded RNA was unlabeled. However, when label was added 15 min before CAM (Fig. 7) the increased synthesis of 5*S* and 23*S* RNA was partially (5*S* RNA) or completely (23*S* RNA) masked by the breakdown of the molecules labeled immediately before CAM addition. Only if 5*S* or 23*S* RNA were labeled one to two generations before the addition of CAM (^{3}H label in the experiments of Fig. 6 and 7) did these species remain completely stable in CAM (experiments not shown here).

In these experiments the labeling of DNA was followed to monitor the specific activity of the pyrimidine nucleotide pool. After CAM treatment, the rate of DNA synthesis continues to increase exponentially for 10 to 20 min (23). Therefore, the DNA isotope ratios from the CAM-treated culture must be plotted versus the expanded time scale (Fig. 6b, open triangles) rather than versus the linear time scale (Fig. 6b, open circles). If this is done (i.e., if the values for both the treated and untreated culture are compared using the same time scale), it is apparent that the labeling of DNA is not immediately changed upon the addition of CAM. This suggests that the specific radioactivity of the pyrimidine nucleotide pool also is not immediately affected by CAM. Thus, the apparent CAM-induced stimulation of rRNA and tRNA synthesis is not a labeling artifact.

The isotope ratio of total RNA increased faster than that of 23*S* rRNA molecules (Fig. 6a and 7a), indicating that much of the rRNA was still present as acid-precipitable fragments, which contribute to the total RNA but not to the intact species. (The labeling of 16*S* rRNA was not followed because the 16*S* region in the gel is always contaminated by breakdown fragments from 23*S* rRNA.)

Growth rate dependency of CAM-induced stimulation of RNA synthesis. The stimulation of tRNA (and thus rRNA) synthesis was measured (in exactly the same way as illustrated in Fig. 7) after the addition of CAM to bacteria growing in six different media: succinate, glycerol, and glucose minimal medium and the same three media supplemented with a mixture of 20 amino acids. In general, the stimulation decreased with increasing growth rate of the bacteria measured before the addition of CAM, as was expected (see above), but this correlation is not strict. However, if the minimal media and the amino acid-supplemented media are considered separately, then the correlation between growth rate and stimulation (within each group of media) is strictly valid (Fig. 8).

Synthesis of mRNA. The rate of mRNA synthesis relative to total RNA synthesis was measured by analyzing pulse-labeled RNA with a DNA-RNA hybridization competition assay. RNA synthesized during exponential growth or 5 min after the addition of CAM was labeled for 1 min with [^{3}H]uridine and mixed with a known amount of ^{14}C-labeled 16*S* and 23*S* rRNA (as an internal standard for measuring the hybridization efficiency) and with increas-

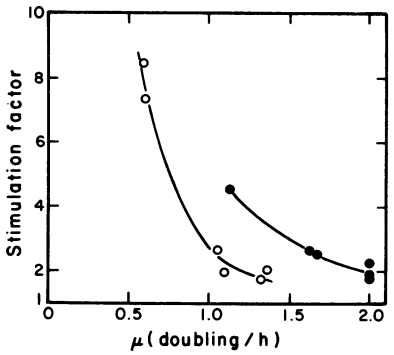

FIG. 8. *CAM-induced stimulation of the rate of tRNA synthesis as a function of the bacterial growth rate (μ; measured in the absence of CAM). Experiments as shown in Fig. 7 were done in six different growth media. Stimulation factor = ratio of the slopes of the isotope ratio curves (+CAM/−CAM) for tRNA. For the CAM-treated cultures, the slopes were determined between 5 and 10 min after CAM. Symbols: Open, cultures grown in minimal media; closed, amino acid-supplemented cultures (see text for composition of media).*

