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Distinctive nucleotide sequences of promoters recognized by RNA polymerase containing a phage-coded "σ-like" protein

(*Bacillus subtilis* phage SP01/DNA and RNA sequence determination)

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ABSTRACT **We report the nucleotide sequences of two promoters for bacteriophage SP01 "middle" genes. These promoters are recognized by a modified form of *Bacillus subtilis* RNA polymerase that contains a phage-coded "σ-like" regulatory protein (gp*28*) in place of the bacterial σ factor. Both promoters shared the identical hexanucleotide 5'A-G-G-A-G-A at about 35 base pairs preceding the start point of transcription and the identical heptanucleotide 5'-*T*-*T*-*T*-A-*T*-*T*-*T* (*T* is the thymine analog 5-hydroxymethyluracil in SP01 DNA) located about 10 base pairs preceding the transcriptional start point. The significance of these sequences in comparison with nucleotide sequences of promoters recognized by σ-containing RNA polymerases is discussed.**

Bacillus subtilis phage SP01 expresses its genes in a well-defined temporal sequence that appears to be controlled at the level of promoter recognition (1). Unmodified bacterial RNA polymerase selectively recognizes and binds to promoters for phage "early" genes (1–4). The activation of phage genes expressed at a middle time after infection requires the product of regulatory gene *28* (5). This "σ-like" protein binds to the host core RNA polymerase and directs it to bind to and initiate transcription from promoters for "middle" genes (1, 4, 6, 7). Finally, the products of regulatory genes *33* and *34* bind to the host core polymerase to direct the transcription of phage genes that are expressed late in the lytic cycle (8, 9). This scheme implies that promoters for phage SP01 early, middle, and late genes contain distinctive nucleotide sequences that can be distinguished by different forms of RNA polymerase. What are these sequences and how are they recognized by σ and σ-like regulatory proteins? Here we report the nucleotide sequence of two middle gene promoters recognized by RNA polymerase containing the gene *28* protein and compare these sequences to that of an early gene promoter recognized by unmodified bacterial RNA polymerase containing σ factor.

RESULTS

Isolation of Restriction Fragments Containing Middle Gene Promoters. In a previous study, we utilized the ability of RNA polymerase to form stable binary complexes with promoter sites on DNA to identify a number of *Eco*RI* restriction fragments that contain promoters for the SP01-modified RNA polymerase containing regulatory gene *28* protein (gp*28*) (1). Because all of these *Eco*RI* fragments were too large for direct sequence determination, we have repeated these filter-binding experiments with a *Hpa* II or *Hha* I restriction digest of SP01 DNA and have identified several small fragments that specifically bound polymerase containing gp*28*. Two fragments, a *Hpa* II fragment of approximately 660 base pairs (bp) and a *Hha* I fragment of about 510 bp were selected for further study. Neither of these fragments was bound by the unmodified bacterial RNA polymerase.

First we verified that these fragments were derived from regions of the genome that had previously been shown to contain middle genes. Each of the fragments was radioactively labeled and hybridized to nitrocellulose strips containing electrophoretically separated *Eco*RI* fragments of SP01 DNA. *Hpa* II-660 hybridized to *Eco*RI* fragment 12, and *Hha* I-510 hybridized to *Eco*RI* fragment 19 (not shown). Both of these *Eco*RI* fragments are located on the left arm of SP01 DNA (Fig. 1) in a region containing middle genes (11) and both have previously been shown to contain promoters recognized by phage-modified polymerase (1).

Next, to locate the promoter sites on the *Hpa* II-660 and *Hha* I-510 fragments, we cleaved these DNAs into subfragments with *Hha* I, *Hin*fI, *Kpn* I, and *Sau* 3A. The *Hpa* II-660 fragment was cut into fragments of 190 and 470 bp by *Hha* I (Fig. 2*a*, lane 1), into two fragments of approximately 330 bp each by *Hin*fI, and into two fragments of 440 and 220 bp by *Kpn* I (Fig. 2*a*, lane 5). Filter binding assays showed that phage-modified RNA polymerase bound tightly and selectively to the 470-bp *Hha* I fragment (Fig. 2*a*, lanes 1–4), a 330-bp *Hin*fI fragment (not shown), and the 220-bp *Kpn* I fragment (Fig. 2*a*, lanes 5–8). Thus, the middle gene promoter is within 220 bp of the right end of the *Hpa* II-660 map (Fig. 3).

