Molec. gen. Genet. 178, 483–486 (1980)

MGG

Short Communication

Autogenous Regulation of the Synthesis of Ribosomal Proteins, L10 and L7/12, in *Escherichia coli*

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Summary. The in vitro synthesis of *Escherichia coli* ribosomal proteins, L10 and L7/12, is specifically repressed by the addition of the L10-L7/12 complex, while that of other ribosomal proteins encoded by the neighboring operons is not affected. Thus the expression of the *rpoBC* operon is controlled by two autorepression systems, one for the two ribosomal proteins and the other for RNA polymerase β and β' subunits, both operating probably at the translational level.

The genes encoding the DNA-dependent RNA polymerase (ribonucleoside 5′-triphosphate: RNA nucleotidyltransferase [EC 2.7.7.6]) core enzyme subunits have been identified in the clusters of ribosomal protein genes in the *E. coli* chromosome (Nomura et al., 1977; Yura and Ishihama, 1979). The structural genes for the β and β' subunits (*rpoB* and *rpoC*) constitute an operon (*rpoBC* operon) at 88 min on the genetic map together with the genes for two ribosomal proteins, L10 and L7/12 (*rplJ* and *rplL*, respectively), and the gene order of the operon is *rpoP*$_\beta$ (promoter)→*rplJ*→*rplL*→*rpoB*→*rpoC*→ terminator (Post et al., 1979). In addition, the possible existence of an attenuator between *rplL* and *rpoB* is discussed (Blumentahl and Dennis, 1978; Post et al., 1979). Reflecting such a gene organization, the synthesis of RNA polymerase and ribosomal proteins is regulated coordinately under various growth conditions of wild-type *E. coli* (Ishihama et al., 1976; Maaløe, 1979). However, this coordination is dissociated under certain growth conditions, e.g., during the stringent response (Mahr and Dennis, 1977), during the transition into stationary phase (Kawakami et al., 1979), and after the addition of RNA polymerase inhibitors (Hayward et al., 1973) or DNA-intercalating dyes (Chao, 1977), and particularly in some mutants which have defects in the assembly of RNA polymerase (Taketo et al., 1976; Yura and Ishihama, 1979). Thus it is feasible to postulate the presence of an additional control mechanism specific for the ribosomal proteins or for the RNA polymerase subunits. Indeed, the autogenous regulation has been proposed, as a control mechanism specific for the synthesis of RNA polymerase, in which RNA polymerase is directly involved in modulating the expression of its own genes (Scaife, 1975; Taketo et al., 1978).

As reported previously we have established an in vitro transcription-translation coupling system, in which the synthesis of β subunit is specifically repressed by either holoenzyme or $\alpha_2\beta$ complex (Fukuda et al., 1978), an intermediate in the assembly of RNA polymerase. Furthermore, we found that the autogenous repression operated at the post-transcriptional level, probably by restricting the translation of *rpoBC* mRNA (Fukuda et al., 1979; Kajitani, Fukuda and Ishihama, submitted for publication). Another RNA polymerase-specific control mechanism operates at the transcriptional level, where, the transcription of the promoter-distal *rpoBC* genes is controlled by changing the frequency of the transcription termination at the proposed attenuator site between *rplL* and *rpoB* (Blumenthal and Dennis, 1978). On the other hand, the observation that the increase of mRNA level for some ribosomal proteins by multiplying the gene copies is not accompanied by a concomitant increase of respective polypeptides (Geyl and Böck, 1977; Dennis and Fiil, 1979; Fallon et al., 1979)

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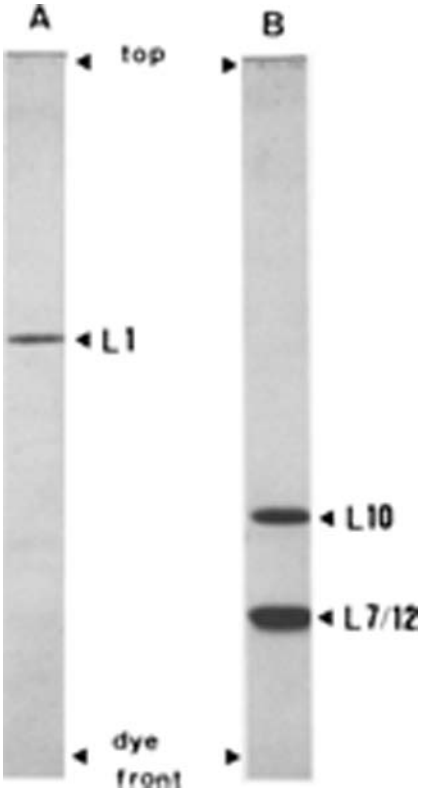

