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Attenuation and processing of RNA from the *rpsO-pnp* transcription unit of *Escherichia coli*

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ABSTRACT

Ribosomal protein S15 and polynucleotide phosphorylase of E.coli are encoded by two adjacent genes, rpsO and pnp, respectively. Analysis of in vivo transcripts from these two genes shows that they are within the same operon (S15 operon). By correlating the 5' and 3' ends of their in vivo transcripts with the DNA sequence, we have identified several features of the operon structure. These features include a promotor upstream from rpsO, an attenuator downstream from rpsO and an RNA processing site between these two genes.

INTRODUCTION

Ribosomal protein S15 is one of the essential proteins which bind specifically to rRNA at an initial stage in ribosomal assembly (1). Polynucleotide phosphorylase is an enzyme that is presumably involved in polynucleotide metabolism (2, 3). Nevertheless, it seems that this enzyme is not essential for the bacteria since mutants completely devoid of this activity have been isolated (4). The genes, rpsO and pnp, have been shown to be adjacent and located near 69 min on E. coli chromosome (5). According to the sequence analysis of this region, these two genes are transcribed in the same direction and they are separated by 250 base pairs which includes probable RNA polymerase termination site and a stem-loop structure (6, 7). Co-expression of these two genes was also suggested by deletion analysis (7) ; deletion of the promotor upstream from rpsO decreases the expression of pnp to 40% of the parent strain.

The studies reported in this paper were performed to identify the transcript from the rpsO-pnp region. By the use of S1 nuclease mapping technique, the precise ends of the transcripts which include rpsO or pnp were determined. Furthermore, attenuation and processing of the transcripts from this region were demonstrated from the experiments using RNase III deficient strain.

MATERIALS AND METHODS

Bacteria and plasmid

E. coli strain JC1553 (*argG*, *metB*, *his*, *leu*, *recA*, *mtl*, *xyl*, *malA*, *gal*, *lacY*, *str*^{r}, *tonA*, *tsx*, *supE*) was obtained from Coli Genetic Stock Center, Yale University, USA. The *rnc*$^{+}$ and *rnc105* (RNase III^{-}) isogenic strains (N2076 and N2077) were gifts from Dr. Apirion (8). Recombinant plasmid A127S was used as a source of the 2.1-kilobase DNA fragment which includes *rpsO* and the beginning of *pnp* (6).

Preparation of end-labeled and uniformly labeled probes

Plasmid DNA and DNA fragment were prepared according to Maniatis *et al.* (9). Restriction endonucleases and other enzymes were purchased from Takara Shuzo Co., Japan. DNA restriction fragments were 5'-end-labeled with polynucleotide kinase or 3'-end-labeled with terminal transferase as described (10). Then these fragments were digested with the second restriction nuclease to prepare the DNA probe which was end-labeled at one 3' or 5' end. Preparation of uniformly labeled plasmid DNA with ^{32}P orthophosphate was according to Studier (11).

S1 nuclease mapping

S1 nuclease mapping was performed mainly according to Maniatis *et al.* (9). Total *E. coli* RNA was prepared from mid-log culture of bacteria in LB medium by the hot phenol method of Salser *et al.* (12). In DNA-RNA hybridization reaction, 150 µg of *E. coli* RNA was mixed with 0.1-0.5 µg of labeled DNA probe in 30 µl of the hybridization buffer and denatured at 72°c. Then the reaction mixtures were incubated for 3 hr at 50°C or cooled slowly to 37°C for 5 hr. S1 nuclease (Takara Shuzo Co., Japan ; 300 units per ml) digestion was carried out for 40 min at 43°C or for 1 hr at 37°C. After nuclease treatment, the hybrids were separated on 5% polyacrylamide gels or 8% polyacrylamide gels containing 8M urea with chemical cleavage ladders of the same DNA fragment. Chemical cleavage of the end-labeled DNA fragments was performed according to the method of Maxam and Gilbert (10).

