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Biochemistry

In vitro regulation of DNA-dependent synthesis of *Escherichia coli* ribosomal protein L12

(transducing phage/gene cloning/RNA polymerase)

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ABSTRACT **The DNA of the transducing phage λrif^{d}18 contains, among others, the genes for the ribosomal proteins L11, L1, L10, and L12 and the β and β′ subunits of RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6). In a coupled *in vitro* protein-synthesis system, λrif^{d}18 DNA directs the synthesis of about four to five molecules of L12 per molecule of L10. This is consistent with the finding that there are four copies of L12 per ribosome. The ratio of L12/L10 was also examined from an *Eco*RI fragment of λrif^{d}18 that contains the L10 gene and about 50% of the L12 gene. A significantly lower ratio of truncated L12/L10 was observed compared to the intact phage. The binding of RNA polymerase to various λrif^{d}18 DNA restriction fragments was used to locate possible promoter sites. These binding experiments suggest that the β and β′ subunits of RNA polymerase are cotranscribed with at least ribosomal protein L12 and, also, that there may be an additional promoter site for the L12 gene within the structural gene for L10.**

In *Escherichia coli* the synthesis of ribosomal RNA and ribosomal proteins is under coordinate control (1). All of the ribosomal proteins exist on the ribosome in stoichiometric quantities except for ribosomal protein L12, which is present in four copies. Other results indicate that protein L12 is the product of a single gene located at 88 min on the *E. coli* chromosome (2).

Previous studies have shown that DNA from the transducing phage λrif^{d}18 can be used to direct the *in vitro* synthesis of ribosomal RNA, elongation factor Tu, ribosomal proteins L1, L10, L11, and L12, and also the β and β′ subunits of RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) (3). DNA-directed protein-synthesis systems are well suited to investigate the regulation of gene expression. As an example, guanosine-5′-diphosphate-3′-diphosphate accumulates in a stringent organism when starved for an amino acid, and this nucleotide is believed to mediate the *in vivo* inhibition of the synthesis of ribosomal RNA and ribosomal proteins (4). Likewise, the λrif^{d}18 DNA-directed *in vitro* synthesis of ribosomal RNA and ribosomal proteins is inhibited by guanosine-5′-diphosphate-3′-diphosphate (5–9).

The present study was undertaken to obtain information concerning the regulation of the synthesis of L12, the only ribosomal protein that is present in greater than stoichiometric amounts on the ribosome.

MATERIALS AND METHODS

Materials. *E. coli* H105, lysogenic with λrif^{d}18 phage, was obtained from J. B. Kirschbaum (Harvard University, Cambridge, MA), and was used as the source of λrif^{d}18 DNA. *E. coli* NF1201 (thr$^-$, leu$^-$, his$^-$, arg$^-$, thiamine$^-$), carrying plasmid pcc 703 (10), was provided by J. Friesen (York University, ON). Charon 3 and Charon 3r11 phages were a gift of B. Williams (Cetus Corporation, Berkeley, CA). Purified ribosomal proteins L1, L10, and L11 were provided by H. G. Wittmann (Max-Planck-Institut fur Molekulare Genetik, Berlin), and ribosomal protein L12 was purified as described (11).

RNA polymerase holoenzyme was purchased from Enzo Biochemicals, Inc. (New York, NY); [^{35}S]methionine was from Amersham/Searle, and the various restriction enzymes were obtained from New England BioLabs. Ribosomal wash, washed ribosomes, and the 0.25 M and 1.0 M salt eluates from a DEAE-cellulose fractionation of an S-100 extract were obtained as described (12). Antisera to L12 were raised in rabbits with the aid of Freund's adjuvant.

Phage and Plasmid DNA. Phage and plasmid DNA were isolated and the DNA was digested with the various restriction enzymes as described (13, 14). After digestion the DNA was extracted with phenol, precipitated with ethanol, and subjected to agarose gel electrophoresis (15).

