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Coupling of Rates of Transcription, Translation, and Messenger Ribonucleic Acid Degradation in Streptomycin-Dependent Mutants of *Escherichia coli*

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The growth rates of streptomycin-dependent mutants varied in proportion to the level of streptomycin supplied; growth also varied characteristically from one dependent strain to another at a given streptomycin concentration. When cells growing at different rates (over a threefold range) were treated with rifampin, direct proportionality was observed for three parameters: (i) the rates of shutoff of transcription of total ribonucleic acid (RNA) and ribosomal RNA, as measured by pulse labeling at later times; (ii) the translation time for molecules of β-galactosidase; and (iii) the rate of chemical degradation of messenger RNA. In contrast, the rate of functional inactivation of both total and β-galactosidase messenger RNA was about the same at all growth rates. None of the variations of growth or other parameters were observed in an otherwise isogenic streptomycin-resistant strain treated with streptomycin. Since the mutational change in str^d mutants and the site of action of streptomycin are in the 30*S* ribosomal subunits, it is suggested that the rate of ribosome function is set by the dependent lesion (and the level of streptomycin). One possibility is that the other correlated effects are mechanistically "coupled" to ribosome function, but the apparent coupling could also be an indirect result of differential effects of streptomycin on variables such as ribosomal miscoding and nucleotide pool size. However, since the rate of functional inactivation of messenger RNA is constant even when the RNA is broken down two- to fourfold more slowly, translation yield tends to be proportional to the growth rate of the dependent strains.

When translation is strongly inhibited, messenger ribonucleic acid (mRNA) transcription and degradation in *Escherichia coli* are both affected. The accumulation of some mRNA species is selectively shut off in vivo (2, 14, 15), and degradation of newly formed mRNA is inhibited in cells (5, 12) and extracts (4, 17, 21). Ribosome function might, therefore, be required for normal transcription and mRNA decay; however, this apparent "coupling" (33, 37) might, instead, be a result of independent events after the sharp arrest of protein synthesis.

Lesions that affect the rate of the ribosome cycle without blocking steady-state growth should give more information about the possible effects of ribosome function on RNA formation or breakdown. One approach is to use low levels of certain inhibitors of ribosome function (1, 13). In the approach used here, strains bearing different, dependent alleles at the *str* locus (or the same mutant studied at different levels of streptomycin) have been employed to give a controlled rate of ribosome function. The results show correlated effects on transcription, translation, and mRNA degradation and are consistent with a role for ribosomes in coupling the processes.

MATERIALS AND METHODS

Bacteria and culture conditions. The parental strain C3 employed in these studies is an *E. coli* K-12 derivative, from strain D10 (8), cured of λ and made trp^- by ultraviolet light mutagenesis. Bacteria were grown with aeration at 31 C in minimal salts medium (contains [per liter]: Na_2HPO_4, 7 g; KH_2PO_4, 3 g; NH_4Cl, 1 g; Na_2SO_4, 0.8 g; $FeCl_3$, 1.75 mg; $MgCl_2$, 48.8 mg; and $CaCl_2$, 2.75 mg) supplemented with 1% Difco technical-grade Casamino Acids, 0.25% glucose, tryptophan (50 μg/ml), and thiamine (10 μg/ml). For streptomycin-dependent strains, the medium also contained 100 μg of streptomycin per ml except where noted. Growth of cultures in all experiments was monitored by absorbancy at 420 nm (A_{420}) in a Gilford spectrophotometer. In experiments in which bacteria were transferred from a medium of one streptomycin

concentration to another, an exponentially growing culture was centrifuged, washed once with streptomycin-free medium, and resuspended in 1/5 volume of streptomycin-free medium. The washed cells were then inoculated (1:50 to 1:100) in a medium containing the desired concentration of streptomycin. Bacteria were then grown for at least two generations in the new medium before the start of any experiment.

Mutant isolation. Chemical mutagenesis was not required to produce the mutant phenotypes, since consistent results were found with spontaneous *str*[d] mutants, obtained with a frequency of about 10^{-8} by direct plating of 10^{9} cells on a plate containing 100 μg of streptomycin per ml (see below). Mutagenesis, when used, was by the method of Miller (23). In these trials, cells of *E. coli* C3 were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (50 μg/ml) for 30 min at 37 C. Bacteria were then centrifuged, washed once with 0.1 M potassium phosphate buffer (pH 7.0), and suspended in 10 ml of L broth. After 2 h of growth at 37 C in this medium, various dilutions of the mutagenized culture were plated on plates containing 100 μg of streptomycin per ml. The plates were incubated at 30 C for 48 h. Streptomycin-dependent mutant colonies were then identified by replica plating and were purified by repeated streaking and retesting. Strains a1, a2, a4, a6, a10, and a20 are all *str*[d]; strain b13 is *strA* (resistant).