RESULTS

The *in vivo* transcripts from the *rpsO-pnp* region were analyzed by S1 nuclease mapping. For our initial experiments, we used uniformly labeled *HpaI* fragment as a probe (Fig. 1, B-1), which includes entire *rpsO* and the beginning of *pnp* as reported previously (6, 7). Schematic presentation of the probe and the results are shown in Fig. 1. Hybridization of the probe with RNA extracted from strain JC1553 and successive S1 nuclease digestion

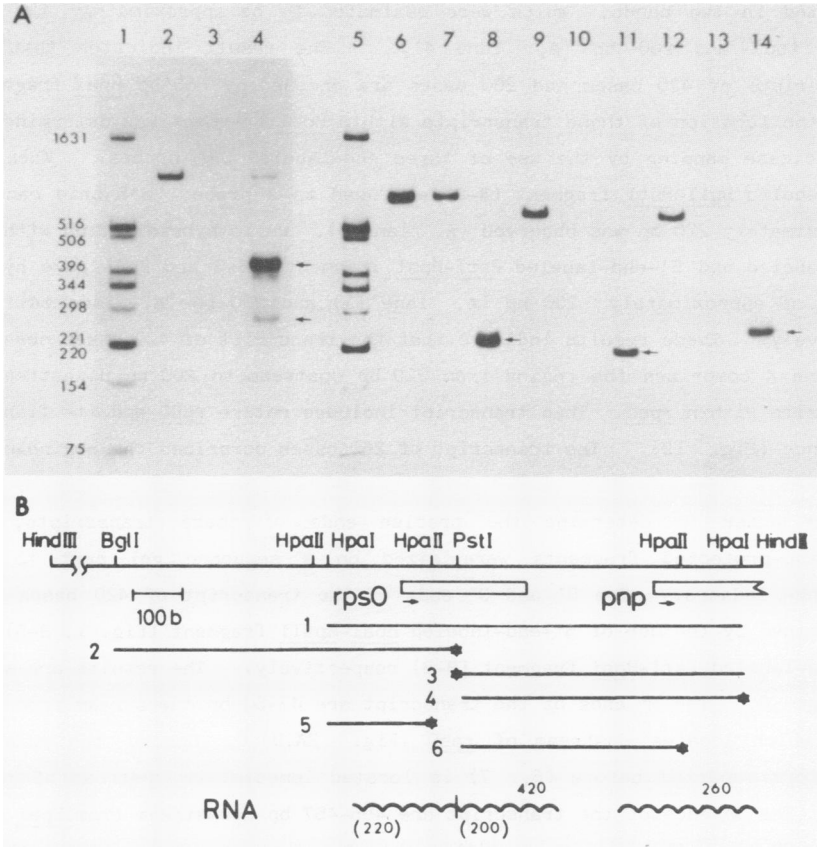

Fig. 1. S1 nuclease mapping of the transcript from rpsO and pnp region on the E. coli genome. A. Hybrids formed between uniformly labeled or end-labeled DNA probe (see B section) and in vivo RNA. Lanes 2, 3, 4 ; experiments with probe B-1. Lanes 6, 7, 8 ; experiments with probe B-2. Lanes 9, 10, 11 ; experiments with probe B-3. Lanes 12, 13, 14 ; experiments with probe B-4. Lanes 1, 5 ; size marker (^{32}P-labeled HinfI fragments of pBR322). Lanes 2, 6, 9, 12 ; DNA probe only. Lanes 3, 7, 10 and 13 are controls in which DNA and yeast RNA were subjected to the hybridization and nuclease treatment. Lanes 4, 8, 11 and 14 show the hybrids formed between DNA probes and E. coli RNA. Major hybrids are indicated by arrows. B. DNA probes used in this experiment. Structure of the 2.1-kilobase fragment isolated from plasmid A127S and the location of rpsO and pnp are shown in the upper section (6, 7). The direction of transcription is indicated by arrows below each gene. B-1 ; uniformly labeled 850 bp HpaI fragment. B-2 ; 5'-end-labeled 700 bp BglI-PstI fragment. B-3 ; 3'-end-labeled 580 bp PstI-HpaI fragment. B-4 ; 5'-end-labeled 580 bp PstI- HpaI fragment. B-5 ; 5'-end-labeled 210 bp HpaI-HpaII fragment. B-6 ; 5'-end-labeled 470 bp PstI-HpaII fragment. The position of ^{32}P radiolabel is indicated by asterisks. The probes, B-5 and B-6, were used to determine precise ends of the transcript. The transcripts detected in these experiments are shown at the bottom of the figure. Approximate size of the transcript is given in bases. The number in parenthesis indicates distance from PstI site.

resulted in two bands, which were estimated to be approximately 420 base pairs (bp) and 260 bp (A, lane 4). The result indicates that two transcripts of 420 bases and 260 bases are encoded by 850 bp HpaI fragment. Then the location of these transcripts within HpaI fragment was determined by S1 nuclease mapping by the use of three end-labeled DNA probes. When 5'-end-labeled BglI-PstI fragment (B-2) was used as a probe, a hybrid band of approximately 220 bp was observed (A, lane 8), while hybridization with 3'-end-labeled and 5'-end-labeled PstI-HpaI fragments (B-3 and B-4) gave hybrid bands of approximately 200 bp (A, lane 11) and 260 bp (A, lane 14) respectively. These results indicate that the transcript of 420 bases observed in lane 4 comprizes the region from 220 bp upstream to 200 bp downstream of PstI site within rpsO. This transcript includes entire rpsO and its flanking sequence (Fig. 1B). The transcript of 260 bases comprizes the beginning of pnp.

