The ability of the core isolated from *Escherichia coli* RNA polymerase to interact with specificity-determining subunits isolated from *Bacillus subtilis* RNA polymerase has been determined by measuring the transcription of "early" and "middle" genes of phage SP82. Two specificity-determining subunits were tested: the σ subunit and a 28,000 dalton (28 K) peptide isolated from a modified polymerase produced at approximately 8 min after infection of *B. subtilis* with SP82. Earlier experiments (Spiegelman, G. B. and Whiteley, H. R. (1978) *Biochem. Biophys. Res. Commun.* 81, 1058-1065) demonstrated that σ and the 28K peptide are required for the recognition of early and middle gene promoters, respectively, by the *B. subtilis* core assembly. The present investigation showed that *E. coli* core interacted more efficiently with the *B. subtilis* σ than with the 28K peptide, as judged by the rate of RNA synthesis. Early RNA was produced by the *E. coli* and *B. subtilis* holoenzymes and by *E. coli* core supplemented with *B. subtilis* σ and only minor differences were found in comparisons of transcripts by hybridization and by electrophoretic analysis. Measurements of template specificity, the formation of stable enzyme-DNA complexes, and the hybridization of DNA with fragments of SP82 DNA produced by digestion with restriction endonucleases indicated that *E. coli* core supplemented with the 28K peptide produced "middle RNA." Electrophoretic comparisons of the major transcripts produced by the 28K-supplemented *E. coli* core with those synthesized by the modified polymerase extracted from *B. subtilis* 8 min after infection with SP82 suggest that both preparations recognized the same initiation and termination sequences.

Several lines of evidence indicate that the specificity of transcription is altered by changes in the subunit composition of RNA polymerase after infection of *Bacillus subtilis* with phage SP82 (1-4) or the related phage, SP01 (5-12). Two modified forms of the enzyme have been isolated from SP82-infected cells, each having a different composition and a different transcriptional specificity (3, 4). Comparisons of the unmodified and modified forms show that all three polymerases consist of the same core subunits (β′βαω) and auxiliary peptides (δ, ω1, ω2). In addition, the unmodified polymerase contains the σ subunit, the polymerase isolated from cells 8 min after infection (8 min enzyme) contains peptides of 28,000 and 18,000 daltons (referred to as the 28K and 18K peptides) and the enzyme isolated 20 min after infection (20 min enzyme) contains peptides of 26,000 and 13,000 daltons (referred to as the 26K and 13K peptides). Experiments with unmodified polymerase, with the 8 min enzyme and with *B. subtilis* core supplemented with σ or the 28K peptide showed that σ specifies the interaction of polymerase with early gene promoters and that the 28K peptide is required for recognition of middle gene promoters (13); the function(s) of the 18K peptide have not been determined. Although promoter-binding studies have not been made, analyses of RNAs synthesized *in vitro* by the 20 min enzyme are compatible with the idea that the 26K and 13K peptides also function in promoter recognition (2).

The observation that recognition of different promoters by the unmodified polymerase and the 8 min enzyme depends on the presence of the σ subunit or the 28K peptide raises the question of whether these regulatory peptides can interact with core assemblies from polymerases extracted from other, unrelated bacteria, thereby directing the mixed complex of subunits to transcribe promoters specified by the *B. subtilis* components. The *Escherichia coli* RNA polymerase was selected as the source of an unrelated core assembly; this enzyme does not contain a σ peptide and the molecular weight of the σ subunit is significantly different from that of the *B. subtilis* σ subunit. The primary goal of the present investigation was to study the interaction of *E. coli* core with SP82 DNA in the presence of the 28K peptide. A secondary aim was to determine if the *B. subtilis* σ subunit endowed the *E. coli* core with a specificity which differed from that of the *E. coli* holoenzyme.

**EXPERIMENTAL PROCEDURES**

*Isolation of Polymerases and Subunits—*RNA polymerases were purified from uninfected *B. subtilis*, from SP82-infected *B. subtilis*, and from *E. coli* strain MRE 600 as described by Spiegelman et al. (14). Core preparations were isolated from *B. subtilis* and *E. coli* holoenzymes by chromatography on columns of phosphocellulose (13) and Bio-Rex 70 (15). Traces of the σ subunit were removed from the *E. coli* core preparations by chromatography through columns of polycydiolate cellulose. 1 The *B. subtilis* σ subunit was purified by subjecting a fraction, obtained during the isolation of the core assembly, to sequential chromatography on phosphocellulose, DEAE-cellulose, and DNA cellulose (14). The 28K and 18K peptides were isolated from preparations of the 8 min enzyme according to Spiegelman and Whiteley (13). The subunit compositions of the enzyme preparations and the purity of the isolated subunits were determined.