Protocol for determining the chemical and functional decay of RNA. The experimental design was modeled after that of Pato and von Meyenberg (30), the fate of various cellular components being followed after the arrest of transcription initiation by rifampin. Bacteria were grown at 31 C, and growth of the culture was followed by monitoring its A_{420}. When the A_{420} of the culture reached 0.2 to 0.5, 5 to 10 ml of the culture was transferred to a 50-ml flask. After some time elapsed, for the determination of bulk mRNA decay, [^{3}H]uridine (30 Ci/mmol; 2 μCi/ml) was added to the culture. One minute later, rifampin (10 mg/ml in 50% ethanol), nalidixic acid (1 mg/ml), and cold uridine (10 mg/ml) were added to the culture to give final concentrations of 300, 20, and 200 μg/ml, respectively. The time of addition of rifampin was considered to be time zero. At subsequent times, 0.2-ml aliquots of culture were removed and pipetted directly into 1 ml of cold 10% CCl_3COOH. The acid precipitates were filtered through glass-fiber filters and were washed with cold 5% CCl_3COOH in 50% ethanol to remove all traces of rifampin. The filters were dried and counted in toluene-based scintillation fluid.

Because virtually every result given below is based on measurements in which rifampin was added to block the initiation of RNA synthesis, the efficacy of its action was especially important to verify. For these strains, trials have verified that the action is extremely rapid (e.g., Fig. 2 of reference 10). Furthermore, in all the physiological states tested, the decline in pulse-labeling of total RNA began within 30 s of antibiotic addition (see Fig. 2 and 4; also, unpublished data).

Experiments comparable to those done with [^{3}H]uridine were done using [^{14}C]phenylalanine (0.1 μCi/ml; 460 mCi/mmol; Schwarz/Mann) (as in Fig. 7). In this case, the labeled amino acids were added at the same time as rifampin, and the decay of protein-forming capacity was observed.

To determine the rate of functional inactivation of an mRNA (β-galactosidase), isopropyl-[β,D]-thiogalactopyranoside (IPTG) was added to an exponentially growing culture at a final concentration of 5×10^{-4} M. One minute later, rifampin (300 μg/ml) was added to the culture. Samples (0.2 ml) were removed at subsequent times and added to 0.8 ml of buffer (per liter: $Na_2HPO_4 \cdot 7H_2O$, 16.1 g; $NaH_2PO_4 \cdot H_2O$; 5.5 g; KCl, 0.75 g; $MgSO_4 \cdot 7H_2O$, 0.246 g; β-mercaptoethanol, 2.7 ml; pH adjusted to 7.0) containing 100 mg of chloramphenicol per ml. When all samples had been taken, each was once again diluted fivefold in the assay buffer. A drop of toluene was added to each tube, which was then vortexed for 15 s and transferred to a water bath held at 30 C. After 15 min, 0.2 ml of substrate (*o*-nitrophenyl-β-D-galactopyranoside [ONPG], 4 mg/ml in 0.1 M phosphate buffer, pH 7.0) was added to each tube. When sufficient yellow color developed, the reaction was terminated by the addition of 0.5 ml of 1 M Na_2CO_3. The times of addition of ONPG and Na_2CO_3 were recorded. A_{420} of the samples was determined, and the increase of optical density per minute was calculated.

Labeling of ribosomal RNA (rRNA) in kinetic experiments. Cultures of *E. coli* C3 and a10 (15 ml) were grown in the presence of [^{14}C]uracil (0.1 μCi/ml; specific activity, 61 mCi/mmol) for 1 to 2 h to prelabel the cellular RNA. At the start of the experiment, a 2-ml portion was sampled and pulse-labeled for 30 s with [^{3}H]uridine (100 μCi/ml; specific activity, 30 Ci/mmol) to measure the rate of RNA synthesis in the absence of the drug. Rifampin was then added to the cultures (zero time) to give a final concentration of 300 μg/ml. At subsequent times, 2-ml portions of the cultures were similarly pulse-labeled for 30 s with [^{3}H]uridine. At the end of each pulse period, unlabeled uridine (final concentration, 500 μg/ml) was added to the cultures, which were further incubated for 15 min to permit degradation of all pulse-labeled mRNA. Cells were then harvested by centrifugation and washed twice with minimal medium containing 20 mM sodium azide and 150 μg of chloramphenicol per ml. The washed cells were suspended in 2 ml of a solution containing 0.02 M sodium acetate (pH 5.5), 1 mM ethylenediaminetetraacetic acid, and 0.5% sodium dodecyl sulfate (Matheson, Coleman and Bell). RNA was extracted twice with phenol for 20 min, first at 55 C and then at room temperature. The aqueous phase was made 0.1 M in NaCl, and the RNA was precipitated with 2.5 volumes of ethanol at −20 C.

Gel electrophoresis of the RNA samples was carried out in agarose-polyacrylamide gels as described previously (27).

RESULTS

The availability of a class of mutants in a single ribosomal locus, the *str*[d] mutants, permits physiological effects to be assayed either in a number of strains all tested at the same level

of streptomycin or with a single strain tested at different levels of streptomycin. The two methods gave overlapping results on growth rate and RNA metabolism, as follows.