Fig. 3 also shows the cleavage sites of restriction enzymes *Sau* 3A and *Hin*fI on the 510-bp *Hha* I fragment. In this case, RNA polymerase containing gp*28* bound specifically to the 260-bp *Sau* 3A fragment (not shown) indicating that the promoter is in the left half of the *Hha* I-510 fragment.

Localization of the Middle Gene Promoters. To locate more precisely the promoters on these DNA fragments, we examined the size of the RNA synthesized from each fragment by polymerase containing gp*28*. The transcript copied from *Hpa* II-660 was about 170 bases long (Fig. 2*b*, lane 2). When the *Kpn* I, *Hha* I, or *Hin*fI digest of the fragment was used as template, the RNA species did not vary in size (Fig. 2*b*, lanes 3, 5, and 6). Therefore, either RNA synthesis proceeds rightward, initiating about 170 bp from the right end of the *Hpa* II-660 fragment and terminating at the right end of the fragment, or RNA synthesis proceeds leftward, initiating somewhere within 50 bp of the right end of *Hpa* II-660 and terminating at an internal stop site to the right of the *Kpn* I site.

Next, the exact site of the middle gene promoter on *Hpa* II-660 was determined by "DNase footprinting" (13). *Hpa* II-660 was radioactively labeled at its 5′ ends with polynucleotide kinase and then cleaved with *Kpn* I to generate a 220-bp promoter-containing subfragment (as identified in Fig. 2*a*, lane 5) that was now labeled at only one of its 5′ ends. The radioactive 220-bp subfragment was purified by gel electrophoresis,

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Abbreviations: gp*28*, regulatory gene *28* protein; bp, base pair(s); *T*, 5-hydroxymethyluracil.

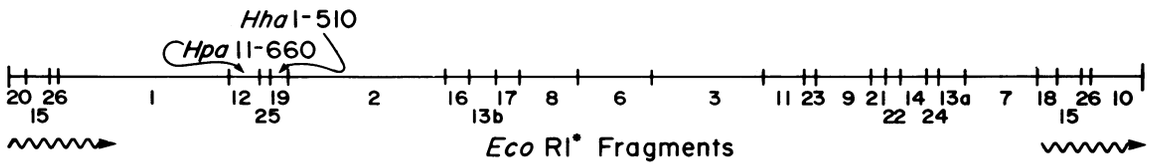

FIG. 1. *Eco*RI* restriction map of the SP01 genome showing the location of fragments *Hpa* II-660 and *Hha* I-510. *Eco*RI* fragments are numbered according to size (10, 11). The positions and orientation of the terminally repeated sequences in SP01 DNA are indicated by wavy arrows.

isolated, and then treated with DNase I in the presence and absence of prebound RNA polymerase containing gp28. A concentration of DNase was chosen that nicked the DNA an average of once per fragment. Because nicking occurred nearly randomly and because the fragment was labeled at only one end, electrophoresis of the partial digest through a denaturing gel generated a "ladder" of radioactive DNA pieces generally differing in size from their neighbors by one nucleotide (13). DNA digested in the absence of RNA polymerase yielded an uninterrupted ladder of fragments representing cleavage at most sites in the 220-bp DNA (Fig. 4). However, when the DNA first was bound to phage-modified RNA polymerase, a stretch of approximately 60–70 bp was protected from DNase I action. This region extended from about 150 to 210–220 bp from the labeled end of the 220-bp fragment. Combined with the RNA synthesis experiment of Fig. 2*b*, this placed the middle gene promoter recognized by gp28-containing polymerase about 170 bp from the right end of the *Hpa* II-660 fragment (see Fig. 3).

