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mRNA-dependent *in vitro* synthesis of ribosomal proteins L12 and L10 and elongation factor Tu

(mRNA translation/guanosine 5′-diphosphate 3′-diphosphate)

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ABSTRACT **RNA extracted from growing *Escherichia coli* can direct the *in vitro* synthesis of ribosomal proteins L12 and L10 and elongation factor Tu when an *E. coli* system is used. The synthesized L12 can be bound to L12-depleted ribosomes and the synthesized elongation factor Tu can form complexes with both elongation factor Ts and GDP. Guanosine 5′-diphosphate 3′-diphosphate has no effect on the synthesis of these proteins from an RNA template but inhibits their synthesis when a DNA template is used.**

Specialized transducing phages containing different clusters of bacterial genes in conjunction with DNA-directed cell-free protein synthesis systems have proven to be useful tools for studying the synthesis and regulation of specific proteins in bacteria (1–7). Although RNA phages have been used as templates to study the *in vitro* synthesis of various phage proteins (reviewed in ref. 8), the use of bacterial mRNA has been limited. It has been shown that mRNA extracted from *Escherichia coli* as well as mRNA obtained from the *in vitro* transcription of transducing phage DNA can direct the *in vitro* synthesis of several *E. coli* proteins and peptides, including an outer membrane lipoprotein (9), alkaline phosphatase (10), gene products of the galactose operon (11), and the NH_2-terminal portions of tryptophanase (12) and β-galactosidase (13).

It is known that *E. coli* ribosomal protein L12* and elongation factor (EF) Tu are produced in large amounts relative to other proteins. Multiple copies of L12 are present on the ribosome (14–18) in addition to the amount of L12 found in the postribosomal supernatant (19–21). It has been shown that there are two genes coding for EF-Tu (22) and that this protein may constitute >5% of the soluble protein of *E. coli* (23). Little is known about the regulation of the synthesis of these proteins. The synthesis of L12 and EF-Tu is under stringent control, because both proteins (as well as ribosomal RNA and other ribosomal proteins) are decreased during amino acid starvation in a stringent but not in a relaxed organism (24–26). Guanosine 5′-diphosphate 3′-diphosphate (ppGpp), a nucleotide that accumulates in a stringent organism during amino acid starvation (27), is thought to mediate the decrease in the synthesis of L12 and EF-Tu. Recent studies (28, 29) using DNA from the transducing phage λrif^{d}18 (which contains genes for EF-Tu, L10, and L12) as template for the *in vitro* synthesis of both L12 and EF-Tu have corroborated the *in vivo* results in that the DNA-directed cell-free synthesis of both proteins was inhibited by ppGpp. Indirect evidence suggested that the inhibition by ppGpp was at the level of transcription (29).

The present communication describes the preparation of an *E. coli* RNA fraction capable of directing the *in vitro* synthesis of ribosomal proteins L10 and L12 and EF-Tu. As opposed to the DNA-directed synthesis of these proteins (28–30), the RNA-directed synthesis of these proteins is unaffected by ppGpp.

MATERIALS AND METHODS

Materials. Uniformly ^{14}C-labeled L amino acid mixture (54 mCi/milliatom of carbon) was purchased from Amersham/Searle. RNA polymerase was purchased from Enzo Biochemicals, New York, NY; DNase and pancreatic RNase were from Worthington Biochemical Corp., Freehold, NJ; and rifampicin was from Sigma Chemical Co., St. Louis, MO; ppGpp was provided by Alan Cook of Hoffmann-La Roche. *E. coli* H105, obtained from J. B. Kirschbaum, was used as the source of λrif^{d}18 DNA (31). Ribosomal protein L12, EF-Tu, and EF-Ts were purified from *E. coli* as described previously (16, 32, 33). Purified L10 was obtained from H. G. Wittmann. [^{3}H]L12, [^{3}H]Tu, and [^{3}H]L10 were prepared by reductive methylation of the purified proteins (34), using ^{3}H-labeled formaldehyde (New England Nuclear Corp.). Ribosomal wash, washed ribosomes, and the 0.25 M and 1.0 M salt eluates from a DEAE-Sephadex fractionation of an S-100 extract were prepared as described elsewhere (35). Protein concentration was assayed by the method of Lowry *et al.* (36). Antisera to L10 and L12 were raised in rabbits with the aid of Freund's complete adjuvant. EF-Tu antiserum was prepared as described previously (29).