Growth rates of the mutants. In salts-amino acids medium containing 100 μg of streptomycin per ml, various streptomycin-dependent mutants grew at characteristic rates. Strains a1, a2, a6, a10, and a20 had mass-doubling times of 96, 110, 132, 180, and 90 min, respectively, compared with 50 min for the parental strain C3. These mutants were chosen for further study because the effect on growth rate was appreciable and covered a considerable range.

When an exponentially growing culture of any one of these strains was transferred to media containing different concentrations of streptomycin, the steady-state growth rate was found to be proportional to the streptomycin concentration over the range adequate to support exponential growth of each strain. Results for strain a10 are shown in Fig. 1 and its inset. A similar relationship between growth rate and streptomycin concentration was observed for spontaneously occurring *str*[d] mutants, which corroborates earlier studies by Spotts (35).

Effect of genetic lesions and streptomycin on the rate of transcription. (i) Shutoff of total RNA synthesis in the presence of rifampin. To get an estimate of the rate of transcription, we followed the shutoff of RNA synthesis by rifampin in strains C3 and a10 (growing in the presence of different concentrations of streptomycin). In exponentially growing cells, the synthesis of RNA is nonsynchronous, and RNA polymerase may be assumed to be randomly distributed along the corresponding genes. When the initiation of transcription is blocked with rifampin, fewer and fewer molecules remain unfinished with time. The amount of label incorporated into RNA during successive brief pulses then decreases steadily with time during the last transit by RNA polymerases.

In these experiments, rifampin was added to the cultures at zero time and, at subsequent times, samples of the cultures were withdrawn and pulse-labeled with [^{3}H]uridine for 1 min to determine the extent of residual RNA synthesis. These estimates of the rate of shutoff (Fig. 2) are of limited accuracy, for two reasons.

First, they depend, in part, on the relative amounts of short transfer RNA, longer mRNA, and still longer rRNA chains. These might vary moderately with growth rate, although they would probably remain near 0.2:0.4:0.4 (see,

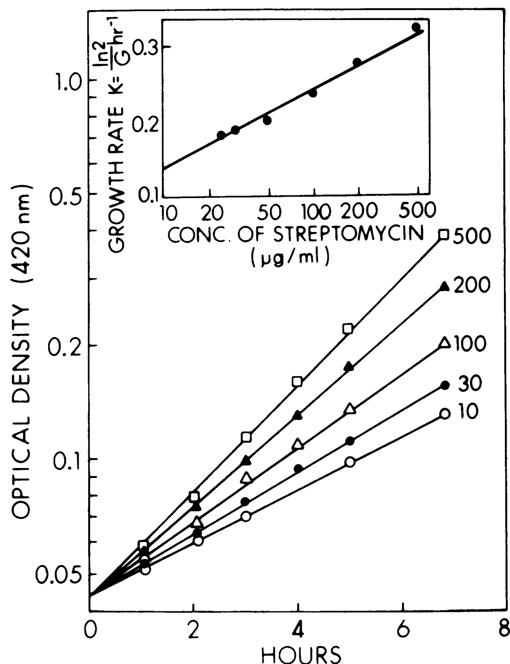

FIG. 1. *Growth of streptomycin-dependent bacteria at 31 C in media containing different concentrations of streptomycin. An exponentially growing culture of E. coli a10 was transferred to fresh media containing 10, 30, 100, 200, and 500 μg of streptomycin per ml, respectively. Subsequent growth of the cultures was followed by monitoring their A_{420}. The inset shows the relationship between the growth rate constant k (k = ln2/G per h, where G is doubling time in hours) and the concentration of the streptomycin in the medium. Numbers on the figure refer to the concentration of the streptomycin in the medium (10, ○; 30, ●; 100, △; 200, ▲; and 500 μg/ml, □).*

e.g., reference 26). Second, they depend on the specific activity of the RNA precursor pools. Cold nucleotides from internal pools and from mRNA degraded in the rifampin-treated cells will progressively dilute the added label (22, 26, 29). However, the time course of this dilution is also likely to be comparable in the various cultures, since the transcription times in the sections below are comparable to literature values (11, 28).

Qualitatively, then, as shown in Fig. 2, RNA synthesis in strain C3 dropped to 10% of the initial level in 2.75 min; in strain a10 growing in a medium containing 250, 50, or 10 μg of streptomycin per ml, the corresponding times were 3.25, 4.33, and 5.33 min, respectively. Thus, it took an increasingly longer time in these mutants for the apparent completion of RNA synthesis: the rate of overall transcription

was proportional to the growth rate of the cells.

(ii) Shutoff of rRNA synthesis. The results for the kinetics of transcription of total RNA were extended to a particular long species of RNA, rRNA. In an extension of the technique described above, bacterial cultures were prelabeled with [^{14}C]uracil and received rifampin at time zero. At later times, aliquots of the cultures were pulse-labeled with [^{3}H]uridine, followed by a long chase with unlabeled uridine. RNA was extracted from these samples and analyzed by electrophoresis on agarose-polyacrylamide gels (Fig. 3). Gels shown in this figure have been normalized with respect to their ^{14}C counts. From these gels, the ratio ^{3}H/^{14}C in 16*S* and 23*S* rRNA at different times was computed.

