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Autogenous control of *Escherichia coli* ribosomal protein L10 synthesis *in vitro*

(ribosomal protein synthesis/autogenous regulation/translational control)

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ABSTRACT **The DNA-dependent *in vitro* synthesis of *Escherichia coli* ribosomal protein L10 was inhibited when L10 was added to the protein-synthesizing incubations. Addition of L10 had little or no effect on the synthesis of ribosomal protein L12, elongation factor Tu (*tufB*), or the β and β′ subunits of RNA polymerase. In addition, ribosomal protein L12 did not inhibit its own synthesis or the synthesis of L10. Experiments using a mRNA-directed system showed that the inhibition of the synthesis of L10 by itself is at the level of translation of protein synthesis. The mechanism of inhibition does not appear to be due to increased degradation of L10 mRNA.**

The synthesis of *Escherichia coli* ribosomal proteins occurs as a coordinate event (1). This synthesis provides for the assembly of ribosomes containing stoichiometric amounts of all the ribosomal proteins with the exception of protein L12. Four copies of L12 (or its acetylated congener L7 or both) are present on the ribosome (2, 3). The gene for ribosomal protein L12 is adjacent to the gene for L10 and is clustered among the genes for ribosomal proteins L1 and L11 and the β and β′ subunits of RNA polymerase (Fig. 1). DNA-directed *in vitro* protein-synthesizing systems have been used to study the synthesis of L12 and L10, and it has been shown that *in vitro* the ratio of L12/L10 formed is 4–6 (4). This value agrees with the results *in vivo* and indicates that the normal regulatory mechanism that maintains the L12/L10 ratio is functioning in the *in vitro* system. However, very little is known about the regulation of the expression of these genes. *In vivo* experiments have suggested that the regulation of L12 and L10 synthesis is at the level of both transcription and translation (5–7). Recent *in vitro* experiments have indicated that L12 has its own promoter and that its overproduction relative to L10 may be, in part, due to increased mRNA synthesis (8). The present report shows that ribosomal protein L10 can specifically inhibit its own synthesis in an *in vitro* protein-synthesizing system and that this inhibition is at a post-transcriptional step of protein synthesis.

MATERIALS AND METHODS

Materials. *E. coli* H105 lysogenic with λ*rif*^{d}*18* phage was obtained from J. B. Kirschbaum (Harvard University, Cambridge, MA). *E. coli* JF943 carrying plasmid pNF1337 was a generous gift of J. Friesen (York University, Ontario, Canada). Ribosomal protein L12 was purified as described (9), and ribosomal protein L10 was a generous gift of H. G. Wittmann (Max-Planck-Institut für molekulare Genetik, Berlin). Antiserum to L12 was raised in rabbits with the aid of Freund's adjuvant.

Phage and Plasmid DNA. Phage and plasmid DNAs were isolated as described elsewhere (10).

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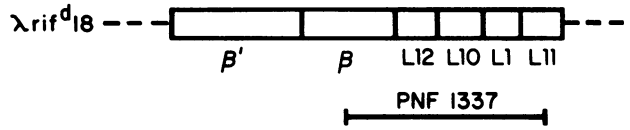

FIG. 1. Schematic map of a portion of λ*rif*^{d}*18* and pNF1337 DNA.

***In Vitro* Protein Synthesis.** The complete system (35 μl) containing ribosomal wash, washed ribosomes, and the 0.25 M and 1.0 M salt eluates from a DEAE-cellulose fractionation of an S-200 extract was similar to that previously described (4, 11). λ*rif*^{d}*18* DNA, pNF1337 DNA, or RNA prepared from transcription of pNF1337 DNA *in vitro* (see below) was used as template, and incubations were for 50 min at 37°C. After centrifugation at 7000 × *g* for 10 min, an aliquot of the supernatant was assayed for incorporation of radioactivity into total protein by precipitation of the protein with hot Cl_3CCOOH and collection on a nitrocellulose disc. The filter was assayed for radioactivity in a scintillation spectrometer.

