Proc. Natl. Acad. Sci. USA
Vol. 75, No. 3, pp. 1185–1189, March 1978
Biochemistry

Promoter recognition by phage SP01-modified RNA polymerase

(positive regulatory protein/restriction endonuclease fragments/filter binding assay)

CAROL TALKINGTON AND JANICE PERO

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Communicated by Jack L. Strominger, December 27, 1977

ABSTRACT **A modified form of *Bacillus subtilis* RNA polymerase containing a phage SP01-coded regulatory protein (the gene *28* product) selectively transcribes "middle" genes of the phage genome *in vitro*. In this paper, we identify a subset of restriction endonuclease fragments of SP01 DNA that promote specific transcription by the phage-modified polymerase. In the absence of nucleoside triphosphates, RNA polymerase containing the gene *28* protein selectively binds to these DNA fragments thereby forming stable binary complexes that can be isolated on nitrocellulose filters. In contrast, unmodified RNA polymerase containing sigma factor selectively binds to and transcribes a subset of phage DNA fragments that contain "early" sequences and that are in large part distinct from the fragments recognized by the phage-modified transcriptase. Our results strongly suggest that phage "early" and "middle" genes are transcribed from distinct promoters and that the RNA polymerase containing the gene *28* protein binds to sites that are located at or near promoters for SP01 "middle" genes.**

The specificity of gene transcription by RNA polymerase in *Bacillus subtilis* is modified by infection with bacteriophage SP01 (1–4). The unmodified RNA polymerase holoenzyme of the host bacteria specifically copies only "early" genes on the phage genome (1, 3). Early in the lytic cycle the product of SP01 regulatory gene 28 binds to the host core polymerase and directs the transcription of SP01 "middle" genes (5, 6). This modified RNA polymerase, known as enzyme B, contains the 26,000-dalton gene *28* protein and lacks the host sigma component of polymerase (1, 2). Then, at an intermediate time after infection, the products of regulatory genes *33* and *34* bind to the host core RNA polymerase and direct the transcription of phage "late" genes (2, 7, 8).

How do these phage-coded regulatory proteins alter the specificity of gene transcription by RNA polymerase? One possibility is that they act in a manner analogous to host sigma factor and direct the core enzyme to recognize specific promoters on the phage DNA (for a discussion of this and other possibilities, see ref. 9). To investigate this question we have examined the ability of RNA polymerase containing the gene *28* protein to selectively bind restriction fragments of the SP01 genome that have promoters for middle genes. Here we report that unmodified RNA polymerase holoenzyme selectively binds DNA fragments containing phage early gene promoters, and that the SP01-modified polymerase, enzyme B, selectively binds DNA fragments containing middle gene promoters.

MATERIALS AND METHODS

Bacterial and Phage Strains. Wild-type *Bacillus subtilis* strain NCTC 3610 was used for all experiments. Wild-type SP01, obtained from D. Shub, was grown and partially purified as described (10).

Preparation and Restriction of SP01 DNA. SP01 DNA was extracted with phenol from wild-type phage that had been grown and purified as described (11). SP01 DNA and *Eco*RI endonuclease (10 units/μg of DNA) were incubated for 2 hr under optimal conditions for *Eco*RI* activity: 37°, 25 mM Tris·HCl, pH 8.5/2 mM $MgCl_2$ (12). DNA fragments were extracted with phenol and then with ether, precipitated by ethanol, and resuspended in 10 mM Tris·HCl, pH 8.0/1 mM EDTA.

RNA Synthesis and Hybridization *In Vitro*. *B. subtilis* RNA polymerase (holoenzyme) from uninfected cells and RNA polymerase containing the gene *28* protein (enzyme B) from phage-infected cells were purified as described (11). Using an *Eco*RI* restriction digest of SP01 DNA as template, RNA was synthesized, extracted, and hybridized (2×10^5 cpm of SP01 [^{32}P]RNA) to nitrocellulose strips containing SP01 DNA fragments (1 μg/strip) as described (11).

End-Labeling of SP01 DNA Restriction Fragments. DNA restriction fragments were treated with Worthington bacterial alkaline phosphatase (25 units/nmol of 5′-ends) for 1 hr at 50° in 10 mM Tris·HCl, pH 8.0/1 mM EDTA, extracted with phenol three times, and precipitated with 0.3 M sodium acetate and 2.5 volumes of 95% ethanol. The dephosphorylated fragments were resuspended in 75 μl of 5 mM Tris·HCl, pH 9.5/0.01 mM EDTA/0.1 mM spermidine and 10 μl of 500 mM Tris·HCl, pH 9.5/100 mM $MgCl_2$/50 mM dithiothreitol (13). One hundred picomoles of adenosine [γ-^{32}P]triphosphate (specific activity 1000 Ci/mmol) and 5 units of polynucleotide kinase (New England Biolabs) were added. The reaction mixture (final volume 100 μl) was incubated for 30 min at 37°; the reaction was terminated by addition of 300 μl of 2 M ammonium acetate. The labeled fragments were extracted with phenol, precipitated by ethanol, and resuspended in 10 mM Tris·HCl, pH 8.0/1 mM EDTA. The kinase reaction did not label all fragments equally (Fig. 3*c*).

