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Expression of *arg* Genes of *Escherichia coli* during Arginine Limitation Dependent upon Stringent Control of Translation

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The transcription and translation of operons for arginine biosynthetic enzymes after arginine removal (arginine down shift) were studied in *relA* and *relA*$^+$ strains of *Escherichia coli*. After arginine down shift, derepression of synthesis of the arginine biosynthetic enzymes ornithine carbamoyltransferase (*argF*) and argininosuccinate lyase (*argH*) began at about 15 min in *relA*$^+$ cells but was delayed in *relA* cells for more than 2 h. However, both *relA*$^+$ and *relA* cells accumulated high levels of *argCBH* mRNA, as shown by dot blot hybridization, after arginine down shift. After 15 min of arginine limitation, the proportion of ribosome-bound *argCBH* mRNA was equivalent in both *relA*$^+$ and *relA* cells. During the 15 min after the arginine down shift, *relA*$^+$ cells produced a significant burst of *argF* and *argH* enzyme synthesis when arginine was added back to the culture, whereas *relA* cells did not produce this burst of enzyme synthesis. The *relA* cells regained the ability to produce a burst of *argF* and *argH* enzyme synthesis when α-methylglucose-induced glucose starvation was combined with arginine limitation. Significant guanosine 5′-diphosphate 3′-diphosphate accumulated in *relA* cells under this condition. Our results support the view that during periods of severe amino acid limitation guanosine 5′-diphosphate 3′-diphosphate acts in some way to ensure the translation of *argCBH* mRNA.

The stringent response by *Escherichia coli* to amino acid starvation has been known for some time to cause a variety of major adjustments in cellular metabolism, including a rapid, transient accumulation of guanosine 5′-diphosphate 3′-diphosphate (ppGpp) and a downshift in the production of the cellular protein-synthesizing machinery (rRNA, ribosomal proteins, and tRNA) (for reviews, see references 10, 21, and 27). The expression of the operons of the protein-synthesizing system is negatively controlled by ppGpp at the level of initiation of transcription (17, 21) and recent evidence suggests that ppGpp-dependent regulation depends upon binding of ppGpp to the β subunit of RNA polymerase (13).

In contrast to the protein-synthesizing system, the expression of a number of *E. coli* genes persists during amino acid limitation, and this expression is correlated with the ability of cells to accumulate ppGpp. The mechanism whereby ppGpp might exert a positive effect on expression of these genes is unknown. Early cell-free studies suggested that ppGpp regulates the expression of *trp*, *lac*, *ara*, and *his* operons at the transcription level (35, 39, 44). However, a more recent study indicates that there is little or no effect of ppGpp on the transcription of *trp* and *lac* mRNA in a purified cell-free system (17). Studies with whole cells have also led to confusion about the proposed effects of ppGpp on the transcription of these operons. Whereas some investigators have suggested that ppGpp enhances the transcription of *his* (39) and *lac* (8, 34) mRNA, others have found no effect of ppGpp on *bla* transcription (22). Unfortunately, many of these studies inferred the effects of ppGpp on transcription from measurements of enzyme activities (34, 35, 39, 44). Very few whole-cell studies have reported the effects of stringent control on both transcription and translation.

Gallant and co-workers (12, 15) suggested that ppGpp affects mRNA translation in amino acid-starved *E. coli* cells. Observations that the decrease in the rate of total protein synthesis is much greater than the decrease in the rate of peptide elongation by amino acid-starved *relA*$^+$ cells led O'Farrell (29) to conclude that the rate of translation initiation may be inhibited by high levels of ppGpp. Results of studies of polyribosome stability in amino acid-starved cells may either support (3, 24) or not support (9, 37, 38) the hypothesis that translation initiation is inhibited by ppGpp. Other investigations have focused on the interaction of ppGpp with the factors involved in peptide elongation (5, 42, 43). More recent whole-cell (29, 31) and cell-free (42, 43) studies indicate that the accumulation of ppGpp is associated with the suppression of translational errors during amino acid limitation. However, a recent review (12) states that the mechanism of translation control by ppGpp is still a mystery.

We reported previously that the addition of ppGpp to a cell-free extract caused a small (ca. 50%) increase in the transcription of *argECBH* mRNA, whereas the synthesis of *argE* and *argH* enzymes was increased up to 20-fold (46), suggesting that the effect of ppGpp on the transcription of the *argECBH* gene cluster is negligible compared with its effect on translation. In this report we describe experiments with *E. coli* cells subjected to transient arginine starvation. The levels of ppGpp, *argCBH* mRNA, and *argF* and *argH* enzymes were measured in *relA*$^+$ and *relA* cells to determine the separate effects on transcription and translation of the stringent response in *E. coli*. Our results strengthen the view that ppGpp regulates the expression of the *arg* genes at some step in translation.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* W-1A and W-11A are spontaneous *argE*$^+$ revertants of strains W-1 and W-11, which were kindly provided by J. Gallant. These strains are isogenic for the following markers: *argR*$^+$, *thi-1*, *his-4*, *proA*, *thr-1*, *leu-6*, *mtl-1*, xyl-5, *ara-14*, *galK2*, *lacY*, and *str-31*. In most experiments, the cells were grown in the MOPS (morpholinepropanesulfonic acid) minimal medium of Neidhardt et al. (25), containing the following constitu-

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ents: KH_2PO_4, 200 mM; glucose, 0.5% (wt/vol); L-arginine, 200 μg/ml; and a defined, arginine-free supplement of vitamins and amino acids (AF medium) (28). For experiments involving ppGpp quantitation, the cells were grown in MOPS minimal medium containing KH_2PO_4 (200 mM) and glucose (0.5% [wt/vol]), with the following additions (per liter): L-arginine, when added, 200 mg; L-proline, 140 mg; L-histidine, 270 mg; L-threonine, 200 mg; L-leucine, 270 mg; and thiamine hydrochloride, 1 mg. For experiments using α-*O*-methylglucose (α-MG) to promote carbon-energy downshift, cells were initially grown in MOPS medium containing glucose (0.1% [wt/vol]) and the additions listed above. The α-MG was added to a final concentration of 2.5% (wt/vol) at the times indicated. Bacterial cultures were grown with aeration at 37°C, and turbidities were monitored with a Klett-Summerson colorimeter at 600 nm. Mean culture generation times were 65 to 70 min in the MOPS minimal medium and 35 to 45 min in supplemented medium (AF medium).