Assay for L10 and L12 by Immunoprecipitation. The amounts of L10, L12, elongation factor Tu (EF-Tu), and the β and β′ subunits of RNA polymerase synthesized were determined by immunoprecipitation and subsequent gel electrophoresis of the immunoprecipitated products (12, 13).

Preparation of RNA. pNF1337 DNA (≈10 μg) was incubated for 20 min at 37°C in an *in vitro* protein-synthesizing system described above with the following modifications. The incubations (without tRNA and amino acids) were scaled up 6-fold to a final volume of 210 μl and contained 6 μCi of [^{3}H]UTP (1 Ci = 3.7 × 10^{10} becquerels). Under these conditions RNA can be synthesized in the absence of protein synthesis. The reaction was terminated by addition of DNase (4 μg/ml) for 30 sec at 0°C and of tRNA (150 μg) followed by EDTA and sodium dodecyl sulfate, final concentrations 5 mM and 1%, respectively. The mixture was extracted twice with an equal volume of phenol, followed by two extractions with ether. The aqueous layer was removed and brought to a final concentration of 0.3 M sodium acetate and 70% ethanol. This mixture was kept at −20°C overnight. The precipitate containing the RNA was centrifuged, dissolved in a small volume of sterile water, and then dialyzed against sterile water for 2–4 hr.

RESULTS

In experiments designed to investigate the possibility that ribosomal protein synthesis is regulated autogenously, ribosomal proteins L10 and L12 were added to an *in vitro* protein-synthesizing system containing either λ*rif*^{d}*18* or pNF1337 DNA as template. Although both of these DNAs contain the genetic information for ribosomal proteins L1, L10, and L12, pNF1337

Abbreviation: EF-Tu, elongation factor Tu.

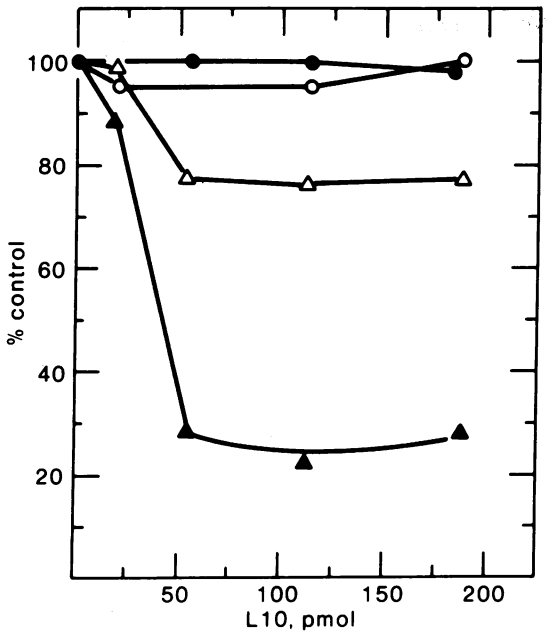

FIG. 2. Effect of L10 on the DNA-directed *in vitro* synthesis of L10, L12, and the β and β' subunits of RNA polymerase. L10 was added to reaction mixtures (35 μl) containing 1.5 μg of $\lambda rif^d 18$ DNA and the other components of the *in vitro* protein-synthesizing system. The amounts of the various proteins synthesized were determined as described in the text. ●, β and β' subunits of RNA polymerase; ○, EF-Tu; △, L12; ▲, L10.

DNA contains only parts of the genes for L11 and the β subunit of RNA polymerase (Fig. 1). Fig. 2 shows the effect of the addition of ribosomal protein L10 to an *in vitro* incubation containing $\lambda rif^d 18$ DNA. When 55 pmol of L10 (1.6 μM) was added, there was about a 70–80% inhibition of the synthesis of L10 whereas the synthesis of L12 was inhibited about 20%. In addition, the synthesis of EF-Tu and the β and β' subunits of RNA polymerase were unaffected by the addition of L10. In similar experiments L12 was added to the *in vitro* system, and the results are shown in Fig. 3. Protein L12 had no effect on either its own synthesis or on the synthesis of ribosomal protein L10. It appears from these results that the effect of exogenous L10 on the synthesis of L10 is quite specific.