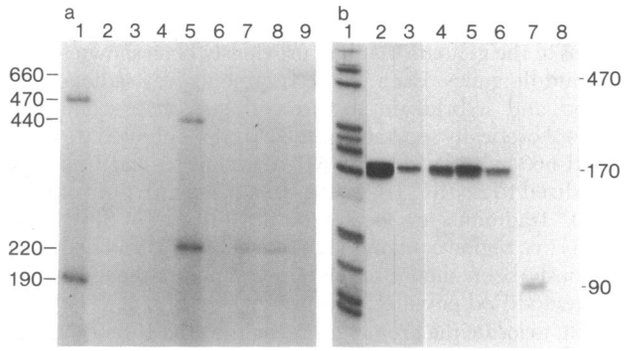

FIG. 2. (*a*) Autoradiograph of *Hha* I and *Kpn* I subfragments of *Hpa* II-660 bound by SP01-modified RNA polymerase. ^{32}P-End-labeled *Hha* I subfragments (lane 1) or ^{32}P-end-labeled *Kpn* I subfragments (lane 5) of *Hpa* II-660 (lane 9) (0.5 pmol each) were incubated with 2.0 pmol of gp28-containing enzyme, and stable binding complexes were isolated on a nitrocellulose filter as described (1). After elution from the filter, fragments were separated by polyacrylamide gel electrophoresis and visualized by autoradiography (exposure time, 1 wk). The binding reactions were as follows: lane 2, *Hha* I subfragments, no enzyme; lanes 3 and 4, *Hha* I subfragments, gp28-containing polymerase; lane 6, *Kpn* I subfragments, no enzyme; and lanes 7 and 8, *Kpn* I subfragments, gp28-containing polymerase. The sizes (bp) of these restriction fragments are indicated at the left; these sizes were computed by using the *Hae* III fragments of the plasmid pMB9 as standards. However, because pMB9 DNA contains thymine whereas SP01 DNA contains hydroxymethyluracil (12) and because DNA containing the latter migrates slightly slower than DNA containing the former (unpublished data), the sizes presented here are not exact. Restriction reactions were performed as described (11). (*b*) RNA species synthesized by SP01-modified RNA polymerase from *Hpa* II-660 and *Hha* I-510. The *in vitro* transcription reactions (20 μl) contained binding buffer (40 mM Tris·HCl, pH 7.9/0.4 mM potassium phosphate/10 mM $MgCl_2$/0.1 mM EDTA/1 mM dithiothreitol/500 μg of bovine serum albumin per ml), 100 μM cytidine, adenosine, and guanosine triphosphates, and 3 μM uridine [α-^{32}P]-triphosphate [specific activity, 400 Ci/mmol (1 Ci = 3.7×10^{10} becquerels); Amersham], 2 pmol of SP01-modified RNA polymerase containing gp28, and about 0.5 pmol of template. Templates were: lane 2, undigested *Hpa* II-660; lane 3, *Kpn* I-digested *Hpa* II-660; lane 4, undigested *Hpa* II-660; lane 5, *Hha* I-digested *Hpa* II-660; lane 6, *Hin*fI-digested *Hpa* II-660; lane 7, *Hin*fI-digested *Hha* I-510; lane 8, undigested, *Hha* I-510. Enzyme and DNA were preincubated for 10 min at 37°C. After addition of nucleotide triphosphates, incubation was continued for 20 min at 37°C; the reaction was terminated by addition of 10–20 μg of tRNA and ammonium acetate (pH 7.1) to 2.5 M. The RNA was then ethanol-precipitated, resuspended in loading buffer with formamide and dyes (80% deionized formamide/1 mM EDTA/50 mM Tris borate, pH 8.3/0.1% xylene cyanol and bromophenol blue), heated at 90°C for 2 min, and subjected to electrophoresis on an 8% polyacrylamide 7 M urea gel. The transcripts were visualized by autoradiography (exposure time, 2 hr); their sizes (in bases) are indicated on the right. As molecular weight markers, lane 1 contained denatured DNAs from a *Hae* III digest of the plasmid pMB9.

Similar methods were used to locate the promoter for the SP01-modified RNA polymerase on the *Hha* I-510 fragment. Filter binding assays had located the promoter on the 260-bp *Sau* 3A fragment depicted at the left end of the map of the *Hha* I-510 fragment (Fig. 3). The RNA synthesized from the *Hha* I-510 fragment was approximately 470 bases long (Fig. 2*b*, lane 8), placing the start point of RNA synthesis about 40 bases from the left end of *Hha* I-510. As predicted from this location, when either a *Hin*fI or a *Sau* 3A digest of *Hha* I-510 was used as template, the resultant RNA species was either 90 or 220 bases long, respectively [Fig. 2*b*, lane 7 (*Sau*3A data not shown)]. A "DNase footprinting" experiment verified that this middle gene promoter mapped very close to the end of the *Hha* I-510 fragment; RNA polymerase containing gp28 protected the DNA up to about 60 nucleotides from the left end (not shown).

