# *INCREASED STABILITY OF POLYSOMES IN AN ESCHERICHIA COLI MUTANT WITH RELAXED CONTROL OF RNA SYNTHESIS**

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*Communicated by Herman M. Kalckar, June 28, 1966*

When cells of *Escherichia coli* auxotrophs are starved for a required amino acid, the net synthesis of RNA ordinarily ceases at once. However, a fortuitously encountered mutant continues to accumulate RNA, at a substantial fraction of the normal rate, when starved for methionine[1] or for various other amino acids,[2] and this mutant has been designated[2] as "relaxed" in the control of RNA synthesis ($RC^{rel}$). The mechanism responsible for this control, and for its alteration in the relaxed mutant, has not yet been satisfactorily established. This problem, and our recent observation of a stimulatory effect of methionine on RNA synthesis in certain strains,[3] has led us to explore the possibility that the level of free ribosomes (i.e., those not engaged in polysomes) might control by direct feedback the synthesis of ribosomal RNA (rRNA). This approach was encouraged by the recent report of Weber and DeMoss,[4] which we have confirmed, that starvation of an *E. coli* auxotroph for a required amino acid results in a substantial shift of ribosomes from polysomes to the monosome peak. The present paper shows that in an $RC^{rel}$ strain, in contrast, a high polysome level is maintained during starvation for an amino acid. Following the completion of this work, we learned (personal communication) that similar observations have recently been made by Morris and DeMoss.[5]

This finding, and others also described below, supports the conclusion that the $RC^{rel}$ mutation increases the stability of polysomes, probably by causing an alteration in the ribosomes.

*Materials and Methods.—Strains and growth:* Mutant 687 of *E. coli* K12, which is $RC^{rel}$ $arg^-$ $met^-$, was kindly provided by M. Lubin; strain B-90 ($arg^-$) by L. Gorini; strain 15TA$^-$($thy^-arg^-$) by R. Rudner; and mutant 45A-25 ($arg^-$) of the W strain was from the collection of B. D. Davis. All cultures were grown at 37° with aeration in minimal medium A[6] with 0.5 per cent glucose (unless otherwise specified), supplemented with 0.1 mg/ml of amino acids and 0.01 mg/ml of thymine as required.

Polysomes were extracted by a method employing freezing and thawing in the presence of lysozyme, followed by treatment with deoxycholate (Ron, Kohler, and Davis[7]). Because polysomes are subject to a somewhat variable degree of degradation during their extraction and measurement, the variously treated cells, with radioactively labeled ribosomes, were extracted together with an excess of unlabeled, untreated carrier cells. [The ribosomes of the latter, measured by optical density (OD), thus provided an internal standard for the ribosomes of the treated cells, measured by radioactivity.] The extracts were sedimented on a linear (15–30%) sucrose gradient in a Spinco centrifuge at 0°C. The OD at 260 mμ was determined using a Gilford continuously recording spectrophotometer. Fractions of 10 drops each were precipitated with 10 per cent trichloroacetic acid, collected on Millipore filters, dried, and measured for radioactivity in a Nuclear-

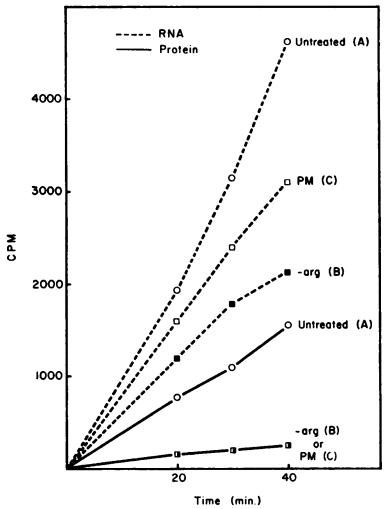


FIG. 1.—RNA and protein synthesis in "relaxed" mutant 687 of *E. coli*. A sample removed from an exponentially growing culture (the "carrier cells" of the following experiments) was filtered rapidly on a 47-mm Millipore filter and washed with 3 vol of medium A. The cells were resuspended in medium A containing glucose, methionine, $C^{14}$-leucine (0.01 $\mu$c + 20 $\mu$g per ml), and $H^3$-uracil (0.25 $\mu$c + 10 $\mu$g per ml), and were distributed into tubes containing arginine (*untreated:* curves *A*), arginine plus puromycin (400 $\mu$g per ml) (*PM*, curves *C*), or no addition (*-arg*, curves *B*). The tubes were aerated at 37° by shaking. Samples of 0.5 ml each, precipitated with an equal volume of 10% trichloroacetic acid, were analyzed as described.[3]