***In Vitro* Protein Synthesis.** The complete system for protein synthesis with a partially fractionated *E. coli* extract was similar to that described (16). Each incubation mixture (70 μl) contained 1 μmol of Tris acetate (pH 8.0), 0.7 μmol of magnesium acetate, 2.5 μmol of ammonium acetate, 5.5 μmol of potassium acetate, 0.14 μmol of dithiothreitol, 0.052 μmol each of GTP, CTP, and UTP, 0.21 μmol of ATP, 2.0 μmol of phospho*enol*-pyruvate, 0.5 μg of pyruvate kinase, 50 μg of tRNA, 3 μg of calcium leucovorin, 2.5 mg of polyethylene glycol, 1.6 A_{260} units of ribosomes, 100 μg of ribosomal wash, 120 μg of 0.25 M eluate, 16 μg of 1 M eluate, 2–5 μg of λrif^{d}18, Charon 3r11, or pcc 703 DNA, 125 μM 19 amino acids (minus methionine), and 14 μM [^{35}S]methionine (38 Ci/mmol, 1 Ci = 3.7×10^{10} becquerels). The various DNA templates, as described in the legends to the figures and tables, were incubated for 50 min at 37°C and then chilled and centrifuged at 7000 × *g* for 10 min. An aliquot of the supernatant was assayed for incorporation of radioactivity into total protein by precipitation with hot Cl_3CCOOH and collecting the precipitate on a nitrocellulose filter. The filter was assayed for radioactivity with a scintillation spectrometer.

Assay for L12 and L10 by Immunoprecipitation. An aliquot of the reaction mixture was removed and immunoprecipitated with antiserum to ribosomal protein L12 as described (16). The precipitate was dissolved and subjected to sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis with a 15% gel. The gels were sliced into 1-mm sections with a Gilson Aliquogel fractionator, extracted with 0.1% $NaDodSO_4$, and then assayed for radioactivity in 5 ml of Instabray. The levels of L12 and L10 synthesized were calculated from the amount of radioactivity under the respective peaks on the gel by using values of 3 and 5 mol of methionine per mole of L12 and L10, respectively (17, 18). All of the methionine residues of L12 are situated within the first 26 amino acids.

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

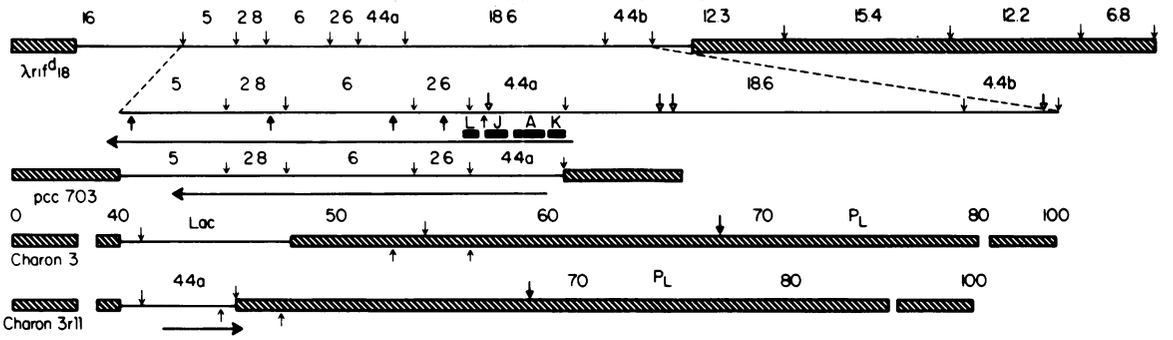

FIG. 1. Structures of λrif^{d}18, Charon 3, and Charon 3r11 phages and pcc 703 plasmid DNAs. Solid lines represent bacterial DNA; broken lines represent phage DNAs. All *Eco*RI (3) restriction sites are shown on λrif^{d}18, Charon 3, and Charon 3r11 phages and the pcc 703 plasmid. *Hin*dIII (3) restriction sites are shown on the expanded map of λrif^{d}18 DNA and on the maps for both Charon phages. The *Sma* I (3) and *Sal* I cleavage map is presented only on the expanded map of λrif^{d}18 DNA; the *Xho* I cleavage site is shown on the maps for the Charon phages. The sizes of the restriction fragments are shown in percent of the λ phage DNA molecule. The orientation of the 4.4%a *Eco*RI fragment of λrif^{d}18 DNA in the Charon 3r11 hybrid phage was determined by agarose gel electrophoresis of the restriction fragments obtained after digestion with *Hin*dIII and *Eco*RI. Horizontal arrows show the direction of transcription. Boxes (K, A, J, and L) in the expanded map represent the positions of ribosomal protein genes L11, L1, L10, and L12, respectively, and are based on previous studies (3). Genes for the β and β′ subunits are located to the left of the L12 gene. ↓, *Eco*RI; ↑, *Sal* I; ↓, *Sma* I; ↑, *Hin*dIII; ↓, *Xho* I.

