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Organization and Transcription of the Principal σ Gene (*rpoDA*) of *Pseudomonas aeruginosa* PAO1: Involvement of a σ^{32}-Like RNA Polymerase in *rpoDA* Gene Expression

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S1 nuclease mapping and Northern (RNA) hybridization revealed that the *rpoDA* gene encoding the principal σ subunit of *Pseudomonas aeruginosa* PAO1 is transcribed as a monocistronic mRNA of 2 kb and that the transcription from the *rpoDA* promoter (P_C) starts 32 bases upstream from the first nucleotide of the initiation codon during the steady-state growth condition at a low temperature (30°C). The transcript terminates 31 bases downstream from the last nucleotide of the termination codon. When the growth temperature was shifted to 42°C, the synthesis of *rpoDA* mRNA from a heat shock promoter was transiently induced, although transcription was still occurring from P_C during the heat shock period. The transcription initiation site of the heat shock promoter (P_{HS}) is located about 220 bases upstream of the initiation codon of *rpoDA*. In addition, both promoters were utilized in vitro by RNA polymerase partially purified from heat-shocked cells of *P. aeruginosa* PAO1. When the *rpoDA* was introduced into *Escherichia coli*, the transcription patterns of *rpoDA* at 30 and 42°C were similar to those observed for *P. aeruginosa*. These results suggested that the transcription of *rpoDA* in *P. aeruginosa* is regulated by the principal RNA polymerase and the heat shock RNA polymerase in response to the environmental temperature.

RNA polymerases in eubacteria are generally complex oligomeric species consisting of at least four different subunits, β′, β, α, and σ. The core enzyme, with the subunit structure $\alpha_2\beta\beta'$, is capable of RNA polymerization but requires the presence of a σ subunit for specific transcription initiation at the promoter (22).

Global switches in transcription patterns corresponding to changes in circumstances are known to be attributable mainly to modulation of promoter selectivity of multiple RNA polymerase holoenzymes composed of the same RNA polymerase core with different σ factors. Each σ confers on RNA polymerase the ability to initiate transcription at specific sites but also to use a specific class of cognate promoters (21, 22, 26). Under normal physiological growth conditions, eubacterial cells use RNA polymerase holoenzyme containing a principal σ factor which is responsible for the promoter recognition of housekeeping genes (18).

Genes encoding principal σ factors of several eubacterial species, such as *Escherichia coli* (7), *Salmonella typhimurium* (11), *Bacillus subtilis* (15, 33), *Myxococcus xanthus* (19), *Streptomyces coelicolor* (9, 27), and *Anabaena* sp. (5) have been isolated and sequenced. Recently, Tanaka et al. reported detection of two hybridization signals in the DNA of *Pseudomonas aeruginosa* by using a synthetic DNA probe designed from the common amino acid sequence of the principal σ factors of *E. coli* and *B. subtilis* (29). The gene for one of these signals, *rpoDA*, was cloned and sequenced (30). The DNA region, corresponding to another hybridization signal, has also been cloned and has been proved to be a homolog of the *E. coli* stationary-phase σ factor gene *katF* (*sigS*) (23, 31). The amino acid sequence deduced from the nucleotide sequence of *rpoDA* showed extensive homology with that of the principal σ factor of *E. coli*, and the *rpoDA* was able to complement a temperature-sensitive mutation of the *E. coli rpoD* gene. These results indicated that the *rpoDA* gene product has a structure and a function homologous to those of the *rpoD* gene product of *E. coli*.

In this paper, we report the organization of the *rpoDA* transcriptional unit and its transcription pattern. Our results indicate that *rpoDA* is transcribed as a monocistronic mRNA. We also describe a heat shock response of *rpoDA* transcription and the identification of a heat shock promoter upstream of the constitutive promoter.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and culture conditions. *P. aeruginosa* PAO1 (wild type) was used. *E. coli* HB101 was used as a host strain for plasmid pASB3, which is pTZ18R (Pharmacia) carrying a 2.3-kb *Pst*I DNA fragment containing *rpoDA* (30). All the strains were grown in Luria-Bertani medium, and for *E. coli* harboring the plasmid, the Luria-Bertani medium was supplemented with 50 μg of ampicillin per ml. For temperature shift experiments, cultures of *P. aeruginosa* and *E. coli* harboring pASB3 grown at 30°C were split in the exponential-growth phase (A_{650} = 0.6), and one half was cultured at 30°C and the other was cultured at 42°C.

