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The existence of two genes between *infB* and *rpsO* in the *Escherichia coli* genome: DNA sequencing and S1 nuclease mapping

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ABSTRACT

A number of genes encoding proteins involved in transcription and translation are clustered between 68 and 69 minutes on the *Escherichia coli* genome map and are transcribed clockwise as two operons: the *metY* operon, containing *metY*, P15A, *nusA*, *infB*; and about a kilobase further downstream, the *rpsO* and *pnp* operon. The DNA sequence between *infB* and *rpsO* was determined and two open reading frames were detected which code for proteins of 15,200 (P15B) and 35,091 (P35) daltons. Maxicell analysis showed a relatively strong expression of P15B whereas P35 was synthesized more weakly. An overlap of the termination codon of P15B and the initiator codon for P35 suggests that translation of P15B and P35 may be coupled. S1 nuclease mapping of *in vivo* transcripts between *infB* and *rpsO* provided no evidence for major promoters but detected a moderately efficient rho-independent terminator between *infB* and P15B. The results indicate that P15B and P35 are expressed as part of the *metY* operon, but that some transcriptional read through into the *rpsO* operon also occurs, thereby, functionally linking the expression of these two complex systems.

INTRODUCTION

Genes for proteins involved in translation and transcription frequently are clustered on the bacterial genome and are expressed from large polycistronic operons. Such a gene cluster is located between 68 and 69 minutes on the *Escherichia coli* genomic map and contains in the following order: *metY*, coding for a minor form of the initiator tRNA, $tRNA^{Metf}_2$; an open reading frame coding for a 15,471 dalton protein of unknown function, called P15A or P21; *nusA*, for a transcriptional termination factor; *infB*, for protein synthesis initiation factor IF2; *rpsO*, for ribosomal protein S15; and *pnp*, for polynucleotide phosphorylase. The region from *metY* through *infB* has been cloned and sequenced (1-6), as has the region from *rpsO* through *pnp* (7-9), and it has been shown that all of the genes are transcribed clockwise on the genome (from *metY* toward *pnp*). However, restriction enzyme mapping indicates that about a kilobase of unsequenced DNA lies between *infB* and *rpsO*. Expression of the cloned genes *in vitro* or in maxicells indicates that *metY*, the gene for P15A, *nusA*, *infB*, and a gene for another 15 kDa protein (named

P15B) downstream from *infB*, are all expressed from a promoter located upstream of *metY* (10). In contrast, expression of *rpsO* and *pnp* is not dependent on the *metY* promoter but is due primarily to a promoter upstream of *rpsO* and another between the two genes. S1 nuclease mapping of *pnp* and *rpsO* transcripts suggests that a small amount of transcription may arise by read through from regions further upstream from *rpsO* (11). In order to better characterize the distal end of the *metY* operon and to evaluate its expression in relationship to *nusA*, *infB*, *rpsO* and *pnp*, we sequenced the DNA between *infB* and *rpsO* and characterized transcripts from this region by S1 nuclease mapping. We describe two open reading frames downstream from *infB* which code for 15 kDa and 35 kDa proteins (P15B and P35, respectively) and demonstrate that the genes are expressed from the *metY* promoter and are cotranscribed with *rpsO*.

METHODS

DNA sequencing.

Overlapping fragments of 1.5 and 1.4 kb were obtained by digestion respectively of pBP280 (7) with *Dra*I, and pB15-6 (12) with *Pst*I and *Hind*III. Each fragment was purified by polyacrylamide gel electrophoresis and was digested with *Hpa*I, *Hpa*II, *Sau*3A, *Alu*I, *Rsa*I or *Fnu*DII, and the subfragments were "shotgun" cloned into bacteriophage M13mp8 and M13mp9 grown in *E. coli* strain JM103. Appropriate inserts were identified and sequenced by the dideoxynucleotide chain termination method of Sanger et al. (13) such that complete sequences for both strands of the 1.4 and 1.5 kb fragments were obtained.

Nuclease mapping of *in vivo* transcripts.