RNA Polymerase Binding. RNA polymerase binding was carried out essentially as described (19). Each 100-μl incubation mixture contained 40 mM Tris·HCl (pH 7.9), 50 mM KCl, 8 mM $MgCl_2$, 1 mM GTP and ATP, 1.5 pmol of digested λrif^{d}18 DNA, and 100 pmol of RNA polymerase holoenzyme (1000 units/mg). The reaction mixture was incubated for 20 min at 30°C and filtered through a nitrocellulose filter (13 mm diameter) at a flow rate of 1 ml/min. The filter was washed with 4 ml of buffer (1 M KCl/8 mM $MgCl_2$/40 mM Tris·HCl, pH 7.9) and then with 2 ml of 40 mM Tris·HCl, pH 7.9/8 mM $MgCl_2$. DNA retained on the filter was eluted with 3 ml of 0.2% $NaDodSO_4$/20 mM Tris·HCl (pH 7.9) for 2 hr at 37°C with shaking. The resulting solution was extracted with phenol, concentrated by ethanol precipitation, and subjected to agarose gel electrophoresis.

RESULTS

***In Vitro* Products with Different DNAs as Templates.** In an attempt to understand the mechanism for the overproduction of L12 relative to other ribosomal proteins, we have studied the *in vitro* synthesis of L12 and L10 directed by various DNA species containing the genetic information for these ribosomal proteins. Fig. 1 shows the structure of the DNAs used in this study. As noted above, λrif^{d}18 DNA contains the genetic information for ribosomal RNA, elongation factor Tu, ribosomal proteins L1, L10, L11, and L12, and the β and β′ subunits of RNA polymerase. The location of the ribosomal protein genes is shown in the expanded portion of the bacterial chromosome in Fig. 1. The composite plasmid pcc 703, reported by Collins *et al.* (10), was obtained by the cloning of a partial digest of λrif^{d}18 DNA in a ColE1 vector. The DNA of this recombinant plasmid has the genetic information for L1, L10, L11, and L12 and the β and β′ subunits of RNA polymerase. The hybrid phage Charon 3r11 is a clone containing the 4.4%a *Eco*RI fragment of λrif^{d}18 DNA carried by the Charon 3 phage vector (20). Charon 3r11 contains the genetic information for L1, L10, and L11 and about 170 base pairs coding for the amino-terminal portion of L12. These three types of DNA were extracted and incubated in an *in vitro* protein-synthesizing system, and the products were precipitated with antiserum to ribosomal protein L12. Fig. 2 shows a display of the radioactive patterns obtained after $NaDodSO_4$ gel electrophoresis of the solubilized immunoprecipitates. It has been shown (16) that L10 binds to L12, and thus L12 antiserum precipitates both L12 and L10. Although ribosomal protein L10 was synthesized from all three DNA templates, a peak corresponding to ribosomal protein L12 was observed only when DNA from λrif^{d}18 (Fig. 2*A*) and the plasmid (Fig. 2*B*) were used as templates. However, no protein corresponding to the molecular weight of L12 could be immunoprecipitated from the incubations containing the DNA of Charon 3r11 (Fig. 2*C*). Instead, a new peak of radioactivity was observed migrating with a molecular weight of about 6000. This is consistent with the observation that the 4.4%a *Eco*RI restriction fragment contains only enough information for the first 52 amino acids of the L12 molecule.

To identify the other ribosomal proteins that were synthesized when the various DNAs were used as templates, we subjected an aliquot of each incubation to slab gel electrophoresis and radiofluorography. Table 1 summarizes the results of these experiments and shows that L1, L10, and L12, or a truncated L12, are synthesized from λrif^{d}18, pcc 703, and Charon 3r11 DNA, whereas L11 is synthesized only from λrif^{d}18 DNA. In addition, the amount of L1 synthesized (relative to L10) appears to be reduced in the incubations containing Charon 3r11 or pcc 703 DNA.