RNA Polymerase Binding to DNA Fragments. The binding reaction mixture (25 μl) consisted of binding buffer (40 mM Tris·HCl, pH 7.9/10 mM $MgCl_2$/0.1 mM EDTA/1 mM dithiothreitol/0.4 mM potassium phosphate), 50 mM KCl (unless otherwise indicated), 12.5 μg of bovine serum albumin, 0.5 μg of 5′-[γ-^{32}P]phosphorylated *Eco*RI* SP01 DNA restriction fragments, and the indicated units of RNA polymerase. (A unit of enzyme incorporates 1 nmol of ATP in 10 min at 37°, with SP01 DNA as template. For holoenzyme, 1 unit was equal to 1.7 μg of polymerase; for enzyme B, 1 unit equalled 8 μg of polymerase. For SP01 DNA, 1 μg equals 10^{-2} pmol.)

After incubation at 37° for 10 min, the binding reaction was terminated by the addition of 5 μg of unlabeled calf thymus DNA and incubation was continued for another 10 min. [When heparin was used as a competitor, the binding reaction was terminated by the addition of 62 ng of heparin (Upjohn) in place of calf thymus DNA and the incubation was continued for another 10 min.] The reaction mixture was diluted to 500 μl with binding buffer that had been warmed to 37°. The solution was filtered with gentle suction through Millipore HA nitrocellulose filters (24 mm diameter) that had been soaked for 30 min in binding buffer. The filters were rinsed with 500

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

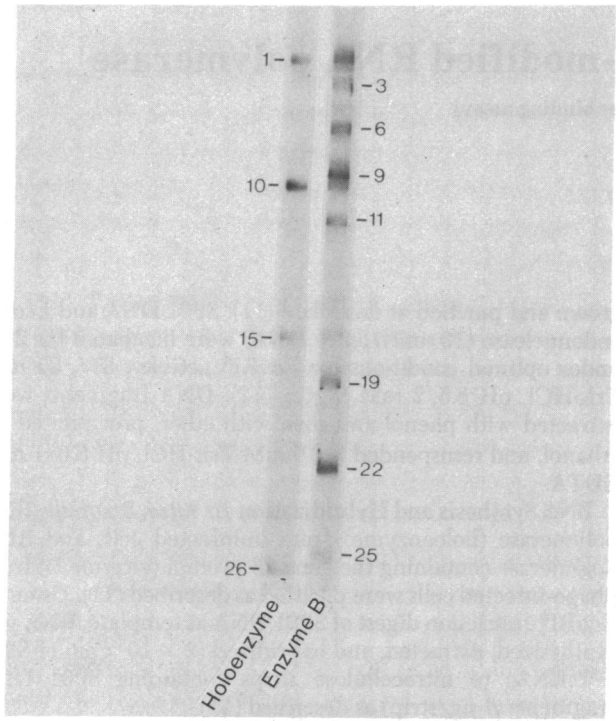

FIG. 1. Hybridization pattern of RNA synthesized from *Eco*RI*-digested SP01 DNA. Nitrocellulose filter strips containing *Eco*RI* restriction fragments of SP01 DNA (1 μg) were annealed with [^{32}P]RNA (2 × 10^5 cpm) synthesized *in vitro* by holoenzyme (10 units/pmol of DNA) and by enzyme B (15 units/pmol of DNA), with an *Eco*RI* digest (10 μg) of the SP01 genome as template. About 10% of the input RNA hybridized to the strips. Hybrids were visualized by exposure to x-ray film (Kodak SB5) for 2 days.

μl of binding buffer, dried, and subjected to Cerenkov counting.

Retained DNA fragments were eluted from the filter with 1% sodium dodecyl sulfate/40 mM Tris·HCl, pH 7.9, precipitated by ethanol, and resuspended in 60 μl of sample buffer (0.01% bromophenol blue/4 mM Tris·HCl, pH 7.8/0.5 mM sodium acetate/0.1 mM EDTA/0.1% sodium dodecyl sulfate) to be analyzed by agarose gel electrophoresis as described (11), except the gel was 0.7% agarose (wt/vol). The DNA fragments were visualized by exposing the dried gel to x-ray film (Kodak XR5 with Quanta II intensifying screens, at −70°) for approximately 1 day.