Preparation of mRNA-Containing Fraction. *E. coli* CP79 (*his*$^-$, *leu*$^-$, *thr*$^-$, *arg*$^-$, *rel*$^-$) was grown in antibiotic medium 3 (Difco) and harvested at the midlogarithmic phase of growth. Total RNA was extracted from the cells according to the method of Salser *et al.* (37). Briefly, this involved heating the cells at 60° in the presence of sodium dodecyl sulfate (NaDodSO$_4$) followed by a phenol extraction. The aqueous layer was removed and the RNA was precipitated with ethanol overnight. The precipitate was dissolved in water and dialyzed, and this preparation of total RNA was used as the source of mRNA.

Cell-Free System of Protein Synthesis. The complete system (70 μl) for protein synthesis using a partially fractionated *E. coli* extract as the source of translation factors was the same as that previously described (28) (except that cyclic AMP was omitted) with either λrif^{d}18 DNA or RNA as template. The synthesis reaction was carried out at 37° and terminated by adding 1 μg of pancreatic ribonuclease. The reaction mixture was chilled and then centrifuged at 7000 × *g* for 10 min to remove insoluble material. Five microliters of the clarified supernatant was assayed for the incorporation of radioactivity into total proteins

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Abbreviations: EF, elongation factor; ppGpp, guanosine 5′-diphosphate 3′-diphosphate; NaDodSO$_4$, sodium dodecyl sulfate.

* The term "L12" refers to either or both ribosomal proteins L7 and L12, L7 being an acetylated form of L12.

Table 1. Comparison of *E. coli* total RNA- and λrif^{d}18 DNA-directed incorporation of ^{14}C-labeled amino acids into protein

Additions to complete system	Total protein, cpm	
	RNA	DNA
—	5410	6970
DNase	7500	460
RNase	138	156
Rifampicin	6033	474

Protein synthesis was carried out with *E. coli* total RNA (100 μg) or λrif^{d}18 DNA (15 μg) as template. Aliquots of the reaction mixture (5 μl) were precipitated with hot CCl_3COOH after the addition of 30 μg of bovine serum albumin as carrier. One microgram each of DNase and RNase and 25 μg of rifampicin were added to the incubations where indicated.

by precipitation with hot CCl_3COOH (20 min at 90°) and collecting the precipitate on a nitrocellulose filter. The filter was assayed for radioactivity.

Assay for L12, L10, and EF-Tu by Radioimmunoprecipitation. To 50 μl of the clarified reaction mixture at 37° in buffer A (0.05 M Tris·HCl at pH 7.4/0.5 M NaCl/1% Triton X-100) were added antisera (either 50 μl of anti-L12, 100 μl of anti-L10, or 400 μl of anti-Tu) and the respective ^{3}H-labeled carrier proteins (5 μg of [^{3}H]L12, 1000 cpm/μg; 5 μg of [^{3}H]L10, 700 cpm/μg; or 3 μg of [^{3}H]EF-Tu, 3000 cpm/μg). The total volume was between 125 and 600 μl. After flocculation had occurred (1–2 hr), the reaction mixtures were chilled at 4° for 15–30 min. The resulting immunoprecipitates were washed four times in 0.5 ml of buffer A, suspended in 40 μl of 4 M urea containing 50% (vol/vol) acetic acid, and heated for 2 min at 90° to dissolve the precipitate. A 10-μl aliquot was assayed for radioactivity in a Beckman liquid scintillation counter. The remainder of the solubilized immunoprecipitate was lyophilized and analyzed by gel electrophoresis as described below.