The available evidence indicates that the synthesis of the ribosomal proteins L10 and L12 directed by Charon 3r11 DNA is regulated by a bacterial promoter(s) and not by the P_L phage promoter.* Since no synthesis of L11 was detected in the incubations containing Charon 3r11 or pcc 703 DNA, the gene coding for L11 is most likely on a separate transcription unit, with its promoter located to the right of the 4.4%a *Eco*RI restriction fragment of λrif^{d}18 DNA (Fig. 1). These results suggest that the 4.4%a *Eco*RI restriction fragment contains the promoter(s) for ribosomal proteins L10 and L12 but not for L11 (and probably L1).

Ratio of L12 to L10 Synthesized *In Vitro*. Since L12 is overproduced *in vivo* relative to the other ribosomal proteins, it was of interest to ascertain the ratio of L12/L10 synthesized in the *in vitro* incubations. Similar to the *in vivo* situation, an L12/L10 ratio between 4 and 5 was obtained (Table 2) in those incubations that contained either the λrif^{d}18 DNA or plasmid DNA. In contrast when Charon 3r11 DNA (which contains the genetic information for only the first 52 amino acids of L12)

* The structure of Charon 3r11 DNA was determined by restriction enzyme analysis with *Eco*RI and *Hin*dIII. It was found that the transcription of the ribosomal protein gene cluster proceeds in a direction opposite to the transcription initiated from the P_L phage promoter. This implies that the synthesis of ribosomal proteins L10 and L12 in this phage is under control of a bacterial promoter.

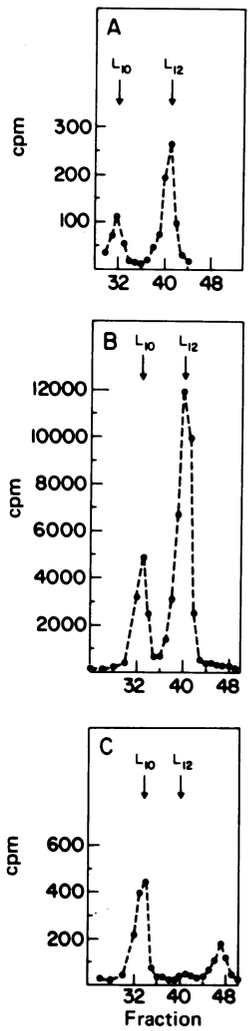

FIG. 2. Disc gel electrophoresis of ribosomal proteins L10 and L12 synthesized in a DNA-directed *in vitro* system. An aliquot of the *in vitro* reaction mixture containing [^{35}S]methionine was immunoprecipitated with L12 antiserum, and the precipitate was solubilized and analyzed on NaDodSO$_4$/polyacrylamide gel electrophoresis. The gels were sliced and assayed for radioactivity. *In vitro* protein synthesis was directed by λrif^{d}18 DNA (*A*), pcc 703 DNA (*B*), and Charon 3r11 phage DNA (*C*).

was used as template, the amount of truncated L12 formed was usually in the same range as the amount of L10. Kinetic experiments showed that at longer incubation times the truncated L12/L10 ratio occasionally went up to 2.0–2.5. However, these values were significantly less than that obtained with the λrif^{d}18

Table 1. Synthesis of ribosomal proteins L1, L10, L11, and L12 by using various DNA templates

DNA	L1	L10	L11	L12
λrif^{d}18	+	+	+	+
Charon 3r11	±	+	−	+*
pcc 703	±	+	−	+

The DNA species were incubated in an *in vitro* protein-synthesizing system. An aliquot was removed and electrophoresed in a 12% polyacrylamide gel. The gel was then subjected to radiofluorography and the ribosomal proteins were identified by comparison with pure proteins. It was observed from the intensity of the radiofluorogram that the amount of L1 synthesized (relative to L10) was reduced when either the Charon or plasmid DNA was used.

* Truncated L12, identified by immunoprecipitation and gel electrophoresis.