RNA Chain Initiation on DNA Fragments. RNA polymerase was bound to SP01 DNA fragments as described in the preceding section. Unlabeled calf thymus DNA and the four nucleoside triphosphates (final concentration 100 μM) were added, increasing the reaction volume to 50 μl. Initiation with ATP was assayed by reducing the GTP concentration to 1 μM; GTP initiations were assayed at 1 μM ATP (14). After incubation for 1 min at 37°, RNA synthesis was terminated by the addition of EDTA to a final concentration of 20 mM. In order to select for initiation complexes, KCl was added to 250 mM and the reaction mixture was chilled to 0° for 30 min. After dilution to 500 μl, the reaction mixture was filtered and the initiation complexes were analyzed as above.

RESULTS

Transcription of Endonuclease Restriction Fragments *In Vitro*. The restriction endonuclease *Eco*RI* cleaves SP01 DNA into about 26 fragments which range in size from 1 to 21 kilobases (Fig. 3*c*). To determine which fragments contained promoters for unmodified and phage-modified forms of RNA polymerase we used *Eco*RI*-restricted DNA as a template for transcription *in vitro*. Fragments that promoted RNA synthesis were identified by annealing [^{32}P]RNA synthesized *in vitro* to strips of nitrocellulose that had been imprinted with electrophoretically separated *Eco*RI* DNA fragments (11). Hybrids were visualized by autoradiography. *Eco*RI* fragments that served as specific templates for transcription *in vitro* were presumed to contain promoters for modified or unmodified forms of RNA polymerase.

RNA synthesized *in vitro* by RNA polymerase from uninfected bacteria (holoenzyme) hybridized primarily to fragments 1, 10, 15, and 26 (Fig. 1). These DNA segments contain sequences that are actively transcribed at early times in the SP01 lytic cycle (11). In contrast, a form of phage-modified RNA polymerase containing the SP01 regulatory gene *28* product (termed enzyme B) preferentially synthesized RNA from fragments 1, 3, 6, 9, 11, 19, 22, and 25 (Fig. 1). These fragments contain sequences that are transcribed at a middle time in the phage lytic cycle (11). At least certain DNA segments (e.g., fragments 21 and 23) that contain middle sequences and that are transcribed from *uncut* SP01 DNA by enzyme B *in vitro* were not copied when *Eco*RI*-restricted DNA was used as a template; *Eco*RI* cleavage apparently severed middle genes in these segments from their respective promoters for middle transcription.

Our laboratory has recently mapped the location of most of the *Eco*RI* restriction fragments on the SP01 genome. The positions of those *Eco*RI* fragments that supported early and middle transcription *in vitro* are shown in Fig. 2. Although the details of our endonuclease restriction mapping will be published elsewhere (J. Pero, N. Hannett, and C. Talkington, unpublished data), it is relevant to note here that the fragments most actively transcribed by holoenzyme are clustered in terminally redundant ends of the phage genome, while the fragments apparently containing the most active promoters for enzyme B are clustered in two groups: one group adjacent to the redundant region at the left end of the map and the other group clustered just to the right of the center of the map.

A complication in identifying promoters on fragments by *in vitro* transcription of *Eco*RI*-cut DNA is that our *Eco*RI* restriction pattern does not represent a limit digest. *Eco*RI* restriction endonuclease does not cleave all recognition sites in the hydroxymethyluracil-containing phage DNA with equal efficiency (11). Thus, certain fragments that appeared to promote transcription *in vitro* may actually have been transcribed as part of a larger promoter-containing segment of SP01 DNA. However, our mapping data, based on the two-dimensional hybridization procedure of Hutchison (cited in ref. 15), have shown that none of the putative promoter-containing segments identified in Fig. 2 contains sequences in common with a larger, partially cleaved *Eco*RI* fragment. In fact, only two fragments (4 and 5) are due to incomplete cleavage, and neither of these

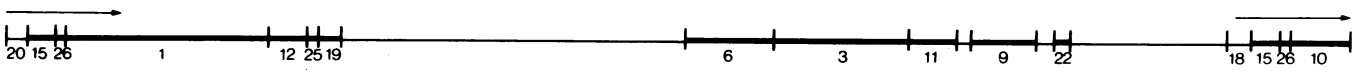

FIG. 2. Schematic map of the SP01 genome showing the location of those *Eco*RI* restriction fragments that contain the most prominent promoters for early and middle genes. Fragments presumed to contain early or middle gene promoters are indicated with a bold line and numbered as in the text. Also included are the fragments in the 12-kilobase region of terminal redundancy; the orientation and location of these repeated sequences are indicated by the arrows. A detailed account of our endonuclease restriction mapping will be published elsewhere (J. Pero, N. Hannett, and C. Talkington, unpublished data).