Mid-exponential-phase cultures (2×10^8 to 4×10^8 cells per ml) were subjected to arginine downshift by rapidly centrifuging the cells and suspending them in prewarmed AF medium, as described by Faanes and Rogers (6).

Preparation of extracts and assay of enzyme activities. Cell sonic extracts were prepared from 10-ml culture samples, as described previously (19). Ornithine carbamoyltransferase (EC 2.1.3.3) (OTC) activity in cell sonic extracts was determined using reaction conditions described previously (36). Citrulline was assayed colorimetrically by the method of Prescott and Jones (33). Argininosuccinate lyase (EC 4.3.2.1) (ASL) was assayed by using the conditions described by Krzyzek and Rogers (19). One unit of enzyme activity was defined as 1 μmol of product per h under the assay conditions. Specific enzyme activity is reported as units per milligram of protein. Protein determinations were made by the method of Lowry et al. (23).

Labeling, extraction, and quantitation of ppGpp. Cultures grown at 37°C in MOPS medium supplemented with AF medium were labeled with $^{32}P_i$ (35 μCi/ml; 175 mCi/nmol of PO_4), and nucleotides were extracted essentially as described by Bochner and Ames (1). ppGpp was resolved from other cellular nucleotides by two-dimensional thin-layer chromatography by using the Tb/Sb solvent system of Bochner and Ames (2). ppGpp spots were cut out, and the radioactivity was quantitated by liquid scintillation spectrometry.

Extraction and blot hybridization of *argCBH* mRNA. Cells from 5-ml culture samples were harvested as described by Krzyzek and Rogers (18) for subsequent RNA extraction. Total cellular RNA was isolated essentially as described by Okamoto et al. (30), as modified by Stubbs and Hall (40). Lysis of the cells was performed in a total volume of 0.5 ml, and phenol extractions were performed in 1.5-ml Eppendorf tubes (Brinkmann Instruments, Inc.). The RNA was precipitated with ethanol and stored at −70°C. RNA samples prepared by this method consistently gave 260 nm-to-280 nm ratios of greater than 2.0. Northern dot blots (0.1 to 2.0 μg of RNA per spot) were prepared as described by Thomas (41).

Recombinant M13 bacteriophage DNA containing the entire *argECBH* gene cluster (M. G. Williams and P. Rogers, manuscript in preparation) was used to prepare probe DNA for DNA-RNA hybridization assays. The phage DNA was labeled with $^{32}P_i$ and was extracted as described elsewhere (Williams and Rogers, in preparation). Prehybridization, hybridization, and washing conditions were described by Thomas (41). The hybridization reaction mixtures contained 10% (wt/vol) sodium dextran sulfate (Pharmacia), and the total RNA:DNA mass ratio was less than or equal to 50:1. Hybridized blots exposed Kodak XAR film (Eastman Kodak Co.) at −70°C with a Dupont Cronex Lightning-Plus intensifier screen. Quantitative scanning densitometry was performed with a Beckman DU-8 spectrophotometer at 600 nm.

Preparation and fractionation of lysates containing polyribosomes. Exponential-phase cultures of *E. coli* (130 ml) in MOPS medium plus arginine (200 μg/ml) supplemented with AF medium were labeled with [^{3}H]uridine (0.3 μCi/ml; 5.5 pmol of uridine per ml) for 15 min before the culture was split into two 65-ml aliquots. Growth was terminated in the first aliquot by pouring the culture over an equal volume of frozen and crushed TM buffer (18) containing 20 mM sodium azide and chloramphenicol (250 μg/ml). The cells were immediately pelleted at 4°C. The second aliquot was quickly shifted to AF medium and incubated for 15 min at 37°C, and the cells were subsequently harvested as described for the first aliquot. The pellets were suspended, and the bacteria were lysed by the method of Godson and Sinsheimer (14). All operations were performed at 2 to 4°C. The lysates were layered onto 15 to 30% linear sucrose gradients, centrifuged in an SW27 Rotor (Beckman Instruments, Inc.) at 25,000 rpm for 225 min, and 1-ml fractions were collected from the top of each gradient with an Instrument Specialties Co. model 5 gradient fraction collector. Adjacent fractions were pooled in pairs, and polysomes were precipitated with ethanol, as described by Godson and Sinsheimer (14). The precipitates were collected by centrifugation and suspended in a sodium acetate buffer, and the RNA was extracted twice with phenol, as described by Krzyzek and Rogers (20). The recovery of [^{3}H]RNA per pooled fraction was determined by the difference between the acid-insoluble counts per minute remaining in the RNA extracts and the acid-insoluble counts per minute in the original pooled fractions. Quantitative measurements of hybridizable *argCBH* mRNA in each pooled polysome fraction were adjusted proportionately to account for differences in the percent recovery of [^{3}H]RNA from each sample.

Materials. [5,6-^{3}H]uridine (55 Ci/mmol) and $^{32}P_i$ (carrier free, in 0.02 N HCl) were obtained from ICN Chemical and Radioisotope Division. Chromatographic standards including ppGpp and hexalithium salt were from P-L Biochemicals, Inc. L-Amino acids and vitamins used in growth media and for substrates in enzyme activity assays were of the highest purity available from Calbiochem-Behring, Sigma Chemical Co., or U.S. Biochemical Corp. Chloramphenicol and rifampin were from Sigma. Brij detergent was obtained from Atlas Chemical Co., and sodium deoxycholate was from Mann Research Laboratories.

