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Translational regulation by ribosomal protein S8 in Escherichia coli: structural homology between rRNA binding site and feedback target on mRNA

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ABSTRACT

It has been previously shown that ribosomal protein synthesis in Escherichia coli is regulated at the level of translation by certain key ribosomal proteins. In the spc operon, S8 regulates the expression of L5 and some of the subsequent genes, while the first two genes (L14 and L24) are regulated independently. We therefore determined the DNA sequence at the junction of the L24 and L5 genes, which corresponds to the putative feedback target for S8. We show that there is a striking homology between the structure of the mRNA for this region and the known binding site for S8 on 16S rRNA. These results support the theory that the regulation of ribosomal protein synthesis is based on competition between rRNA and mRNA for regulatory ribosomal proteins.

INTRODUCTION

The synthesis of ribosomal proteins[1] in Escherichia coli is closely coordinated with ribosome assembly, such that there is virtually no free pool of r-proteins in the cell. Studies of several r-protein operons has led to a model for the regulation of synthesis of r-proteins (2, 3): certain key r-proteins act as negative feedback regulators ("translational repressors"), inhibiting the translation of mRNA coding for themselves and certain other proteins in the same operon. It has been shown that L1 is a translational repressor in the L11 operon (4, 5), S4 in the α operon (4, 5), L4 in the S10 operon (6, 7, 8), S7 in the str operon (3, 9), S8 in the spc operon (4, 10), and L10 in the β operon (11, 12). It is postulated that there is competition between rRNA and mRNA for regulatory proteins, and that these r-proteins have a higher affinity for rRNA than mRNA. Consequently, while rRNA is present in excess, these proteins should enter the ribosome assembly reaction without inhibiting translation. Supporting this notion, it was found that the presumed target sites for S4 and S7 on mRNA have structural homologies to their respective binding sites on the 16S rRNA (3).

The regulation of the spc operon was recently investigated both in vivo

and in vitro (4, 10). It was found that S8 acts as a translational repressor for many of the r-proteins of the operon but not the first two, L14 and L24 (see Figure 1). S8 is thought to inhibit the initiation of translation of L5, the third cistron of the operon, and this in turn leads to the inhibition of the expression of distal genes by a polar effect (see Discussions in previous papers, References 3, 7 and 10).

If the regulation involves competition between rRNA and mRNA for S8, we would expect some structural homology between the S8 binding site on rRNA and the S8 target site on mRNA, as was found for S4 and S7 (3). The S8 binding site on 16S rRNA is known from the work by various investigators (see the Discussion). We therefore determined the DNA sequence around the start of the L5 gene, since the mRNA for this region presumably includes the putative target site for S8. Comparing this mRNA structure with the known S8 binding site on 16S rRNA, we found a striking structural homology between the two S8 sites as reported in this paper.

MATERIALS AND METHODS

Two different hybrid plasmids were used as a source of DNA for sequencing the region between the L24 and L5 genes. One is pNO1001 which is an RSF2124 ("ColE1-Amp") derivative and carries the 10% EcoRI fragment from λfus3 transducing phage (13; see Figure 1). The other is pNO1507 which is a derivative of pBR322 and carries a BamHI-SalI fragment from λspc1 transducing phage (14). This fragment encompasses from the BamHI site which is in the right arm of λ DNA to the SalI site which is within the L5 gene (see Figure 1). The coli-λ junction in λspc1 is to the right of the promoter for the spc operon. Thus the BamHI-SalI fragment, which is about 1.9 Kb long, carries the L14, L24 genes and part of the L5 gene. The plasmid pNO1507 was constructed by D. Dean in this laboratory and the plasmid DNA used was a gift from him. The pNO1001 DNA was purified by G. Baughman in this laboratory. The method of purification of the plasmid DNAs was described previously (15).