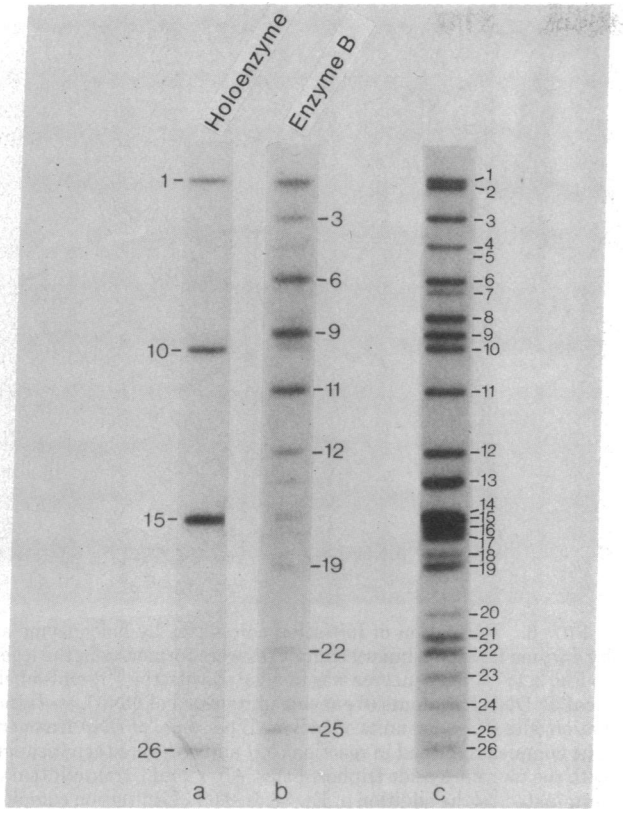

FIG. 3. Agarose gel electrophoresis of SP01 DNA fragments retained on nitrocellulose filters by holoenzyme and by enzyme B. ^{32}P-End-labeled *Eco*RI* DNA fragments were incubated with either holoenzyme (6 units/pmol of DNA) or enzyme B (6 units/pmol of DNA), and stable binding complexes were isolated by the filter binding assay. DNA fragments were subsequently eluted from the filters with sodium dodecyl sulfate and separated by agarose gel electrophoresis. Radioactive fragments were visualized by autoradiography. Fragments retained on the filters by holoenzyme and enzyme B are displayed in *a* and *b*, respectively. *c*, The total pattern of ^{32}P-end-labeled *Eco*RI* fragments.

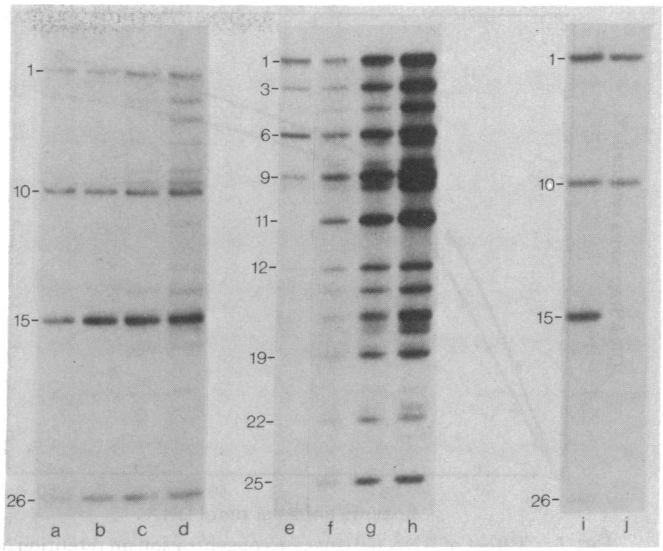

FIG. 4. Effect of RNA polymerase concentration and heparin on the formation of stable binding complexes by holoenzyme and enzyme B. DNA fragments were selectively bound by RNA polymerase, filtered, eluted, and separated by agarose gel electrophoresis as described in the legend to Fig. 3. (*a–d*) Fragments bound by holoenzyme at concentrations of (*a*) 3, (*b*) 7.5, (*c*) 15, and (*d*) 30 enzyme units per pmol of DNA. (*e–h*) Fragments bound by enzyme B at concentrations of (*e*) 3, (*f*) 6, (*g*) 12, and (*h*) 30 enzyme units per pmol of DNA. (*i* and *j*) Effect of heparin on the ability of holoenzyme to form stable binding complexes. Holoenzyme was mixed with ^{32}P-end-labeled *Eco*RI* fragments (6 enzyme units/pmol of DNA) under the binding conditions described in *Materials and Methods*. The reaction depicted in *i* was carried out in the absence of any competitor. The binding complexes depicted in *j* were competed with heparin (2.5 μg/ml).

fragments contains a major promoter for early or middle transcription. (Fragment 4 contains segments 7 and 18, while fragment 5 is composed of segments 8 and 17.)