Preparation of RNA. Samples (30 ml each) of cultures of *P. aeruginosa* PAO1 and *E. coli* HB101 harboring pASB3 were harvested after culture for 2, 5, 10, 20, and 60 min at 30 and 42°C, and total RNAs were prepared by the hot-phenol method (1). Total RNAs were used for S1 nuclease mapping and Northern (RNA) hybridization.

S1 nuclease mapping. S1 nuclease mapping experiments were carried out by the method of Aiba et al. (1). RNA (20 μg) was hybridized with probes (10,000 cpm, ca. 0.02 pmol) and treated with S1 nuclease. The S1 nuclease-protected DNA fragments were analyzed by 8 M urea–8% polyacryl-

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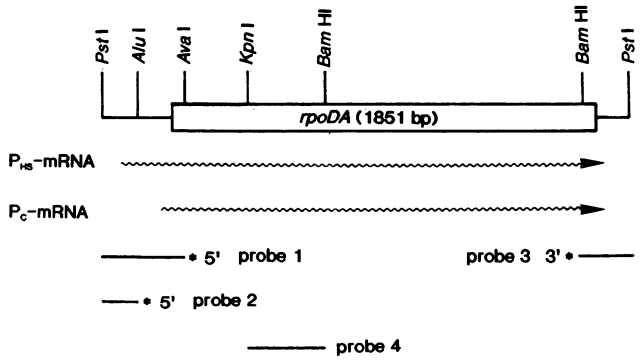

FIG. 1. Locations of probes used for S1 nuclease mapping and Northern hybridization of the *rpoDA* gene. The upper part of the figure shows the 2.3-kb *Pst*I DNA fragment inserted in pTZ18R. The open box represents the coding region of *rpoDA*. The thin lines indicate the noncoding regions of *rpoDA* derived from *P. aeruginosa* (30). The wavy lines below the restriction map of *rpoDA* indicate the direction and lengths of P_C mRNA and P_{HS} mRNA. The lower part of the figure shows the DNA fragments used as probes. Details of experiments are described in Materials and Methods.

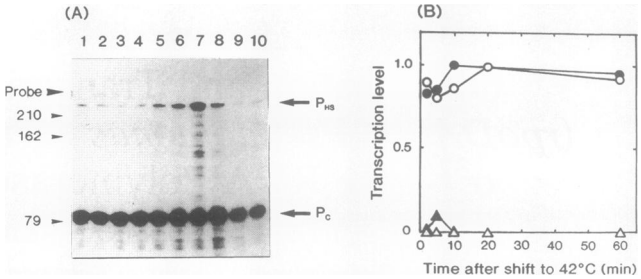

FIG. 2. S1 nuclease mapping of *rpoDA* transcripts. RNA extraction and S1 nuclease mapping conditions are described in Materials and Methods. (A) *P. aeruginosa* PAO1 was grown at 30°C (lanes 1 to 5) and at 42°C (lanes 6 to 10). The cells were harvested, and RNAs were extracted 2 min (lanes 1 and 6), 5 min (lanes 2 and 7), 10 min (lanes 3 and 8), 20 min (lanes 4 and 9), and 60 min (lanes 5 and 10) after temperature shiftup. Probe 1 was used. The positions of the fragments protected by P_C and P_{HS} mRNAs are marked on the right. The positions of the probe and DNA size markers (in bases) are indicated on the left. (B) Quantitation of relative synthesis rates. The transcriptional levels were normalized to the maximum level and expressed in arbitrary units. Symbols: ○, P_C mRNA at 30°C; ●, P_C mRNA at 42°C; △, P_{HS} mRNA at 30°C; ▲, P_{HS} mRNA at 42°C.