Restriction enzymes were obtained from New England Biolabs (Beverly, Massachusetts), Bethesda Research Laboratories (Rockville, Maryland), or Biotec (Madison, Wisconsin). Calf-intestine alkaline phosphatase was purchased from Boehringer-Mannheim (New York, New York). Polynucleotide kinase was prepared according to a published method (16) by G. R. Davis in this laboratory. Dimethylsulphate, hydrazine and piperidine were purchased from Aldrich (Milwaukee, Wisconsin), Eastman-Kodak (Rochester, New York) and Fisher (Fair Lawn, New Jersey), respectively.

DNA sequencing was performed according to the Maxam and Gilbert method (17). Labeled DNA fragments were recovered from polyacrylamide gels by electro-elution (18). The method used involved electrophoresing the DNA through a plug of 5% polyacrylamide in the end of an Eppendorf pipette tip. The material was collected in a small dialysis bag secured over the end of the Eppendorf tip, and recovered by ethanol precipitation in the presence of carrier tRNA. The recovery of the labeled DNA was usually greater than 95%.

RESULTS

The fragment covering from the BamHI site in the L16 gene to the SalI site in the L5 gene (see Figure 1) was purified from the plasmid pNO1001. This fragment was digested with HaeIII and 5'-end labeled with ^{32}P (17). The 600 base pair HaeIII fragment carrying the L24-L5 intercistronic region (determined by preliminary experiments) was purified by polyacrylamide gel electrophoresis and then cleaved with HhaI. One of the resulting fragments carrying the intercistronic region was isolated and used to obtain the DNA sequence of the sense strand as shown in Figure 1.

The BamHI-SalI fragment from pNO1507 was used to sequence the comple-

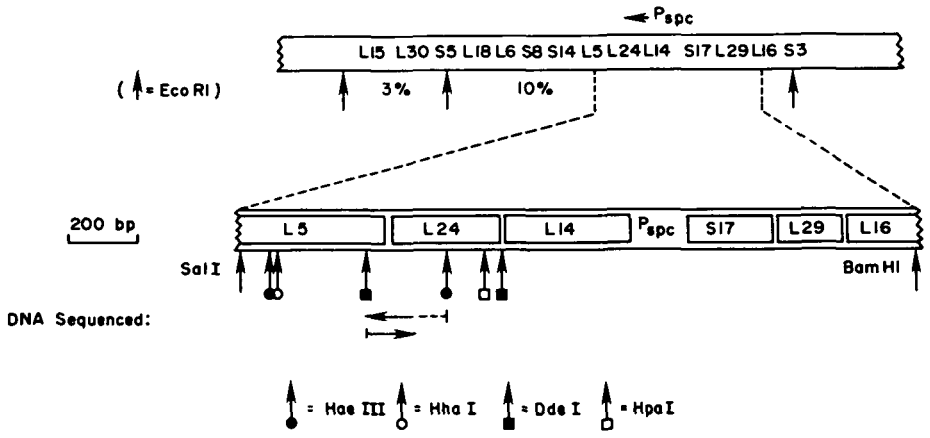

Figure 1. Map of the spc operon and part of the S10 operon. The enlargement shows the HaeIII, DdeI, HhaI and HpaI sites which were used for sequencing. Other HaeIII, DdeI, HhaI and HpaI sites are not shown. Horizontal arrows represent the region sequenced (see the text). The promoter for the spc operon is indicated as P_{spc}. The genes are indicated by the name of proteins encoded. Approximate sizes of the EcoRI fragments are shown in percentage of the λ genome (1% λ = 480 base pairs).

mentary strand. The fragment was digested with DdeI and 5'-end labeled with ^{32}P. One of the DdeI fragments carrying the L24-L5 intercistronic region was purified, and a singly-labeled fragment obtained after secondary digestion with HpaI was used for sequencing as indicated in Figure 1. A typical sequencing-gel is shown in Figure 2.