Fig. 1A and B. Polyacrylamide gel electrophoresis of the isolated ribosomal proteins. The CsCl split proteins from 50S ribosomes were applied onto a DEAE Sephadex A50 column and eluted by a linear gradient of KCl (0.15 M to 0.6 M). The L1 protein was eluted at 0.1 M KCl in the flow-through fraction and the L10-L7/12 complex at 0.3 M KCl. The isolated L1 (**A**, 0.8 μg) and the L10-L7/12 complex (**B**, 4.9 μg) were analysed by SDS-15% polyacrylamide gel electrophoresis according to Laemmli (1970)

indicates the existence of a post-transcriptional mechanism which restricts translation of excess mRNA for ribosomal proteins. But this is not always the case, because a gene dosage effect was observed on the synthesis of proteins, L21, L27 and S21 (Takata, 1978). The absence of a gene dosage effect seems to be analogous to the autogenous regulation for the *rpoBC* genes, and therefore, its possibility was examined for the expression of *rplJ* and *rplL* genes.

For this purpose, I isolated the ribosomal proteins L10 and L7/12 and examined their effect on the in vitro synthesis of the respective proteins. Since it is known that the L10-L7/12 complex prepared from 50S ribosomes under non-denaturing conditions are capable of rebinding 23S rRNA (Dijk et al., 1977; Brimacombe et al., 1978), a procedure to isolate the native L10-L7/12 complex was established (Fukuda, manuscript in preparation); the CsCl split proteins (Traub et al., 1971) from 50S ribosomes were applied onto a DEAE-Sephadex A50 column, and eluted with a linear gradient of KCl in the absence of protein denaturants. The ribosomal protein L1 was recovered in the flow-through fractions (the first protein peak) while a pure L10-L7/12 complex was eluted in the third peak. As shown in Fig. 1, the L10-L7/12 complex thus isolated was almost pure. The addition of the L10-L7/12 complex or the L1 protein gave no appreciable influence on the total protein synthesis in the in vitro transcription-translation coupled system directed by $\lambda rif^{d}18$ DNA. However, when the products were analyzed by SDS-polyacrylamide gel electrophoresis, it was found that, in the presence of the complex, the synthesis of L10 and L7/12 was reduced to 39% and 49% respectively (Fig. 2A). At the saturation level of the inhibition (2.5 μg L10-L7/12 complex and 3.4 μg $\lambda rif^{d}18$ DNA), the molar ratio of the protein complex versus the DNA is calculated to be 400, assuming that a monomeric form of the complex composed of one L10 and four L7/12 proteins is the functional molecule with repressor activity (Dijk et al., 1977). In contrast, little effect was observed on the synthesis of the elongation factor Tu (EF-Tu) and the ribosomal protein L11, both encoded by the genes in the neighboring operons on the DNA used. The ribosomal protein L1, which is known to form a tight complex with 23S rRNA (Zimmermann, 1974), gave no effect on the synthesis of individual proteins encoded by the DNA (Fig. 2C).

In another set of experiments, DNA lacking the promoter ($rpoP_{\beta}$) was used to analyze the DNA sequence required for the autogenous repression of L7/12 synthesis. The composite plasmid pLBC-1 contains the *Hind*III 21.8 fragment (Lindahl et al., 1977) of $\lambda rif^{d}18$ DNA, which includes the genes *rplL*, *rpoB* and *rpoC* but lacks $rpoP_{\beta}$, the leader region between $rpoP_{\beta}$ and *rplL*, and the promoter-proximal 90% of *rplJ* (Fukuda et al., 1979). The transcription of the remaining *rpoBC* operon in pLBC-1 seems to be initiated from a promoter in the vector pBR322 (Fukuda, unpublished observation). When pLBC-1 DNA was used in place of $\lambda rif^{d}18$ DNA, the synthesis of L7/12 was not repressed by the addition of the L10-L7/12 complex (Fig. 2B), indicating that the deleted sequences, probably the leader region or the promoter, is required for the repression. The lack of repression of pLBC-dependent L7/12 synthesis was confirmed by an in vivo observation (Fukuda, manuscript in preparation), i.e., cells carrying the pLBC-1 plasmid over-produce L7/12 five fold compared to the amounts synthesized in cells carrying pBR322. This is in contrast with the absence of the gene dosage effect in the synthesis of L10 and L7/12 in the cells carrying multicopies of the complete *rpoBC* operon (Dennis and Fiil, 1979).