amide gel electrophoresis. The probes were prepared as follows. For preparation of probe 1 (Fig. 1), pASB3 was digested with *Kpn*I and a 650-bp *Kpn*I fragment was purified. The left *Kpn*I site is in a multiple cloning site on pTZ18R (data not shown), and the right *Kpn*I site is in the inserted DNA (Fig. 1). The 650-bp *Kpn*I fragment was digested with *Ava*I, and the 5′ ends of the resulting fragments were labeled with [γ-^{32}P]ATP by using polynucleotide kinase after dephosphorylation with bacterial alkaline phosphatase. Then, the end-labeled DNAs were restricted with *Pst*I, the fragments produced were separated on a 5% polyacrylamide gel, and the band corresponding to the 343-bp labeled *Ava*I-*Pst*I DNA fragment was cut out from the gel and eluted. For preparation of probe 2, the purified 650-bp *Kpn*I fragment was digested with *Alu*I and the 5′ ends of the resulting fragments were labeled as described above. The end-labeled fragments were digested with *Pst*I, the fragments produced were separated on a 5% polyacrylamide gel, and a 140-bp labeled *Alu*I-*Pst*I DNA fragment was cut out from the gel and eluted. For preparing probe 3, pASB3 was digested with *Bam*HI and *Pst*I, the fragments produced were separated on a 5% polyacrylamide gel, and a 201-bp *Bam*HI-*Pst*I fragment was cut out from the gel and eluted. The purified 201-bp *Bam*HI-*Pst*I fragment was 3′ end labeled with [α-^{32}P]dCTP by using the Klenow fragment of DNA polymerase I. Probes 1 and 2 were used for 5′-end mapping, and probe 3 was used for 3′-end mapping of mRNA (Fig. 1). For quantitative analysis, the autoradiograms were traced with a densitometer.

In vitro transcription. In vitro transcription assays of a single-round reaction with truncated DNA templates and partially purified RNA polymerase were carried out as described before (12, 13). The 343-bp nonlabeled *Ava*I-*Pst*I DNA fragment and the 140-bp nonlabeled *Alu*I-*Pst*I DNA fragment (Fig. 1), which carry the P_C and P_{HS} promoters and the P_{HS} promoter, respectively, were used as the template DNAs for in vitro runoff transcription.

Purification of RNA polymerase. *P. aeruginosa* PAO1 was harvested 5 min after a temperature shift from 30 to 42°C. RNA polymerase was prepared from the heat-shocked cells by the method of Burgess and Jendrisak (6) with minor modifications (2, 12, 13). RNA polymerase fractions containing the transcribing activities of both the P_C and the P_{HS} promoters were pooled and used for in vitro transcription.

Northern hybridization. RNA was separated by electrophoresis on 1% agarose-formaldehyde gels in MOPS (morpholinepropanesulfonic acid) buffer, blotted onto GeneScreen Plus (Du Pont), and hybridized with probe 1 and probe 4. Probe 4 (Fig. 1) was obtained by labeling a purified 336-bp *Kpn*I-*Bam*HI fragment with [α-^{32}P]dCTP by using a random-primed DNA labeling kit from Takara Shuzo. rRNAs were used as molecular size standards.

Enzymes and chemicals. [α-^{32}P]dCTP (110 TBq/mmol) and [γ-^{32}P]ATP (185 TBq/mmol) were purchased from Amersham. [α-^{32}P]UTP (29.6 TBq/mmol) was purchased from Du Pont. Restriction enzymes, the Klenow fragment of DNA polymerase I, alkaline phosphatase, and T4 polynucleotide kinase were from Takara Shuzo.

RESULTS

Transcription of *rpoDA*. The transcription of *rpoDA* was analyzed by S1 nuclease mapping. In *E. coli* (16, 24, 32) and *P. aeruginosa* (3), the principal σ factors of the RNA polymerases are heat shock proteins. Therefore, we tested for synthesis of *rpoDA* mRNA in cultures of *P. aeruginosa* PAO1 at 30 and 42°C by S1 nuclease mapping using probe 1 (Fig. 1). When *P. aeruginosa* PAO1 was cultured at 30°C, a protected DNA corresponding to an RNA transcript (P_C mRNA) was identified (Fig. 2A, lanes 1 to 5). When cultures were shifted from 30 to 42°C, another mRNA (P_{HS} mRNA) increased (Fig. 2A, lanes 6 to 10). A faint band of P_{HS} mRNA also can be seen at 30°C. The transcription level of P_C mRNA was not affected by the temperature shift (Fig. 2B). To examine the transcription pattern of P_{HS} mRNA after temperature shiftup, we used probe 2 (Fig. 1). During the early period after temperature shiftup (5 to 10 min), the level of P_{HS} mRNA increased dramatically, but later (60 min), P_{HS} mRNA disappeared, reflecting adaptation of the heat shock response (Fig. 3). Comparison of the activities of P_{HS} and P_C indicated that P_C was strong enough as a promoter to make a major contribution to σ synthesis during growth at 30 and