An uncoupled protein synthesizing system was used to determine whether the added L10 was inhibiting transcription or translation. In these experiments the DNA was transcribed

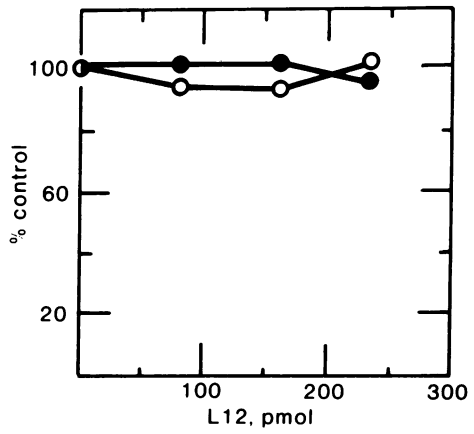

FIG. 3. Effect of L12 on the DNA-directed *in vitro* synthesis of L12 and L10. L12 was added to reaction mixtures as described in the legend to Fig. 1. The amounts of L12 (●) and L10 (○) synthesized were determined as described in the text.

Table 1. Effect of L10 or L12 on synthesis of L10 in an uncoupled DNA-directed protein-synthesizing system

Ribosomal protein added	Addition at Transcription (1st incubation)	Addition at Translation (2nd incubation)	L10 synthesized, pmol
None	−	−	2.0
L10	+	−	0.6
L10	−	+	0.7
L12	+	−	2.0
L12	−	+	1.9

$\lambda rif^d 18$ DNA (1.5 μg) was incubated at 37°C in the protein-synthesizing reaction mixture (11) except that the tRNA and amino acids were omitted. After 20 min of incubation, rifampicin was added (28 μg/ml) to stop RNA synthesis. Protein synthesis was then started by adding tRNA and amino acids and the incubation was continued for 40 min. L10 (55 pmol) or L12 (200 pmol) was added, where indicated, either at the start of the first incubation or in the second incubation when rifampicin was added. The amount of L10 synthesized was determined as described (12). The results are expressed as pmol of L10 synthesized per 10^6 cpm incorporated into total protein.

for 20 min in the absence of tRNA and amino acids. After 20 min, rifampicin was added to stop RNA synthesis, and protein synthesis was then initiated by adding tRNA and amino acids. L10 was added either at the beginning of the incubation or after rifampicin addition to determine whether L10 was acting during transcription or translation. The results of these experiments are summarized in Table 1. It is clear that L10 is equally effective as an inhibitor of its own synthesis when added either before or after transcription. These results indicate that the L10 inhibition in the *in vitro* system is occurring at a post-transcriptional step of protein synthesis. Protein L12 had no effect in the uncoupled system (Table 1).

A mRNA-directed protein-synthesizing system was used to obtain direct evidence that L10 was inhibiting its own synthesis post-transcriptionally. The mRNA in these experiments was obtained by isolating the RNA transcribed *in vitro* from pNF1337 DNA (see *Materials and Methods*). It can be seen in