Chicago gas-flow counter as previously described.[3] In order to focus on shifts in the polysome region, the OD and radioactivity curves were normalized to yield similar monosome peaks; the curves thus provide a comparison of the ratios of polysomes to monosomes, and the polysome patterns, but do not reflect the increase in monosomes that accompanies a decrease in polysomes under certain conditions.

*Results.—Effect of amino acid starvation:* Cells of relaxed strain 687, auxotrophic for arginine and methionine, were grown on minimal medium supplemented with the required amino acids, with or without radioactive uracil. The prelabeled cells were filtered during exponential growth and then resuspended and incubated in minimal medium lacking arginine. Control cultures with $C^{14}$-leucine plus $H^3$-uracil showed that under these conditions, protein synthesis was immediately slowed to the low rate expected from turnover (5%), while RNA synthesis continued at $^2/_3$ of the normal rate during the 20–60 min period of observation (Fig. 1, curves *A* and *B*). After 20 min of amino acid starvation, the prelabeled cells were chilled, harvested, and extracted together with unstarved, unlabeled carrier cells of the same organism, and the sedimentation distribution of the labeled and unlabeled ribosomes in the extract was determined. Figure 2 shows that the extract of the

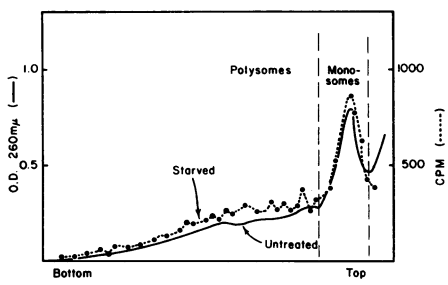


FIG. 2.—Effect of amino acid starvation on the distribution of ribosomes between polysomes and monosomes in the relaxed mutant. To prelabel its ribosomes, *E. coli* mutant 687 was grown for several generations in 5 ml of adequately supplemented medium containing $C^{14}$-uracil (0.015 $\mu$c + 7 $\mu$g per ml). The cells were filtered, washed with 15 ml of medium A, resuspended in 10 ml of this medium containing glucose and methionine (but no arginine), and incubated for 20 min at 37°. These arginine-starved cells, at *ca.* 5 × $10^8$ per ml, were harvested together with 50 ml of a culture of "carrier" cells at a similar density, exponentially growing in the same medium plus arginine. An extract containing polysomes was prepared as described in *Materials and Methods*, and 1 ml was placed on a 26-ml linear sucrose gradient and was centrifuged for 135 min at 25,000 rpm. Analyses were performed as described in *Materials and Methods*.

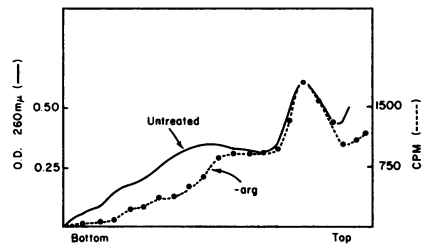


FIG. 3.—Effect of amino acid starvation on the distribution of ribosomes between polysomes and monosomes in a "stringent" strain of *E. coli*. Experimental details as described in Fig. 2, except that strain 15TA⁻ was used, and thymine (10 μg/ml) was added instead of methionine. The extract (0.5 ml) containing polysomes was placed on a 5-ml linear sucrose gradient and centrifuged for 60 min at 39,000 rpm.

relaxed cells harvested after 20 min of amino acid starvation had at least as high a level of polysomes (60% of the total ribosomes) as the unstarved cells. The polysomes persisted for even longer periods of starvation, but with a shift in the direction of smaller polysomes.