In order to determine the precise ends of these transcripts, S1 nuclease-protected fragments were sized on a sequence gel next to DNA sequence ladders. The 5' and 3' ends of the transcript of 420 bases were determined by the use of 5'-end-labeled HpaI-HpaII fragment (Fig. 1, B-5) and 3'-end-labeled PstI-HpaI fragment (B-3) respectively. The results are shown in Fig. 2. The 5' ends of the transcript are 43-50 bp downstream from HpaI site which locates upstream of rpsO (Fig. 2A,D). One of the putative promotors reported before (6, 7) is located immediately upstream of these ends. The 3' ends of the transcript are 455-457 bp downstream from HpaI site (Fig. 2B,D). These ends are within the stem-loop structure which probably functions as an RNA polymerase termination site (6, 7). The 5' ends of the transcript of 260 bases were determined by the use of 5'-end-labeled PstI-HpaII fragment (Fig. 1, B-6). The 5' ends of this transcript is 554-557 bp downstream from HpaI site (Fig. 2C,D). These ends are within the large stem-loop structure preceding pnp as shown in Fig. 2D. We could not find any consensus promotor sequence within the region from 10 to 35 bp upstream of our determined 5' ends.

Absence of the promotor sequence in -10 and -35 region of the transcript of 260 bases suggests that it arises from processing of the longer transcript. To test this possibility, S1 nuclease mapping was carried out by the use of RNase III deficient strain (rnc^-), since processing of mRNA from other ribosomal protein operon (beta operon) by RNase III has been reported before (13). When 5'-end-labeled BglI-PstI fragment (Fig. 1, B-2) was used as a probe, a hybrid band of the same size was obtained with RNA extracted