S1 nuclease mapping was performed by the method of Burton et al. (14) as described by Regnier and Portier (11). Various amounts of RNA (see Figure legends) were isolated from *E. coli* strain BL322 (*thi*-1, *argH*-1, *sup*44) or the isogenic strain BL321 carrying the *rnc*-105 allele which confers a deficiency in RNase III (15). The RNA preparations (up to 50 μg) were hybridized in 50 μl with about 0.1 μg of single stranded or denatured double stranded DNA probes labeled either at 5'-termini with [^{32}P]phosphate by polynucleotide kinase or at 3'-termini by chain extension with Klenow fragment. Following S1 nuclease digestion for 30 minutes at 37°, resistant DNA fragments were analyzed on 8% polyacrylamide gels containing 8 M urea or were glyoxylated and separated on 1.2% agarose gels. The dried gels were subjected to autoradiography with Kodak X-omat film as described in the figure legends. Exonuclease VII digestions were carried out essentially as described for S1

nuclease mapping except that hybridization mixtures (50 μl) were transferred to 450 μl buffer containing 10 mM Tris-HCl, pH 7.4, 10 mM KCl, 30 mM EDTA, and 0.45 units/ml exonuclease VII and incubated 1 hr. at 37° before gel analysis of DNA fragments.

RESULTS

DNA sequencing between *infB* and *rpsO*.

The reported sequence of the gene for IF2, *infB*, includes an additional 392 base pairs downstream from the structural gene, ending at a *Hind*III restriction site (5). We extended the knowledge of this region of the *E. coli* genome further by sequencing a 1.4 kb DNA fragment from the *Hind*III restriction site downstream to a *Pst*I site, as well as an overlapping 1.5 kb *Dra*I fragment which begins just upstream from the *Hind*III site (see Figure 3 and Methods). Both fragments also overlap the reported DNA sequence which begins upstream from *rpsO* (8). Figure 1 reports the antisense strand sequence (1861 bp) and the deduced amino acid sequences for the putative proteins. This completes DNA sequencing in this region of the genome and enables us to link physically at the nucleotide level the *nusA-infB* operon to the *rpsO-pnp* operon. To simplify this and any subsequent analyses, we have used a base pair numbering system that begins with the *metY* promoter (see legend to Figure 1).

Two open reading frames are apparent in the sequence, coding for putative proteins with 133 amino acids (P15B: 15,200 Da) and 314 amino acids (P35: 35,091 Da). The gene for P15B contains an AUG initiator signal (5198-5200) preceded by the Shine-Dalgarno sequence, AGGAG (5185-5189), an A at -3, and a favored sequence, GCGA at +4 to +7 (16). The coding sequence for P35 begins two base pairs following P15B and therefore lies in a different reading frame. The AUG initiator codon (5599-5601) overlaps the UAA termination codon for P15B and is preceded by the Shine-Dalgarno sequence, AGGAGG (5589-5594). Both cistrons possess features that are typical of strong translational start signals (16), although the spacing of the Shine-Dalgarno and initiation codon regions for P35 is rather close. The proximity of the termination and initiation codons for the P15B and P35 cistrons suggests that their translation may be coupled.

The sequence shown in Figure 1 also contains part of the structural gene for S15, which begins at nucleotide 6692. Computer-assisted analysis of the DNA sequence between *infB* and *rpsO* for transcriptional signals indicates that a possible weak promoter (16) may be present in the region 4999-5031 (see ref.