The sequence results obtained from both strands were consistent and are shown in Figure 3. The sequence represents the strand corresponding to mRNA

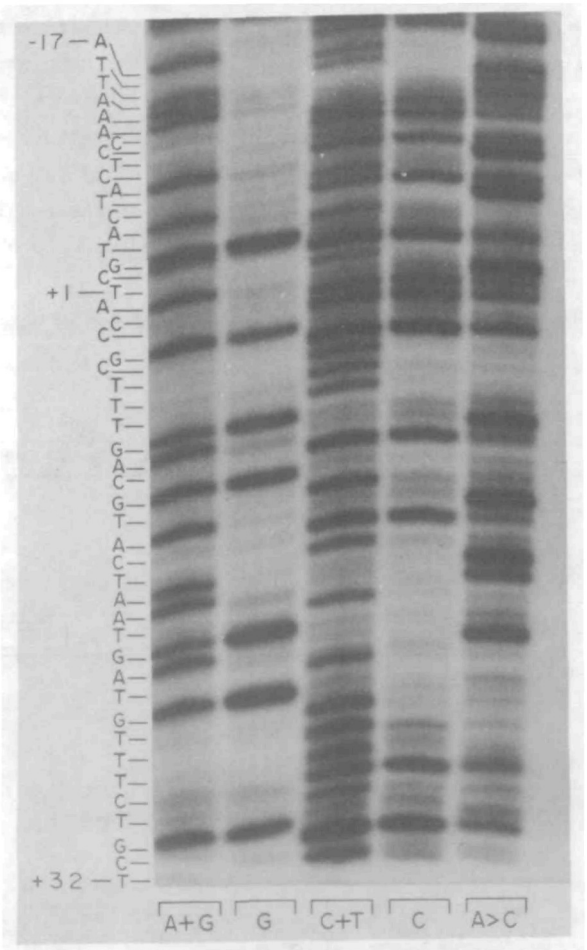

Figure 2. Portion of a Maxam and Gilbert DNA sequencing gel encompassing the start of the L5 gene. The numbering scheme is the same as in Figure 3. The DdeI end was labeled, so the sequence shown is complementary to the spc mRNA.

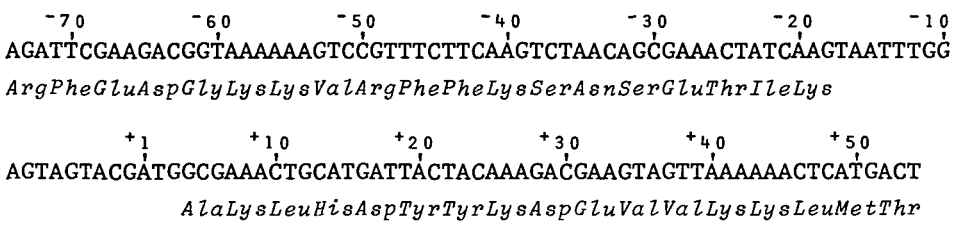

Figure 3. DNA sequence of the end of the L24 gene (amino acid residue 85) through the start of the L5 gene (amino acid residue 17). Only one strand, that with the polarity of the mRNA, is shown. The first nucleotide of the initiation codon for L5 is designated "+1".

and covers amino acid residue 85 in L24 through residue 17 in L5. The amino acid sequence deduced from the DNA sequence agrees exactly with the published structures of L24 (19) and L5 (20). As can be seen, there are only 14 intercistronic nucleotides between the L24 and L5 genes. No sequence resembling known promoter structures (21) is found in the region sequenced. The results are consistent with the previous conclusion that the L5 gene is co-transcribed with the preceding L14 and L24 genes (22, 23), even though the synthesis of L14 and L24 is regulated independently from that of L5 and other proteins from the rest of the operon (10).