Table 2. Ratio of L12/L10 synthesized *in vitro* with various DNA templates

DNA	L12/L10
λrif^{d}18	3.8–5.2
pcc 703	4.3–5.2
Charon 3r11	0.7–1.1
Charon 3r11 + *Xho* I	0.8–0.9
λrif^{d}18 + *Eco*RI	0.8–1.2
λrif^{d}18 + *Sal* I	3.8

The experimental procedure is described in the legend to Fig. 2 and in the text. DNA was digested to completion, as judged by agarose gel electrophoresis, extracted with phenol, and precipitated with ethanol. The amount of L12 and L10 synthesized was determined by the amount of radioactivity found in each peak.

DNA. These kinetic experiments also showed that the decreased ratio was not due to preferential degradation of the truncated L12. In addition, the decreased L12/L10 ratio from the Charon DNA was not due to incomplete immunoprecipitation of the truncated L12 since very little or no radioactivity was detected when the supernatant from the first immunoprecipitate was subjected to a second immunoprecipitation.

As noted above, ribosomal proteins L10 and truncated L12 appear to be transcribed from a bacterial promoter(s) present on the 4.4%a *Eco*RI restriction fragment. If transcription originating from the P_L phage promoter of Charon 3r11 and proceeding in the opposite direction reads through into the bacterial DNA insert, it might interfere with the transcription of L10 and L12, thus preventing the overproduction of truncated L12. Digestion of the phage DNA with *Xho* I restriction enzyme was used to examine this point. *Xho* I cleaves the DNA at a restriction site between the P_L promoter and the bacterial DNA insertion. Thus, the digestion of Charon 3r11 DNA with *Xho* I eliminates the effect of transcription starting from the P_L promoter. As shown in Table 2, this treatment does not restore the overproduction of truncated L12. In addition when the 4.4%a fragment was prepared directly from λrif^{d}18 DNA by digestion with *Eco*RI and incubated in the *in vitro* protein-synthesizing system, an L12/L10 ratio of about 1 was also observed (Table 2). These results suggest that the DNA coding for the carboxyl moiety of L12 might be involved in maintaining the overproduction of L12 relative to L10. In order to obtain more information on this point, λrif^{d}18 DNA was digested with several different restriction enzymes to screen for a restriction site closer to the carboxyl end of the structural gene of L12. Digestion of the λrif^{d}18 DNA with *Sal* I yielded a 22% DNA fragment coding for all four ribosomal proteins, the end of which was about 300 nucleotides to the left of the end of the structural gene of L12 (Fig. 1). When λrif^{d}18 DNA was digested to completion with *Sal* I and this DNA was incubated in the *in vitro* protein-synthesizing system, an L12/L10 ratio of about 4 was obtained. This is comparable to the value observed when the intact λrif^{d}18 DNA was used (Table 2).

RNA Polymerase Binding to Restriction Fragments of λrif^{d}18 DNA. In order to obtain further information on the location of promoter sites for the ribosomal proteins and β and β′ subunits, the binding of RNA polymerase to various restriction fragments was determined. RNA polymerase holoenzyme was incubated with *Eco*RI plus *Hin*dIII or *Eco*RI plus *Sma* I digests of λrif^{d}18 DNA at a ratio of 60 pmol of polymerase per pmol of digested λrif^{d}18 DNA. After the incubation, the reaction mixtures were filtered and washed as described in *Materials and Methods*. The restriction fragments retained on the filters were eluted with NaDodSO$_4$ and subjected to agarose gel electrophoresis. No DNA was bound