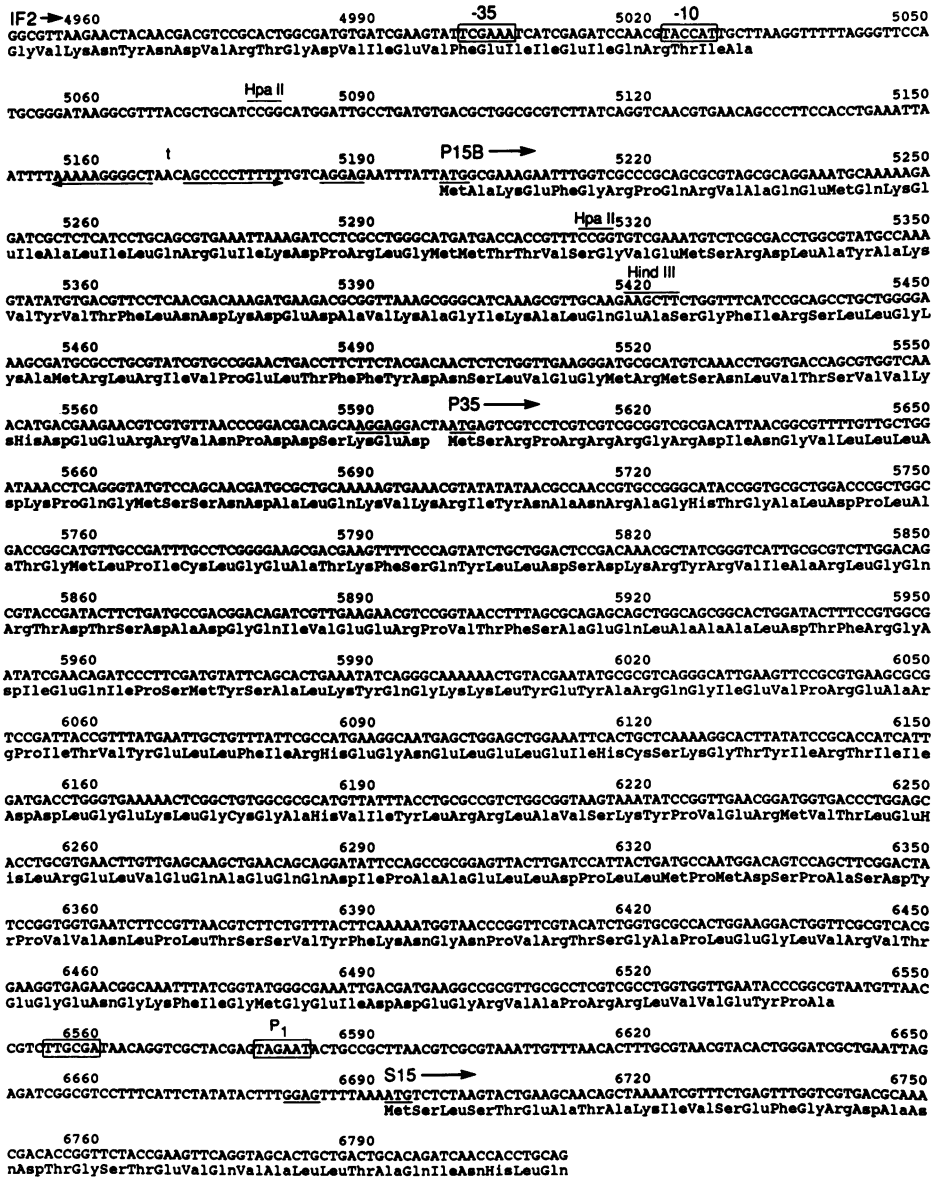

Figure 1. Nucleotide sequence of the P15B and P35 genes between *infB* and *rpsO*. The DNA sequence shown begins within the coding region of *infB* (IF2) and terminates at the *Pst*I site within *rpsO* (S15). Since the sequenced DNA is part of the *metY* operon, a numbering system was used which begins two bp before the *Pst*I site near the *metY* promoter (1) and runs through the sequences reported for *metY*, P15A, *nusA*, and *infB* (1-3,5,6). The first base pair

reported here, 4951, corresponds to bp 2614 in the sequence reported for *infB* (5). Base pair 6545 corresponds to the first base pair reported for the sequence containing *rpsO* (8). The sequence shown includes: the end of the coding region of *infB*; a weak promoter at the end of *infB*, whose -10 and -35 regions are boxed; the transcriptional terminator (t) between *infB* and P15B, underlined by diverging arrows; the open reading frames for P15B and P35; the P_1 promoter of the *rpsO-pnp* operon, whose -10 and -35 regions are boxed; and the beginning of the ribosomal protein S15 coding sequence. The amino acid sequences for all coding regions are given beneath the nucleotide triplets from which they are deduced. Initiation codons and Shine-Dalgarno sequences are underlined and relevant restriction enzyme sites are overlined and labeled.

(5), nucleotides 2779-2807), which lies just at the end of the coding region for IF2. A rho-independent termination signal just upstream from the P15B gene (5156-5180), also is apparent, as noted previously (5). However, no other obvious promoters or terminators near the P15B and P35 genes were detected.

Maxicell analysis.

In order to determine whether or not proteins corresponding to P15B and P35 are expressed by genomic DNA between *infB* and *rpsO*, we employed the maxicell assay of Sancar et al. (17). The 1.5 kb *Dra*I fragment (see Methods) was cloned into the *Sma*I site of pUC12 and a number of independent isolates in both orientations were analyzed. As shown in Figure 2, plasmid pD31 expresses a 15 kDa protein (P15B) and more weakly a 35 kDa protein (P35) which are essentially absent in the pUC12 control. pD31 contains the *Dra*I fragment oriented such that transcription from the vector's *lac* promoter could express P15B and P35. Plasmid pD6, which expresses P15B and P35 much less efficiently, contains the *Dra*I fragment in the opposite orientation; transcription could arise either from a promoter in the *Dra*I fragment (unlikely) or by read through from the Amp^r promoter of pUC12. Another plasmid, pB15-6, a pBR322 derivative that contains a DNA insert beginning at the *Hind*III site within the P15B gene and ending at an *Eco*RI site downstream from *pnp* (12), expresses PNPase, S15, and P35. P15B expression is not seen, as expected, since only the C-terminal portion of the structural gene is included in pB15-6. We conclude that P15B is rather strongly expressed, whereas P35 is synthesized less efficiently.