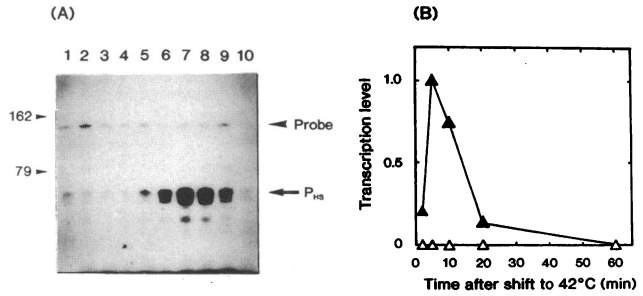

FIG. 3. S1 nuclease mapping for detection of P_{HS} mRNA. (A) Lanes are labeled as for Fig. 2A. Probe 2 was used. The positions of the fragments protected by P_{HS} mRNA, the probe, and DNA size markers (in bases) are indicated. (B) Quantitation of relative synthesis rates of P_{HS} mRNA. Symbols are labeled as for Fig. 2B.

42°C (Fig. 2): even after temperature shiftup (5 min), the P_{HS} activity was only about 10% of the P_C activity (Fig. 2B).

Location of the 5′ end of *rpoDA* mRNA. The 5′ end of *rpoDA* mRNA was located precisely by high-resolution S1 nuclease mapping. As shown in Fig. 4, the 5′ end of P_C mRNA was 32 bases upstream from the initiation codon (ATG) of *rpoDA*. P_{HS} mRNA had two 5′ ends, 220 and 221 bases upstream from the initiation codon of *rpoDA*. Some faint bands were also detected below the larger ones, but we do not know whether these smaller transcripts were processed from larger ones or whether they represent starts from independent promoters. The DNA sequences that precede the 5′ ends of *rpoDA* mRNAs were compared with the consensus promoter sequence (−35, TTGACA, and −10, TATAAT, separated by 17 bases) recognized by σ^{70} (26). Upstream of the P_C mRNA initiation site, the sequences CTGGCG and TATAAT were found as −35 and

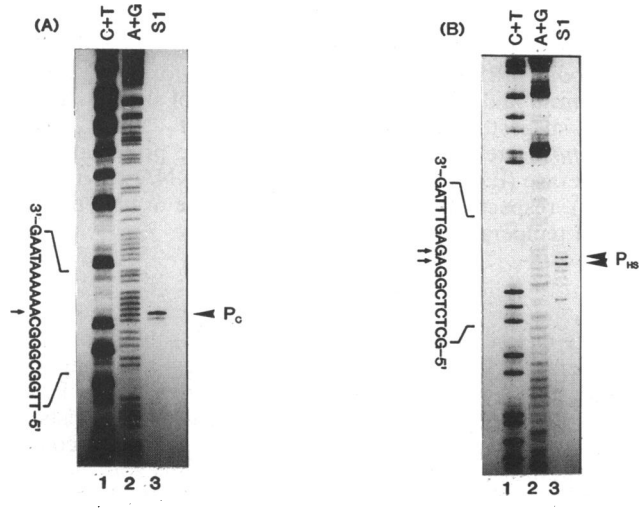

FIG. 4. Determination of 5′ ends of P_C mRNA and P_{HS} mRNA by S1 nuclease mapping. (A) The 5′ end of P_C mRNA. Lane 1, C+T, base-specific chemical cleavage; lane 2, A+G cleavage; lane 3, DNA fragment protected by P_C mRNA from S1 nuclease digestion. Probe 1 was used. (B) The 5′ end of P_{HS} mRNA. Lane 1, C+T cleavage; lane 2, A+G cleavage; lane 3, DNA fragment protected by P_{HS} mRNA from S1 nuclease digestion. Probe 2 was used. DNA fragments protected by P_C and P_{HS} mRNAs are indicated on the right of each panel. The 5′ ends of the *rpoDA* transcripts are indicated by arrows on the left of each panel.