Nucleotide Sequence of Promoters. The strategy for sequencing the promoter located on the *Hpa* II-660 fragment was to isolate the 330-bp *Hin*fI subfragment that contained the promoter (see Fig. 3) and to label both of its 5′ ends with ^{32}P by using polynucleotide kinase. The two strands of this fragment were then separated and their sequences were determined by the method of Maxam and Gilbert (15, 16). [SP01 DNA differs from genomes previously sequenced because it contains hydroxymethyluracil in place of thymine (12). Although this analog did not interfere with the chemical sequencing reactions, hydroxymethyluracil was more susceptible to the hydrazine/piperidine reaction than was cytosine.] Fig. 5 presents the nucleotide sequence of the promoter region determined from the strand whose ^{32}P-labeled 5′ terminus was at the *Hin*fI end of the DNA. This sequence was confirmed by sequence determination of the promoter region of the complementary strand.

To localize precisely the start point of RNA synthesis on this stretch of DNA, we determined the sequence of the 5′ terminus of the RNA copied by gp28-containing polymerase from the *Hpa* II-660 fragment. First, we identified the starting nucleotide of this 170-base RNA as being ATP [decreasing the ATP concentration to 20 μM almost completely inhibited synthesis of the 170-base RNA, whereas 20 μM CTP, GTP, or UTP did not appreciably affect synthesis (data not shown); higher con-

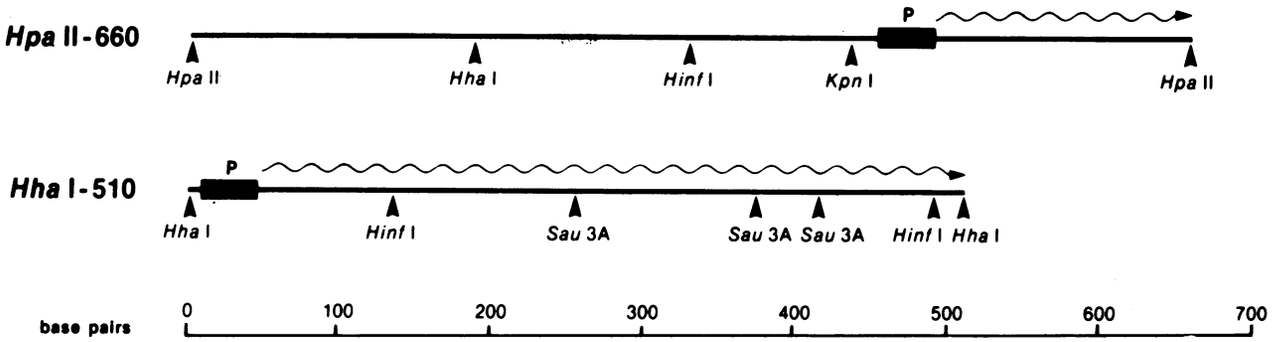

FIG. 3. Restriction endonuclease maps of fragments *Hpa* II-660 and *Hha* I-510. The thick black bars labeled P denote the promoter regions. The location of transcripts copied from *Hpa* II-660 and *Hha* I-510 are designated by the wavy arrows.

centrations of nucleotide are known to be required for initiation than for elongation]. Thus, in order to determine the sequence of the RNA, the 5′ terminus was labeled by initiating transcription with adenosine [γ-^{32}P]triphosphate. The resulting 170-base transcript was isolated and sequenced by the RNase cleavage method of Donis-Keller *et al.* (17). As shown in Fig. 6, the first 14 bases of the RNA transcript were 5′pppA-A-U-U-G-C-G-G-U-A-A-G-U-G which matched exactly with a DNA sequence in the promoter region of *Hpa* II-660. The start point of RNA synthesis identified by this sequence is marked by a large arrowhead in Fig. 5.