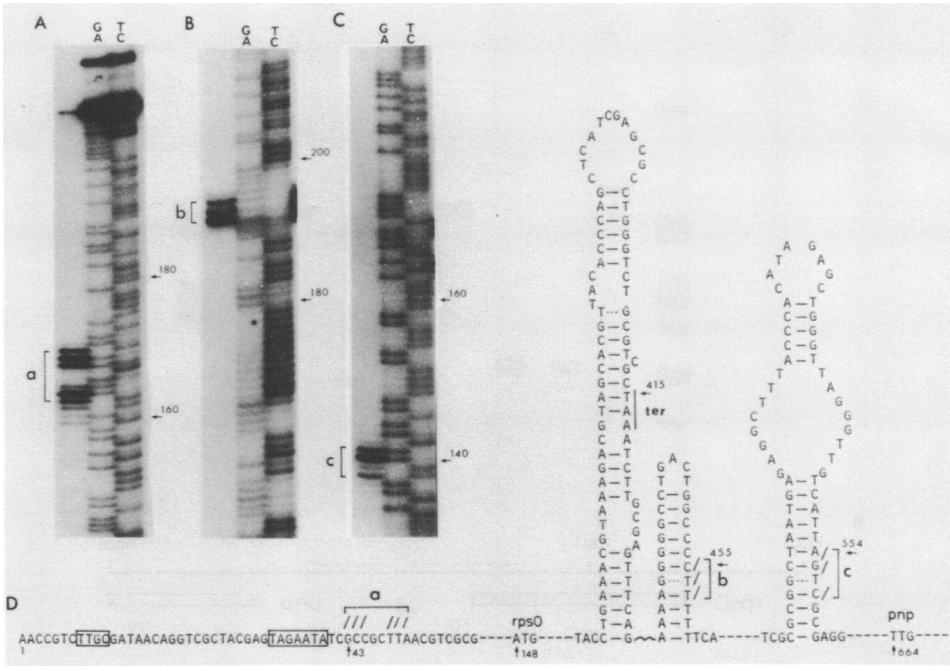

Fig. 2. S1 nuclease mapping of the 5' and 3' ends of the transcript. The hybrid bands are formed between end-labeled DNA probes and RNA, and they are shown in the left lane next to chemical cleavage ladders in each section. The 5' and 3' ends are indicated by letters a, b and c. Only G + A (GA) and T + C (TC) sequencing reactions were performed. A. The 5' ends of the transcript of 420 bases by the use of probe B-5 (see Fig. 1B). B. The 3' ends of the transcript of 420 bases by the use of probe B-3. C. The 5' ends of the transcript of 260 bases by the use of probe B-6. The number in the right margin indicates distance in bases from labeled end of the probe. D. The 5' and 3' ends detected in these experiments on the DNA sequence of the rpsO-pnp region (indicated by letters a, b, c and slash marks). These letters correspond to those in section A, B and C. The nucleotide sequence and its secondary structure are from our previous report (6) and Portier and Regnier (7). The S15 promotor (open box), initiation codon of rpsO (ATG) and pnp (TTG) and termination codon of rpsO (TAA) are shown in the figure. The number indicates distance in bases from HpaI site upstream of rpsO.

from rnc^+ and rnc^- strains (Fig. 3A, lane 3,4). However hybridization pattern between two strains differed when other two probes were used. Hybridization of 3'-end-labeled PstI-HpaI fragment (Fig. 1, B-3) with rnc^- RNA resulted in a new band of approximately 520 bp in addition to 220 bp band (Fig. 3A, lane 6,7). This transcript covers rpsO, pnp and the intercistronic region of the two genes. The 520 bp band observed is slightly smaller in size than the full-length protected probe. Since processing by RNase III

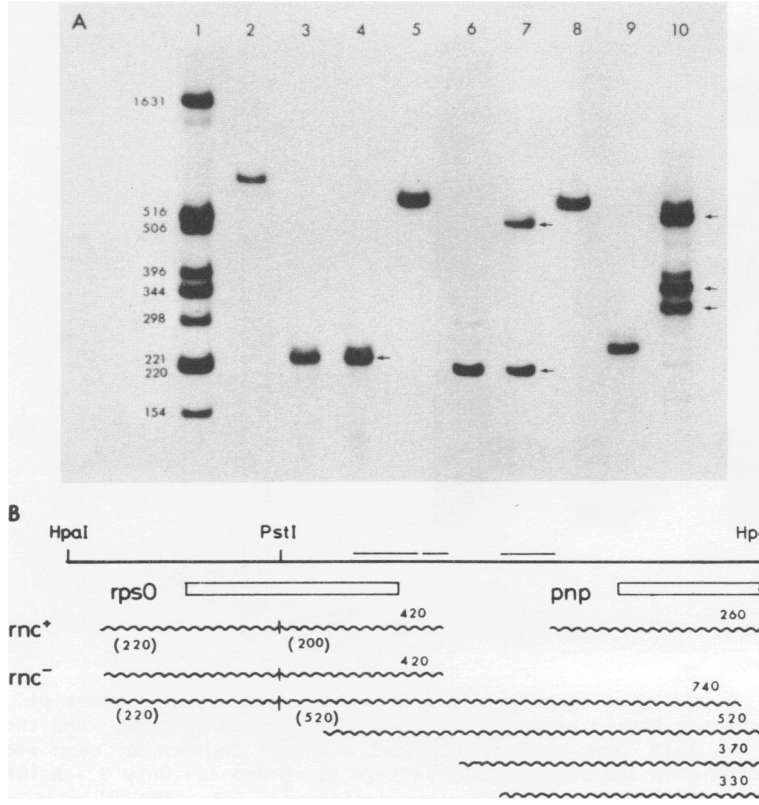

Fig. 3. Analysis of the transcript from RNase III deficient strain. A. Hybrids formed between end-labeled DNA probes and RNA extracted from rnc$^+$ (N2076) or rnc$^-$ (N2077) strain. Lane 1 ; size marker (^{32}P-labeled HinfI fragments of pBR322). Lanes 2, 5, 8 ; DNA probe only. Lanes 3, 4 ; hybrids formed between probe B-2 (see Fig. 1B) and RNA from rnc$^+$ and rnc$^-$ strains respectively. Lanes 6, 7 ; hybrids between probe B-3 and RNA from rnc$^+$ and rnc$^-$ strains respectively. Lanes 9, 10 ; hybrids between probe B-4 and RNA from rnc$^+$ and rnc$^-$ strains respectively. Major hybrid bands formed with rnc$^-$ RNA are indicated by arrows. B. The transcripts detected in these experiments. Approximate size of the transcript is given in bases. The number in parenthesis indicates distance from PstI site. Location of the possible stem-loop structure is shown above cleavage map by solid lines.