RESULTS

Effects of transient arginine starvation on *arg* enzyme derepression and ppGpp synthesis by *E. coli*. The effect of transient arginine starvation on the derepression of *argF* enzyme (OTC) and *argH* enzyme (ASL) synthesis is shown in Fig. 1. The *relA*$^+$ strain synthesized *arg* enzymes at a higher rate than that of total protein synthesis (hence, an increase in specific enzyme activities) immediately after arginine removal, as observed previously (18). Peak levels of OTC and ASL specific activity were attained within 40 min after arginine removal from *relA*$^+$ cells. Maximum derepressed levels of OTC and ASL specific activities in *E. coli* W-1A extracts were approximately 100-fold and 10-fold higher, respectively, than the prestarvation levels. In con-

trast, the specific activities of both OTC and ASL increased only slightly in *relA* cells during the 80 min immediately after the removal of exogenous arginine (Fig. 1). In fact, *relA* cells did not attain the high derepressed level of these enzymes until after 4 h.

Previous studies demonstrated that amino acid starvation results in the accumulation of intracellular ppGpp by *relA*$^+$ cells (11). The effect of arginine downshift on ppGpp accumulation by *E. coli* W-1A (*relA*$^+$) and W-11A (*relA*) was examined, and the results (not shown) were in general agreement with those of previous studies.

Effect of transient arginine starvation on *argCBH* transcription by *E. coli*. We used dot blot hybridization assays as a direct measurement of the synthesis of hybridizable *argCBH* mRNA in arginine-limited *relA*$^+$ and *relA* cells (Fig. 2). After the removal of exogenous arginine, the amount of hybridizable *argCBH* mRNA increased to peak levels in about 15 min, and the maximum reached was roughly equivalent in both *relA*$^+$ and *relA* cells. Subsequently, the level of

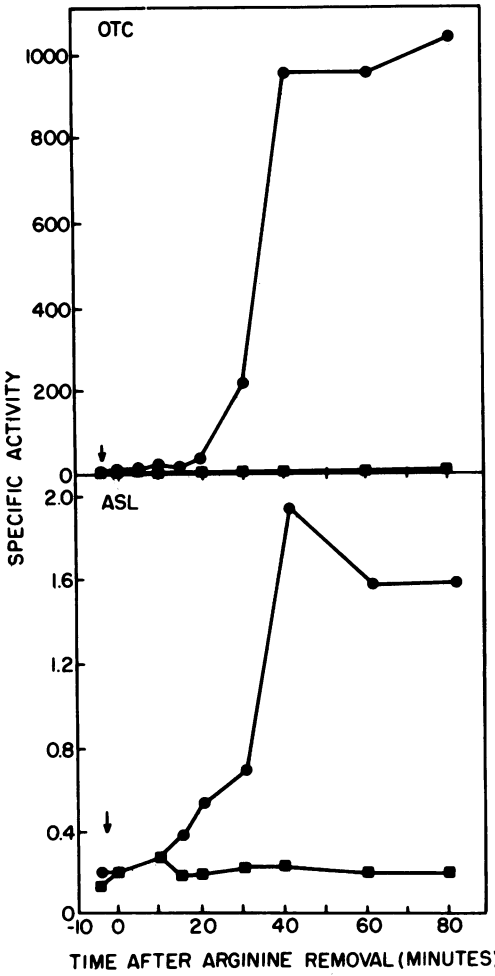

FIG. 1. Derepression of *arg* enzyme synthesis after arginine downshift in *E. coli relA* and *relA*$^+$. Cells of *E. coli* W-1A (*relA*$^+$) and W-11A (*relA*) grown exponentially in AF medium plus L-arginine (200 μg/ml) were washed and suspended in prewarmed AF medium at the time shown by the arrows. At the times indicated, samples were withdrawn and the specific activities (micromoles of product per hour per milligram of mg protein) were determined for OTC and ASL. Symbols: ●, *E. coli* W-1A (*relA*$^+$); ■, *E. coli* W-11A (*relA*).

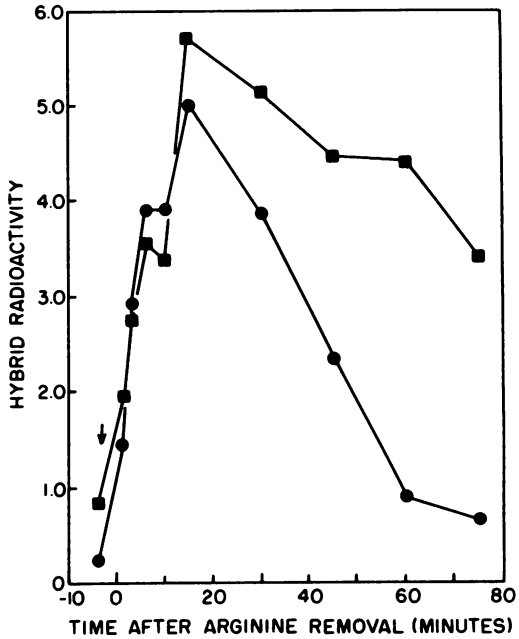

FIG. 2. Derepression of *argCBH* mRNA after arginine downshift. *E. coli* W-1A (*relA*$^+$) and W-11A (*relA*) were grown in MOPS medium supplemented with AF medium and containing added arginine (200 μg/ml). During mid-exponential-phase growth, the cells were quickly shifted (shown by arrow) to prewarmed AF medium. At the times indicated, culture samples were withdrawn for the quantitation of *argCBH* mRNA by dot blot hybridization. The values for hybrid radioactivity were derived from the integrated absorbance peaks of a densitometer scan of the exposed autoradiogram. Symbols: ●, *E. coli* W-1A (*relA*$^+$); ■, *E. coli* W-11A (*relA*).