The genes for P15B and P35 are co-transcribed with *infB*.

In order to determine whether or not *infB* and P15B are linked transcriptionally, we used S1 nuclease mapping of the 5'-termini of RNA transcripts expressed *in vivo* from this region of the genome. A 5'-labeled dsDNA probe (505 bp) was prepared which extends from a *Hpa*I site in *infB* to the *Hind*III site in P15B (see Figure 3). As shown in Figure 4A (lanes 2-4),

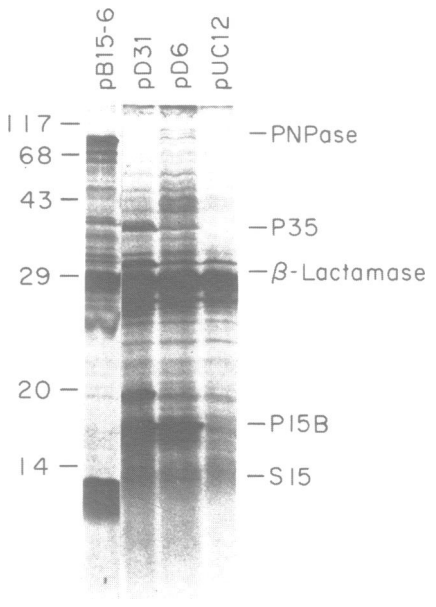

Figure 2. Maxicell synthesis of P15B and P35. *E. coli* strain CSR603 was used in the maxicell assay (17) following transformation with plasmids (see text). Lysates containing plasmid-encoded proteins labeled with [^{35}S]methionine were analyzed by electrophoresis on 12% polyacrylamide gels in sodium dodecylsulfate (20), and the gels were then dried and exposed to Kodak X-Omat film for two days. The plasmids used to transform the cells are shown at the top of the figure. Migration positions for PNPase, P35, β-lactamase, P15B and S15 are labeled on the right, whereas molecular weight markers are shown on the left.

the most intense band of protected DNA is the full-length probe (band A), which indicates that most of the transcripts entering the P15B gene originate upstream from the *Hpa*I site in *infB*. Numerous much less intense bands (B-H) are seen also, ranging in size from 170 to 375 nucleotides. As shown in Figure 3, these bands suggest that low-abundant RNAs have 5'-termini mapping in the intercistronic region between *infB* and P15B (bands B-D) and within the structural gene for P15B (bands E-H).

Since bands B-D end in regions that are rich in A-T base pairs (Figure 1, nucleotides 5144-5212) and therefore may have arisen artifactually by S1 nuclease cleavage in melted A-T rich regions, the S1 degradations were carried out at 25° rather than 37° (Figure 4A, lanes 6 and 7). Except for Bands D and E, which are substantially reduced in intensity, a nearly identical pattern of