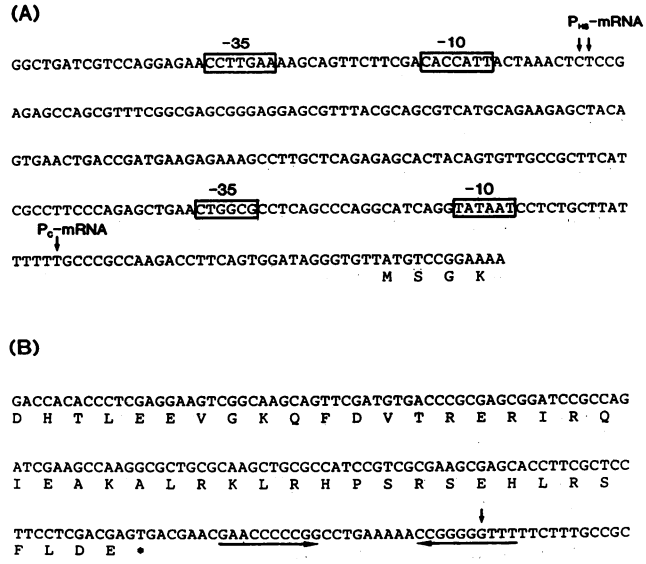

FIG. 5. Nucleotide sequences of the *rpoDA* promoter and terminator regions. These sequences were determined previously (30). (A) The vertical arrows indicate the start sites from which P_C mRNA and P_{HS} mRNA are transcribed. The proposed −10 and −35 recognition sequences are boxed. (B) The vertical arrow indicates the termination site of *rpoDA* mRNA. The results of experiments are shown in Fig. 6. The horizontal arrows represent palindromic sequences. The amino acid sequence of *rpoDA* is shown in single letters.

−10 potential promoter sequences. The −10 sequence had complete homology to the σ^{70}-type consensus sequence, but the −35 sequence and the spacing (19 bases) between −35 and −10 show poor homology to the σ^{70} consensus sequence (Fig. 5A).

For P_{HS}, the sequences CTTGAA and CACCATT separated by 14 bases were found as potential promoter sequences, comparable to the consensus sequences of the heat shock promoters (−35, CCCTTGAA, and −10, CCCCATT, separated by 13 to 15 bases) recognized by *E. coli* σ^{32} (Fig. 5A) (10, 26).

Location of the 3′ end of *rpoDA* mRNA. The 3′ end of *rpoDA* mRNA was determined by S1 nuclease mapping. The 3′-end-labeled probe 3 (Fig. 1) was hybridized with total RNA extracted from cells cultured at 30°C and shifted to 42°C for 5 min, and the probe was treated with S1 nuclease. The gel electrophoresis pattern showed that the 3′ end of *rpoDA* mRNA was located 31 bp downstream of the termination codon not only under the steady-state condition but also under the temperature shiftup condition (Fig. 6). The 3′ end of *rpoDA* mRNA was followed by T stretches (Fig. 5B). This region contains a self-complementary structure for a potential ρ-independent termination signal for *rpoDA* mRNA synthesis as indicated previously (30). Thus, we conclude that this self-complementary structure is actually a terminator for synthesis of the *rpoDA* transcript.

Transcriptional unit of *rpoDA*. Total RNA was separated by electrophoresis on an agarose gel, blotted onto a filter, and probed with ^{32}P-labeled probes 1 and 4 (Fig. 1) by Northern analysis. A band corresponding to an RNA transcript of approximately 2 kb was observed (Fig. 7). The probes hybridized to 23S and 16S rRNA nonspecifically. Thus, as the *rpoDA* is 1,851 bp (30), the gene is thought to be transcribed as a monocistronic mRNA.