Polyacrylamide Gel Electrophoresis. For disc-gel electrophoresis in the presence of $NaDodSO_4$, the dried immunoprecipitate was dissolved by heating for 2 min at 90° in 30 μl of 1% $NaDodSO_4$ and subjected to polyacrylamide gel electrophoresis in 0.1% $NaDodSO_4$ (38). A 15% gel was used for L12 and L10 and a 10% gel for EF-Tu.

For electrophoresis at pH 4.5, the dried immunoprecipitate was dissolved in 30 μl of H_2O and electrophoresed in 7.8% polyacrylamide gels containing 6 M urea (39).

The gels were sliced into 1-mm ($NaDodSO_4$ gels) or 2-mm (urea gels) sections with the Gilson Aliquogel fractionator, extracted with 0.7 ml of 0.1% $NaDodSO_4$ for 1 hr at 80°, and then assayed for radioactivity in 5 ml of Instabray (Yorktown Research, NJ).

For two-dimensional gel electrophoresis, a modification of the system of Kaltschmidt and Wittmann (40, 41) was used. Because both L12 and L10 are acidic, only proteins moving to the anode in the first dimension were examined. Electrophoresis in the first dimension was carried out in 4% gels for 4 hr at 5 mA per gel and in the second dimension in 12% gels at 30 V for 14 hr. After electrophoresis, the slab gels were either stained with Coomassie blue or subjected to radiofluorography (42). In the former case, the stained spots were punched out, digested in 0.5 ml of 30% (wt/vol) H_2O_2 at 55° for 6 hr, and assayed for radioactivity in 10 ml of Aquasol (New England Nuclear). The recovery as estimated from coelectrophoresis of the tritiated standards was between 15 and 20%.

Ribosome Binding of Synthesized L12 and L10. ^{14}C-Labeled L12 and L10 synthesized *in vitro* were assayed for their ability to bind to ethanol/NH_4Cl-extracted 70S ribosomes (deficient in L12 and L10) as described previously (28). The binding was allowed to proceed at 37° for 5 min and the ribosomes were recovered by centrifugation at 200,000 × *g* for 90 min through 1 ml of 10% sucrose. The bound [^{14}C]L12 and L10 products were extracted from the ribosomes with ethanol/NH_4Cl (43) and the amount per A_{260} of ribosomes was quantitated by immunoprecipitation with L12 antiserum and subsequent analysis of the immunoprecipitate by $NaDodSO_4$/polyacrylamide gel electrophoresis.

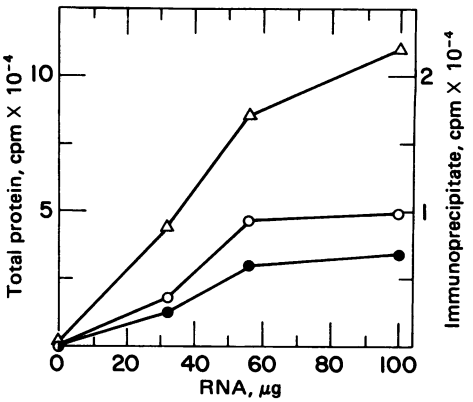

FIG. 1. The effect of RNA concentration on the incorporation of ^{14}C-labeled amino acids into total protein and material immunoprecipitable by antisera to L12 and EF-Tu. Various amounts of RNA were added to the incubations and each sample was assayed for the amount of radioactivity incorporated into total protein (△) and into L12 (○) and EF-Tu (●) immunoprecipitable material. A conversion factor of 40 μg of RNA/A_{260} unit has been used.

Interaction of Synthesized EF-Tu with EF-Ts and GDP. ^{14}C-Labeled EF-Tu synthesized *in vitro* was assayed by its ability to react with EF-Ts and GDP as previously described (29). Briefly, this involves assaying for changes in the chromatographic behavior of the *in vitro* synthesized EF-Tu when it is incubated with EF-Ts or GDP. These changes in the elution profile of EF-Tu are dependent upon its ability to interact reversibly with EF-Ts and GDP.