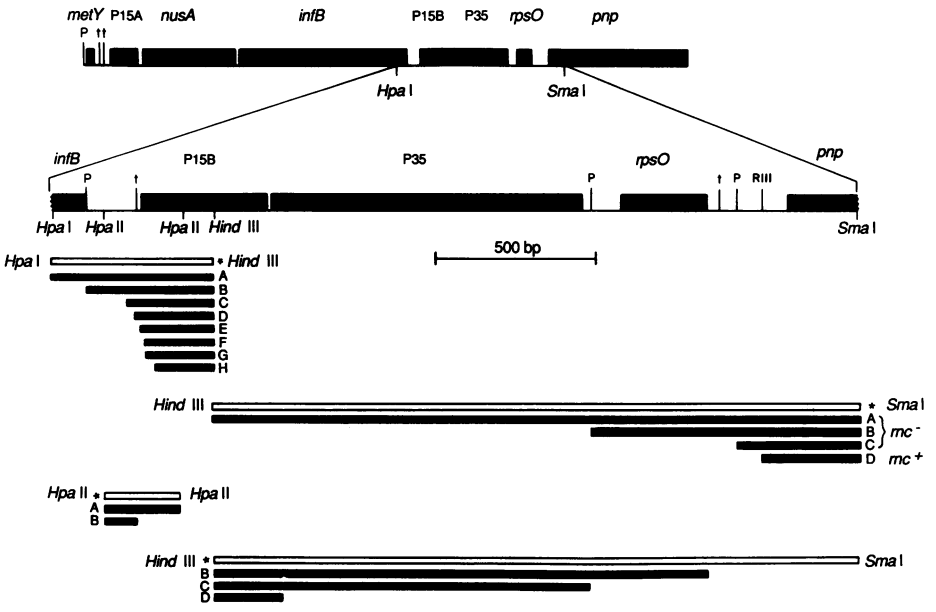

Figure 3. Transcription of the P15B and P35 genes. The map of the *E. coli* genome is based on DNA sequences reported for the *metY-nusA-infB* operon (1,2,3,5), the P15B and P35 genes reported in Figure 1, and the *rpsO-pnp* operon (8,9) Identified promoters (P), terminators (t), and RNase III maturation signal (RIII) are labeled. The results of S1 nuclease and exonuclease VII experiments are summarized below the map. The DNA probes are shown as open bars and the site of end-labeling is indicated by an asterisk. Protected probe fragments are shown by solid bars and are identified by letters that correspond to the bands labeled in Figures 4 and 5. In the case of the *Hind*III-*Sma*I fragment 5'-labeled at the *Sma*I site, the *rnc*$^-$ and *rnc*$^+$ strains used are indicated on the right.

bands from A through H was obtained. This suggests that localized melting of heteroduplexes followed by S1 nuclease digestion is not the cause of most of these bands. In an alternate approach to the same problem, cellular mRNAs were tested by exonuclease VII treatment with the same *Hpa*I-*Hind*III probe (Figure 4A, lanes 8-12). The band pattern is strikingly similar to that with S1 nuclease, again suggesting that the bands exhibited in these experiments are generated by true 5'-termini of mRNAs present in the cell in low abundance.

The above results indicate that P15B is mostly co-transcribed with *infB*, likely from the *metY* promoter, but that a low level of either promoter activity or RNA processing of these transcripts is occurring in the vicinity