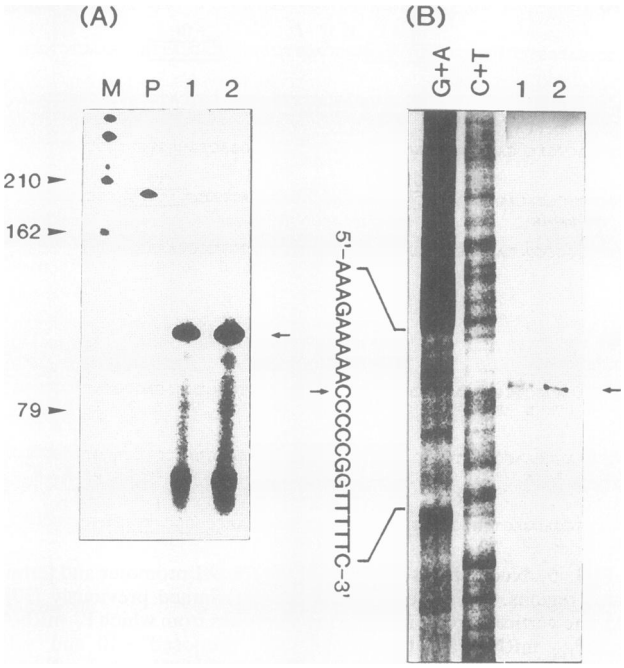

FIG. 6. Determination of the 3′ end of *rpoDA* mRNA by S1 nuclease mapping. RNAs were extracted from cells after 5 min at 30°C or shiftup to 42°C. (A) Low-resolution analysis. Lane M, DNA size markers; lane P, probe 3; lane 1, DNA fragment protected by P_C mRNA at 30°C from S1 nuclease digestion; lane 2, DNA fragment protected by P_C and P_{HS} mRNAs at 42°C from S1 nuclease digestion. Bands migrating at lower positions are oligonucleotides digested by S1 nuclease. Positions of DNA size markers (in bases) are indicated on the left. The position of protected DNA is indicated on the right. (B) High-resolution analysis. Lanes G+A and C+T, products of chemical cleavage; lanes 1 and 2, the same samples as in panel A. Probe 3 was used. The position of protected DNA is indicated on the right. The arrow on the left indicates the 3′ end of *rpoDA* mRNA (P_C and P_{HS} mRNAs).

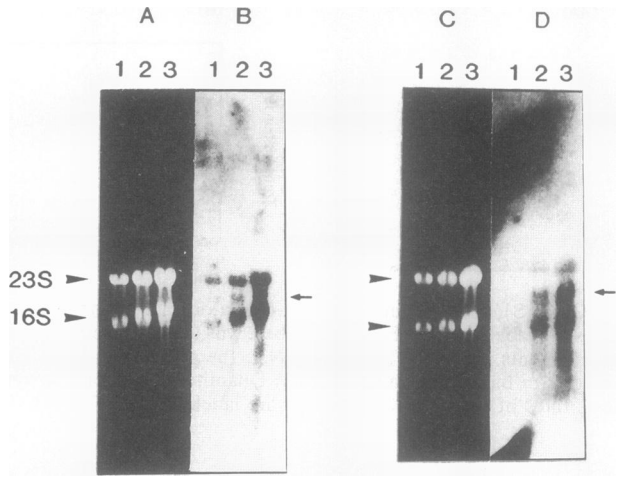

FIG. 7. Identification of the transcript of *rpoDA* by Northern hybridization. Samples of RNA (5, 10, and 20 μg for lanes 1, 2, and 3, respectively) from cells shifted to 42°C for 5 min were electrophoresed on a 1% agarose-formaldehyde gel. Panels A and C are photographs before autoradiography. Panels B and D are hybridizations with probe 1 and probe 4, respectively. The arrow on the right of each panel indicates the position of *rpoDA* mRNA (2 kb). The positions of rRNAs are indicated on the left of each panel.

In vitro transcription of *rpoDA*. Transcription of *rpoDA* was carried out in vitro by using partially purified RNA polymerase obtained from heat-shocked *P. aeruginosa* PAO1 and DNA fragments containing the putative P_C and P_{HS} promoters. If transcription of the truncated templates is initiated from the positions corresponding to the 5′ ends of *rpoDA* mRNAs and terminated at the end of the templates, each promoter will give the following transcript: 82-base RNA for P_C and 271- and 270-base RNAs for P_{HS} from template A and 59- and 58-base RNAs from template B (Fig. 8, upper part). When the 0.84-kb *Sma*I-*Ava*I fragment (template A, Fig. 8, upper part) was used as a template, two major transcripts of about 270 and 80 bases in length were found (Fig. 8, lower part, lane A), whereas when the 0.14-kb *Pst*I-*Alu*I fragment (template B, Fig. 8, upper part) was used as a template, a single major transcript of about 60 bases in length was found (Fig. 8, lower part, lane B). In addition to these major products, some other bands representing the end-to-end transcripts of the template DNAs were observed. From these results, we concluded that in vitro transcriptions by RNA polymerase prepared from heat-shocked *P. aeruginosa* are initiated from two promoters, P_C and P_{HS}, and that the positions of the transcriptional start sites are consistent with the 5′ ends of in vivo-synthesized mRNAs.