Another complication in identifying promoter-containing fragments is that SP01 DNA is terminally redundant (16). Thus, fragment 20, which is located at one end of the SP01 genome, has sequences in common with fragment 18 while fragment 10, which is located at the other end, has sequences in common with fragment 1. Therefore, we could not be certain whether fragment 10 contained its own promoter(s) for early transcription or that RNA which hybridized to fragment 10 was actually copied from fragment 1. However, as will be shown below, both fragments 1 and 10 (as well as the other fragments that were transcribed *in vitro*) contained tight binding sites for RNA polymerase.

As a further test to identify fragments that contain promoters, fragments 25 and 26 have recently been purified by elution from a polyacrylamide gel. In confirmation of our identification of an early promoter on fragment 26 and a middle promoter on fragment 25, fragment 26 specifically promoted transcription by holoenzyme, while fragment 25 preferentially stimulated transcription by enzyme B (G. Lee, personal communication).

RNA Polymerase–DNA Fragment Binding Complexes. How do holoenzyme and enzyme B selectively transcribe different segments of the SP01 genome? One possibility is that phage early genes and phage middle genes are controlled by distinct promoters and that RNA polymerase containing sigma factor recognizes promoters for early sequences while RNA polymerase containing SP01 gene 28 protein recognizes middle promoters. As a test of this idea, we have taken advantage of the ability of RNA polymerase to bind tightly to promoter sites on the DNA in the absence of nucleoside triphosphates, thereby forming stable binary complexes of enzyme and DNA. Fragments of DNA that form such stable binding complexes with RNA polymerase can be isolated by their ability to be retained on nitrocellulose filters (17). Therefore, if holoenzyme and enzyme B recognize different promoters, then these two forms of *B. subtilis* RNA polymerase ought to bind tightly to, and hence retain on nitrocellulose filters, distinct subsets of the 26 *Eco*RI* restriction fragments of SP01 DNA. We have examined here the ability of holoenzyme and enzyme B to complex with specific SP01 DNA fragments; fragments retained on nitrocellulose were identified by elution from the filters and electrophoresis through agarose.

Holoenzyme and enzyme B bound distinct but overlapping subsets of the 26 *Eco*RI* fragments (Fig. 3). At low concentrations of polymerase, holoenzyme selectively retained on filters fragments 1, 10, 15, and 26 (Fig. 3*a*) while enzyme B retained fragments 1, 3, 6, 9, 11, 12, 13, 14 (or 15), 19, 22, 25, and to a lesser extent certain other fragments (Fig. 3*b*). Fragments bound by holoenzyme corresponded to those that contain phage early sequences (11) and that were selectively transcribed *in vitro* by unmodified bacterial RNA polymerase (Fig. 1). Similarly, all the fragments bound by enzyme B contain DNA sequences that are expressed at a middle time in the phage lytic cycle (11). These fragments were transcribed *in vitro* by phage-modified polymerase containing the gene 28 protein (Fig. 1).

Fig. 4 displays the effect of varying the concentration of

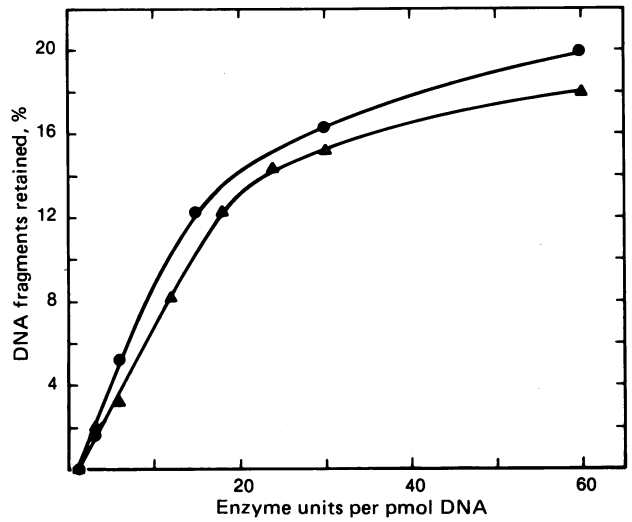

FIG. 5. Effect of RNA polymerase concentration on retention of DNA fragments on nitrocellulose filters. *Eco*RI* SP01 DNA fragments (1.1×10^5 cpm/0.5 μg) were incubated with increasing amounts of RNA polymerase under the binding conditions described in *Materials and Methods*. The binding reaction mixtures were passed through nitrocellulose filters, and the percentages of DNA fragments retained by holoenzyme and enzyme B were determined from the amount of radioactivity remaining on the filters. A blank (2% of the input radioactivity), representing the amount of labeled DNA fragments retained in the absence of RNA polymerase, has been subtracted from all points. ●, Binding curve for holoenzyme; ▲, binding curve for enzyme B.