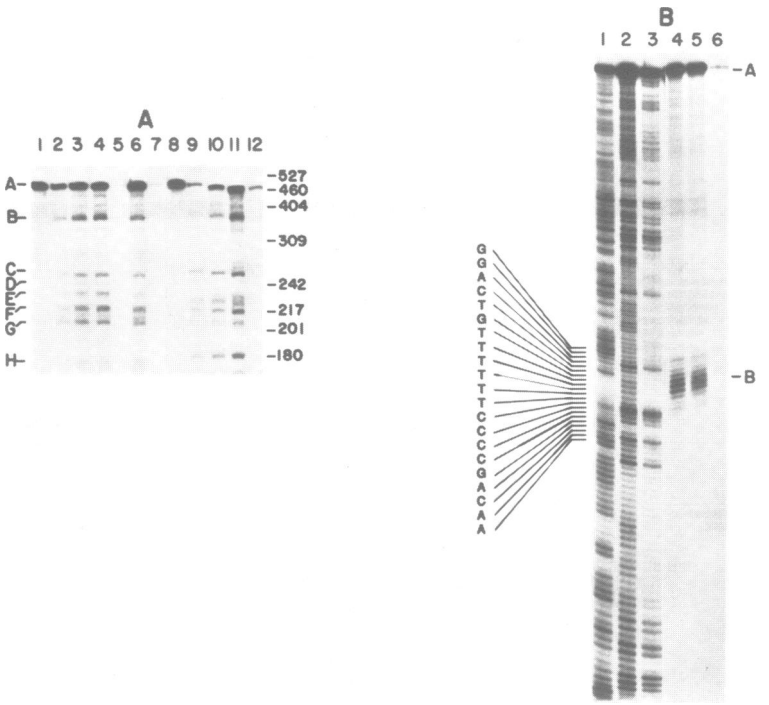

Figure 4. Mapping of *infB*-P15B transcript termini. Panel A: Identification of 5'-termini of P15B transcripts. The DNA probe is the 5'-labeled 505 bp *Hpa*I-*Hind*III fragment covering the *infB*-P15B intercistronic region (Figure 3). It was denatured and hybridized at 52° for 16 h with variable amounts of RNA from strain BL322 as described in Methods: 12.5 μg RNA (lanes 2 and 9); 25 μg RNA (lanes 3 and 10); and 50 μg RNA (lanes 4,6, and 11). Control reactions contained 50 μg tRNA (lanes 5,7, and 12). S1 nuclease digestion was carried out for 30 min at 37° (lanes 2 to 5) or at 25° (lanes 6 and 7); exonuclease VII digestion was at 37° for 1 h (lanes 9 to 12). Resistant DNAs were analyzed by electrophoresis on 6% polyacrylamide gels next to the probe (1/20 of that used for hybridization with mRNA) (lanes 1 and 8). Positions of migration of marker DNA fragments are indicated to the right of the figure. Bands corresponding to protected DNA fragments are indicated by letters from A to H on the left. Panel B: Identification of 3'-termini of *infB* transcripts. The DNA probe was a 237 nucleotide single-stranded *Hap*II-*Hap*II fragment labeled at the 3'-end. It spans the putative transcriptional terminator located downstream of *infB* (Figure 3). The probe was hybridized for 16 h at 45° to 25 μg (lane 5) and 50 μg (lane 4) of RNA of strain BL322 and to 50 μg tRNA (lane 6). The temperature was decreased to 30° over three hours to stabilize the AT-rich region of the heteroduplex located upstream of the transcriptional terminator. S1 nuclease digestion was carried out for 30 min at 37° and resistant DNA fragments were analyzed on an 8% polyacrylamide gel next to DNA sequencing ladders generated by chemical cleavage (21) of the probe at C+T (lane 1), G+A (lane 2), and G (lane 3). The sequence of a portion of the coding strand, complementary to that of the probe, is shown on the left (nucleotides 5167 to 5187).

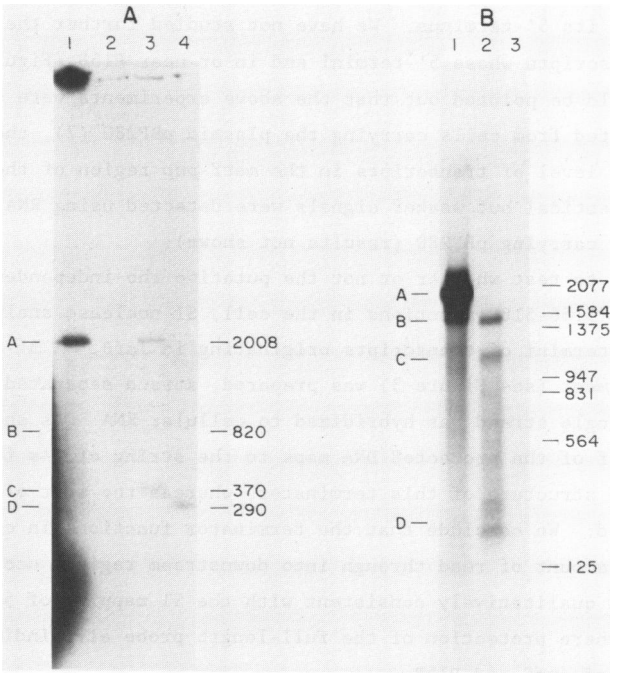

Figure 5. Mapping of P15B-P35-*rpsO* transcript termini. Panel A: Identification of 5'-termini of *pnp* transcripts. S1 nuclease mapping of 5'-termini was performed as described in Methods by using a 5'-labeled *Hind*III-*Sma*I probe (2008 bp) as shown in Figure 3. The probe was denatured and hybridized at 52° for 5 h to: 50 μg tRNA (lane 2); 50 μg RNA from strain BL321 (*rnc*$^-$) (lane 3); and 50 μg RNA from strain BL322 (*rnc*$^+$) (lane 4). After S1 nuclease digestion at 37°, resistant DNA fragments were analyzed by electrophoresis on 1.2% agarose gel. Lane 1 contains the undigested probe. Bands corresponding to protected DNA are labeled on the left and their lengths (in nucleotides) are shown on the right. Panel B: Identification of 3'-termini of transcripts from the P15B gene. The same probe as in Panel A, except labeled at the 3'-terminus, was denatured and hybridized at 52° for 16 h to 50 μg RNA from strain BL322 (lane 2) and to 50 μg tRNA (lane 3), then subjected to S1 nuclease digestion and gel electrophoresis as described for Panel A. Lane 1 contains the untreated probe at 1/20 the amount used in the hybridizations. Protected DNA bands are labeled on the left; lengths of marker DNAs are shown on the right.