Heat shock response of *rpoDA* transcription in *E. coli*. S1 nuclease mapping was used to see whether mRNA synthesis corresponding to the temperature shiftup occurred in *E. coli* as in *P. aeruginosa*. pASB3 carrying *rpoDA* was introduced into *E. coli* HB101, and total RNAs were prepared from cells grown at 30°C or in parallel cultures shifted to 42°C for 2, 5, 10, 20, and 60 min. When *E. coli*(pASB3) was cultured at 30°C, a protected DNA corresponding to P_C mRNA was identified, and when the cultures were shifted to 42°C, a protected DNA corresponding to P_{HS} mRNA appeared (Fig. 9). The transcription patterns of P_C and P_{HS} mRNAs were similar to those observed for *P. aeruginosa* (Fig. 2 and 3). The probes used in this experiment did not hybridize to *E. coli rpoD* mRNA as the G+C contents of DNA in *P. aeruginosa* and *E. coli* are different (data not shown). These results suggested that the transcriptions of P_C and P_{HS} in *P. aeruginosa* are regulated by analogs of the principal RNA polymerase (E σ^{70}) and of the heat shock RNA polymerase (E σ^{32}), respectively, in *E. coli*, in response to the environmental temperature.

DISCUSSION

The *rpoD* gene encoding the principal σ subunit of RNA polymerase is cotranscribed with two genes preceding it in *E. coli* (8), *S. typhimurium* (11), and *B. subtilis* (33). Regulation of the *E. coli rpoD* (σ^{70}) operon, which consists of *rpsU*, encoding ribosomal protein S21; *dnaG*, encoding DNA primase; and *rpoD*, must be very complex as the gene products in a single operon are involved in translation, replication, and transcription (8). The structure and organization of the *rpoD* (σ^A) operon of *B. subtilis* resemble those of the *E. coli* σ^{70} operon except for the first gene, *P23*, the function of which is unknown (33). S1 nuclease mapping (Fig. 2, 3, and 6), in vitro transcription (Fig. 8), and Northern blot hybridization (Fig. 7) indicated that the *rpoDA* mRNA in *P. aeruginosa* is transcribed from its own promoter (P_C or P_{HS}), extends to the *rpoDA* terminator (Fig. 5B), and is about 2 kb in size (Fig. 7). The precise sizes of P_C mRNA

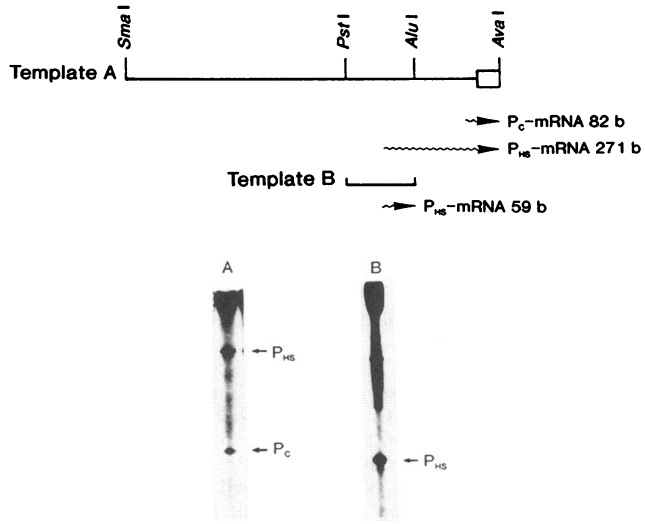

FIG. 8. Structure of truncated DNA templates and RNA products directed by the templates. Truncated DNA fragments carrying P_C and P_{HS} promoters were prepared as described in Materials and Methods. The P_C promoter is located between the *Alu*I and *Ava*I sites. The P_{HS} promoter is located between the *Pst*I and *Alu*I sites. The open box represents the coding region of *rpoDA*. In vitro transcription directed by each DNA fragment was carried out in the single-round transcription system. Wavy lines represent accurate transcripts directed by the respective DNA templates. The expected nucleotide lengths of RNA transcripts are also shown on the right sides of the wavy lines. RNA products were analyzed by 8% polyacrylamide gel electrophoresis, and the autoradiograms are shown in the lower part. Lanes A and B, templates A and B, respectively. Arrows to the right of the autoradiograms show expected transcripts for the templates.

and P_{HS} mRNA calculated from the results of S1 nuclease mapping were 1,917 and 2,106 bases, respectively. Therefore, we conclude that *rpoDA* is transcribed as a monocistronic mRNA. Recently, a gene encoding the principal σ factor of the vegetative cell RNA polymerase from a cyanobacterium, *Anabaena* sp., has been cloned and the gene has been demonstrated to be transcribed as monocistronic mRNAs (5).