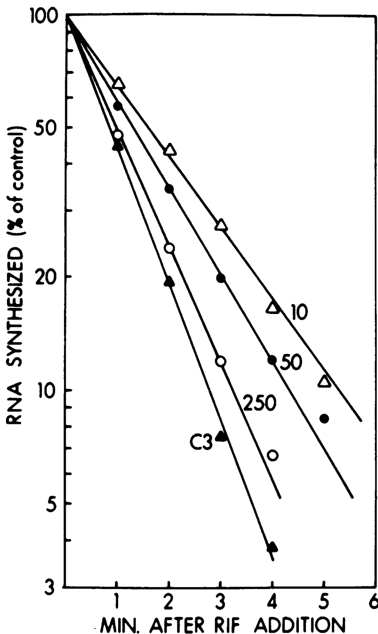

FIG. 2. *Kinetics of shutoff of total RNA synthesis in strains C3 and a10 after the addition of rifampin (RIF). Strain a10 was grown in a medium containing 10, 50, or 250 μg of streptomycin per ml. Rifampin (300 μg/ml) and nalidixic acid (20 μg/ml) were added to the bacterial cultures at zero time. At various times thereafter, 1-ml portions of the cultures were pulse-labeled with [^{3}H]uridine (25 μCi/ml) for 1 min. At the end of each pulse, cold 10% CCl_3COOH was added. Zero-time values (100%) in these experiments were obtained by pulse-labeling the cells just before rifampin addition, and were 160,000 counts/min for strain C3 and 52,000, 50,000, and 48,000 counts/min for strain a10 growing in media containing 10, 50, and 250 μg of streptomycin (Sm) per ml, respectively. Symbols: △, a10 (10 μg of streptomycin per ml); ●, a10 (50 μg of streptomycin per ml); ○, a10 (250 of streptomycin per ml); ▲, C3.*

A plot of these ratios versus time, for strains C3 and a10 (grown in presence of 50 and 250 μg of streptomycin per ml) is shown in Fig. 4. At different times after rifampin addition, the ratio ^{3}H/^{14}C decreased roughly linearly with time; the intercept of this line on the abscissa represents the time after which no ^{3}H pulse label entered an RNA species. This was taken as an estimate of the transcription time of that species; the basis for choosing this method of estimation is detailed in reference 27. The apparent transcription times for 16*S* and 23*S* rRNA were 60 s and 120 s, respectively, for strain C3, comparable to literature values (11, 27, 28). In strain a10, the times were prolonged in proportion to growth rate: 95 s and 190 s at 250 μg of streptomycin per ml and 140 and 280 s at 50 μg of streptomycin per ml. Since the synthesis time of the well-defined transcripts varied progressively in response to the level of streptomycin in the medium, it was evident that RNA chain growth rate was affected in these mutants.

Rate of movement of ribosomes. To begin to extend the analysis to possible effects of *str*^{d} lesions on mRNA function and metabolism, the translation time of a polypeptide chain and the rate of loss of functional capacity of mRNA were measured for β-galactosidase after a brief pulse of induction.

Figure 5 shows results of a trial in which 1-min pulses of inducer (5×10^{-4} M IPTG) were given to cultures of strain a20 growing in media containing different levels of streptomycin. At the end of the pulse, rifampin was added to the cultures, and at subsequent times samples were taken and their content of β-galactosidase was estimated. The time at which β-galactosidase began to appear gave an estimate of the translation time of the enzyme. In strain a20, the translation time for β-galactosidase was 3.5, 4.5, and 5.5 min, respectively, at 100, 30 and 10 μg of streptomycin per ml as compared with 2.0 min for the parental strain C3 (Fig. 5).

Rate of functional inactivation of mRNA. The above experiment also gave an estimate of the rate of functional inactivation of the β-galactosidase mRNA. At various times after the induction pulse, the residual enzyme-forming capacity is a measure of the functionally active mRNA in the cell. The rate at which this capacity is lost gives an estimate of the functional half-life of the mRNA (Fig. 6).

The functional decay rate of β-galactosidase mRNA did not show appreciable variation in strain a20 growing in media containing different concentrations of streptomycin (Fig. 6). This is