of P15B. An *rnc*$^-$ strain generates the same pattern of bands seen in Figure 4A (results not shown); therefore the transcripts in the *infB*-P15B region are not processed by RNase III. The weak promoter identified in the DNA sequence at the end of *infB* (4999-5031) may be responsible for generating band B, which maps to the same region. Band E maps very near the hairpin structure of the terminator, which could be responsible for protecting P15B mRNA from

degradation at its 5'-terminus. We have not studied further the nature of the low-level transcripts whose 5'-termini end in or near P15B (Figure 4A, bands D-H). It should be pointed out that the above experiments were carried out with RNA isolated from cells carrying the plasmid pBP280 (7), thereby increasing the level of transcripts in the *metY-pnp* region of the genome. Essentially identical but weaker signals were detected using RNA preparations from cells not carrying pBP280 (results not shown).

In order to test whether or not the putative rho-independent terminator at nucleotides 5156-5180 functions in the cell, S1 nuclease analyses were used to map the 3'-termini of transcripts originating in *infB*. A 3'-labeled *Hap*II-*Hap*II DNA fragment (see Figure 3) was prepared, strand separated, and the appropriate single strand was hybridized to cellular RNA. As shown in Figure 4B, nearly half of the protected DNA maps to the string of T's following the stable hairpin structure of this terminator, whereas the rest of the probe is fully protected. We conclude that the terminator functions in cells, but that a substantial amount of read through into downstream regions occurs also. This result is qualitatively consistent with the S1 mapping of 5'-termini (Figure 4A), where protection of the full-length probe also indicated co-transcription of *infB* and P15B.

Analysis of the P15B-P35-*rpsO* region.

The proximity of P15B to P35 and the failure to find a rho-independent termination site between P15B and *rpsO* imply that transcription coming out of P15B may not terminate before entering the *rpsO-pnp* operon. However, evidence suggests that *rpsO* and *pnp* are expressed independently of the *nusA-infB* operon from a promoter located 14 bp downstream from P35 (8,11). S1 nuclease mapping was used to determine whether or not P15B, P35, *rpsO* and *pnp* are linked transcriptionally and to search for possible promoters in the P15B-P35 region. As shown in Figure 5A (lane 3), RNA from an *rnc*$^-$ strain deficient in RNase III protects three different fragments of a DNA probe labeled at a *Sma*I site in *pnp* and extending upstream to the *Hind*III site in P15B (see Figure 3). Band A corresponds to the full-length probe, indicating that transcripts originating upstream from the *Hind*III site in P15B continue into *rpsO* and *pnp*. Bands B and C map to the *rpsO* and *pnp* promoters previously identified (11). No protected DNAs are detected between bands A and B, which suggests that there are no promoters active at the distal end of P15B and throughout P35. As expected, RNA from an *rnc*$^+$ strain generates a single band (lane 4, band D) which maps to the RNase III maturation site described elsewhere (11).

In order to identify sites at which P15B-P35 transcripts terminate *in vivo*, mRNA was hybridized with a *Hind*III-*Sma*I probe (see Figure 3) 3'-labeled

at the *Hind*III site in P15B and analyzed by S1 nuclease digestion (Figure 5B). A number of bands are detected: band B maps to the previously characterized terminator directly following *rpsO* (11); band C maps to the end of the P35 gene; and a general smear of protected DNA down to a group of bands labeled D maps throughout the P35 gene, ending at the P15B-P35 junction. These results indicate that a significant amount of P15B transcription continues into the *rpsO-pnp* operon, consistent with the results in Figure 5A. They also indicate that many P15B transcripts end within or near the P35 gene, either through transcriptional termination or through RNA processing and partial degradation. In particular, band B may be due to a rho-dependent termination event since computer-assisted calculations suggest little secondary structure in the distal part of the P35 gene.

DISCUSSION

We report here new DNA sequence information about the *E. coli* genome, providing 1127 bp of sequence from the *Hind*III site downstream from *infB* (5) to the *Hpa*I site upstream from *rpsO* (8). The new sequence enables us to construct a precise physical map of the region and thereby link the *metY-infB* operon to the *rpsO-pnp* operon (see Figure 3). The sequence reveals two open reading frames between *infB* and *rpsO* which code for proteins of unknown function, named P15B and P35. Both proteins are expressed from plasmids in maxicell assays (Figure 2), although P35 synthesis is relatively low. A protein corresponding to P15B was detected previously and its expression was shown to be linked to the *nusA-infB* operon (10).