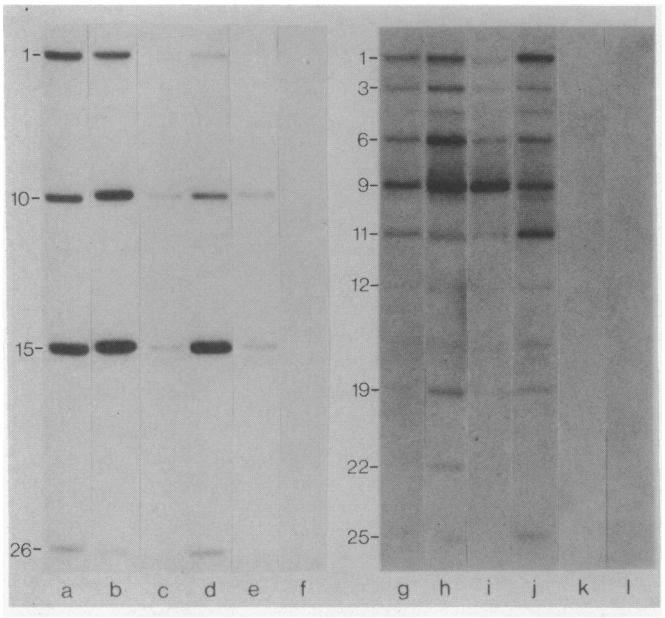

FIG. 6. Formation of initiation complexes by holoenzyme and by enzyme B. Stable binary complexes were formed as in the legend to Fig. 3. (*a–f*) Holoenzyme was incubated with the ^{32}P-end-labeled *Eco*RI* DNA fragments (6 enzyme units/pmol of DNA). (*g–l*) Incubation with 6 enzyme units of enzyme B per pmol of DNA fragments. The complexes formed in reactions *b–f* and *h–l* were then incubated with the four nucleoside triphosphates. After 1 min, transcription was terminated by the addition of EDTA to 0.02 M. Initiation complexes were isolated and the resultant DNA fragments were separated by agarose gel electrophoresis. The final concentrations of NTPs added to each reaction mixture were: (*b* and *h*) 100 μM all NTPs; (*c* and *i*) 1 μM ATP and 100 μM GTP, CTP, and UTP; (*d* and *j*) 1 μM GTP and 100 μM ATP, CTP, and UTP; (*e* and *k*) 1 μM ATP and GTP and 100 μM CTP and UTP; (*f* and *l*) no NTPs. (*a* and *g*) Fragments forming stable binary complexes with holoenzyme and enzyme B, respectively.

polymerase on the pattern of fragments retained on nitrocellulose filters. Holoenzyme preferentially bound to fragments 1, 10, 15, and 26 over a wide range of polymerase-to-DNA ratios (Fig. 4 *a–d*). Enzyme B also exhibited preferential binding to specific DNA fragments [fragments 1, 3, 6, 9, 11, 12, 13, 14 (or 15), 19, 22, and 25] over a range of polymerase-to-DNA ratios (Fig. 4 *e–f*). However, individual DNA fragments were retained with different affinities; at the highest enzyme-to-DNA ratios, nonspecific binding by enzyme B to a large number of *Eco*R1* fragments could be observed (Fig. 4 *g* and *h*).

The amount of [^{32}P]DNA retained by holoenzyme and enzyme B at each enzyme-to-DNA ratio is shown in Fig. 5. Although in terms of activity units the two enzymes were able to bind DNA with almost equal efficiency, our preparation of enzyme B was of lower specific activity than the unmodified bacterial transcriptase. Therefore, in terms of the amount of protein in the binding reaction, enzyme B was less efficient than holoenzyme in retaining DNA on filters.