RESULTS

Dependence of Cell-Free Protein Synthesis on *E. coli* RNA. Table 1 compares the incorporation of ^{14}C-labeled amino acids into hot CCl_3COOH-insoluble material in the cell-free system directed by either *E. coli* RNA or λrif^{d}18 DNA. As expected, amino acid incorporation directed by the RNA template is unaffected by DNase and rifampicin, but is almost completely inhibited by pancreatic RNase. On the other hand, when DNA from the transducing phage λrif^{d}18 (which contains the genes for EF-Tu, L10, and L12) is used as template, all three agents inhibited protein synthesis. Fig. 1 shows that protein synthesis increases with RNA concentration up to about 60 μg of RNA per incubation and that maximal amounts of radioactive material immunoprecipitable by antisera to L12 and EF-Tu are also obtained at this RNA concentration. Both total protein synthesis and the amount of anti-L12 and anti-EF-Tu immunoprecipitable radioactivity were linear during 20 min of incubation and the incorporation was generally complete within 30 min (data not shown).

Gel Analysis of Products Immunoprecipitated by L12 Antiserum. The L12 immunoprecipitate was resolved into two major radioactive peaks by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 2*A*). One peak comigrated with authentic L12 with a molecular weight of 12,000, whereas the other peak

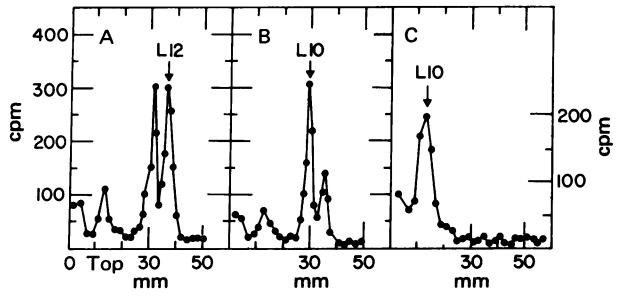

FIG. 2. Gel electrophoresis of the products synthesized *in vitro* that are precipitated by L12 and L10 antisera. The arrows show the positions of authentic L12 and L10. (*A*) NaDodSO$_4$ gel electrophoresis of anti-L12 immunoprecipitable products; (*B*) NaDodSO$_4$ electrophoresis of anti-L10 immunoprecipitable products; (*C*) pH 4.5 electrophoresis of anti-L12 immunoprecipitable material.

migrated with a molecular weight of about 17,000. The amounts of radioactivity in each peak were approximately equal.

It is to be noted that we have previously shown (30) in a cell-free protein synthesis system using λrif^{d}18 DNA as template that antiserum against protein L12 precipitates both L12 and L10 (which is about 17,000 daltons). This protein forms a tight complex with L12 and is coprecipitated with the L12 by L12 antiserum. Fig. 2*B* shows that when the products of the RNA-directed system are immunoprecipitated with L10 antiserum and subjected to gel electrophoresis, 70–80% of the radioactivity comigrates with authentic L10. In addition, a small peak of radioactivity is observed comigrating with L12 at 12,000 daltons. The immunoprecipitation of the 17,000-dalton protein by both L12 and L10 antisera appears to be quantitative because no further radioactivity is precipitated by a second immunoprecipitation with L10 antiserum. In addition, the 17,000-dalton protein comigrates with L10 when electrophoresed at pH 4.5 (Fig. 2*C*).

Additional proof that the 17,000-dalton protein is L10 was obtained by electrophoresing the solubilized immunoprecipitate in duplicate, in a two-dimensional gel system (20 μg of [^{3}H]L10 added as carrier). One gel was stained and the proteins corresponding to L10, L12, and L8 were punched out of the gel and assayed for radioactivity, while the other gel was subjected to radiofluorography. Radioactivity was found to be concentrated in the protein spots corresponding to L10, L12, and L8 in both assays (data not shown). It is to be noted that ribosomal protein L8 has been found to be a mixture of L12 and L10 (44).