The expression of NusA and IF2 involves transcriptional initiation at one or more promoters near the tRNA gene, *metY;* no important promoters have yet been identified between P15A and *infB*, although a weak promoter may exist in front of *infB* (12). The S1 nuclease mapping of the 5'-termini of *in vivo* transcripts reported here reveals no major promoters between *infB* and the end of the P35 gene; low levels of transcripts with 5'-termini mapping between *infB* and P15B suggest that only minor promotion of transcription and/or mRNA processing may occur in this region. Therefore, the majority of P15B and P35 expression is transcriptionally linked to *infB*, likely from the *metY* promoter. This conclusion is in agreement with that of Nakamura and Mizusawa (10) concerning P15B expression. Analyses of 3'-termini in the same region demonstrate that the rho-independent terminator between *infB* and P15B presumably functions *in vivo*, since many transcripts end in the T-rich region following the terminator structure. In addition, a significant amount of read

through occurs at this site, resulting in co-transcription of *infB*, P15B, P35, *rpsO* and *pnp* genes. Since promoter activity within or immediately downstream of *infB* is weak, transcripts containing P15B should be less abundant than those containing *infB*. The S1 nuclease analyses (Figure 5) also indicate that mRNA in the P35 region may be relatively unstable. No stabilizing secondary structures are found in or directly following the P35 gene, except near the 5'-proximal end (see below). These hairpin structures could serve to protect the P15B mRNAs from 3'-exonucleolytic cleavage.

The continuation of transcripts from the P15B-P35 region through *rpsO* implies that the *nusA-infB* operon is transcriptionally linked, at least in part, to the *rpsO-pnp* operon. Other S1 mapping analyses (11) have shown that the vast majority of *rpsO* transcripts begins at a single promoter just upstream from *rpsO*, but small amounts of RNA originating further upstream were detected as well. This is consistent with the analyses reported here, where about 10-40% of the *rpsO* transcripts originate upstream from its own promoter. Readthrough from one operon into another may be a general phenomenon, since terminators function with variable efficiencies. An example is the *in vivo* read through of class I transcripts of bacteriophage T7 into the class II and class III regions (22). Only in special cases, such as ribosomal RNA operons, is termination near-absolute, and this appears to require a tandem arrangement of two very strong terminators. Many genes for translational components such as rRNAs, tRNAs and ribosomal proteins are transcribed from two promoters which are differentially regulated. Since *rpsO* has only a single, nearby promoter, the low level of read through from the *metY-infB* operon into *rpsO* may serve to express minor amounts of S15 under special circumstances. Adaptation of growing cells to a cold shock may be such a case. The relative overexpression of NusA, IF2 and PNPase observed under these conditions (18) might be due to an increased co-transcription of the *metY-infB* and *rpsO-pnp* operons. Analysis of bacterial cells under a variety of physiological conditions is needed to elucidate possible roles for the transcriptional linkage of these two large operons.

The separation of the coding regions of P15B and P35 by only two base pairs suggests that the synthesis of the two proteins may be translationally coupled. However, the expression of P15B is more efficient than that for P35, as detected by maxicell analysis. Both sequences contain strong ribosomal binding sites, and both utilize a rather high frequency of optimal codons (ϵ = 0.83 for P15B; ϵ = 0.73 for P35). The low efficiency of expression of P35 may be due to differential mRNA stability, as discussed above. Another

contributing factor may be secondary structure in the initiation region of the mRNA. A hairpin structure (5574-5622; ΔH = -16 kcal/mol) may form which places the P35 Shine-Dalgarno sequence in the stem and the AUG initiator codon in the loop. The structure could also serve to assure coupling of P35 synthesis to P15B synthesis.

Comparison of the amino acid sequences of P15B and P35 with those of other proteins in the GenBank and EMBL data banks reveals no striking homologies to other proteins and suggests no functional role for these proteins in the cell. However, the requirement of P15B and P35 expression for cell growth was addressed in work reported elsewhere (19). A strain was constructed in which P15B and P35 were placed under control of the *lac*-UV5 promoter, and only a small decrease in growth rate was detected when cells were grown in the absence of the *lac* inducer, IPTG. Since a low level of expression of P15B and P35 from the *lac*-UV5 promoter or by read through cannot be ruled out, it is not possible to conclude rigorously that either gene is dispensable. However, maximal growth rate was restored when the strain contained a plasmid carrying an intact P15B gene and a truncated P35 gene. These results suggest that P15B is required for maximal growth rate but that P35 may not be required.

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