argCBH mRNA in *relA*$^+$ cells decreased to approximately four times the prestarvation value, whereas the level of *argCBH*-hybridizable mRNA in *relA* cells remained relatively high (at least 60% of peak levels) for at least 70 min after arginine removal. The rapid disappearance of *argCBH* mRNA from *relA*$^+$ cells between 15 and 70 min after arginine depletion (Fig. 2) is consistent with the view that the *relA*$^+$ cells synthesized sufficient endogenous arginine to partially repress the transcription of *arg* mRNA. This view is supported by the observation that *relA*$^+$ cells synthesized enough endogenous arginine within 25 min after arginine removal to support a rate of [^{3}H]leucine incorporation into protein equivalent to the rate observed for a control culture with added arginine (data not shown).

We also examined the stability of *argCBH* mRNA accumulated during arginine depletion. Arginine-grown cells of *relA* and *relA*$^+$ strains were shifted and incubated without arginine for 15 min. Rifampin (200 μg/ml) was added, cell samples were withdrawn at various times, and RNA was extracted and assayed for specific *argCBH* mRNA by dot blot hybridization. The half-life of this mRNA was about 5.5 min for both strains (data not shown), suggesting that the overall rate of *argCBH* mRNA decay was also unaffected by the level of ppGpp present in the cells.

Effect of transient arginine starvation on the capacity to translate accumulated *argCBH* or *argF* mRNA. Since we observed that arginine-limited *relA* cells were able to derepress *arg* mRNA synthesis in 15 min (Fig. 2) but were unable to derepress the synthesis of *arg* enzyme activities for more than 2 h (Fig. 1), we tested whether the *arg* mRNA accumulated by *relA* cells during arginine starvation could be

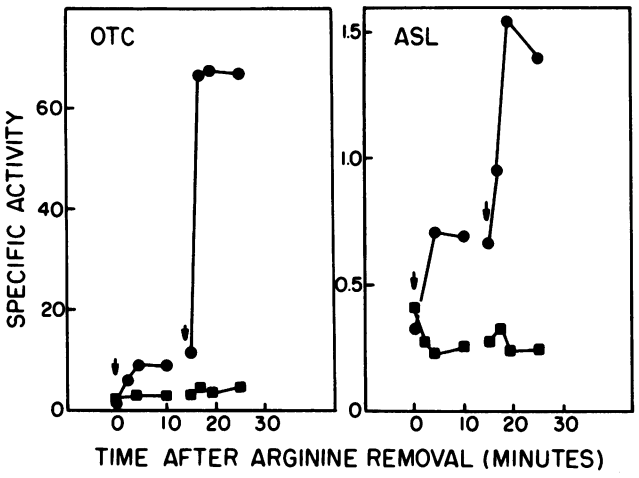

FIG. 3. Translation capacity of *arg* mRNA after arginine downshift, as shown by rapid enzyme synthesis upon adding back arginine. Cells of *E. coli* W-1A and W-11A were subjected to arginine downshift and were divided into two subcultures at 0 min. Arginine (final concentration, 200 μg/ml) was added back to one subculture at 0 min and to the other subculture at 15 min, as shown by the arrows. At the times shown after arginine readdition, samples were removed and specific activities were determined for OTC and ASL. Symbols: ●, *E. coli* W-1A (*relA*$^+$); ■, *E. coli* W-11A (*relA*).

translated by whole cells into active enzymes when arginine was restored to the culture medium. The experimental approach used was developed by Faanes and Rogers (6, 7) to examine the amounts of translatable *argE*, *argF*, and *argH* mRNA in *E. coli* cultures subjected to various growth conditions. The immediate rapid increase in *arg* enzyme specific activity after arginine readdition is due to the translation of *arg* mRNA that accumulates during the period of arginine limitation (7). The data presented in Fig. 3 show the translation of accumulated *argF* and *argH* mRNA into active enzymes when arginine was added back to a portion of the cultures at 0 or 15 min after the cells were shifted to AF medium. There was a relatively small burst of *argF* and *argH* enzyme synthesis when arginine was added back to *relA*$^+$ cells immediately after the arginine downshift (0 min). This was probably due to the accumulation of some *arg* mRNA during the 3-min washing step when the cells were shifted to AF medium, as shown by the 5- and 0-min samples in Fig. 2. When arginine was added back after 15 min of arginine limitation, there were relatively large bursts of synthesis of ASL (threefold) and OTC (sevenfold) enzyme activities by *relA*$^+$ cells (Fig. 3). In contrast, there was no significant increase in the synthesis of OTC and ASL enzyme activites when arginine was added back to *relA* cells 0 and 15 min after arginine removal (Fig. 3), even though the *relA* cells accumulated large amounts of *argCBH* mRNA under these conditions (Fig. 2).

This experiment was similar in principle to an experiment reported by Gallant et al. (11). However, their results were the opposite of our data reported above. Whereas Gallant et al. observed that *relA* cells synthesized an equal or greater amount of *argF* enzyme (relative to that synthesized by *relA*$^+$ cells) when arginine was added back to the cultures, we found that *relA* cells were unable to synthesize *argF* or *argH* enzymes when arginine was added back (Fig. 3). Our results were reproduced identically when we tested *E. coli* CP78 (*relA*$^+$) and CP79 (*relA*) (data not shown). The interpretation of our observations is dicussed below.