1 R. Kingston and M. Chamberlin, personal communication.
by electrophoresis through sodium dodecyl sulfate polyacrylamide slab gels as described earlier (4). Scans of stained gels showed that the relative activities of the isolated preparations of B. subtilis α and 28K peptide, and 18K peptide was >95%. The possible contamination of cores by traces of the α subunit was monitored by comparing the patterns of hybridization of RNAs synthesized in vitro by supplemented cores with the patterns of hybridization produced by RNAs synthesized by the holoenzymes (9). Protein was determined according to Bradford (16).

Synthesis of RNA—RNA polymerase activity was assayed by measuring the incorporation of [γ-32P]ATP into trichloroacetic acid-precipitable material (14); a low salt buffer (14) was used in experiments to investigate the effect of NaCl. The concentration of active polymerase molecules in the E. coli holoenzyme preparation was 20 to 50%, as determined by the method of Chamberlin et al. (17). The relative activities of the E. coli and B. subtilis holoenzyme and the 8 min enzyme were estimated by comparing RNA synthesis from T7 and SP82 DNAs. Assays of template specificity, the formation of stable enzyme-DNA complexes and the synthesis of RNAs for analysis of transcripts were performed with enzyme preparations having equivalent activities. For experiments with supplemented cores, each core preparation was titrated with B. subtilis α or 28K peptide to determine the core:subunit ratio which provided the maximum stimulation (e.g. as in Fig. 3 of "Results"). Unless otherwise noted, all reactions containing holoenzymes or cores derived from B. subtilis were performed in the presence of 0.09 M NaCl; the concentration of NaCl used in experiments with E. coli holoenzyme or supplemented E. coli core was 0.20 M. Previously described methods were used for the purification of phage DNAs (1).

RNAs were labeled during phage infection by adding 90 Ci of 32P-orthophosphate (carrier-free, Amersham)/ml to cultures grown in a medium containing 0.12 M Tris, 0.017 M sodium citrate, 0.008 M ammonium sulfate, 0.028 M glucose, 1.22 mM MgSO4, 0.02 mM MnCl2, and 0.05% Casamino acids, pH 7.0. After the appropriate labeling period at 37°C, the cultures were "chased" for 30 s by adding one-half volume of the same medium containing 0.05 M potassium phosphate and rifampicin (to give a final concentration of 10 μg/ml). The cells were harvested, converted to protoplasts (18), lysed with detergent, and the RNAs were extracted (3).

RNAs were synthesized in vitro (3) in the presence of [γ-32P]ATP (540 Ci/mmol, Amersham), unlabeled nucleotides, buffer, and the indicated concentration of NaCl at a polymerase:DNA molar ratio of 1000 and the samples were processed for gel electrophoresis according to Gegenheimer et al. (19). The sizes of the transcripts were estimated from the known sizes of 32P-labeled T7 RNA extracted from E. coli infected with wild type T7 and with a H1 mutant and 32P-labeled ribosomal RNA from E. coli. These preparations were contributed by Susan Strome (Department of Biochemistry, University of Washington). Procedures used for the hybridization of labeled RNA to fragments of DNA transferred to nitrocellulose membrane filters have been described (3).