With various stringent arginine auxotrophs (B-90, 15TA⁻, 45A-25), in contrast (e.g., Fig. 3), during 20 min of deprival of the required amino acid, the fraction of ribosomes present in polysomes decreased by 20–40 per cent, and the remainder were mostly small polysomes. This decrease in polysomes must be accompanied by an equivalent increase in monosomes, since the RNA content of the cells remained constant, as anticipated. Figure 3 does not reveal this increase in monosomes because the two curves are arbitrarily normalized to a constant monosome peak (see *Materials and Methods*).

*Effect of stepdown in energy supply:* It has been reported that a relaxed mutant, while differing from stringent strains in its response to amino acid deprival, resembles these strains in its response to a "stepdown" in energy supply.[8] When the relaxed strain was shifted from glucose to acetate, the rate of protein synthesis in several experiments, measured over a 50-min period, was only 15–20 per cent of the control value, while the net RNA synthesis was reduced to less than 5 per cent. This deceleration of RNA synthesis, out of proportion to the deceleration of protein synthesis, is seen to be associated with a decrease in polysome level from 55 to 30 per cent (Fig. 4). A similar drop in polysome level, and in protein and RNA synthesis, was observed after a stepdown with stringent strain B-90.

Since the relaxed and the stringent strains differed in the stability of their polysomes under amino acid starvation, their similar behavior in a stepdown suggested that the decrease in polysomes under the latter conditions might have a different cause, such as a shortage of mRNA. This possibility could be tested by the use of chloramphenicol, since this drug causes immedi-

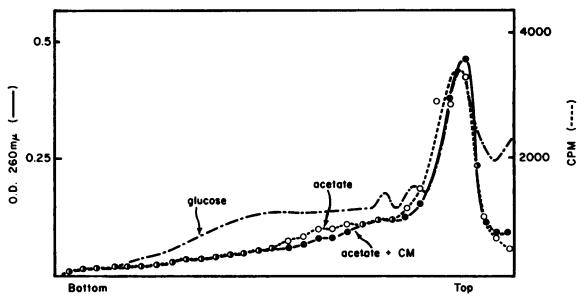


FIG. 4.—Effect of stepdown on the distribution of ribosomes between polysomes and monosomes in the relaxed strain. Experimental details as described in Fig. 2, except that the labeled culture was resuspended in growth medium containing methionine, arginine, and sodium acetate (0.5%) instead of glucose. To half of this culture 50 μg/ml of chloramphenicol (*CM*) was added 2 min before harvesting. These cells were harvested and extracted together with another portion of the carrier cells, whose OD curve was found to be essentially identical to the one shown.

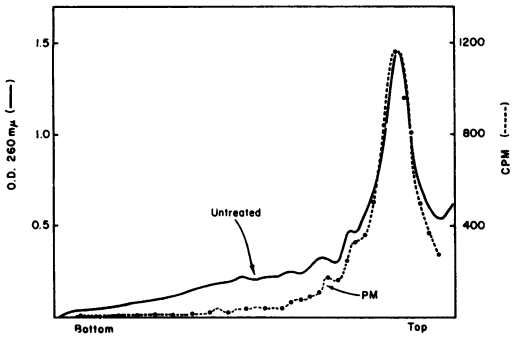


FIG. 5.—Effect of puromycin on the distribution of ribosomes between polysomes and monosomes in the relaxed strain. Experimental details as described in Fig. 2, except that the labeled culture was exposed to puromycin (*PM*) (400 μg per ml) for 20 min in a growth medium, containing methionine, arginine, and glucose.

ate formation of small polysomes in amino acid-deprived cells,[4] which evidently contain much free mRNA, whereas it has no such effect in cells deprived of an energy source,[9] which presumably lack free mRNA. As is shown in Figure 4, addition of chloramphenicol to a stepped-down culture of the relaxed mutant, 2 min before harvesting, did not increase the polysome level. In amino acid-starved stringent cells, as expected,[4] the treatment with chloramphenicol did restore the polysomes to a normal level. This result suggests that in the stepdown, the decrease in polysome level is due to a limited supply of mRNA.

*Effect of puromycin:* There is much evidence that puromycin interferes with protein synthesis by causing the release of peptidyl tRNA as peptidyl puromycin and free tRNA.[10, 11] Figure 1 (curves *A* and *C*) shows that in the relaxed strain, puromycin, at a level that essentially completely blocked protein synthesis, nevertheless permitted RNA synthesis to continue at a high rate. In the stringent strain essentially identical results were obtained.