ing amounts of non-radioactive 16*S* and 23*S* competitor rRNA. This RNA mixture was allowed to hybridize to a 500- to 1,000-fold excess of *E. coli* DNA (the excess of rDNA over rRNA was only one- to twofold). With increasing amounts of competitor rRNA, the hybridization of ^{14}C-labeled rRNA approached zero, but the hybridization of ^{3}H pulse-labeled RNA reached a plateau (Fig. 9a). The height of this plateau is assumed to represent the fraction of RNA, synthesized at any instant, that is mRNA and tRNA. The level does not significantly depend on the hybridization efficiency (13 to 30%), the DNA-RNA ratio (between 300:1 and 1,500:1), or the duration of the pulse (0.5 to 1.0 min) within the range tested (data not shown). The contribution of tRNA to the plateau level was calculated by assuming the rate of tRNA synthesis to be 15/85 (17.5%) of the rate of rRNA synthesis (9). The results of such experiments were expressed as the fraction of total RNA synthesis that is stable RNA (Fig. 9b, left ordinate) or as the complement fraction of total RNA synthesis that is mRNA (Fig. 9b, right ordinate) and plotted versus the growth rate. Since the values were measured as fractions of pulse-label in pyrimidine-labeled RNA, they had to be corrected for the different mole fraction of pyrimidines in mRNA (0.5) and stable RNA (0.43) to give the fractional synthesis rates (see legend to Fig. 9). They were further corrected for the presence of unstable spacer regions in the rRNA precursor (see legend to Fig. 9).

In exponentially growing cultures, the fractional synthesis of stable RNA increased with growth rate (Fig. 9b, dashed curve), in agreement with previously determined values from this laboratory (Fig. 9b, × symbol) obtained with the rifampin method (7) but higher than values obtained with the histidine method (4).

In Fig. 9b the values obtained from bacteria growing in succinate-amino acids medium appear to be too high for the growth rate and thus do not fit the pattern obtained from the other media (dashed curve). This means that succinate-amino acids-grown bacteria contain either more ribosomes that are less active than bacteria grown in glycerol minimal medium (which show about the same growth rate) or less mRNA per ribosome. This question was answered by measuring the RNA-protein ratio. The results (data not illustrated) showed that succinate-amino acid- and glycerol-grown bacteria produce the same amount of rRNA and tRNA, i.e., the same number of ribosomes, per given amount of protein. Hence, succinate-amino acid-grown bacteria produce less mRNA in comparison to glycerol-grown bacteria.

If it is again assumed that the results from minimal media and from amino acid-supplemented media represent two separate groups (Fig. 9b, thin lines, open and solid circles), then the low fractional synthesis of mRNA in succinate-amino acid-grown bacteria would not be an exception. Viewed according to this interpretation, the data suggest that for exponential growth the synthesis rate of stable RNA and the synthesis of mRNA are determined separately by a mechanism that depends on both the carbon source and the presence or absence of amino acids in the growth medium.

Addition of CAM always produced an increase in the fraction of rRNA and tRNA synthesis and a corresponding decrease in the fraction of mRNA synthesis (Fig. 9c). Again, the results from minimal and from amino acid-supplemented media fall into two groups: without amino acids, 10 to 15% of the RNA synthesis rate corresponds to mRNA, whereas with amino acids the fraction is 0 to 10%.

DISCUSSION

Absolute rates of synthesis of rRNA, tRNA, and mRNA. For the bacterial strain used here, the rate of stable RNA synthesis per genome equivalent of DNA has been determined previously as a function of growth rate (9); it increases with growth rate as indicated by the parabolical curve (open circles) in Fig. 10b and e. Combining this curve with the stimulation factors of Fig. 8 gives the synthesis rates of stable RNA during the first 10 min after CAM in