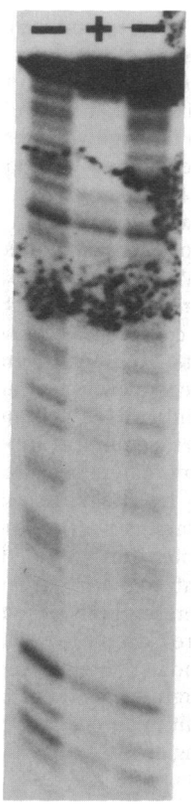

FIG. 4. Nucleotides protected from the nicking action of DNase I by phage-modified enzyme containing gp28. The 220-bp *Kpn* I fragment of *Hpa* II-660, labeled at the 5′-*Hpa* II-end by the exchange kinase reaction (14), was incubated (37°C, 10 min) either with or without gp28-containing polymerase (1.5 pmol of enzyme per 0.5 pmol of DNA in 20 μl of binding buffer plus 2 mM $CaCl_2$) and subsequently treated with DNase I (Worthington) the final nuclease concentration was 1 ng/ml. After 1 min at 37°C the reaction was terminated by a DNase stop solution (final concentrations: 50 mM EDTA, 2.5 M ammonium acetate, and 20 μg tRNA). The fragments were precipitated by ethanol, resuspended in the loading buffer with formamide and dyes, incubated at 90°C for 2 min, and subjected to electrophoresis on a 10% polyacrylamide/8.3 M urea gel. −, Reactions without RNA polymerase; +, reaction with polymerase.

To determine the sequence of the promoter contained on *Hha* I-510, the fragment was treated with kinase and digested with *Sau* 3A, and the 260-bp subfragment with a 5′-^{32}P-labeled *Hha* I end was isolated and its sequence was determined. The nucleotide sequence from the *Hha* I end through the promoter region is presented in Fig. 5. This sequence was confirmed by sequencing in from the 5′-^{32}P-labeled *Hha* I end of the 130-bp subfragment generated by *Hin*fI digestion of *Hha* I-510. To determine the exact location of the initiation site for RNA synthesis on *Hha* I-510, we sequenced the 5′ terminus of the RNA copied by gp28-containing enzyme. When RNA synthesis was measured by incorporation of either adenosine [γ-^{32}P]-triphosphate or guanosine [γ-^{32}P]triphosphate, the initiating nucleotide of the 90-base transcript was found to be uniquely GTP. (Other experiments had indicated that neither CTP nor UTP was the starting nucleotide because decreasing the concentration of either nucleoside triphosphate to 5 μM did not inhibit synthesis of this RNA.) Radioactive RNA with γ-^{32}P was isolated and its sequence was determined as described above. The first 15 bases of the 90-base transcript were 5′pppG-A-A-C-X-A-G-U-U-C-X-A-G-G-G (X is either A or U), which matched the DNA sequence starting 44 nucleotides from the left end of *Hha* I-510 (not shown). The initiation site of transcription on *Hha* I-510 as identified by this sequence is indicated by the large arrowhead in Fig. 5.

DISCUSSION

We have determined the nucleotide sequences of two SP01 promoters recognized by *B. subtilis* RNA polymerase containing a phage-coded "σ-like" regulatory protein (gp28). These polymerase binding sites were approximately 60–70 bp long as determined by the ability of the phage-modified enzyme to protect DNA in the vicinity of the start point of transcription from the nicking action of DNaseI (18). The polymerase-protected DNA region extended from about 40–50 bases (position −40 to −50) preceding the start point of RNA synthesis to about 20 bases (position +20) past this site. Little or no DNA upstream from this protected stretch of nucleotides was essential for binding and transcription *in vitro* because gp28-containing RNA polymerase initiated on a restriction fragment (*Hha* I-510) whose terminus was 43 bp from the start point of RNA synthesis.

How do the nucleotide sequences of the promoters recognized by gp28-containing RNA polymerase compare with the nucleotide sequences of promoters recognized by σ-containing RNA polymerase? The binding sites recognized by *Escherichia coli* RNA polymerase containing σ factor are about 60–75 bp long (18, 19) and exhibit two regions of homology: one region from about −35 to −30 which is sometimes called the −35 re-