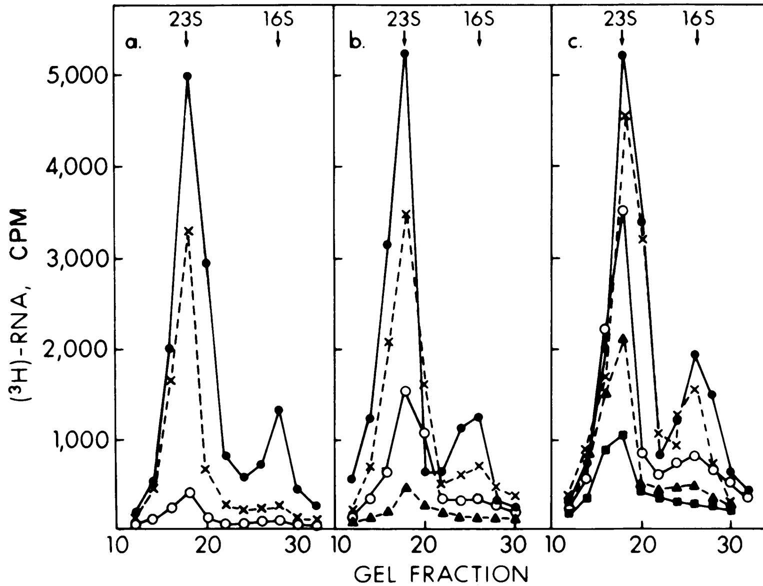

FIG. 3. *Gel electrophoretic analysis of rRNA in strains C3 and a10 pulse-labeled at various times after rifampin addition. Cultures were prelabeled with [^{14}C]uracil, and rifampin was added at time zero. At subsequent times (i.e., 0.5, 1.25, 2.0, 3.0, and 4.33 min), portions of the cultures were pulse-labeled with [^{3}H]uridine (100 μCi/ml; specific activity, 30 Ci/mmol) for 30 s. At the end of the pulse, a large excess of cold uridine (500 μg/ml) was added, and the cultures were further incubated for 15 min. RNA extracted from these samples was analyzed on agarose-polyacrylamide gels. For clarity in presentation, the curves for ^{14}C-labeled rRNA are not included. However, the ratio of ^{14}C in 23S and 16S rRNA in all the experiments was always 2 ± 0.05. Curves for various time points in each panel have been normalized to a constant amount of ^{14}C-labeled rRNA. Panels (a), (b) and (c) correspond to strains C3, a10 (250 μg of streptomycin per ml), and a10 (50 μg of streptomycin per ml), respectively. Symbols: ●, 0.5 min; ×, 1.25 min; ○, 2.0 min; ▲, 3.0 min; ■, 4.33 min.*

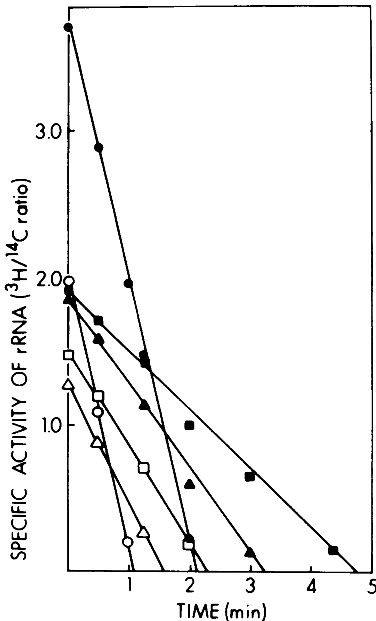

FIG. 4. *Kinetics of shutoff of 16S and 23S rRNA synthesis in the presence of rifampin (RIF) in E. coli C3 and a10 (grown in the presence of 50 and 250 μg of streptomycin per ml). Data for these curves have been obtained from Fig. 3. Time zero points and the 1.0-min point for C3 have been taken from a different experiment. Amounts of ^{3}H and ^{14}C radioactivities in 16S and 23S rRNA were obtained by summing up the corresponding gel fractions. Symbols: ○ and ●, 16S and 23S rRNA in C3; △ and ▲, 16S and 23S rRNA in a10 (250 μg of streptomycin per ml); □ and ■, 16S and 23S rRNA in a10 (50 μg of streptomycin per ml).*

to be contrasted with the chemical half-life of mRNA in this strain, which showed wide variation in the same conditions (see below).

A comparable experiment was done for the shutoff of total protein synthesis after rifampin addition. In those trials (Fig. 7), the incorporation of radioactive amino acids was determined as a function of time after rifampin addition. From the extent of radioactive amino acid incorporation at any time, and its final levels, the residual functional capacity of bulk mRNA was determined. Once again, the functional inactivation rates were comparable at all streptomycin levels and in all strains tested.

Chemical decay of mRNA. (i) Effect of ribosomal mutations on mRNA metabolism. To determine whether any of the mutants were