Formation of Enzyme-DNA Complexes—Fragments of SP82 DNA were generated by digesting 25 μg of DNA with 8 units of restriction endonuclease Hha I (New England Biolabs) in the presence of 6 mM Tris, pH 7.4, 12 mM MgCl2, 50 mM NaCl, 7 mM 2-mercaptoethanol, and the reaction mixtures were incubated at 37°C for 10 h. Enzyme preparations were incubated for 8 min at 37°C with 2 or 3 μg of digested DNA, 40 mM Tris, pH 7.9, 20 mM MgCl2, 10 mM β-mercaptoethanol, and the indicated concentration of NaCl in a total volume of 0.5 ml; GTP, CTP, and ATP were added to give a final concentration of 6.4 mM with respect to each nucleotide and the reaction mixtures were incubated an additional 5 min at 37°C. Complex formation was stopped by the addition of 2 ml of an ice-cold solution of Tris-Mg buffer (40 mM Tris, pH 7.9, 20 mM MgCl2) containing 1 mM NaCl and the reaction mixture was filtered through nitrocellulose membrane filters which had been soaked in Tris-Mg buffer at 4°C overnight. The filters were washed with 2 ml of ice-cold Tris-Mg, 1 M NaCl, and 2 ml of room temperature Tris-Mg buffer at a flow rate of 1 ml/10 s. The filters were eluted for 10 min at 58°C with 0.5 ml of a solution containing 0.05% sodium dodecyl sulfate, 10 mM Tris, pH 7.9, and 10 mM MgCl2 and the eluates were lyophilized. The separation of restriction fragments by electrophoresis through agarose (3) has been described (13). To quantitate the extent of binding of individual fragments, gels were stained with 6.5 μg/ml of ethidium bromide, photographed using an ultraviolet lamp, the photographic negatives were scanned with a Joyce-Loeble densitometer, the areas corresponding to each band were determined with an Electronic Graphics Calculator (Numonics Corp.) and divided by the molecular weight of the fragments. To compare results from different experiments, data were normalized to values obtained with a small fragment which consistently showed efficient binding (fragment 32, M = 0.62 × 106, for the holoenzymes and α-supplemented cores and fragment 22, M = 1.01 × 106, for the 8-min enzyme and the 28K-supplemented cores).

RESULTS

Subunit Structures of Polymerases—The subunit structures shown in lanes A and C of Fig. 1 confirm earlier observations (20) that the E. coli and B. subtilis polymerases differ in the following respects: 1) the subunits making up the core assembly (ββ'α α) in the two enzymes have different molecular weights, 2) the B. subtilis and E. coli α subunits are significantly different in size (M = 56,000 and 82,000, respectively), 3) the B. subtilis holoenzyme, isolated by the method of enzyme purification used in these investigations, contains the 8 peptide (14, 21) which is not found in the E. coli enzyme, and 4) the B. subtilis polymerase contains two ω peptides, whereas the E. coli enzyme has only one. After removal of the α subunit, the E. coli core (lane B, Fig. 1) retained the ω peptide; the B. subtilis core preparation (lane E, Fig. 1) contained the δ peptide and both ω peptides. The purities of typical preparations of the regulatory peptides used to supplement the cores are shown in lanes F, G, and H of Fig. 1; the subunit composition of the 8 min enzyme from which the 28K and 18K peptides were isolated is shown in lane E of Fig. 1.

Transcription by Supplemented Core Preparations: Salt Requirements, Regulatory Peptide:Core Ratios, and Template Specificities—In addition to differences in subunit structure, it is known that the B. subtilis and E. coli polymerases have different salt requirements for the synthesis of RNA (20, 22). Fig. 2A presents data on the effects of NaCl on the transcription of SP82 DNA by the polymerases shown in lanes A, C, and E of Fig. 1. Although both the E. coli and B. subtilis RNA polymerases and purified subunits. A photograph of a sodium dodecyl sulfate polyacrylamide slab gel (15% acrylamide, 0.4% N,N′-methylenebisacrylamide) stained with Coomassie blue showing the following: A, 22.8 μg of the E. coli holoenzyme; B, 19.5 μg of the E. coli core preparation; C, 23 μg of the B. subtilis holoenzyme; D, 15.8 μg of the B. subtilis core; E, 18 μg of the 8 min enzyme; F, 8.4 μg of a B. subtilis α preparation; G, 5 μg of the 28K peptide; H, 2.5 μg of the 18K peptide.
**Subtilis** holoenzymes show comparable effects at concentrations of NaCl lower than 0.1 M, the *E. coli* enzyme is more resistant to the inhibitory effect of higher concentrations of NaCl than the *B. subtilis* polymerase or the 8 min enzyme. As described below (Fig. 3), the *E. coli* core can transcribe SP82 DNA when supplemented with either the *B. subtilis* subunit or the 28K peptide. Fig. 2B presents data indicating that preparations of *E. coli* core plus σ or the 28K peptide showed maximal activity under these conditions of assay in the presence of 0.2 M NaCl suggesting that the salt optimum is a function of the core assembly. However, the specificity-determining subunits, or the interaction of these subunits with the core, may also be influenced by NaCl (i.e. the curves shown in Fig. 2B differ considerably from the curves obtained with the 8 min enzyme and the *B. subtilis* holoenzyme shown in Fig. 2A). As detailed elsewhere (22), ionic strength can affect several steps of RNA synthesis and the optimum salt concentration varies not only with the polymerase but also with the template, the nucleotide concentration, the concentration of divalent cations, and the temperature. Studies of the interaction of polymerase with individual promoters (23–25) have shown that one of the effects of NaCl is to restrict the formation of nonspecific binary complexes or complexes with "weak promoters." Based on this consideration as well as the data shown in Fig. 2 and in a later figure (Fig. 5), a concentration of 0.2 M NaCl was selected for RNA synthesis employing *E. coli* core or holoenzyme preparations and 0.09 M NaCl was chosen for reactions with the *B. subtilis* holoenzyme, 8 min enzyme, and supplemented *B. subtilis* core.