Effect of transient arginine starvation on ribosome-bound *argCBH* mRNA. Since these results suggested that the *argCBH* mRNA accumulated in *relA* cells was not translated, we next determined the amount of this mRNA bound to ribosomes by measuring the percentage of total hybridizable *argCBH* mRNA within the cellular fractions that contain polyribosomes. The effects of transient arginine starvation on the distribution of *argCBH*-hybridizable mRNA in the ribosome-containing fractions of *relA*$^+$ and *relA* cells is shown in Table 1. Arginine-limited *relA*$^+$ cells showed a relatively small (10%) increase in the proportion of total *argCBH* mRNA present in fractions containing polysomes compared with the cells grown with arginine. After arginine removal from *relA* cells there was approximately 20% less *argCBH* mRNA present in fractions containing ribosomes. Nevertheless, the proportions of free and polysome-bound *argCBH* mRNA were roughly similar in *relA*$^+$ and *relA* cells after arginine removal (Table 1).

Effect of combined glucose starvation and arginine limitation on ppGpp synthesis and the capacity of *relA* cells to translate accumulated *arg* mRNA. As shown by Hansen et al. (16), *relA* cells accumulate higher levels of ppGpp when subjected to carbon-energy source starvation caused by α-MG addition (Fig. 4A). A peak ppGpp (approximately 34 pmol/10^8 cells) was reached within 10 min after α-MG addition, and the level remained above the basal concentration for 30 min after α-MG addition. We then measured the accumulation of ppGpp by glucose-starved *relA* cells also limited for arginine. Although it is technically impossible to limit growth with more than one limiting substrate, it should be possible to examine the effect of such double limitation on the physiology (e.g., nucleotide pools and mRNA translation) of the cells. The results of a representative experiment in which ppGpp levels were examined in arginine-limited *relA* cells before and after α-MG addition are shown in Fig. 4B. As shown by others, there was a reduction in the level of ppGpp in arginine-limited *relA* cells, whereas *relA* cells

TABLE 1. Polysome-bound *argCBH* mRNA[a]

E. coli strain	Condition	Amt of *argCBH* mRNA (% of total)[b]	
		Free	Polysome bound
W-1A (*relA*$^+$)	Growth with arginine	34	66
	15 min after arginine downshift	25	75
W-11A (*relA*)	Growth with arginine	13	87
	15 min after arginine downshift	33	67

[a] Amount of polyribosome-bound *argCBH* mRNA in *E. coli* before and after arginine downshift. Cells of *E. coli* W-1A and W-11A were grown in MOPS medium supplemented with AF medium and containing L-arginine (200 μg/ml). During mid-exponential-phase growth, total cellular RNA was labeled for 15 min with [^{3}H]uridine, and the cultures were divided into two aliquots. One aliquot from each culture was shifted to medium lacking arginine, and the cells were incubated for 15 min (arginine downshift). The remaining aliquots were controls (growth with arginine). The cells in each aliquot were gently lysed and the RNA was fractionated in 15 to 30% sucrose gradients. The RNAs from each sequential pair of 1-ml fractions were pooled, extracted with phenol, denatured, and dot blotted to a nitrocellulose filter. The RNA was hybridized with ^{32}P-labeled M13mg5A DNA, and the *argCBH* mRNA was quantitated as described in Materials and Methods.

[b] The percent free *argCBH* mRNA was calculated as the fraction of the total hybridizable *argCBH* mRNA found in the pooled fractions eluted before the 70S RNA peak. The percent polysome-bound *argCBH* mRNA was the hybridizable *argCBH* mRNA in the remaining fractions, including the 70S ribosome peak.

accumulated ppGpp during combined arginine and glucose limitation (Fig. 4B).

We examined next whether *relA* cells could translate *arg* mRNA into active enzymes during simultaneous arginine and glucose limitation. Cells grown with arginine were transferred rapidly to medium without arginine and divided into two aliquots, and α-MG (2.5%) was added to one. After various periods of arginine limitation with or without α-MG, the translation of accumulated mRNA was facilitated by adding both arginine and glucose to subcultures (Fig. 5, flasks A through F), as in the experiment for which results are shown in Fig. 3. Samples were withdrawn and assayed for OTC and ASL. The data demonstrate that the translation of *argF* and *argH* mRNA into OTC and ASL activities by *relA* cells increased as the time of glucose starvation increased (Fig. 5, flasks C through F). Meanwhile, arginine-limited *relA* cells did not translate accumulated *arg* mRNA (Fig. 5, flasks A and B). Furthermore, RNA dot blot experiments demonstrated that the levels of total hybridizable *argCBH* mRNA were approximately equal (within 10%) throughout the course of the experiment in both the arginine-limited and arginine- and glucose-limited cultures (data not shown). These results suggest that the accumulation of ppGpp by glucose-starved *relA* cells correlated with their ability to translate *arg* mRNA into functional enzymes.