In contrast to the pattern observed on deprival of an amino acid, the high rate of RNA synthesis in the presence of puromycin was not accompanied, in the relaxed strain, by a high level of polysomes. As is seen in Figure 5, puromycin treatment for 20 min caused the level of polysomes to drop from 50 to 20 per cent. A similar decrease, from 40 to 10 per cent, was seen in a stringent strain under the same conditions.

*Discussion.*—The results presented in this paper show that the $RC^{rel}$ mutant differs from stringent strains in maintaining its level of polysomes during amino acid starvation. The decrease in polysome level seen in stringent strains under these conditions suggests that when a ribosome encounters a codon on the mRNA for which an aminoacyl RNA (aRNA) is not available, it normally tends to drop off sooner or later. In the relaxed mutant, however, the peptidyl tRNA-ribosome-mRNA complex is evidently more stable and does not require the additional stabilizing contribution of aRNA. Since the relaxed mutant exhibits greater stability of the polysome complex regardless of which kind of aRNA is limiting, and since the ribosomes bind to a great variety of mRNA species, it seems reasonable to suppose that the ribosome itself or an associated enzyme is altered by the $RC^{rel}$ mutation, in a way that stabilizes polysomes specifically in the absence of aRNA.

Alternatively, mRNA might be more stable in the relaxed strain, and therefore retain the attached ribosomes during amino acid deprival; while in the stringent strain the mRNA would turn over faster, and the formation of new complexes would be limited by the limitation of protein synthesis. However, the mRNA in the relaxed strain appears to be normally unstable, since in a stepdown of energy supply

the relaxed and stringent strains appear to be similar in their loss of polysomes and of free mRNA.

The small content of polysomes in extracts of cells treated with puromycin suggests that the complex is also unstable when it lacks peptidyl tRNA. This instability is not overcome by the effect of the $RC^{rel}$ mutation, which emphasizes the specificity of the stabilizing effect of the mutation for complexes lacking aRNA.

The greater stability of polysomes in the relaxed strain deprived of an amino acid, compared with the stringent strain, supports the hypothesis that the rate of net synthesis of RNA (predominantly rRNA in a glucose medium) is regulated by a feedback response to the level of free ribosomes. The normal behavior of the relaxed strain in a "stepdown" is also explained by this hypothesis, since the decrease in RNA synthesis under these conditions is associated with a drop in polysome level, and the chloramphenicol test (Fig. 4) provides strong evidence that this drop is due to a mechanism (reduced supply of mRNA) that is equally present in relaxed and in stringent strains.

The continued accumulation of RNA in the relaxed strain in the presence of puromycin, despite a low polysome level, appears to conflict with the proposed regulatory role of free ribosomes. This evidence, however, is far from decisive: the polysomes in the cell may well be less stable in the presence of puromycin, and therefore less completely recovered in the extracts; or many of the monosomes may not be free ribosomes but may be attached to a mRNA molecule. Further work on the role of ribosomes in regulating RNA synthesis, and on the *in vitro* properties of ribosomes from relaxed strains, is in progress.

*Summary.*—In "stringent" strains of *Escherichia coli*, deprival of a required amino acid causes a shift of ribosomes from polysomes to monosomes, but in a mutant with "relaxed" control of RNA synthesis the polysome level is maintained. The addition of puromycin, or a stepdown from glucose to acetate, causes a drop in the polysome level in both stringent and relaxed strains. It therefore appears that in the relaxed mutant the ribosomes, or an associated enzyme, are altered, yielding stable complexes with peptidyl tRNA plus mRNA even in the absence of aminoacyl RNA. This property of the mutants supports the hypothesis that the rate of rRNA synthesis is controlled by the level of free ribosomes.

* This investigation was supported by research grant GB-1307 from the National Science Foundation. E.Z.R. is supported by USPHS training grant GM-177-07, and R.E.K. by a USPHS postdoctoral fellowship. This work is to be submitted by E.Z.R. in partial fulfillment of the requirements for a Ph.D. degree at Harvard University.

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