results in a strong stabilization of mRNA (14), the large primary transcript, which includes rpsO and pnp, might be subjected to endonucleolytic cleavage in rnc$^-$strain resulting in the 520 bp band. When 5'-end-labeled PstI-HpaI fragment (Fig. 1, B-4) was used, the 260 bp band which was observed in hybridization with rnc$^+$ RNA, disappeared and instead new five bands of the larger size (three major and two minor bands) were observed. Two out of

these five bands have the length to cover not only pnp but also a part of rpsO. Absence of the transcript of 260 bases in rnc⁻ RNA suggests that this transcript arises by processing of the primary transcript which includes both rpsO and pnp. These results indicate that there are at least two primary transcripts in the rpsO-pnp region. The 5' ends of these transcripts would be the same or very close (a region in Fig. 2D). Both transcripts initiate at the promotor upstream of rpsO and one of the transcripts is terminated immediately behind rpsO. The other transcript extends to pnp beyond the intercistronic region and then it is processed as shown in Fig. 3B. In supporting this conclusion, hybridization between uniformly labeled HpaI fragment and rnc⁻ RNA resulted in two major hybrid bands of approximately 420 bp and 740 bp (data not shown). Five transcripts observed in rnc⁻ RNA (Fig. 3, lane 10) might arise from endonucleolytic cleavage of unprocessed transcript in rnc⁻ strain.

DISCUSSION

The results presented in this paper show co-expression of the rpsO and pnp genes. We tentatively designate this region as S15 operon. The transcription starts at the promotor upstream of rpsO (rpsO promotor) and attenuation occurs downstream of rpsO. Non-attenuated transcript extends to pnp beyond the intercistronic region and it is processed between rpsO and pnp. The 5' ends of the pnp transcript is within the stem-loop structure which seems characteristic of the recognition site by RNase III (for a review, see ref. 15) and it would represent processing site by this enzyme. It is unclear if processing event gives rise to the transcript other than the transcript of 260 bases derived from pnp. Since there are only two major bands of 420 bp and 260 bp in hybridization with uniformly labeled fragment (Fig. 1A, lane 4), the remaining part other than the transcript of 260 bases might be subjected to nuclease digestion without leaving any sequence detectable on the gel. In this case, the transcript of 420 bases is produced only by attenuation. Alternatively, the transcript of 420 bases might be produced not only by attenuation but also by processing. If so, the attenuation site and processing site on the primary transcript should be the same.

The presence of a putative promotor for pnp (pnp promotor) was suggested by Portier and Regnier (7, see INTRODUCTION). The -10 region of this promotor is located about 250 bp upstream from HpaI site within pnp. This implies that hybridization with 5'-end-labeled PstI-HpaI fragment (Fig. 1,

B-4) should give an approximately 240 bp band on the gel. Absence of such a band in our experiment might mean that pnp promotor is used for transcription only when rpsO promotor is deleted or inactive.

Co-transcription of ribosomal protein genes and other genes have been reported in several operons (13, 16, 17). Sigma operon starts with rpsU followed by dnaG and rpoD. Beta operon starts with rplK and two genes, rpoB and rpoC, are located at a distal part of this operon. The features of S15 operon described here show remarkable similarity to these operons. All these operons start with a ribosomal protein gene(s) successively followed by a terminator structure which probably functions as an attenuator, a possible RNase III processing site and a promotor permitting potential independent expression of a gene in RNA metabolism. Biological meaning of co-transcription of rpsO and pnp is unclear, but it would be noteworthy that the function of these gene products is directly related to RNA. Ribosomal protein S15 binds to 16S rRNA and polynucleotide phosphorylase degrades mRNA in vivo (2, 3). The same is true with the gene products of sigma and beta operons.

The regulatory mechanism for expression of rpsO and pnp is still unknown. Translational autoregulation of S15 synthesis was suggested by the observation that S15 synthesis is not subject to gene dosage (18) and there exists strong structural homology between rRNA binding site for S15 and a distal part of the rpsO transcript (6). On the other hand, pnp shows gene dosage effect ; strains carrying hybrid plasmid of pBR322 and pnp overproduce polynucleotide phosphorylase up to 20 fold (5). Such differential expression of these two genes might be explained by attenuation, processing of the transcript or the presence of intercistronic promotor. It would be also interesting to know if S15 operon includes other genes in addition to rpsO and pnp. The details are now under investigation.

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