Based on these observations a working hypothesis

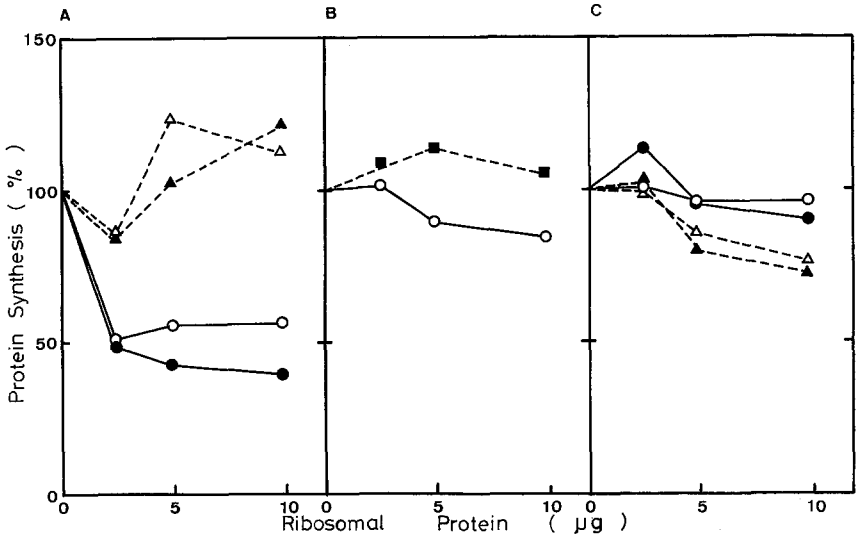

Fig. 2A. Effect of the ribosomal proteins in the in vitro synthesis of ribosomal proteins. In vitro protein synthesis was carried out according to Fukuda et al. (1978). The reaction mixture contained the followings: **A** 3.4 μg of $\lambda rif^d 18$ DNA and the indicated amount of the L10-L7/12 complex; **B** 5.3 μg of pLBC-1 DNA and the L10-L7/12 complex; **C** 3.4 μg of $\lambda rif^d 18$ DNA and the L1 protein. Determination of the product proteins was performed by the procedure previously described with the following modifications. The concentration of L-methionine was reduced to 0.1 mM. [^{35}S]methionine (2–3 μCi/reaction) was used as a labelled amino acid. After 40 min reaction at 37° C, the ribosomal proteins were supplemented, where necessary, to adjust their amounts in each sample. The reaction mixtures were treated with nucleases and then precipitated with 5% TCA. The precipitates were collected by centrifugation (10,000 g × 10 min), washed successively with 5% TCA and cold ethyl ether, and finally dissolved by heating at 95° C for 5 min in SDS sample solution. The samples were subjected to SDS-polyacrylamide (15%) slab gel electrophoresis (Laemmli, 1970). The slab gels were treated for fluorography (Laskey and Mills 1975). The fluorograms were traced with a Joyce-Loebl microdensitometer for quantitative determination of the respective proteins. The radioactivity in each protein was divided by the total radioactivity incorporated into hot-acid-insoluble materials. The differential synthesis rate thus obtained was compared with the control values without the addition of the ribosomal proteins (on the ordinate). L10 (●), L7/12 (○), L11 (▲), elongation factor Tu (△) and a polypeptide (Mw:27,000 ■) encoded by the plasmid pBR322

is proposed. In addition to the autorepression for the $\beta\beta'$ synthesis, there may be another autorepression system operating for the expression of *rplJ* and *rplL*, the two promoter-proximal genes in the same *rpoBC* operon. Considering the recent observations on the post-transcriptional control for the expression of these genes (Dennis and Fiil, 1979; Fallon et al., 1979), it seems reasonable to assume that the autorepression for the L10 and L7/12 synthesis operates at the translational level. In this respect, it is noteworthy that Fiil et al. (1979) have isolated mutants which are defective in the translation of L10 mRNA sequences, but are not appreciably affected in the transcription of the L10 gene; these mutations are localized in the leader region of the *rpoBC* operon. Thus a regulatory site seems to exist on the transcript of this region, which controls the efficiency of translation of the L10-L7/12 mRNA. The site interacts with the L10-L7/12 complex, and modulates the conformation of the mRNA thereby restricting the translation of the L10-L7/12 mRNA. The structure of the putative regulatory site could be similar to that present on 23S rRNA, with which the L10-L7/12 complex associates specifically. This hypothesis predicts the following control. When rRNA is actively produced, the L10-L7/12 complex associates with rRNA to form ribosomes, resulting in derepression of the *rplJL* expression. On the contrary, decrease in rRNA production leads to accumulation of free complex, which in turn represses L10 and L7/12 synthesis. This control may be one of the mechanisms by which rRNA and ribosomal proteins are synthesized coordinately.

Acknowledgements. I wish to thank Dr. A. Ishihama for valuable comments on the manuscript, Dr. H. Nagasawa for help during the work, and Mrs. M. Tsutsumi for secretarial help. This work was supported in part by grants from the Ministry of Education, Science and Culture, of Japan.

Note Added in Proof

Recently, Lindahl and Zengel (1979) have cloned the genes for ribosomal proteins, L2, L4 and L23 on a plasmid vector that contains a *lac* promoter and operater. Addition of a *lac* inducer to the cells harboring the composite plasmid results in a specific 5- to 10-fold increase in the synthesis of these ribosomal proteins. Shortly after the induction, the synthesis of ribosomal proteins, S3, S19, L3, L16, L22 and L29 stops almost completely without dramatic effect on the synthesis of any other ribosomal proteins. The genes for all those proteins reside in the same chromosomal operon as L2, L4 and L23.

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Communicated by K. Isono

Received January 22/February 29, 1980