to the filters when the same experiment was performed in the absence of RNA polymerase. Agarose gel electrophoresis of the total *Eco*RI and *Hin*dIII digest of λrif^{d}18 is shown in Fig. 3 lane A. After incubation in the presence of RNA polymerase, a number of phage and bacterial DNA fragments were retained by the filter (Fig. 3 lane B). Among these was a 3.7% fragment which carries the genes for L1, L10, and L11 (but not L12). This fragment was a result of the cleavage of the 4.4%a *Eco*RI fragment by *Hin*dIII restriction enzyme (Table 3 and Fig. 1). The *Hin*dIII restriction site is located about 320 nucleotides from the left end of the 4.4%a *Eco*RI fragment. Five other smaller restriction fragments, 0.7%, 1.1%, 1.2%, 2.6%, and 2.8%, generated by the above combination of enzymes, were not retained by the filter. To further localize the polymerase binding site on the 3.7% fragment, we incubated the *Eco*RI digest of λrif^{d}18 DNA with *Sma* I. A total *Eco*RI and *Sma* I digest is shown as a control in Fig. 3 lane C. After the digest was incubated with RNA polymerase, very little or no binding of a 3.5% fragment to the filter was observed (Fig. 3 lane D). This fragment was the product of the cleavage of the 4.4%a *Eco*RI fragment by *Sma* I at a restriction site about 420 nucleotides from the left end of the 4.4%a *Eco*RI fragment (Table 3 and Fig. 1). The significant change in RNA polymerase binding properties observed after cleavage of an additional 100 nucleotides from the left end of the 3.7% fragment (the distance between the *Hin*dIII and *Sma* I cleavage sites on the 4.4%a *Eco*RI fragment) indicates the presence of an RNA polymerase binding site within this short DNA region.

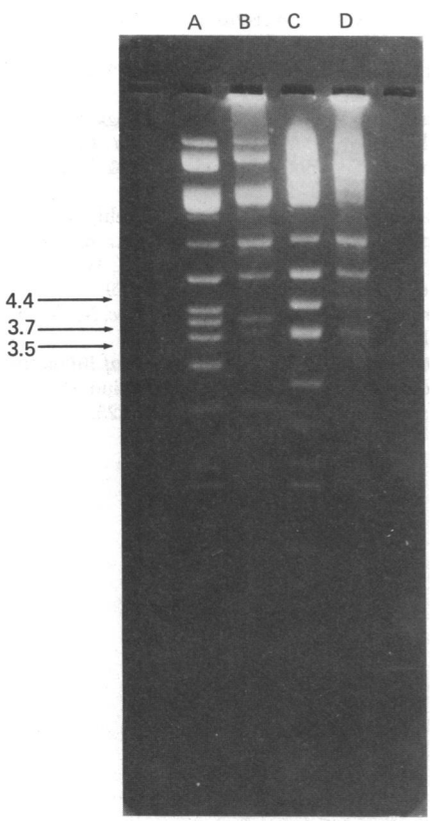

FIG. 3. RNA polymerase binding to the restriction fragments of λrif^{d}18 DNA. λrif^{d}18 RNA was digested with *Eco*RI and *Hin*dIII or *Eco*RI and *Sma* I. Aliquots were subjected to agarose gel electrophoresis directly or were incubated with RNA polymerase for 20 min at 30°C. The latter reaction mixtures were filtered through a nitrocellulose filter and treated as described in *Materials and Methods*. The DNA fragments retained on the filters were eluted and also subjected to agarose gel electrophoresis. Lane A, *Eco*RI, *Hin*dIII, total digest; lane B, *Eco*RI, *Hin*dIII, and RNA polymerase; lane C, *Eco*RI, *Sma* I, total digest; lane D, *Eco*RI, *Sma* I, and RNA polymerase.

Table 3. Digestion of λrif^{d}18 DNA with various restriction enzymes

*Eco*RI	*Eco*RI + *Hin*dIII	*Eco*RI + *Sma* I
4.4a	3.7 + 0.7	3.5 + 0.9
4.4b	4.4	3.1 + 1.3
18.6	18.6	14.2 + 4.4

The numbers represent % of λ DNA. See Fig. 1 for more details of the genetic map.

Fig. 3 lane D does show the presence of an RNA polymerase binding site on a 4.4% fragment that was obtained after digestion of λrif^{d}18 DNA with both *Eco*RI and *Sma* I. This fragment, which was derived from the left end of the 18.6% *Eco*RI fragment, is situated immediately to the right of the 4.4%a *Eco*RI fragment (Fig. 1 and Table 3). It is suggested that this binding site represents the promoter for L11 (and possibly L1) and is consistent with the results discussed above showing that the promoter for L11 (L1) is to the right of the 4.4%a *Eco*RI fragment.

It can also be seen from Fig. 3 lane B that there was no binding of RNA polymerase to an *Eco*RI DNA fragment (2.6%) that contains the genetic information for the carboxyl moiety of L12 and the amino-terminal end of the β subunit of RNA polymerase. The absence of a polymerase binding site on this DNA fragment suggests that this RNA polymerase subunit is cotranscribed with at least ribosomal protein L12. While this manuscript was in preparation, Yamamoto and Nomura (21) reported that the β and β′ subunits of RNA polymerase are cotranscribed with ribosomal proteins L10 and L12.