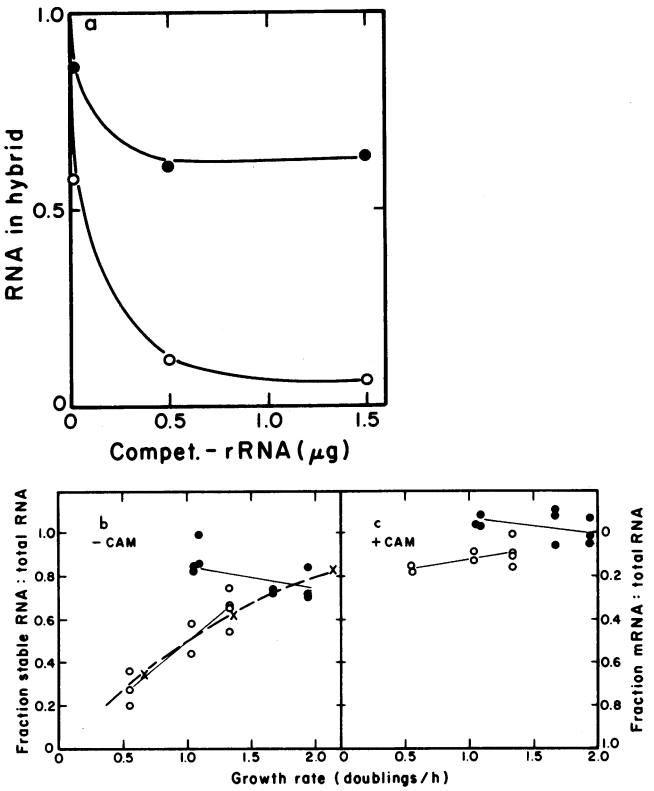

FIG. 9. *Relative rate of synthesis of rRNA and tRNA (left ordinate) and of mRNA (right ordinate) as a function of the bacterial growth rate. (a) Content of rRNA in pulse-labeled RNA. Symbols: (●) ^3H hybrid = RNA pulse-labeled during exponential growth; (○) ^{14}C hybrid = rRNA purified from ribosomal particles. Hybridization efficiency is about 25%. Each point is the average of triplicates. See text for details. (b) Exponential cultures, labeled for 1 min (A_{460} = 0.5) with [^3H]uridine (50 Ci/ml, 22 Ci/mmol). (c) 1 min pulse-labeling 5 min after CAM (100 μg/ml); same cultures as in (b). Symbols: (○) minimal media; (●) amino acid-supplemented media; (×) values from reference 9 multiplied by 1.2 (see below). The curves (——, - - - -) are interpretations; see text. The relative rate ρ (left ordinate) is defined as fraction of the instantaneous rate of RNA synthesis that corresponds to rRNA and tRNA; it was obtained from experiments as in (a), using the relationship: ρ = 1.2 r/[r + (0.85 − r) 0.86], where r is the fraction of pulse-label competed by non-radioactive rRNA; the constants 0.86 and 0.85 account for the different mole fractions of labeled pyrimidines in mRNA and stable RNA and for the synthesis of tRNA relative to the sum rRNA + tRNA, respectively. The factor 1.2 accounts for the fact that the rRNA precursor contains unstable spacer material (27). The value 1 − ρ (right ordinate) is the fraction of the instantaneous rate of RNA synthesis corresponding to mRNA.*

absolute units (nucleotides per minute per genome; closed circles in Fig. 10b and e). These curves can be further combined with the values for the relative rate of mRNA synthesis of Fig. 9 to give the absolute rate of mRNA synthesis (in nucleotides per minute per genome) during exponential growth or immediately after CAM addition (Fig. 10c and f). The sum of the synthesis rates of stable RNA plus mRNA is the synthesis rate of total RNA, again before and after CAM addition (Fig. 10a and d).

A simple expectation was that the total rate of RNA synthesis would not increase in CAM and, therefore, that the increase in the rate of stable RNA synthesis after CAM addition would be accompanied by a compensatory decrease in the rate of mRNA synthesis. The data in Fig. 10 clearly indicate that this is not the case; i.e., CAM causes a 1.3- to 3.5-fold increase in the total rate of RNA synthesis. Only part of this increase (maximally 1.5-fold) might be explained by the difference in the mRNA and rRNA chain growth rates (1, 8, 25). It appears, therefore, that CAM activates RNA polymerase in addition to causing a redistribution of functioning RNA polymerase molecules over