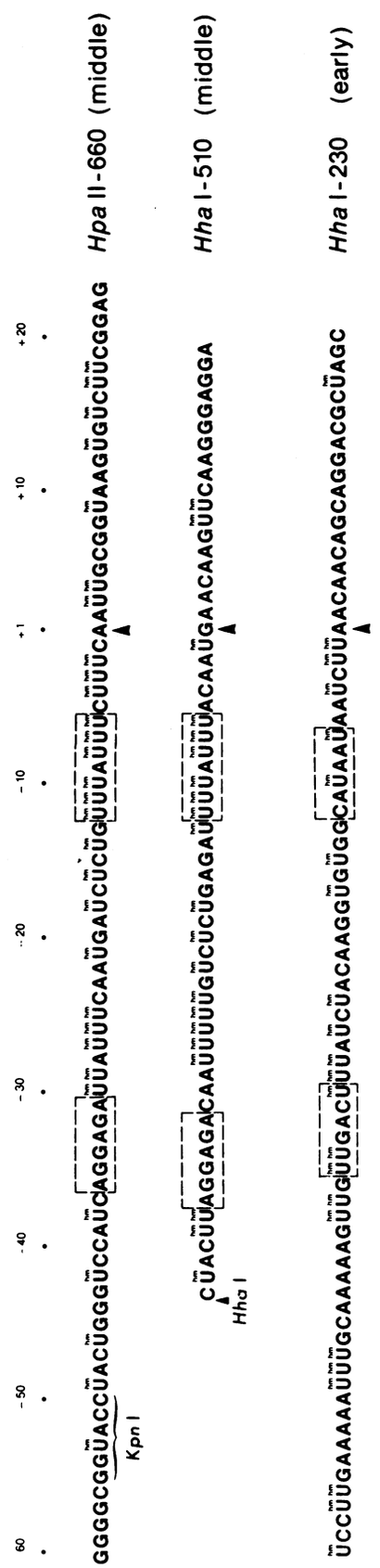

FIG. 5. Nucleotide sequences of two middle gene promoters and one early gene promoter of phage SP01. The sequences of the middle gene promoters contained on *Hpa* II-660 and *Hha* I-510 were derived as discussed in the text. The early gene promoter was contained on *Hha* I-230, a subfragment of *Eco*RI* fragment 26 (see Fig. 1); details of the promoter's localization and sequence determination are in ref. 25 and will be published elsewhere. The sequences on *Hpa* II-660, *Hha* I-510, and *Hha* I-230 were aligned according to their initiation sites (at +1); these sites are denoted by the large vertical arrowheads. Identical hexanucleotide and heptanucleotide sequences shared by the two middle gene promoters of *Hpa* II-660 and *Hha* I-510 in the −35 and the −10 region, respectively, are enclosed by boxes. These homologous sequences were distinct from those located in the −35 region and the Pribnow box of the early gene promoter on *Hha* I-230 (the latter two regions are also in boxes). The *Kpn* I and *Hha* I restriction sites are depicted by the bracket and small arrowhead, respectively.

gion and another region from about −12 to −7 which is referred to as the Pribnow box (20–22). Both of these regions play a critical role in promoter recognition; mutations or chemical modifications in either can interfere with RNA polymerase binding (20, 21, 23, 24). Based on an analysis of 32 promoters, the "most common sequence" of the −35 region is 5′-T-T-G-A-C-A and the most common Pribnow box sequence is 5′-T-A-T-A-A-T (22). The sequence of an SP01 early gene promoter that is recognized by σ-containing *B. subtilis* RNA polymerase has been determined and is shown is Fig. 5 (25). The nucleotide sequence of this SP01 promoter bore a strong homology to promoters recognized by *E. coli* polymerase, differing by only one nucleotide from the most common sequences in both the −35 region and the Pribnow box region. This is not surprising because SP01 early genes are transcribed by both *B. subtilis* and *E. coli* RNA polymerases (ref. 26; unpublished results).

In contrast, the two SP01 middle gene promoters described here exhibited distinctive sequences in both the −35 region and Pribnow box region. In the −35 region, both middle gene promoters contained the identical hexanucleotide 5′-A-G-G-A-G-A. The sequence was quite different from the 5′*T*-*T*-G-A-C-*T* found in the −35 region of the early gene promoter. (*T* represents the thymine analog 5-hydroxymethyluracil.) Both middle gene promoters also contained an identical heptanucleotide, 5′-*T*-*T*-*T*-A-*T*-*T*-*T* centered on position −10. This A·*T*-rich heptamer bore some resemblance to the Pribnow box sequence of promoters recognized by σ-containing RNA polymerases; it matched the most common sequence (5′-T-A-T-A-A-T) in four of six nucleotides. On the other hand, both heptanucleotides lacked the highly conserved As in the second and fifth positions of the Pribnow box sequence. Whether this degree of homology to the Pribnow box sequence is of functional significance remains to be determined.