Binding of *In Vitro* Synthesized L12- and L10-like Protein to Depleted Ribosomes. The ribosome-binding ability of the L12 and L10 synthesized *in vitro* was assayed by measuring the amount of anti-L12 immunoprecipitable radioactivity that could be bound to L12-depleted ribosomes. It is known that ethanol/NH$_4$Cl-extracted ribosomes are depleted of nearly all of their L12 and also lose small amounts of L10 (43). Table 2 shows that when the radioactive products (L12 plus L10) were incubated with depleted ribosomes, anti-L12 immunoprecipitable radioactivity became associated with the ribosomes (Exp. 1). The specificity of binding can be seen by the decreased binding of these products to non-depleted ribosomes (Exp. 2). The immunoprecipitable radioactive products bound to the ribosomes were further analyzed by NaDodSO$_4$ gel electrophoresis, and it was found that L12 and L10 were bound to the ribosome with approximately equal efficiencies (data not shown).

Table 2. Binding of *in vitro* synthesized L12 and L10 to ethanol/NH$_4$Cl-extracted ribosomes

Exp.	Ribosomes	Binding, cpm*
1	Depleted	170
2	Normal	34

Protein synthesis was carried out with *E. coli* total RNA as template. The ribosomes were removed from the assay mixture by centrifugation and an aliquot of the postribosomal supernatant was mixed with 540 pmol of either L12-depleted ribosomes or normal ribosomes. A 460-pmol sample of [^{3}H]L12 was then added and the mixture was incubated for 5 min at 37°. The binding assay and quantitation of the products were performed as previously described (28) and in the text. It was found that about the same percentage of [^{3}H]L12 and ^{14}C-labeled immunoprecipitable products was bound to the depleted ribosomes.

* Immunoprecipitable radioactivity bound per A_{260} unit of ribosomes.

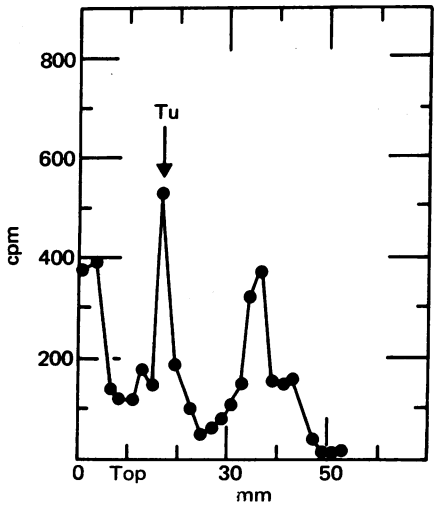

FIG. 3. NaDodSO$_4$ gel electrophoresis of *in vitro* synthesized products immunoprecipitated by EF-Tu antiserum. The arrow shows the position of authentic EF-Tu.

Gel Analysis of Products Immunoprecipitated by EF-Tu Antiserum. The EF-Tu immunoprecipitate was analyzed by NaDodSO$_4$ gel electrophoresis and found to contain radioactivity comigrating with EF-Tu at a molecular weight of about 40,000 (Fig. 3). However, several other peaks were also observed and only about 25% of the immunoprecipitated radioactivity comigrated with EF-Tu. It is not known whether the other peaks are related to EF-Tu.

Interaction *In Vitro* Synthesized EF-Tu with EF-Ts and GDP. In an attempt to further characterize the EF-Tu synthesized *in vitro*, the products of an incubation were mixed with [^{3}H]EF-Tu and GDP and chromatographed on an Ultragel AC44 column (Fig. 4*A*). The fractions comigrating with [^{3}H]EF-Tu were pooled and mixed with a stoichiometric amount of EF-Ts and then rechromatographed. Fig. 4*B* shows that the putative EF-Tu synthesized *in vitro* behaves similarly to authentic EF-Tu in its ability to combine with EF-Ts and form an EF-Tu·EF-Ts complex that, because of its heavier weight, emerged in the peak centered on fraction 48. The fractions containing the EF-Tu·EF-Ts complex were pooled and rechromatographed in the presence of GDP, which dissociates the EF-Tu·EF-Ts complex to form EF-Tu·GDP and EF-Ts (45). Fig. 4*C* shows that both the *in vitro* synthesized [^{14}C]EF-Tu and purified [^{3}H]EF-Tu now elute later from the column, with a peak at tube 55. These results show that the EF-Tu synthesized in the RNA-directed cell-free system can form binary complexes with either EF-Ts or GDP.