Kinetic experiments indicated that both holoenzyme and enzyme B formed tight binding complexes with SP01 DNA fragments in less than 60 sec and that the half-time for dissociation of these complexes was greater than 30 min (data not shown). The stability of the complexes was not significantly affected by the presence of a large excess of heterologous DNA from calf thymus. Although ionic strength had little effect on the efficiency with which holoenzyme retained DNA on filters (over a range from 0.05 to 0.2 M KCl), high salt concentrations markedly inhibited the amount of DNA bound by enzyme B. (Increasing the concentration of KCl from 0.05 to 0.2 M decreased the amount of DNA retained on filters by 60%). Nevertheless, the ionic strength of the binding reaction had little effect on the selectivity of fragments retained by either holoenzyme or enzyme B.

Escherichia coli RNA polymerase is able to form complexes at certain promoter sites that are resistant to the drug heparin, a potent inhibitor of bacterial transcriptases (18). This is not a characteristic of all RNA polymerase–DNA complexes, as holoenzyme bound at certain promoters is known to remain sensitive to the polyanion (19). We found that in binding experiments with SP01 DNA only the complexes formed between *B. subtilis* holoenzyme and fragments 10 and 1 were resistant to heparin (Fig. 4 *i* and *j*). All of the specific binding complexes formed by enzyme B were completely sensitive to the drug (data not shown).

As a control, we also studied the binding of core polymerase to the *Eco*RI* fragments of SP01 DNA. At low ratios of core polymerase to DNA, no binding to fragments could be detected; however, in the presence of purified sigma polypeptide, core polymerase specifically retained "early" fragments 1, 10, 15, and 26 (data not shown). At high concentrations of core polymerase, all 26 *Eco*RI* fragments were bound by the core enzyme with only a slight preference for fragments 1, 10, 15, and 26. (This small amount of selectivity could have resulted from trace amounts of sigma polypeptide contaminating the core enzyme.)

Formation of Initiation Complexes. Are the binding sites on SP01 DNA promoters from which RNA polymerase can initiate transcription? Studies with *E. coli* RNA polymerase have shown that after a brief exposure to ribonucleoside triphosphates, enzyme bound at promoter sites on DNA forms "initiation" complexes of enzyme, DNA template, and nascent RNA chains (20). Such ternary complexes are extremely stable and can be detected by filtration through nitrocellulose membranes after incubation at low temperature (0°) and high ionic strength (0.25 M KCl). These conditions dissociate binary

complexes of RNA polymerase and DNA promoters (14). We asked whether the unmodified and phage-modified forms of *B. subtilis* RNA polymerase that were bound to *Eco*RI* fragments of SP01 DNA would form such initiation complexes. In the presence of ATP, GTP, CTP, and UTP, both holoenzyme and enzyme B formed complexes with SP01 DNA fragments that were stable at low temperature and high ionic strength. The DNA fragments that were retained in these complexes are displayed in Fig. 6 *b* and *h*. These DNA fragments [fragments 1, 10, 15, and 26 for holoenzyme and fragments 1, 3, 4, 6, 9, 11, 12, 14 (or 15), 19, 22, and 25 for enzyme B] correspond to the subsets of *Eco*RI* segments that had formed binary complexes with unmodified and modified polymerases in the absence of ribonucleotides (Fig. 6, *a* and *g*).

The initiating ribonucleotide in RNA synthesis, usually ATP or GTP, is required in much higher concentration than the substrates for RNA chain elongation (reviewed in ref. 21). Thus, at low concentrations of ATP, for instance, the formation of RNA chains that start with ATP is selectively restricted. We have taken advantage of this concentration dependence of initiation to investigate the starting nucleotides in SP01 transcription. Fig. 6 shows that lowering the concentration of GTP from 100 μM (slot *b*) to 1 μM (slot *d*) had only a small effect on the pattern of initiation complex formation by *B. subtilis* RNA polymerase holoenzyme. In contrast, lowering the concentration of ATP (slot *c*) markedly reduced the ability of holoenzyme to initiate on fragments 1, 10, 15, and 26. Lowering the concentration of ATP (slot *c*) or both ATP and GTP (slot *e*) did not, however, completely eliminate initiation on fragments 1, 10, and 15, an observation which we have not yet investigated further. Nevertheless, our results strongly suggest that a large proportion of the transcripts synthesized from SP01 DNA by unmodified *B. subtilis* RNA polymerase are initiated with the purine nucleotide ATP.

A majority of the transcripts generated by phage-modified polymerase also appear to be initiated with ATP. Fig. 6*i* shows that lowering the concentration of ATP prevented ternary complex formation on fragments 12 and 25 and significantly inhibited initiation complex formation with several other fragments. The binding of certain fragments (in particular fragment 9) by enzyme B was, however, relatively unaffected by low ATP concentrations. Low GTP concentrations (slot *j*), on the other hand, had only a small effect on the amount of initiation complex formation, while low concentrations of both ATP and GTP (slot *k*) completely abolished binding to all fragments. We conclude that virtually all of the *Eco*RI* fragments bound by enzyme B specify transcripts that start with ATP, although at least some of these DNA segments also specify RNAs that begin with GTP.