The middle gene promoter sequences 5′-A-G-G-A-G-A at −35 and 5′-*T*-*T*-*T*-A-*T*-*T*-*T* at −10 are particularly interesting features because they are located in exactly the same positions as the two regions of *E. coli* promoters known to be critical for binding by σ-containing RNA polymerase (20, 21, 23, 24). Perhaps, therefore, these sequences play a functional role in the binding of gp28-containing RNA polymerase to middle gene promoters. [Indeed, these sequences appear to be general features of middle gene promoters because preliminary experiments in this laboratory have identified similar hexamers near position −35 and heptamers near position −10 in two other gp28-controlled promoters (unpublished results).]

Finally, we note that, like other promoters recognized by bacterial RNA polymerases, both the early and middle gene promoters of SP01 were extremely A·*T*-rich in the region between −30 and −20 and in the region preceding the initiation site (−12 to +3). A high A·T content in the latter region is thought to be important in polymerase-promoter interactions because local unwinding of DNA near the start site occurs prior to initiation of RNA synthesis (27, 28).

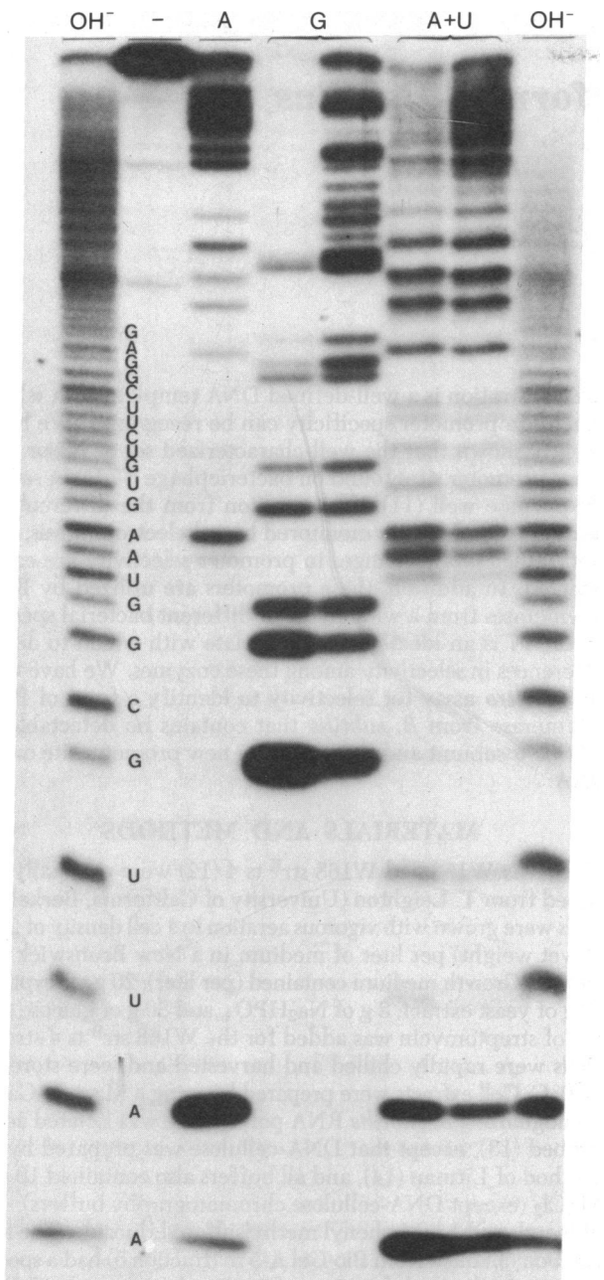

FIG. 6. 5′-Terminal sequence of RNA copied from *Hpa* II-660. RNA was synthesized for sequence determination as described in Fig. 2 except for the following modifications: 2–5 pmol of the *Hpa* II-660 fragment was transcribed by 1.5 pmol of gp*28*-containing RNA polymerase (in a 10-μl reaction volume), at nucleotide concentrations of 100 μM cytidine, uridine, and guanosine triphosphates and 100 μM adenosine[γ-^{32}P]triphosphate (specific activity, 3000 Ci/mmol; New England Nuclear). For sequencing, the 170-base transcript was eluted electrophoretically from a 6% polyacrylamide/7 M urea gel, precipitated by ethanol, and resuspended in double-distilled H_2O. Partial cleavage products of the γ-^{32}P-labeled 170-base RNA species were obtained according to the method of Donis-Keller *et al.* (17) and were separated on a 20% polyacrylamide/7 M urea gel. OH^- lanes, partial alkaline hydrolysis of the RNA; –, undigested sample of the transcript; A lane, partial cleavage products generated by 2 units of RNase U2; G lanes, partial cleavage products generated by 0.2 and 0.05 unit of RNase T1, respectively; A+U lanes show partial cleavage products generated by 1.5 and 0.5 μl, respectively, of RNase purified from *Physarum* (gift from H. Donis-Keller). The letters to the right of the alkaline hydrolysis reaction represent the first 23 nucleotides of the RNA sequence.