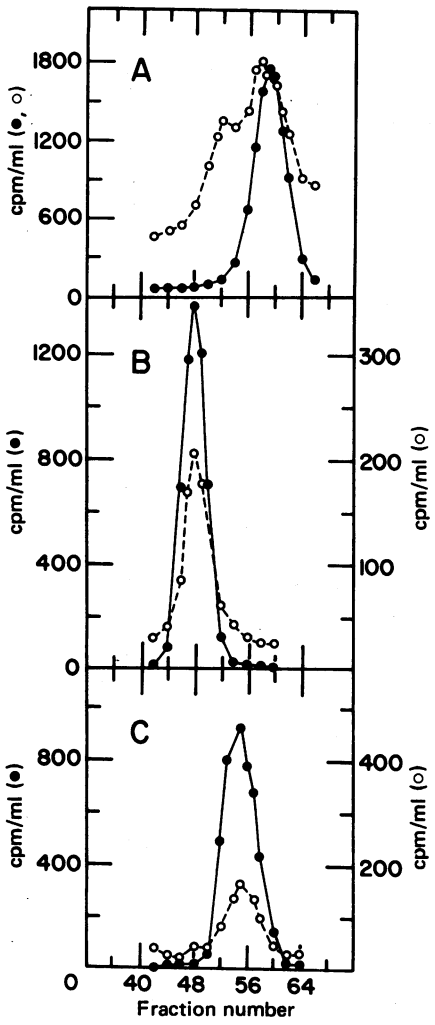

FIG. 4. Interaction of the RNA-directed, *in vitro* synthesized EF-Tu with EF-Ts and GDP. Details of the incubation conditions and chromatography are described in the text. (*A*) Gel filtration of an incubation mixture labeled with ^{14}C-labeled amino acids. (*B*) Gel filtration of EF-Tu·EF-Ts complex. (*C*) Gel filtration of the complex obtained from *B* in the presence of 80 μM GDP. ○, ^{14}C-Labeled proteins; ●, [^{3}H]EF-Tu.

Effect of ppGpp on the RNA-Directed Synthesis of L12, L10, and EF-Tu. It has been shown that the *in vitro* DNA-dependent synthesis of ribosomal RNA (46, 47), ribosomal proteins L12 (28) and L10 (30), and EF-Tu (29) is inhibited by ppGpp. Recent results have indicated that ppGpp appears to inhibit the synthesis of these proteins in the DNA-directed cell-free system at the level of transcription (29). In an attempt to ascertain directly if ppGpp has any effect on the translation of either ribosomal proteins or EF-Tu, the effects of ppGpp on the DNA- and RNA-directed synthesis of these proteins were compared. Fig. 5 shows that although the synthesis of all three proteins was inhibited by the ppGpp in the DNA-directed system, their synthesis in the RNA-directed system was not significantly affected by similar levels of ppGpp. The data provide more direct evidence that physiological concentrations of ppGpp (<1–2 mM) do not affect the translation of the mRNAs for these proteins.

DISCUSSION

The present study presents data which show that RNA extracted from *E. coli* can direct the *in vitro* synthesis of ribosomal proteins L12, L10, and EF-Tu. The synthesized products resemble their cellular counterparts in size and immunological properties, and they also possess similar functional activities. Thus, it has been shown that the L12 and L10 synthesized *in vitro* are able to bind to ribosomes depleted of these two proteins, and that the newly synthesized EF-Tu is able to interact with both EF-Ts and GDP. The availability of an RNA-directed *in vitro* protein synthesis system for these proteins is of special interest because these proteins can also be synthesized in a system directed by DNA from the transducing phage λrif^{d}18. The RNA- and DNA-directed systems offer a useful combination to compare and study the regulation of the synthesis of these proteins.