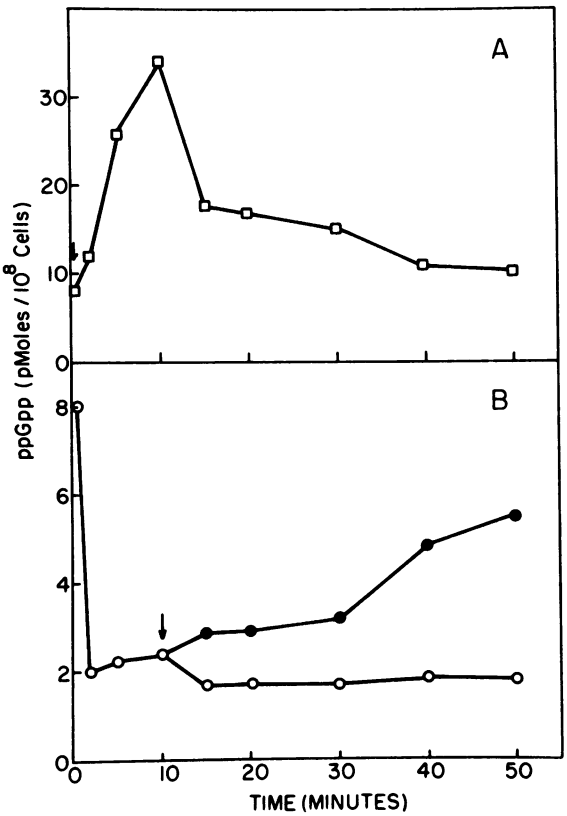

FIG. 4. Effect of α-MG addition on ppGpp accumulation. (A) α-MG (final concentration, 2.5% [wt/vol]) was added to mid-exponential-phase *E. coli* W-11A (*relA*) cells at the time indicated by the arrow, and samples were removed at the times shown for ppGpp quantitation as described in Materials and Methods. (B) A mid-exponential-phase culture of *E. coli* W-11A was shifted from medium containing added arginine (200 μg/ml) to prewarmed AF medium and divided into two subcultures at 0 min. Ten minutes later (indicated by the arrow), α-MG (final concentration, 2.5% [wt/vol]) was added to one of the subcultures (●); the other subculture served as the control (○). Samples of both subcultures were removed at the times shown, and intracellular ppGpp content was determined.

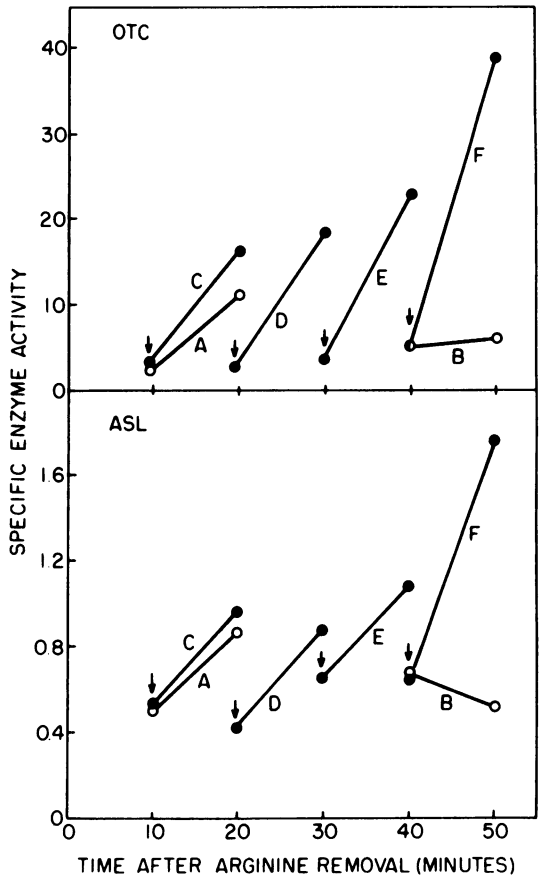

FIG. 5. Effect of added α-MG on translation capacity of *arg* mRNA accumulated by *relA* cells during arginine downshift. A master culture of *E. coli* W-11A (*relA*) was grown in MOPS medium containing added arginine (200 μg/ml). During mid-exponential-phase growth, the cells were shifted to prewarmed arginine-free MOPS medium (0 min), and the culture was divided into six subcultures (A through F). Ten minutes after arginine removal, α-MG (final concentration, 2.5% [wt/vol]) was added to flasks C through F to induce glucose starvation; flasks A and B served as controls. At the times shown by the arrows, one-half of each subculture was removed for the determination of OTC and ASL specific activities, and a mixture containing rifampin (final concentration, 250 μg/ml), arginine (final concentration, 200 μg/ml), and glucose (final concentration, 2% [wt/vol]) was immediately added to the remainder of the subculture. After 10 min of incubation with rifampin-arginine-glucose, the cells were harvested for a second determination of OTC and ASL activities. Symbols: ○, controls; ●, added α-MG.

DISCUSSION

In these experiments, amino acid limitation in *E. coli* *relA*$^+$ and *relA* strains was produced by shifting cells from a medium containing a high concentration of exogenous arginine, causing repression of the arginine biosynthetic pathway, into a medium without added arginine, in which rapid derepression of this pathway occurs (4). During the recovery from arginine limitation, both the pool of specific mRNA (*argCBH*) and a specific enzyme (*argH*) translated from that

message were measured. Although derepression of arginine biosynthetic enzymes was severely impaired in *relA* cells, we present clear evidence that neither specific *argCBH* mRNA synthesis nor binding of this mRNA to ribosomes was affected by the *relA* or *relA*$^+$ background. These results are in direct contrast to reports showing that, in whole cells, severe limitation of β-galactosidase induction during amino acid starvation in *E. coli relA* is accompanied by lowered transcription of *lac* mRNA (8, 32, 34). However, the problem with these earlier studies is that hybridization of pulse-radiolabeled mRNA was the indicator for determination of *lac* mRNA levels (8, 32) or *lac* mRNA was not measured at all (34). The transfer of radioactivity into and out of the nucleotide pool during starvation complicates the interpretation and was not accounted for in these reports (26). Nevertheless, a recent review (21) leaves open the possibility that stringent regulation of some genes, such as *lac* and *his*, occurs at the level of transcription. Our data are consistent with results of in vitro experiments in which added ppGpp had no effect on transcription of *lac* or *trp* mRNA by RNA polymerase from *E. coli* DNA fragments carrying specific promoters (17). Also, we have reported only a very small (15 to 50%) enhancement by ppGpp of transcription of *argECBH* carried on λ phage DNA with purified RNA polymerase (45). With respect to our finding that *argCBH* mRNA bound equally well to ribosomes of *relA* and *relA*$^+$ cells during arginine limitation (Table I), these results agree with other reports showing no significant effect of amino acid starvation on polysome levels in strains with either genotype (9, 37, 38). By inference, initiation of translation of *argCBH* mRNA occurs at roughly equivalent rates in the absence (*relA* strain) or presence (*relA*$^+$ strain) of high levels of ppGpp in vivo.