The σ subunit of *E. coli* RNA polymerase is a heat shock protein (16, 24, 32). The response of σ synthesis to heat shock shows typical kinetics: transient activation followed by adaptation (20, 34). For *P. aeruginosa*, the general properties of the heat shock response have been characterized (3). Synthesis of at least 17 proteins was transiently induced upon temperature shiftup, and the principal σ factor (σ^{87}) of *P. aeruginosa* was shown to be a heat shock protein that was immunologically related to the σ^{70} of *E. coli* (16, 24, 32). These results are in contrast to those for the principal σ factors, σ^{A} of *B. subtilis* (4) and σ^{101} of *Caulobacter crescentus* (25), which are not heat shock proteins.

Under steady-state growth conditions at 30°C, most *rpoDA* transcription is from promoter P_C (Fig. 2). Temperature shiftup from 30 to 42°C induces transient activation of P_{HS}, a promoter located upstream of the P_C promoter (Fig. 2 and 3). But after temperature shiftup, the amount of mRNA from P_{HS} was only 10% of that from P_C (Fig. 2), suggesting that during heat shock P_C still functions as a promoter, making a major contribution to transcription of *rpoDA*.

When the plasmid carrying *rpoDA* was introduced into *E. coli*, the transcription patterns of P_C and P_{HS} mRNAs were similar to those observed for *P. aeruginosa* (Fig. 9). On comparison of the DNA sequences preceding the transcription start of P_C and P_{HS} mRNAs with consensus promoter sequences, possible promoters were found as indicated in Fig. 5A. The sequence for P_C was similar to the σ^{70} consensus sequence, and that for P_{HS} was similar to the σ^{32} consensus sequence. The −10 sequence of the P_C promoter had complete homology to the σ^{70}-type consensus sequence, but the −35 sequence and the spacing (19 bases) between −35 and −10 show poor homology to the σ^{70} consensus sequence. Variations in the recognition specificity of RNA polymerase from *E. coli* and pseudomonads were reported by Gao and Gussin (14). In their studies, *Pseudomonas* enzyme had greater flexibility in recognizing promoters that deviate from consensus sequence than did *E. coli* enzyme. Actually, the transcripts from P_C and P_{HS} promoters could be observed by transcription in vitro (Fig. 8). These results suggest that the P_C promoter is recognized by the RNA polymerase containing the principal σ factor and that the analog of σ^{32}, the heat shock σ factor in *E. coli*, also exists in *P. aeruginosa* in addition to the principal σ factor. Allan et al. reported that a 40-kDa protein associated with the RNA polymerase of heat-shocked *P. aeruginosa* was identified and might be a heat shock σ factor (3). The principal form of RNA polymerase in *P. aeruginosa* (an analog of σ^{70} RNA polymerase in *E. coli*) was purified (2) and shown to be able to transcribe the several *E. coli* promoters recognized by σ^{70} RNA polymerase of *E. coli* (12–14). However, an analog of σ^{32} RNA polymerase in *P. aeruginosa* has not been purified. The increase in *rpoDA* transcription from P_{HS} can easily be explained by an increase in σ^{32} activity during heat shock as observed for *E. coli* (17, 28).

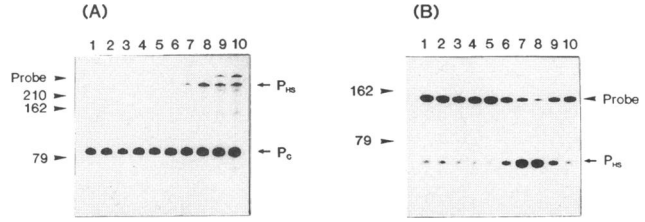

FIG. 9. S1 nuclease mapping of *rpoDA* transcripts in *E. coli* harboring pASB3. The procedures for RNA extraction and S1 nuclease mapping are described in Materials and Methods. (A) P_C and P_{HS} mRNAs were detected with probe 1. *E. coli* HB101 harboring pASB3 was grown at 30°C (lanes 1 to 5) and at 42°C (lanes 6 to 10). Lanes are labeled as for Fig. 2. (B) P_{HS} mRNA was detected with probe 2. Lanes are labeled as for panel A. The positions of fragments protected by P_C mRNAs and P_{HS} mRNAs, the probe, and DNA size markers (in bases) are indicated.

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