The functional integrity of the isolated subunits was tested by measuring the effect of the *B. subtilis* σ, 28K, and 18K peptides on the synthesis of RNA. As shown in Fig. 3A, the incorporation of [3H]UTP into trichloroacetic acid-precipitated material by a *B. subtilis* core preparation was stimulated significantly by the addition of σ or the 28K peptide and there was a small, reproducible stimulation on adding the 18K peptide.

The *B. subtilis* peptides could also interact with the *E. coli* core (Fig. 3B) although less effectively than with the *B. subtilis* core. The finding that the *B. subtilis* σ subunit can stimulate the activity of the *E. coli* core and that the extent of stimulation depends on the template (Table 1) agrees with earlier studies (26–32) of hybrid polymerases produced by mixing *E. coli* core with σ peptides from polymerases derived from unrelated bacteria. In the present experiments, saturation of the activity of the *E. coli* core by σ or the 28K peptide was not achieved even at a high molar ratio (e.g. a 28K:core molar ratio of 14). The maximum stimulation observed on adding the 28K peptide to *E. coli* core was 30 to 50% of the stimulation obtained by adding *B. subtilis* σ. Addition of the 18K peptide produced a slight stimulation in the activity of the *E. coli* core preparation. Since the stimulation by the 18K peptide was significantly lower than that observed on adding σ or the 28K peptide and no detectable effect has been found to date on the specificity of transcription, this peptide was not added to core preparations in the subsequent experiments.

Earlier investigations had shown that the 8-min enzyme had very little or no activity with DNA from the unrelated *B. subtilis* phage, φ29 (14). The data presented in Table I confirm this observation and also show that addition of the 28K peptide to the *E. coli* core preparation stimulated the transcription of SP82 DNA but not the transcription of φ29 or T7 DNAs. As demonstrated previously (14), transcription of templates other than SP82 DNA is inhibited in the presence of either the σ peptide or a moderately high salt concentration. These conditions were met in the experiments shown in Table.

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**Fig. 3.** RNA synthesis by core preparations supplemented with *B. subtilis* σ, 28K, and 18K peptides. Increasing amounts of *B. subtilis* σ (O–O), 28K peptide (△–△), and 18K peptide (□–□) were added to 5 μg of *B. subtilis* core polymerase (A) or to 4.2 μg of *E. coli* core polymerase (B). RNA synthesis was determined with SP82 DNA as the template as described under “Experimental Procedures.”

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**Fig. 2.** The effect of NaCl concentration on RNA polymerase activity. A, the synthesis of RNA from SP82 DNA by 10 μg of *E. coli* holoenzyme (□–□), 9.4 g of *B. subtilis* holoenzyme (△–△), and 9 μg of the 8 min enzyme (○–○). B, the parallel experiment showing the synthesis of RNA by 4.2 μg of *E. coli* core preparation supplemented either with 3.4 μg of *B. subtilis* sigma (●–●) or with 4 μg of 28K peptide (△–△). RNA synthesis was determined at each NaCl concentration as described under “Experimental Procedures.”

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2 E. C. Achberger and H. R. Whiteley, unpublished data.
I: the B. subtilis preparations contained the δ peptide and experiments with the E. coli preparation were performed with 0.2 M NaCl. It should be noted that the data in Table I indicate the relative effectiveness of the added peptide with each core preparation and cannot be taken as a measure of the concentration of active polymerase molecules in each preparation.

Although the 28K-supplemented E. coli core had rather low activity (Fig. 3B and Table I), the finding that this combination of subunits had the same relative activities with three templates as the 8 min enzyme suggested that the hybrid polymerase may possess some specificity in the transcription of SP82 DNA. To test this possibility, RNAs synthesized by the holoenzymes and by the supplemented cores were compared with respect to the formation of enzyme-DNA complexes, hybridization to restriction fragments of SP82 DNA, and the sizes of the transcripts.