We found that the *arg* mRNA which accumulated in *relA* cells during arginine limitation was not translated even when arginine was added back. The enzyme burst experiment, originally used by Faanes and Rogers (6), measures the capacity of *arg* mRNA formed in vivo to act as a template for new enzyme synthesis. Upon adding arginine back after a period of arginine starvation, the *relA*$^+$ cells produced bursts of active *argF* and *argH* enzymes, whereas *relA* cells failed to do so. But when *relA* cells were forced to produce a higher level of ppGpp by subjecting them to glucose depletion during arginine limitation bursts of *argH* and *argF* enzyme activities were observed when arginine and glucose were added. These data support the view that during amino acid starvation successful translation of mRNA is dependent upon (or coincident with) high levels of ppGpp (12, 15). Further, our data are consistent with the current model of ppGpp-mediated reduction of translational error, based on data indicating that ppGpp somehow promoters accurate peptide synthesis in whole cells by reducing the frequency of mistranslation caused by amino acid deprivation (5, 29, 42, 43). On the basis of this model, we visualize that, when arginine is added back to arginine-starved *relA* cells, the peptides that are immediately completed on *arg* mRNA polysomes already contain translational errors and are therefore enzymatically inactive. No new *arg* mRNAs can be initiated due to immediate repression by arginine (4).

Our data appear to agree with our previous observation that an in vitro coupled transcription-translation system produces 20-fold more *argE* (acetylornithinase) and *argH* enzymes when ppGpp is added, whereas *argECBH* mRNA synthesis is enhanced only slightly (46). Curiously, by partial uncoupling of transcription from translation for *argE* and *argH* enzymes (46) or β-galactosidase (35), it has been shown that ppGpp acts very early in the reaction but not during translation of these enzymes that occurs after addition of rifampin. Whether the enhancement by ppGpp of specific enzyme synthesis in the cell-free system reflects the same translational mechanism indicated by the data for whole cells reported here and by others remains unclear and deserves further investigation.

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LITERATURE CITED

1. **Bochner, B. A., and B. N. Ames.** 1982. Selective precipitation of orthophosphate from mixtures containing labile phosphorylated metabolites. Anal. Biochem. **122**:100–107.
2. **Bochner, B. A., and B. N. Ames.** 1982. Complete analysis of cellular nucleotides by two-dimensional thin layer chromatography. J. Biol. Chem. **257**:9759–9769.
3. **Cenatiempo, Y., M. T. M. Sung, and A. J. Cozzone.** 1975. Polysome level and stability in stringent *E. coli* strains under aminoacyl-tRNA deprivation. Biochem. Biophys. Res. Commun. **64**:939–946.
4. **Cunin, R., N. Glansdorff, A. Piérard, and V. Stalon.** 1986. Biosynthesis and metabolism of arginine in bacteria. Microbiol. Rev. **50**:314–352.
5. **Dix, D. B., and R. C. Thompson.** 1986. Elongation factor Tu-guanosine 3-'diphosphate 5'-diphosphate complex increases the fidelity of proofreading in protein biosynthesis: mechanism for reducing translational errors introduced by amino acid starvation. Proc. Natl. Acad. Sci. USA **83**:2027–2031.
6. **Faanes, R., and P. Rogers.** 1968. Roles of arginine and canavanine in the synthesis and repression of ornithine transcarbamylase by *Escherichia coli*. J. Bacteriol. **96**:409–420.
7. **Faanes, R., and P. Rogers.** 1972. Repression of enzymes of arginine biosynthesis by L-canavanine in arginyl-transfer ribonucleic acid synthetase mutants of *Escherichia coli*. J. Bacteriol. **112**:102–113.
8. **Foley, D., P. Dennis, and J. Gallant.** 1981. Mechanism of the *rel* defect in beta-galactosidase synthesis. J. Bacteriol. **145**:641–643.
9. **Friesen, J. D.** 1968. A study of the relationship between polyribosomes and messenger RNA in *Escherichia coli*. J. Mol. Biol. **32**:183–200.
10. **Gallant, J.** 1979. Stringent control in *E. coli*. Annu. Rev. Genet. **13**:393–415.
11. **Gallant, J., H. Erlich, B. Hall, and T. Laffler.** 1970. Analysis of the RC function. Cold Spring Harbor Symp. Quant. Biol. **35**:397–405.
12. **Gallant, J., R. Weiss, J. Murphy, and M. Brown.** 1985. Some puzzles of translational accuracy, p. 92–107. *In* M. Schaechter, F. C. Neidhardt, J. L. Ingraham, and N. O. Kjeldgaard (ed.), The molecular biology of bacterial growth. Jones and Bartlett Publishers, Inc., Boston.
13. **Glass, R. E., S. T. Jones, and A. Ishihama.** 1986. Genetic studies on the β subunit of *Escherichia coli* RNA polymerase. VII. RNA polymerase is a target for ppGpp. Mol. Gen. Genet. **203**:265–268.
14. **Godson, G. N., and R. L. Sinsheimer.** 1967. Use of Brij lysis as a general method to prepare polyribosomes from *Escherichia coli*. Biochim. Biophys. Acta **149**:489–495.
15. **Hall, B., and J. Gallant.** 1972. Defective translation of RC-cells. Nature (London) New Biol. **237**:131–135.
16. **Hansen, M. T., M. L. Pato, S. Molin, N. P. Fiil, and K. von Meyenburg.** 1975. Simple downshift and resulting lack of correlation between ppGpp pool size and ribonucleic acid accumula-