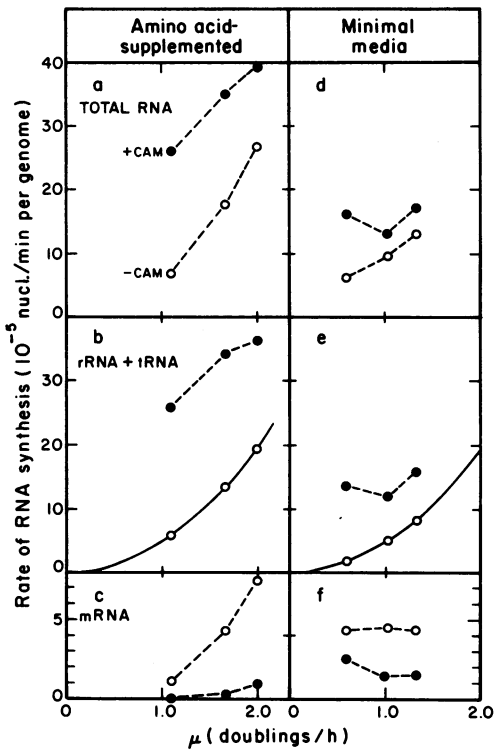

FIG. 10. *Rate of synthesis of mRNA, stable (rRNA and tRNA) RNA, and total RNA (in nucleotides per minute per genome equivalent of DNA) as a function of growth rate, in the presence or absence of amino acids (left and right panels) or of CAM (closed and open symbols), respectively. The data were obtained by combining the results from Fig. 8 and 9 with measurements of the amount of stable RNA, R_s, per genome as a function of the bacterial growth rate, μ. $R_s = 8.2 \times 10^6 \cdot 4.2 \cdot \mu$; the factors 8.2×10^6 and 4.2 are the DNA nucleotides per E. coli genome (3) and the observed factor of proportionality between the numbers of stable RNA nucleotides and DNA nucleotides (from reference 9). The rate of stable RNA synthesis shown (parabolic curves in b and e) is given by $dR_s/dt = R_s(\ln 2/60) \times 1.2\mu = 4.8 \times 10^5\mu^2$. For the factor 1.2, see legend to Fig. 9; for further details, see text.*

mRNA and stable RNA genes, in particular if it is added to slow-growing bacteria.

Effect of amino acids on mRNA synthesis during exponential growth. Two cultures with the same growth rate and with the same rRNA and tRNA synthesis rate per genome can have different rates of mRNA synthesis (Fig. 9). This implies that the amount of mRNA per ribosome is not constant (19). The difference in the rate of mRNA synthesis at a given rate of rRNA and tRNA synthesis (= differences in the rate of total RNA synthesis per genome) might be for one or a combination of the following reasons: (i) differences in the synthesis of RNA polymerase (RNA polymerase per genome); (ii) differences in the activity of RNA polymerase (fraction of active/total polymerase); (iii) differences in the rate of RNA chain elongation. In principle, these possibilities should be distinguishable with available experimental techniques. However, RNA polymerase measurements (i.e., the quantitation of $\beta\beta'$ protein on polyacrylamide gels) and RNA chain growth measurements have in the past been subject to considerable experimental variation such that it is uncertain whether these methods will lead to a convincing distinction between the above alternatives. At present we cannot explain the effect of amino acids on mRNA synthesis.

ACKNOWLEDGMENTS

We are indebted to Horst Brunschede for introducing us to the procedures for hybridization as well as providing us generously with purified DNA. We also wish to thank Ruth Ricamore for her graphics and Joyce Lee for her patience and skill in preparing this manuscript.

This work was supported by Public Health Service grant GM 15142 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Bremer, H., L. Berry, and P. Dennis.** 1973. Regulation of ribonucleic acid synthesis in *Escherichia coli* B/r: an analysis of a shift-up. II. Fraction of RNA polymerase engaged in the synthesis of stable RNA at different steady-state growth rates. J. Mol. Biol. 75:161-179.
2. **Bremer, H., J. Hymes, and P. Dennis.** 1974. Ribosomal RNA chain growth rate and RNA labeling patterns in *Escherichia coli* B/r. J. Theor. Biol. 45:379-404.
3. **Bremer, H., and D. Yuan.** 1968b. Chain growth rate of messenger RNA in *Escherichia coli* infected with bacteriophage T4. J. Mol. Biol. 34:527-540.
4. **Burton, K.** 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323.
5. **Cashel, M.** 1969. The control of ribonucleic acid synthesis in *Escherichia coli*. IV. Relevance of unusual phosphorylated compounds from amino acid-starved stringent strains. J. Biol. Chem. 244:3133-3141.
6. **Colli, W., I. Smith, and M. Oishi.** 1971. Physical linkage between 5s, 16s and 23s ribosomal RNA genes in *Bacillus subtilis*. J. Mol. Biol. 56:117-128.
7. **Dennis, P., and H. Bremer.** 1973. A method for determination of the synthesis rate of stable and unstable RNA in *Escherichia coli*. Anal. Biochem. 56:489-501.
8. **Dennis, P., and H. Bremer.** 1973. Regulation of RNA synthesis in *E. coli* B/r: an analysis of shift-up. I. Ribosomal RNA chain growth rate. J. Mol. Biol. 75:145-159.
9. **Dennis, P., and H. Bremer.** 1974. Macromolecular composition during steady-state growth in *Escherichia coli* B/r. J. Bacteriol. 119:270-281.
10. **Doolittle, F. W., and N. R. Pace.** 1970. Synthesis of 5s rRNA in *E. coli* after rifampicin treatment. Nature (London) 228:125-127.
11. **Ezekiel, D. H., and B. Valuis.** 1965. Increase in functional tRNA during prolonged-incubation in chloramphenicol. Biochim. Biophys. Acta 108:135-136.