Recognition of SP82 DNA Promoters—SP82 DNA was digested with the restriction endonuclease Hha I, yielding the fragments shown in lanes A and L of Fig. 4. The fragments were incubated with enzymes or core preparations supplemented with regulatory peptides and the formation of stably initiated enzyme-DNA complexes was examined using the filter-binding technique of Jones et al. (33). Neither of the two core preparations was able to complex DNA at the concentrations used in these assays (lanes C and H, Fig. 4). Addition of the 28K peptide to E. coli core (lane J, Fig. 4) yielded a pattern which was similar to that obtained with the 8 min enzyme (lanes K and F, Fig. 4) and different from that observed with the E. coli holoenzyme (lane G, Fig. 4). These observations indicate that replacing the σ subunit with the 28K peptide endows the E. coli core with a new specificity which permits the recognition of middle gene promoters. Parallel experiments with the B. subtilis core supplemented with 28K (lane E, Fig. 4) produced a pattern which was identical with the pattern observed with the 8 min enzyme (lanes F and K, Fig. 4). Comparisons of the patterns in lanes J and E with those of lanes F and K revealed quantitative differences in the intensities of fluorescence of individual fragments suggesting that the core assemblies influence the extent of binding (i.e. promoter strength) without affecting the specificity of promoter recognition. For example, scans (not shown) of lanes J and K of Fig. 4 indicate that 24 fragments were complexed as efficiently by both preparations, 4 fragments were complexed less strongly by the 28K-supplemented E. coli core than by the 8 min enzyme (fragments 1, 17-18, 20, and 25), and 7 were complexed more strongly by the E. coli core + 28K preparation (fragments 12, 13, 14, 15, 21, 24, and 32-33).

The effect of adding the B. subtilis σ subunit to the two core preparations is illustrated in lanes D and I of Fig. 4. The fragments seen in these lanes are the same as those complexed by B. subtilis holoenzyme (lane B, Fig. 4). Although many of the same fragments were complexed by the E. coli holoenzyme (lane G, Fig. 4), the latter enzyme also strongly bound a number of fragments which were weakly bound by preparations containing B. subtilis σ (lanes D, B, and I, Fig. 4).

Comprehensive studies of the interaction of E. coli polymerase with other DNAs (23-25) have demonstrated that the formation of binary complexes is influenced by the concentrations of polymerase and NaCl. Accordingly, experiments were performed to determine the effects of increasing concentrations of NaCl on the formation of complexes at three concentrations of each of the holoenzymes. Fig. 5 presents data illustrating the significant features of these experiments. As shown in lane 3 of Fig. 5A, at an enzyme:DNA weight ratio of

![Image](image-url)
complexed fragments were separated by electrophoresis through agarose gels, stained with ethidium bromide, and photographed. The following conditions were used: A: for lanes 1, 2, and 3, 0.10 M NaCl and 2.0, 4.0, and 6.0 µg of E. coli polymerase were used; lanes 4, 5, 6, and 7, the experiments were done with 6.0 µg of E. coli polymerase and 0.15, 0.20, 0.25, and 0.30 M NaCl, respectively; B: for lanes 1, 2, and 3, the experiments were performed in the presence of 0.05 M NaCl with 2.1, 2.4, and 2.0, 4.0, and 6.0 µg of B. subtilis polymerase, respectively; for lanes 4, 5, and 6 the experiments were performed with 6.3 µg of B. subtilis polymerase in the presence of 0.10, 0.15, and 0.20 M NaCl.

2.0 in the presence of 0.1 M NaCl, the E. coli polymerase bound virtually all fragments, suggesting nonspecific binding, possibly due to the formation of “tight-binding complexes” similar to those observed by Kadesch et al. (34, 35). With increasing NaCl concentration, complex formation was progressively restricted and at 0.3 M NaCl (lane 7, Fig. 5A), the pattern of complexed fragments resembled that observed with the same enzyme:DNA weight ratio of B. subtilis polymerase at 0.05 M NaCl (lane 3, Fig. 5B). The data in Fig. 5B permit identification of two groups of fragments: the first (fragments 12, 15, 18, 19, 26, 32, 35, and 37) were strongly bound at an enzyme:DNA weight ratio of 0.67 in the presence of 0.05 M NaCl and were stable in 0.15 M NaCl and the second group (fragments 1, 2, 6, 13, and 25) were less efficiently bound at this enzyme:DNA weight ratio and were dissociated by increasing concentrations of NaCl. The role of the δ peptide of the B. subtilis holoenzyme in the formation of binary complexes with the latter group of fragments will be discussed elsewhere. As shown in the following section, the fragments in the two groups contain “early gene” promoters, i.e. transcription from fragments in the first group begins immediately after infection and significant transcription from fragments in the second group follows shortly thereafter.