DISCUSSION

The present study shows that the *in vivo* pattern of the regulation of synthesis of ribosomal protein L12 can be observed *in vitro* in a DNA-directed protein-synthesizing system. Thus it was found that λrif^{d}18 DNA, which contains a cluster of ribosomal genes (L1, L10, L11, and L12) and the genetic information for elongation factor Tu and the β and β′ subunits of RNA polymerase, can direct the synthesis of four to five molecules of L12 per molecule of L10. This phenomenon is of physiological importance since L12 is the only ribosomal protein found as four copies on the ribosome, whereas the rest of the ribosomal proteins are present in stoichiometric quantities. An L12/L10 ratio of 4–5 was obtained when the DNA used in the *in vitro* protein-synthesizing system contained an intact L12 gene (λrif^{d}18, λrif^{d}18 DNA digested with *Sal* I, and pcc 703 plasmid). However, when DNA carrying the genetic information for the ribosomal gene cluster and a truncated L12 (λrif^{d}18 DNA digested with *Eco*RI, Charon 3r11 DNA, and Charon 3r11 DNA digested with *Xho* I) was used, a much lower ratio of truncated L12/L10 was observed. The decreased L12/L10 ratio was not the result of interference due to the transcription from the P_L phage promoter, to incomplete immunoprecipitation of the truncated L12, or to increased lability of the truncated L12. Further experiments are needed to determine whether the DNA coding for the carboxyl-terminal end of L12 is involved in the regulation of L12 synthesis.

It was previously thought that the genetic information for ribosomal protein L11 is present on the DNA from Charon 3r11 and pcc 703; however, no *in vitro* synthesis of L11 (Table 1) was observed from these templates. This indicated that L11 is synthesized from a separate transcription unit with a promoter to the right of the 4.4%a *Eco*RI restriction fragment. This is

further supported by the finding of an RNA polymerase binding site located to the right of the 4.4%a *Eco*RI fragment. Since the synthesis of L1 from both Charon 3r11 and pcc 703 DNA was decreased, it is possible that L1 and L11 share a common promoter and the low synthesis of L1 could be explained by readthrough from the phage DNA. The lack of synthesis of L11 is compatible with the recent data (21) showing that the right end of the 4.4%a restriction fragment is within the L11 gene.

RNA polymerase binding experiments revealed the presence of a polymerase binding site situated between the *Hin*dIII and *Sma* I restriction sites on the 4.4%a *Eco*RI fragment (Fig. 1). This site is located about 400–500 base pairs from the left end of the 4.4%a *Eco*RI fragment. About 170 base pairs are required to code for the truncated L12. The remaining 250–300 base pairs between the *Sma* I restriction site and the start of the L12 structural gene would be insufficient to accommodate another ribosomal protein gene. Thus it is suggested that this polymerase binding site represents the promoter for ribosomal protein L12. These results agree with previous *in vivo* kinetic experiments (22) in which it was reported that the start of the L12 gene is situated not more than 350 nucleotides away from the promoter.

While this report was in preparation, Yamamoto and Nomura (21) reported that the β and β' subunits of RNA polymerase are cotranscribed with ribosomal proteins L10 and L12. Their conclusion that these proteins share a common promoter was based on genetic experiments and on sequencing data that showed that the *Hin*dIII restriction site in the 4.4%a *Eco*RI fragment is situated within the L10 structural gene. Therefore, a common promoter for the β and β' polymerase subunits and L10 and L12, situated to the right of the L10 gene, was postulated (21). Our experiments, however, show an RNA polymerase binding site within the L10 gene located between the *Hin*dIII and *Sma* I cleavage sites on the 4.4%a fragment. This polymerase binding site could serve as an L12 promoter and suggests an overlapping of the regulatory region of the L12 gene and the structural gene of L10. We cannot exclude the possibility, however, that this is an additional promoter for L12 and that L12 is also cotranscribed with L10 from a second promoter. Additional information is required to determine which of the two putative promoters is used for the transcription of the β and β' genes.

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