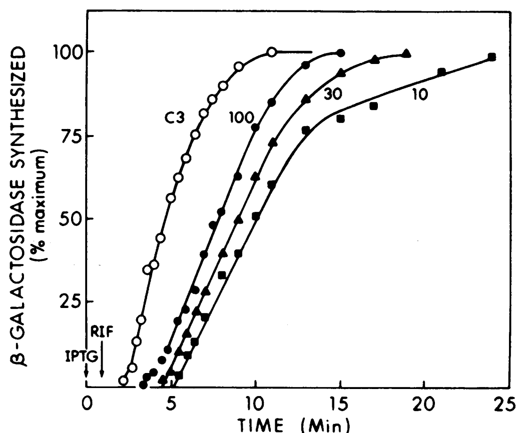

FIG. 5. *Time course of appearance of β-galactosidase in strains C3 and a20 after a pulse of IPTG. An exponentially growing culture of strain a20 was centrifuged, washed, and transferred to media containing 10, 30, and 100 μg of streptomycin per ml, respectively. At a suitable time, cultures were induced with IPTG (final concentration, 5×10^{-4} M) for 1 min, and then rifampin (final concentration, 300 μg/ml) was added. At subsequent times, samples of the cultures were removed, and the amounts of β-galactosidase synthesized were assayed. The maximal amount of β-galactosidase produced in each case (15.0 U for C3; 7.0, 4.0, and 2.2 U for strain a20 growing in the presence of 100, 30, and 10 μg of streptomycin per ml, respectively) has been assumed to be 100%. The basal levels of enzyme in each case was much less than 1% of the final levels. Enzyme synthesis in each case was followed, up to 40 min, and did not increase further than indicated in the figure. Symbols: O, C3; ●, a20 (100 μg of streptomycin per ml); ▲, a20 (30 μg of streptomycin per ml); ■, a20 (10 μg of streptomycin).*

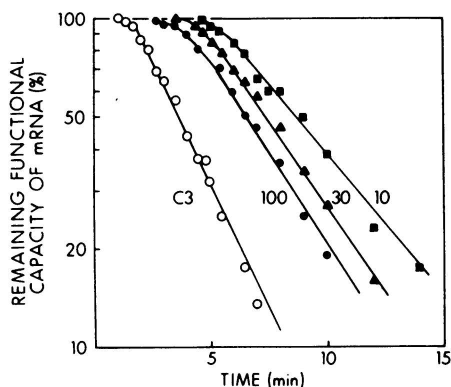

FIG. 6. *Decay of functional capacity of β-galactosidase mRNA in strains C3 and a20 (grown in the presence of different concentrations of streptomycin). Data for these curves have been obtained from Fig. 5. The residual enzyme-synthesizing (functional) capacity of the mRNA at any time is obtained by subtracting the enzyme levels at that particular time from the final enzyme levels, as mentioned above (Fig. 5).*

affected in mRNA metabolism, we studied the decay of pulse-labeled RNA in mutants a1, a2, a4, and a10. $[^3H]$uridine (2 μCi/ml) was added to exponentially growing cultures of the mutant strains. One minute later, rifampin, nalidixic acid, and unlabeled uridine were added to the cultures to prevent most further incorporation of $[^3H]$uridine. At subsequent times, decay of preformed pulse-labeled RNA was followed, until all unstable RNA had disappeared. Decay curves of mRNA for various mutants are shown in Fig. 8: the chemical half-life (time required for 50% of unstable RNA to disappear) of pulse-labeled RNA in strains a1, a2, a4, and a10 was 4.0, 5.5, 7.5, and 9.5 min, respectively (compared with about 3 min for the parental strain C3). mRNA is thus stabilized to different extents (1.5- to 3.0-fold) in various mutants. If we compare the growth rates (see above) and the half-life of mRNA in various mutants, a proportionality between the two appears: the slower the growth rate, the more the mRNA is stabilized (see Discussion and Fig. 10).

(ii) Effect of streptomycin-mediated ribo-

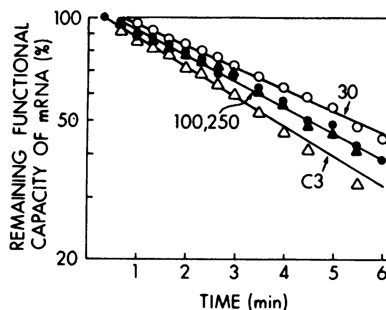

FIG. 7. *Decay of protein-forming (functional) capacity of total mRNA after rifampin treatment. Rifampin (300 μg/ml) and $[^{14}C]$phenylalanine (0.1 μCi/ml) were added to cultures of E. coli C3 and a10 (the latter grown in media containing 30, 100, and 250 μg of streptomycin per ml) at zero time. Samples of the cultures were taken at the indicated times and added to cold 5% CCl_3COOH for the measurement of acid-insoluble radioactivity. Incorporation of ^{14}C-labeled amino acid reached a plateau by 20 min in each case. The maximal counts per minute incorporated were 43,400 for C3; and 16,000, 21,000, and 32,500 for a10 growing in media containing 30, 100, and 250 μg of streptomycin per ml, respectively. The capacity of mRNA to direct protein synthesis at the time of addition of rifampin has been assumed to be 100% in each case. Symbols: Δ, C3; O, a10 (30 μg of streptomycin per ml); ▲, a10 (100 μg of streptomycin per ml); ●, a10 (250 μg of streptomycin per ml).*

Symbols: O, C3; ●, a20 (100 μg of streptomycin per ml); ▲, a20 (30 μg of streptomycin per ml); ■, a20 (10 μg of streptomycin per ml).