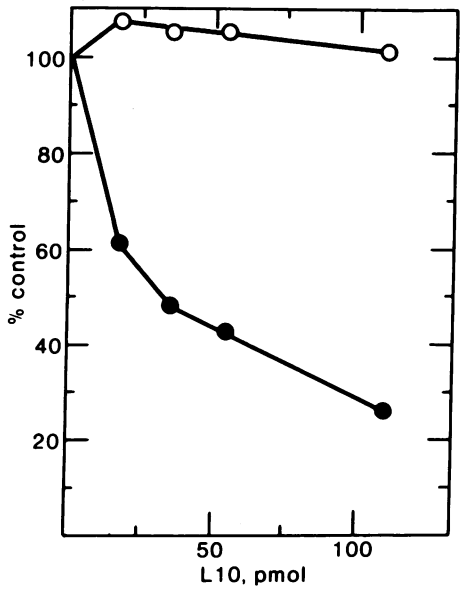

FIG. 4. Effect of L10 on the RNA-directed synthesis of L10 and L12. RNA was synthesized *in vitro* from pNF1337 DNA and isolated as described in the text. L10 was added to reaction mixtures that contained RNA (equivalent to 100 pmol of [^{3}H]UTP incorporated) and the other components of the *in vitro* protein-synthesizing system. The amounts of L12 (○) and L10 (●) synthesized were determined as described in the text.

Fig. 4 that exogenous L10 also inhibits L10 synthesis in this mRNA-dependent reaction.

To determine whether the inhibition by L10 was due to a specific L10-dependent destruction of L10 mRNA, we incubated pNF1337 DNA in an *in vitro* protein-synthesizing system in the presence or absence of L10 (Table 2). An aliquot was removed from each incubation and, as expected, there was about a 70% decrease in the amount of the L10 synthesis in the presence of L10 (Table 2, compare Exps. 1 and 2). The RNA from these first incubations was then extracted and reincubated in a protein-synthesizing system (second incubation) in the absence (Exps. 3 and 4) or presence (Exps. 5 and 6) of L10. The results show that the mRNA-dependent synthesis of L10 is very similar regardless of whether the initial incubations contained L10 (Table 2, compare Exps. 3 and 4). In addition, when L10 was added to the mRNA-dependent second incubation, a decrease in the synthesis of L10 was observed (Table 2, Exps. 5 and 6). These results show that in incubations in which L10 inhibits its own synthesis, the L10 mRNA is not destroyed but can be reisolated and efficiently translated.

DISCUSSION

The present report describes experiments designed to investigate the regulation of the synthesis of ribosomal proteins L10 and L12. Previous *in vivo* studies (5–7) have shown that when *E. coli* is transformed with either a plasmid or a phage containing the genetic information for ribosomal proteins or the β and β' subunits of RNA polymerase, the concentration of mRNAs for these proteins increase without a concomitant increase in the synthesis of the respective proteins. This had led to the suggestion that the synthesis of these proteins is under translational control. Fallon *et al.* (6) have recently proposed a mechanism in which the binding of a ribosomal protein to its respective mRNA allows a nuclease to specifically degrade that mRNA. In these *in vivo* experiments, they found that although the rate of synthesis of the mRNA for various ribosomal proteins was increased when the bacteria contained extra copies of the ribosomal protein genes from the phage, the total amount of these mRNAs remained the same. They concluded that the excess mRNA was degraded. Recently Lindahl and Zengel (14) have shown that when *E. coli* containing a plasmid bearing the genes for L2, L4, and L23 (under control of the *lac* promoter) was induced with isopropylthiogalactoside, synthesis of these proteins increased 5- to 10-fold. In addition, there was almost complete inhibition of the synthesis of ribosomal proteins S3, S19, L3, L16, L22, and L29, which are on the same transcription unit as L2, L4, and L23. The authors suggest that the accumulation of one or more of the latter proteins specifically controls the synthesis of the other proteins in the same operon.

Table 2. Effect of L10 on stability of L10 mRNA

Exp.	Template	L10 present during 1st incubation	L10 present during 2nd incubation	L10 synthesized, pmol
1	DNA	−	None	10.8
2	DNA	+	None	3.3
3	mRNA	+	−	8.3
4	mRNA	−	−	7.5
5	mRNA	+	+	2.4
6	mRNA	−	+	1.8