12. **Gallant, J., G. Margason, and B. Finch.** 1972. On the turnover of ppGpp in *Escherichia coli*. J. Biol. Chem. 247:6055–6058.
13. **Gillespie, S., and D. Gillespie.** 1971. Ribonucleic acid-deoxyribonucleic acid hybridization in aqueous solutions containing formamide. Biochem. J. 125:481–487.
14. **Hayes, F., O. M. Vasseur, N. Nikolaev, D. Schlessinger, J. Sriwidada, A. Krol, and C. Branlant.** 1975. Structure of a 30S pre-ribosomal RNA of *E. coli*. FEBS Lett. 56:85–91.
15. **Helmstetter, C. E.** 1967. Rate of DNA synthesis during the division cycle of *E. coli* B/r. J. Mol. Biol. 24:417–427.
16. **Kurland, C. G., and O. Maaløe.** 1962. Regulation of ribosomal and transfer RNA synthesis. J. Mol. Biol. 4:193–210.
17. **Lazzarini, R. A., M. Cashel, and J. Gallant.** 1971. On the regulation of guanosine tetraphosphate levels in stringent and relaxed strains of *Escherichia coli*. J. Biol. Chem. 246:4381–4385.
18. **Lazzarini, R. A., and E. Santangelo.** 1968. Effect of chloramphenicol on the synthesis and stability of ribonucleic acid in *Bacillus subtilis*. J. Bacteriol. 95:1212–1220.
19. **Maaløe, O.** 1969. An analysis of bacterial growth. Dev. Biol. Suppl. 3:33–58.
20. **McCarthy, B. J., and R. J. Britten.** 1962. The synthesis of ribosomes in *E. coli*. 1. The incorporation of ^{14}C-uracil into the metabolic pool and RNA. Biophys. J. 2:35–47.
21. **Manor, H., D. Goodman, and G. S. Stent.** 1969. RNA chain growth rate in *E. coli*. J. Mol. Biol. 39:1–23.
22. **Massie, H. R., and B. H. Zimm.** 1965. Molecular weight of the DNA in the chromosomes of *E. coli* and *B. subtilis*. Proc. Natl. Acad. Sci. U.S.A. 54:1636–1641.
23. **Messer, W.** 1972. Initiation of deoxyribonucleic acid replication in *Escherichia coli* B/r: chronology of events and transcriptional control of initiation. J. Bacteriol. 112:7–12.
24. **Midgeley, J. E. M., and W. J. H. Gray.** 1971. The control of RNA synthesis in bacteria. The synthesis and stability of RNA in chloramphenicol-inhibited cultures of *E. coli*. Biochem. J. 122:149–159.
25. **Molin, S.** 1976. Control of ribosome synthesis, p. 333–341. *In* N. O. Kjeldgaard and O. Maaløe (ed.), Alfred Benzon Symposium IX, Munksgaard. Academic Press Inc., New York.
26. **Nierlich, D. P.** 1972. Regulation of RNA synthesis in growing bacterial cells. II. Control over the composition of the newly made RNA. J. Mol. Biol. 72:765–777.
27. **Nikolaev, N., D. Schlessinger, and P. K. Wellauer.** 1974. 30S preribosomal RNA of *Escherichia coli* and products of cleavage by RNAse III: length and molecular weight. J. Mol. Biol. 86:741–747.
28. **Norris, T. E., and A. Koch.** 1972. Effect of growth rate on the relative rates of synthesis of messenger, ribosomal and transfer RNA in *Escherichia coli*. J. Mol. Biol. 64:633–650.
29. **Pace, N. P.** 1973. Structure and synthesis of the ribosomal ribonucleic acid of prokaryotes. Bacteriol. Rev. 37:562–603.
30. **Peacock, A. C., and C. W. Dingman.** 1967. Resolution of multiple RNA species by polyacrylamide gel electrophoresis. Biochemistry 6:1818–1827.
31. **Travers, A.** 1976. Modulation of RNA polymerase specificity of ppGpp. Mol. Gen. Genet. 147:225–232.
32. **van Ooyen, A. J. J., M. Gruber, and P. Jorgenson.** 1976. The mechanism of action of ppGpp on rRNA synthesis *in vitro*. Cell 8:123–128.
33. **Yuan, D. and V. Shen.** 1975. Stability of ribosomal and transfer ribonucleic acid in *Escherichia coli* B/r after treatment with ethylenedinitrilotetraacetic acid and rifampin. J. Bacteriol. 122:425–432.