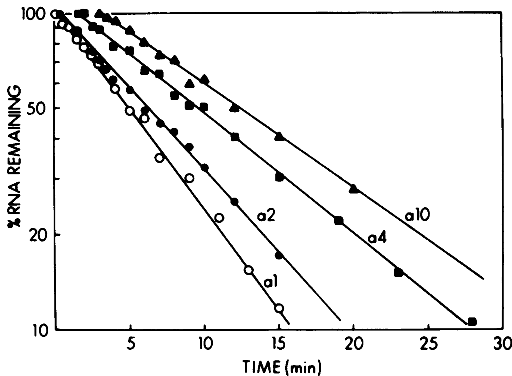

FIG. 8. *Chemical decay of pulse-labeled RNA in various mutants. Bacteria were grown in supplemented minimal medium at 31 C. At an appropriate time, cells were pulse-labeled with [³H]uridine (5 μCi/ml) for 1 min. Rifampin, nalidixic acid, and unlabeled uridine were added at the end of the pulse to give final concentrations of 300, 20, and 200 μg/ml, respectively. Samples were removed at later times into cold 5% CCl_3COOH for measurement of acid-insoluble radioactivity. For the above plots, counts due to stable RNA (i.e., when all unstable RNA had decayed) were subtracted from each earlier time point. Assuming the maximal amount of unstable RNA (reached 1 to 2 min after rifampin addition) to be 100%, the relative amounts of RNA remaining at times thereafter were calculated. Half-lives of the RNA were obtained from the slopes of the curves as the time required for 50% of the RNA to be rendered acid soluble. Symbols: O, strain a1; ●, strain a2; ■, strain a4; ▲, strain a10.*

some alterations on mRNA stability. Once again, the use of graded levels of streptomycin in one mutant gave effects comparable to those in different mutants all tested at the same level. To study the effects of the streptomycin level on mRNA stability, an exponentially growing culture of strain a10 was centrifuged, washed with streptomycin-free medium, and then reinoculated in media containing different concentrations of streptomycin. After about two generations of growth, cells were pulse-labeled with [³H]uridine, and the subsequent decay of pulse-labeled RNA in presence of rifampin, nalidixic acid, and unlabeled uridine was followed (Fig. 9). As the concentration of streptomycin in the medium was reduced, mRNA became increasingly stabilized. The chemical half-lives of mRNA in this strain at 500, 100, and 30 μg of streptomycin per ml were 6.5, 9.5, and 15.5 min, respectively.

A similar experiment was done, with very similar results, using strain a20. The chemical half-lives of mRNA in this strain at 100, 30, and 10 μg of streptomycin per ml were 4.0, 6.5, and 9.0 min, respectively, which is in marked contrast to the invariance of functional half-life (Fig. 5 and 6). Thus, the mass decay rate of mRNA varies in proportion to growth rates and translation time. Spontaneous *str^d* mutants showed a similar variation of chemical half-life and growth rate in response to streptomycin (data not shown).

Control experiments with strain b13 (a streptomycin-resistant strain) in the presence and absence of streptomycin showed no difference in chemical decay kinetics of mRNA at any level of streptomycin (10 to 500 μg/ml). This excludes the possibility of any direct effect of streptomycin on an mRNA decay process independent of ribosome function. (The fact that the chemical half-lives of mRNA varied in proportion to growth rate, as had been observed with different mutants, also argues against a direct effect of streptomycin on a nonribosomal component.)

DISCUSSION

str^d lesions in ribosomes provide a way to study the effects of altered ribosome structure on other processes in growing cells. The degree of ribosome alteration can be affected either by using mutant strains bearing different *str^d* alleles or by using a single mutant grown at different levels of streptomycin. The methods

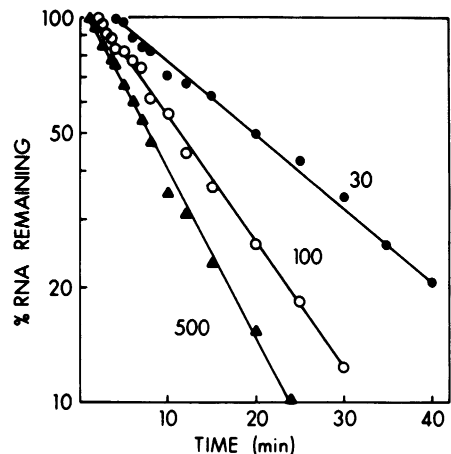

FIG. 9. *Decay of pulse-labeled RNA in E. coli a10 grown in media containing different concentrations of streptomycin. E. coli a10 was grown in media containing 30, 100, and 500 μg of streptomycin per ml, respectively. At a suitable time, cells were pulse-labeled with [³H]uridine for 1 min, and the decay of labeled RNA was followed as described for Fig. 8. Symbols: ●, 30 μg of streptomycin per ml; O, 100 μg of streptomycin per ml; ▲, 500 μg of streptomycin per ml.*

gave similar results and provided a wide range of cellular growth rates. In response to different levels of streptomycin, the rates of cell growth, of apparent RNA polymerase movement (see above; Fig. 2 and 3), of translation (Fig. 5), and of chemical degradation of mRNA (Fig. 8 and 9) all changed coordinately. From the data for rRNA synthesis, one can infer that the rate of transcription is affected everywhere along the RNA chains (proportionally for transcripts containing 16*S* and 23*S* [Fig. 3 and 4]).