In Exps. 1 and 2 pNF1337 DNA was incubated at 37°C in a 6-fold scaled-up protein-synthesizing system (see text). L10 (330 pmol) was present in Exp. 2; at the end of the incubation an aliquot from each incubation was removed and the amount of L10 synthesized was determined. For Exps. 3–6 the remainder of each incubation from Exp. 1 or 2 was extracted with phenol and the RNA was isolated. The RNA used in Exps. 3 and 5 was isolated from Exp. 2, whereas the RNA used in Exps. 4 and 6 was isolated from Exp. 1. An aliquot of the RNA representing 100 pmol of [^{3}H]UTP incorporated into RNA was reincubated in a second protein-synthesizing system in the absence of L10 (Exps. 3 and 4) or in the presence of 55 pmol of L10 (Exps. 5 and 6). After 40 min of incubation, the amount of L10 synthesized was determined as described in the text. The results are expressed as pmol of L10 synthesized per 10^6 cpm incorporated into total protein.

The data presented in this report show a direct effect of a ribosomal protein on its own synthesis *in vitro*. Ribosomal protein L10, when incubated in a protein-synthesizing system with DNA containing the L10 gene, inhibited its own synthesis. The inhibition was very specific; L10 had little or no effect on the synthesis of L12, EF-Tu, or the β and β' subunits of RNA polymerase. In contrast, the addition of L12 to similar incubations had no effect on the synthesis of either L12 or L10, indicating that L12 synthesis is not under autogenous regulation. It was further shown both by uncoupling transcription from translation in the DNA-dependent system and directly in a mRNA-dependent system that the inhibition by L10 was at a post-transcriptional stage of protein synthesis.

Although, as yet, the mechanism of the inhibition of L10 synthesis by L10 in this system is not known, it does not appear to involve selective degradation of L10 mRNA. This was proved by extracting the RNA from an L10-containing incubation (in which a 70% decrease in L10 synthesis was obtained) and showing that it could be translated as well as the RNA isolated from a control incubation in which no L10 was present. In addition, the synthesis of L10 directed by the extracted mRNA was inhibited when L10 was added to the incubations. These data suggest that L10 reversibly inhibits a translational step of protein synthesis and that the stability of the mRNA is probably not involved in the mechanism of regulation. When both L10 and L12 were added to an incubation, about 40% less inhibition was observed than with L10 alone (data not shown). Because L10 and L12 form a complex, we interpret these results as suggesting that L10 is involved in the inhibition and that the L10–L12 complex is either not active or less active as an inhibitor.

It would appear that because a ratio of L12/L10 of about 4 is obtained in the *in vitro* system, the synthesis of these two proteins is being controlled by a normal regulatory mechanism. Although it is possible that part of this regulation may be due to the autogenous regulation by newly synthesized L10, one must be cautious. Relatively high concentrations of L10 (1.6 μM) were needed in the *in vitro* system to obtain a significant inhibition of L10 synthesis (see Fig. 2). Much lower amounts of L10 (1–5 pmol) are made *in vitro* under the conditions used, and these levels would not be expected to inhibit L10 synthesis. This raises the possibility that the mature L10 protein is not involved in the normal regulation of the L12/L10 ratio *in vitro*, but perhaps an incomplete nascent chain or modified form of the protein is active.

In some ways the regulation of the synthesis of EF-Tu (*tufA*) and elongation factor G (EF-G) appear similar to the L12/L10 situation. The *tufA* gene is located at the distal end of a cluster of four genes as follows: S7, S12, EF-G, and EF-Tu. It appears that these four genes are under control of a single promoter (15). Also, about 5 times more EF-Tu is synthesized than EF-G and the latter protein exists in stoichiometric amounts relative to the individual ribosomal proteins.

There are other examples of autogenous regulation in *E. coli*. The synthesis of the β and β' subunits is inhibited by RNA polymerase (16), λ repressor turns off the transcription of its own gene (17), and the synthesis of T4 phage gene 32 product is inhibited by itself (18). In the last case the regulation is also at the translational level of protein synthesis.

Note Added in Proof. We have recently observed that ribosomal protein L1, but not L11, inhibits the *in vitro* DNA-dependent synthesis of both L1 and L11. Preliminary data suggest that, similar to L10, the inhibition by L1 is at the translational level of protein synthesis.

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