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1. Talkington, C. & Pero, J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1185–1189.
2. Pero, J., Nelson, J. & Fox, T. D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1589–1593.
3. Duffy, J. J. & Geiduschek, E. P. (1975) *J. Biol. Chem.* **250,** 4530–4541.
4. Spiegelman, G. B. & Whiteley, H. R. (1978) *Biochem. Biophys. Res. Commun.* **81,** 1058–1065.
5. Fujita, D. J., Ohlsson-Wilhelm, B. M. & Geiduschek, E. P. (1971) *J. Mol. Biol.* **57,** 301–317.
6. Fox, T. D., Losick, R. & Pero, J. (1976) *J. Mol. Biol.* **101,** 427–433.
7. Duffy, J. J. & Geiduschek, E. P. (1977) *Nature (London)* **270,** 28–32.
8. Fox, T. D. (1976) *Nature (London)* **262,** 748–753.
9. Tjian, R. & Pero, J. (1976) *Nature (London)* **262,** 753–757.
10. Talkington, C. & Pero, J. (1977) *Virology* **83,** 365–379.
11. Pero, J., Hannett, N. & Talkington, C. (1979) *J. Virol.* **31,** 156–171.
12. Okubo, S., Strauss, S. & Stodosky, R. (1964) *Virology* **24,** 552–562.
13. Galas, D. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3157–3170.
14. Berkner, K. L. & Folk, W. R. (1977) *J. Biol. Chem.* **252,** 3176–3184.
15. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
16. Maxam, A. M. & Gilbert, W. (1979) *Methods Enzymol.* **65,** in press.
17. Donis-Keller, H., Maxam, A. M. & Gilbert, W. (1977) *Nucleic Acids Res.* **4,** 2527–2538.
18. Schmitz, A. & Galas, D. J. (1979) *Nucleic Acids Res.* **6,** 111–137.
19. Ptashne, M., Backman, K., Humayan, M. Z., Jeffrey, A., Maurer, R., Meyer, B. & Sauer, R. T. (1976) *Science* **194,** 156–161.
20. Gilbert, W. (1976) in *RNA Polymerase,* eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 193–205.
21. Reznikoff, W. S. & Abelson, J. N. (1978) in *The Operon,* eds. Miller, J. H. & Reznikoff, W. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 221–243.
22. Siebenlist, U. (1979) *Nucleic Acids Res.* **6,** 1895–1907.
23. Johnsrud, L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5314–5318.
24. Mozola, M. A., Friedman, D. I., Crawford, C. L., Wulff, D. L., Shimatake, H. & Rosenberg, M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1122–1125.
25. Talkington, C. (1979) Dissertation (Harvard University, Cambridge, MA).
26. Grau, O., Ohlsson-Wilhelm, B. M. & Geiduschek, E. P. (1970) *Cold Spring Harbor Symp. Quant. Biol.* **35,** 221–226.
27. Hsieh, T. & Wang, J. C. (1978) *Nucleic Acids Res.* **5,** 3337–3345.
28. Siebenlist, U. (1979) *Nature (London)* **279,** 651–652.