Many of these parameters also change when growth rates are changed in other ways. For example, direct inhibition of translation inhibits mRNA decay (see Introduction), and rRNA chain growth is slower in cultures growing in minimal media (7). However, in the case of str^d mutants, unlike the other cases, some of the effects can be ordered causally. One can start from the genetic findings that the primary effect of str^d lesions and of the variation of streptomycin levels in str^d mutants is on the 30*S* ribosomal subunit (6). The slowing of the ribosome cycle (19, 31, 38) seems the most likely primary result of the mutational lesion or drug levels on 30*S* ribosomes. This would be sufficient to account not only for streptomycin dependence of rates of growth (Fig. 1), but also for the increasing time required to complete the synthesis of β-galactosidase chains (Fig. 5).

How might the primary alteration in ribosome structure result in the effects on other processes? One possibility is that they vary coordinately because they are mechanistically "coupled," as considered by some authors (14, 16, 33, 34, 37). However, there is no mechanism known at present to account for effects like those on transcription of rRNA, and, especially since the nature of streptomycin dependence is still unclear, many effects could be indirect, resulting perhaps from variable levels of miscoding by streptomycin-dependent ribosomes (9).

More can be inferred about the proportionality of the rate of translation and the rate of chemical degradation of mRNA. First, slower degradation is not itself a trivial consequence of slower transcription. This is clear because decay was specifically followed (Fig. 8 and 9) for RNA segments already pulse-labeled and, therefore by definition, beyond any lag in transcription. (The argument holds in spite of some continued incorporation that continues after the pulse-labeling, even in presence of excess unlabeled uridine; control experiments have shown that the further incorporation is low in these strains [see sample data in reference 10].)

Rather, the variable rates of mRNA decay, which changed to correspond to rates of growth and ribosome movement, strengthen the earlier inferences from cases of extreme blockage of protein synthesis in vivo and in vitro (2, 4, 5, 12, 17–18). Those analyses inferred that the rate of ribosome movement may limit the rate of nuclease activity. The results here (as in Fig. 6) are consistent with bulk decay that follows translating ribosomes along the mRNA (2, 20, 24, 25).

Unlike the rate of chemical decay, inactivation of mRNA chains was not apparently dependent on the rate of the ribosome cycle (Fig. 5 through 7). It is worth noting that the rate of functional inactivation is measured with respect to the total product formed, set at 100% (Fig. 6); thus, it is unaffected by any complex polar effects that lower total protein synthesis (13, 14).

Figure 10 summarizes some of the data showing the very different responses to growth rate of chemical and functional mRNA decay. The results suggest that, during steady-state growth of cells, the loading of competent ribosomes on an mRNA chain is always in jeopardy—independent of the previous extent of translation. This is in agreement with extensive evidence that mRNA can be inactivated independent of its overall chemical lifetime (32). The maximum rate of mRNA decay tends to be observed only after a lag equal to the transit time of ribosomes on the mRNA (Fig. 4 through 8).

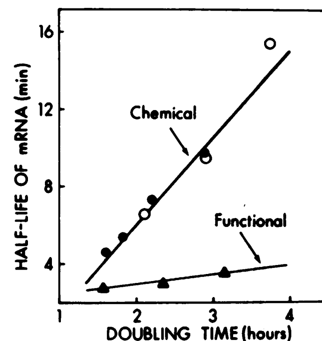

FIG. 10. *Relationship between the growth rate and the half-lives (chemical and functional) of mRNA. Values for functional and chemical half-lives of mRNA have been obtained from Fig. 6, 8, and 9. Doubling times of a10 in media containing different concentrations of streptomycin have been obtained from Fig. 1* (●) *for different mutants* (a_1, a_2, a_4, *and* a_{10} *at the increasing doubling times) and* (○) *for strain a10 growing in media containing different concentrations of streptomycin (as in Fig. 9).* (Δ) *Functional half-life of β-galactosidase production in Fig. 6 (strain a20 at 100, 30, and 10 μg of streptomycin per ml).*

Because of the very different behavior of chemical and functional lifetime of mRNA, streptomycin-dependent cells are in a predicament in low concentrations of streptomycin. Ribosomes are moving along mRNA more slowly and, with the slower ribosome cycle, the rate of initiation of protein chains goes down. However, the functional lifetime of a new mRNA molecule is as short at slow growth rates as it is at fast growth rates. Therefore, in slowly growing cells, proportionally fewer ribosomes would initiate translation on each mRNA before its inactivation. In other words, one would expect the translation yield of mRNA to decrease in inverse proportion to growth rate. This is exactly the observation in the trials of Fig. 5.

Earlier studies on streptomycin-dependent mutants reported that the protein/RNA ratio of the cells decreased in proportion to the growth rate at different levels of streptomycin (35, 36). This observation, previously unexplained, may be another consequence of the proportionally lower translation yield of mRNA at slow growth rates. Perhaps at very slow growth rates, the translation yield becomes too low to support exponential growth, which might account for the "threshold" levels of antibiotic required for the growth of dependent strains.

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