tion. J. Bacteriol. **122**:585–591.
17. **Kajitani, I., and M. Ishihama.** 1984. Promoter selectivity of *Escherichia coli* RNA polymerase: differential stringent control of the multiple promoters from ribosomal RNA and protein operons. J. Biol. Chem. **259**:1951–1957.
18. **Krzyzek, R., and P. Rogers.** 1972. Arginine control of transcription of *argECBH* messenger ribonucleic acid in *Escherichia coli*. J. Bacteriol. **110**:945–954.
19. **Krzyzek, R. A., and P. Rogers.** 1976. Dual regulation by arginine of the expression of the *Escherichia coli argECBH* operon. J. Bacteriol. **126**:348–364.
20. **Krzyzek, R. A., and P. Rogers.** 1976. Effect of arginine on the stability and size of *argECBH* messenger ribonucleic acid in *Escherichia coli*. J. Bacteriol. **126**:365–376.
21. **Lamond, A. I., and A. A. Travers.** 1985. Stringent control of bacterial transcription. Cell **41**:6–8.
22. **Lamond, A. I., and A. A. Travers.** 1985. Genetically separable functional elements mediate the optimal expression and stringent regulation of a bacterial tRNA gene. Cell **40**:319–326.
23. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
24. **Morris, D. W., and J. A. DeMoss.** 1966. Polysome transitions and the regulation of ribonucleic acid synthesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **56**:262–268.
25. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.
26. **Nierlich, D. P.** 1968. Amino acid control over RNA synthesis: a reevaluation. Proc. Natl. Acad. Sci. USA **60**:1345–1352.
27. **Nomura, M., R. Gourse, and G. Baughman.** 1984. Regulation of the synthesis or ribosomes and ribosomal components. Annu. Rev. Biochem. **53**:75–117.
28. **Novick, R. P., and W. K. Maas.** 1961. Control of endogenously synthesized arginine of the formation of ornithine transcarbamylase in *Escherichia coli*. J. Bacteriol. **81**:236–240.
29. **O'Farrell, P. H.** 1978. The suppression of defective translation by ppGpp and its role in the stringent response. Cell **14**:545–557.
30. **Okamoto, K., Y. Sugino, and M. Nomura.** 1962. Synthesis and turnover of phage messenger RNA in *E. coli* infected with bacteriophage T4 and in the presence of chloromycetin. J. Mol. Biol. **5**:527–534.
31. **Parker, J., J. W. Pollard, J. D. Friesen, and C. P. Stanners.** 1978. Stuttering: high-level mistranslation in animal and bacterial cells. Proc. Natl. Acad. Sci. USA **75**:1091–1095.
32. **Pederson, S., and N. O. Kjeldgaard.** 1976. A *rel* gene control of *lac* transcription *in vivo*, p. 164–165. *In* N. O. Kjeldgaard and O. Maaloe (ed.), Control of ribosome synthesis. Alfred Benzon Symposium IX. Munksgaard, International Publishing Ltd., Copenhagen.
33. **Prescott, L. M., and M. E. Jones.** 1969. Modified methods for the determination of carbamyl aspartate. Anal. Biochem. **32**: 408–419.
34. **Primakoff, P.** 1981. In vivo role of the *relA*$^+$ gene in regulation of the *lac* operon. J. Bacteriol. **145**:410–416.
35. **Primakoff, P., and S. W. Artz.** 1979. Positive control of *lac* operon expression *in vitro* by guanosine 5′-diphosphate 3′-diphosphate. Proc. Natl. Acad. Sci. USA **76**:1726–1730.
36. **Rogers, P., and G. D. Novelli.** 1959. Formation of ornithine transcarbamylase in cells and protoplasts of *Escherichia coli*. Biochim. Biophys. Acta **33**:423–436.
37. **Ron, E. Z.** 1971. Polysome turnover during amino acid starvation in *Escherichia coli*. J. Bacteriol. **108**:263–268.
38. **Sells, B. H., and H. L. Ennis.** 1970. Polysome stability in relaxed and stringent strains of *Escherichia coli* during amino acid starvation. J. Bacteriol. **102**:666–671.
39. **Stephens, J. C., S. W. Artz, and B. N. Ames.** 1975. Guanosine 5′-diphosphate 3′-diphosphate (ppGpp): positive effector for histidine operon transcription and general signal for amino acid deficiency. Proc. Natl. Acad. Sci. USA **72**:4389–4393.
40. **Stubbs, J. D., and B. D. Hall.** 1968. Level of tryptophan messenger RNA in *Escherichia coli*. J. Mol. Biol. **37**:289–302.
41. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
42. **Wagner, E. G. H., M. Ehrenberg, and C. G. Kurland.** 1982. Kinetic suppression of translational errors by (p)ppGpp. Mol. Gen. Genet. **185**:269–274.
43. **Wagner, E. G. H., and C. G. Kurland.** 1980. Translational accuracy enhanced *in vitro* by (p)ppGpp. Mol. Gen. Genet. **180**: 139–145.
44. **Yang, H.-L., G. Zubay, E. Urm, G. Reiness, and M. Cashel.** 1974. Effects of guanosine tetraphosphate, guanosine pentaphosphate, and β-γ methylenyl-guanosine pentaphosphate on gene expression of *Escherichia coli in vitro*. Proc. Natl. Acad. Sci. USA **71**:63–67.
45. **Zidwick, M. J., G. Keller, and P. Rogers.** 1984. Regulation and coupling of *argECBH* mRNA and enzyme synthesis in cell extracts of *Escherichia coli*. J. Bacteriol. **159**:640–646.
46. **Zidwick, M. J., J. Korshus, and P. Rogers.** 1984. Positive control of expression of the *argECBH* gene cluster *in vitro* by guanosine 5′-diphosphate 3′-diphosphate. J. Bacteriol. **159**:647–651.