DISCUSSION

Our results show that phage SP01-modified RNA polymerase containing the product of regulatory gene *28* preferentially binds to specific regions of the phage genome (Fig. 3). These DNA segments promote specific transcription by this enzyme *in vitro* (Figs. 1 and 6) and most (if not all) contain sequences whose transcription is controlled by the gene *28* product at middle times in the SP01 lytic cycle (11). Our results also show that the DNA fragments to which phage-modified polymerase binds are largely distinct from the set of DNA fragments that are recognized by unmodified bacterial transcriptase, the form of polymerase that copies phage early genes.

On the basis of these results, we conclude that phage early and middle genes are transcribed from distinct promoter sites and that the gene *28* protein directs RNA polymerase to recognize and bind to sites at or near the start points for middle RNA synthesis. This implies that the mode of action of the gene *28* protein is analogous, at least in part, to that described for sigma factor and that the SP01 regulatory protein should, itself, be regarded as a sigma-like polypeptide (22). The finding of Duffy and Geiduschek (9) that polymerase containing the gene *28* product forms rapidly initiating complexes on intact SP01 DNA is consistent with this model. It is likely that SP01 late genes are also controlled by modification of the promoter recognition properties of RNA polymerase; preliminary results indicate that enzyme containing the products of regulatory genes *33* and *34* preferentially binds to fragments that contain phage late gene sequences.

It will now be of interest to compare the primary structure of SP01 promoters for early, middle, and late genes and to investigate how sigma and sigma-like regulatory proteins direct specific recognition of these sites.

We thank Richard Losick for help in the preparation of this manuscript, G. Lee for *B. subtilis* holoenzyme, and N. Hannett for purifying phage DNA and *Eco*RI restriction endonuclease. C.T. was a predoctoral fellow of the National Science Foundation. This work was supported by National Science Foundation Grant PCM75-00551 A01 to J.P.

1. Pero, J., Nelson, J. & Fox, T. D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1589–1593.
2. Pero, J., Tjian, R., Nelson, J. & Losick, R. (1975) *Nature* **257,** 248–251.
3. Duffy, J. J. & Geiduschek, E. P. (1975) *J. Biol. Chem.* **250,** 4530–4541.
4. Whiteley, H. R., Spiegelman, G. B., Lawrie, J. M. & Hiatt, W. R. (1976) in *RNA Polymerase,* eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Press, Cold Spring Harbor, NY), pp. 587–600.
5. Fox, T. D., Losick, R. & Pero, J. (1976) *J. Mol. Biol.* **101,** 427–433.
6. Duffy, J. J. & Geiduschek, E. P. (1977) *Nature* **270,** 28–32.
7. Fox, T. D. (1976) *Nature* **262,** 748–753.
8. Tjian, R. & Pero, J. (1976) *Nature* **262,** 753–757.
9. Duffy, J. J. & Geiduschek, E. P. (1976) *Cell* **8,** 595–604.
10. Sonenshein, A. L. & Roscoe, D. H. (1969) *Virology* **39,** 265–276.
11. Talkington, C. & Pero, J. (1977) *Virology* **83,** 365–379.
12. Polisky, B., Greene, P., Garfin, D. E., McCarthy, B. J., Goodman, H. M. & Boyer, H. W. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3310–3314.
13. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
14. Seeburg, P. H. & Schaller, H. (1975) *J. Mol. Biol.* **92,** 261–277.
15. Sanger, F., Air, G. M., Barnell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., Hutchison, C. A., III, Slocombe, P. M. & Smith, M. (1977) *Nature* **265,** 687–695.
16. Cregg, J. M. & Stewart, C. R. (1978) *Virology,* in press.
17. Hinkle, D. C. & Chamberlin, M. J. (1972) *J. Mol. Biol.* **70,** 157–185.
18. Walter, G., Zillig, W., Palm, P. & Fuchs, E. (1967) *Eur. J. Biochem.* **3,** 194–201.
19. Kupper, H., Contreras, R., Khorana, H. G. & Landy, A. (1976) in *RNA Polymerase,* eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Press, Cold Spring Harbor, NY), pp. 473–484.
20. Richardson, J. P. (1966) *J. Mol. Biol.* **21,** 115–127.
21. Chamberlin, M. J. (1976) in *RNA Polymerase,* eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Press, Cold Spring Harbor, NY), pp. 17–67.
22. Chamberlin, M. J. (1976) in *RNA Polymerase,* eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Press, Cold Spring Harbor, NY), pp. 159–191.