DISCUSSION

Specific binding of S8 to 16S rRNA was first demonstrated using standard *in vitro* ribosome reconstitution conditions (24, 25). Extensive studies were subsequently carried out to identify the S8 binding site on 16S rRNA. The S8-16S rRNA complexes were digested with RNase A and RNA fragments "protected" by S8 were isolated as S8-RNA fragment complexes and their structures were analyzed. In some experiments, the isolated fragments were shown to be able to re-bind S8 (26, 27). Although slightly different results were obtained in different experiments, all the published data have indicated that the lower part of a hairpin structure shown in Figure 4A represents at least a part of the S8 binding site. The structure for the S8 binding site proposed by Ungewickell *et al.* (27) and Zimmermann *et al.* (28) is a base paired stem comprising nucleotides 587-603 and 635-653 (indicated in the box with a broken line in Figure 4A). This is almost identical to the S8-protected RNA previously found by Schaup *et al.* (26; see also 29, 30). Electronmicrographic analysis of S8-16S rRNA complexes also provided evidence that S8 binds to a hairpin-like structure whose location is consistent with

the S8 binding site described above (31).

The sequence of the mRNA around the L24-L5 intercistronic region determined in this work was compared to the S8 binding site on rRNA. Figure 4B shows a potential stable secondary structure for the L24-L5 region of mRNA, deduced with the aid of a computer program (32). As can be seen, there is striking primary and secondary structural homology between this mRNA structure and the binding site on 16S rRNA shown in Figure 4A. The initiation codon AUG for the L5 gene is base paired with CAU and is included in one of the identical homology structures. The Shine-Dalgarno sequence (GGAG; see Reference 33) preceding this initiation codon is also included in the stem

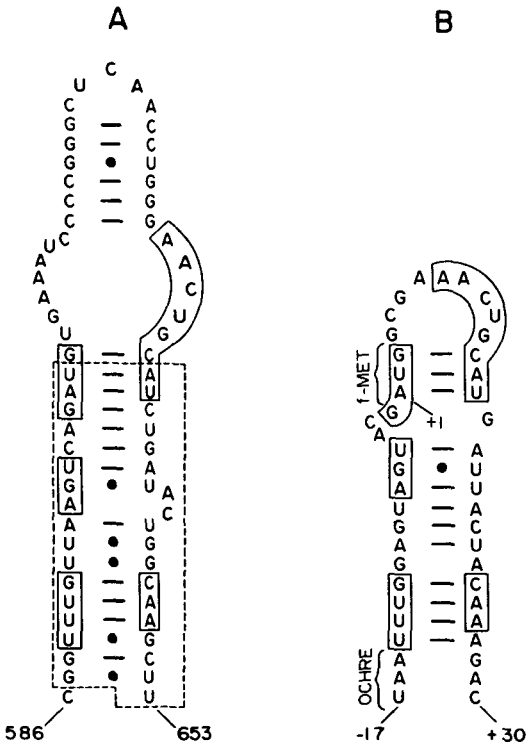

Figure 4. A: Part of the 16S rRNA structure according to the model of Woese et al. (36). The binding site for S8 (27, 28) is delineated by a broken line. B: A potential secondary structure for the mRNA region which presumably includes the target site for the S8 feedback repressor. The sequence encompasses the intercistronic region between the L24 and L5 genes. The initiation codon AUG for the L5 gene and the termination codon UAA for the L24 gene are indicated. Homologies between the two structures which we consider to be significant are shown boxed.

of the proposed hairpin structure. Since the S8 binding site of 16S rRNA displays a specific affinity to S8, it is probable that S8 also recognizes similar structural features of the mRNA shown in Figure 4B. We propose that the secondary structure which blocks the initiator AUG as well as the Shine-Dalgarno sequence is stabilized by the binding of S8, and that this is the basis of the feedback regulation of L5 and distal genes by S8.

The present finding is similar to our previous observations on the homology between the binding sites for S4 and S7 on rRNA and their respective target sites on mRNA. Recently Gourse and his coworkers (34) as well as Branlant and her coworkers (35) noted that there are structural homologies between L1 binding sites on 23S rRNA and the mRNA region where L1 acts as a translational repressor (4, 9). Together, these observations give strong support to the concept that the feedback regulation of r-protein synthesis is based on competition between rRNA and mRNA for the regulatory r-proteins.

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