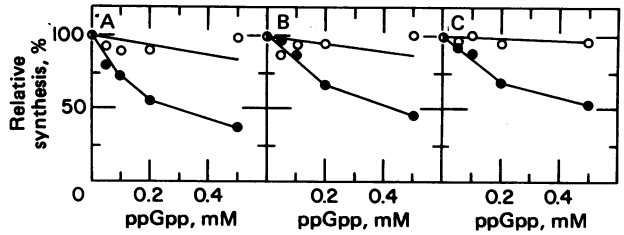

FIG. 5. Effect of ppGpp on the DNA- and RNA-directed *in vitro* synthesis of L12, L10, and EF-Tu. Each incubation contained either λrif^{d}18 DNA (●) or RNA (○) as template. (*A*) L12, (*B*) L10, (*C*) EF-Tu.

It has previously been demonstrated that in a λrif^{d}18 DNA-directed system, the *in vitro* synthesized L10 forms a complex with L12 that is quantitatively precipitated as an L10/L12 complex by L12 antiserum (30). The L10 synthesized in the RNA-directed system is also coprecipitated with L12 by L12 antiserum, suggesting that an L10/L12 complex has been formed. The L10 synthesized *in vitro* is identical to L10 in size (about 17,000 daltons) and immunological behavior (it can be immunoprecipitated by L10 antiserum) and in addition comigrates with authentic L10 in a two-dimensional system.

It has been shown *in vivo* that when a stringent strain of *E. coli* is starved for an amino acid, the syntheses of ribosomal proteins and various elongation factors are depressed (24–26). This inhibition is thought to be mediated by the nucleotide ppGpp, which accumulates during starvation. Direct evidence for this was provided by the observation that ppGpp inhibited the DNA-directed *in vitro* synthesis of ribosomal protein L12 (28). It was later shown that this nucleotide inhibited the DNA-directed *in vitro* synthesis of other ribosomal proteins (30, 48), elongation factors (28, 48), and the α (48) and ββ′ (F. Chu, unpublished observations) subunits of RNA polymerase. It was suggested that ppGpp affected transcription, on the basis of data from experiments utilizing rifampicin to uncouple transcription from translation (29). In the present RNA-directed protein synthesis system, we have demonstrated that ppGpp has little or no effect on the synthesis of L12, L10, and EF-Tu. Thus, these data provide further evidence that ppGpp has no effect on the translation of these proteins and that the inhibition of their synthesis by ppGpp observed in the DNA-directed system most probably occurs at transcription.

F.C. is a Fellow of the National Research Council of Canada.

1. Zubay, G., Chambers, D. A. & Cheong, L. C. (1970) in *The Lactose Operon*, eds. Beckwith, J. R. & Zipser, D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 375.
2. Wetekam, W., Staack, K. & Ehring, R. (1971) *Mol. Gen. Genet.* **112**, 14–27.
3. Kung, H.-F., Fox, J. E., Spears, C., Brot, N. & Weissbach, H (1973) *J. Biol. Chem.* **248**, 5012–5015.

4. Kaltschmidt, E., Kahan, L. & Nomura, M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 446–450.
5. Lindahl, L., Jaskunas, S. R., Dennis, P. P. & Nomura, M. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2743–2747.
6. Schweiger, M. & Gold, L. M. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34,** 763–766.
7. Inouye, H., Pratt, C., Beckwith, J. & Torriani, A. (1977) *J. Mol. Biol.* **110,** 75–87.
8. Lodish, H. L. (1976) *Annu. Rev. Biochem.* **45,** 39–72.
9. Hirashima, a., Wang, S. & Inouye, M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4149–4153.
10. Dohan, F. C., Jr., Rubman, R. H. & Torriani, A. (1971) *J. Mol. Biol.* **58,** 469–479.
11. Schumacher, G. & Ehring, R. (1973) *Mol. Gen. Genet.* **124,** 329–344.
12. Parish, J. H., Khairal Basham, S. A. M., Brown, N. L. & Brown, M. (1971) *Biochem. J.* **125,** 643–653.
13. Reiness, G. & Zubay, G. (1973) *Biochem. Biophys. Res. Commun.* **53,** 967–974.
14. Möller, W., Groene, A., Terhorst, C. & Amons, R. (1972) *Eur. J. Biochem.* **25,** 5–12.
15. Thammana, P., Kurland, C. G., Deusser, E., Weber, J., Maschler, R., Stöffler, G. & Wittmann, H. G. (1973) *Nature New Biol.* **242,** 47–49.
16. Brot, N., Marcel, R., Yamasaki, E. & Weissbach, H. (1973) *J. Biol. Chem.* **248,** 6952–6956.
17. Subramanian, A. R. (1975) *J. Mol. Biol.* **95,** 1–8.
18. Hardy, S. J. S. (1975) *Mol. Gen. Genet.* **140,** 253–274.
19. Morrissey, J. J., Weissbach, H. & Brot, N. (1975) *Biochem. Biophys. Res. Commun.* **65,** 293–302.
20. Ulbrich, B. & Nierhaus, K. H. (1975) *Eur. J. Biochem.* **57,** 49–54.
21. Ramagapol, S. (1976) *Eur. J. Biochem.* **69,** 289–297.
22. Jaskunas, S. R., Lindahl, L., Nomura, M. & Burgess, R. R. (1975) *Nature* **257,** 458–462.
23. Furano, A. V. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4780–4784.
24. Dennis, P. P. & Nomura, M. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3819–3823.
25. Dennis, P. P. & Nomura, M. (1975) *Nature* **255,** 460–465.
26. Furano, A. V. & Wittel, F. P. (1976) *J. Biol. Chem.* **251,** 898–901.
27. Fiil, N. P., Von Meyerburg, K. & Friesen, J. D. (1972) *J. Mol. Biol.* **71,** 769–783.
28. Chu, F., King, H.-F., Caldwell, P., Weissbach, H. & Brot, N. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3156–3159.
29. Chu, F., Miller, D. L., Schulz, T., Weissbach, H. & Brot, N. (1976) *Biochem. Biophys. Res. Commun.* **73,** 917–927.
30. Chu, F., Caldwell, P., Samuels, M., Weissbach, H. & Brot, N. (1977) *Biochem. Biophys. Res. Commun.* **76,** 593–601.
31. Kirschbaum, J. B. & Konrad, E. B. (1973) *J. Bacteriol.* **116,** 517–526.
32. Miller, D. L. & Weissbach, H. (1970) *Arch. Biochem. Biophys.* **141,** 26–37.
33. Hachmann, J., Miller, D. L. & Weissbach, H. (1971) *Arch. Biochem. Biophys.* **147,** 457–466.
34. Rice, R. H. & Means, G. E. (1971) *J. Biol. Chem.* **246,** 831–832.
35. Kung, H.-F., Spears, C. & Weissbach, H. (1975) *J. Biol. Chem.* **250,** 1556–1562.
36. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
37. Salser, W., Gesteland, R. F. & Bolle, A. (1967) *Nature* **215,** 588–591.
38. Laemmli, U. K. (1970) *Nature* **227,** 680–685.
39. Leboy, P. S., Cox, E. C. & Flaks, J. G. (1964) *Proc. Natl. Acad. Sci. USA* **52,** 1367–1374.
40. Kaltschmidt, E. & Wittmann, H. G. (1970) *Anal. Biochem.* **36,** 401–412.
41. Howard, G. A. & Traut, R. R. (1973) *FEBS Lett.* **29,** 177–180.
42. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88.
43. Hamel, E., Koka, H. & Nakamoto, T. (1972) *J. Biol. Chem.* **247,** 805–814.
44. Petterson, I., Hardy, S. J. S. & Liljas, A. (1976) *FEBS Lett.* **64,** 135–138.
45. Miller, D. L. & Weissbach, H. (1969) *Arch. Biochem. Biophys.* **132,** 146–150.
46. Reiness, G., Yang, H. L., Zubay, G. & Cashel, M. (1975) *Proc. NATL/ Acad. Sci. USA* **72,** 2881–2885.
47. Van Ooyen, A. J. J., Gruber, M. & Jorgensen, P. (1976) *Cell* **8,** 123–128.
48. Lindahl, L., Post, L. & Nomura, M. (1976) *Cell* **9,** 439–448.