Hybridization of RNAs Transcribed In Vivo and in Vitro

Fig. 5. The effect of increasing amounts of polymerase and NaCl on the formation of polymerase-DNA complexes. Stably initiated complexes were formed between polymerase and restriction fragments produced by digesting 3 µg of SP82 DNA with Hha I. The complexed fragments were separated by electrophoresis through agarose gels, stained with ethidium bromide, and photographed. The following conditions were used: A: for lanes 1, 2, and 3, 0.10 M NaCl and 2.0, 4.0, and 6.0 µg of E. coli polymerase were used; lanes 4, 5, 6, and 7, the experiments were done with 6.0 µg of E. coli polymerase and 0.15, 0.20, 0.25, and 0.30 M NaCl, respectively; B: for lanes 1, 2, and 3, the experiments were performed in the presence of 0.05 M NaCl with 2.1, 2.4, and 2.0, 4.0, and 6.0 µg of B. subtilis polymerase, respectively; for lanes 4, 5, and 6 the experiments were performed with 6.3 µg of B. subtilis polymerase in the presence of 0.10, 0.15, and 0.20 M NaCl.

Fig. 6. Hybridization of RNAs synthesized during SP82 infection. Procedures used for pulse-labeling SP82-infected B. subtilis. Hybridization to fragments produced by digesting SP82 DNA with Hha I, and radioautography are described under “Experimental Procedures.” The figure shows a photograph of the radioautograms obtained by hybridization of RNAs containing 2 × 10⁶ cpm: the RNAs were extracted from cells pulse-labeled at the following intervals after infection: lane A, 0 to 3 min; lane B, 3 to 5 min; lane C, 5 to 7 min; lane D, 7 to 9 min.

Fig. 7 presents data on the hybridization of RNAs synthesized during SP82 infection (the 0 to 3 min interval of labeling) hybridized to Hha Fragments 12, 18, 19, 26, 32, 35, 39, and 40 and weakly to fragments 15 and 37. Except for fragments 39 and 40, these are the fragments which are strongly bound by B. subtilis holoenzyme and by either core supplemented with B. subtilis a. During the 3 to 5 min labeling interval (Fig. 6), RNAs were produced that hybridized to several additional fragments: fragments 1, 2, 5, 6, 13, and 24. Except for fragments 5 and 24, these are the fragments which were more weakly bound by B. subtilis holoenzyme. These same fragments (i.e. those shown in lanes A and B of Fig. 6) were also hybridized by RNA synthesized by B. subtilis infected with SP82 in the presence of chloramphenicol; chloramphenicol prevents modification of RNA polymerase. RNAs synthesized 5 to 7 min and 7 to 9 min after infection hybridized to additional fragments as well as to some of the fragments hybridized in the earlier labeling intervals. One explanation for the latter observation is that modification of the polymerase may not have been completed in this experiment by the 7 to 9 min interval; hybridization data (36) also indicate that some of the fragments contain both early and middle gene promoters.

Hybridization of RNAs Transcribed In Vivo and in Vitro

3 E. C. Achberger and H. R. Whiteley, manuscript in preparation.

4 A. T. Panganiban, unpublished observations.
the E. coli core supplemented with the 28K peptide (lane E, Fig. 7) was significantly different from that observed with RNAs synthesized by the E. coli holoenzyme (lane D, Fig. 7) and similar to that obtained by hybridizing RNAs produced by the 8-min enzyme (lane F, Fig. 7). Specifically, hybridization of 28 matching fragments could be detected in lanes E and F of Fig. 7. 21 fragments were hybridized with approximately equal intensities, 2 were hybridized less intensely in lane E than in lane F (fragments 2 and 28-29), and 5 were hybridized more intensely in lane E (fragments 12, 17-18, 19, 32-33, and 35). If it is assumed that the modification of polymerase was not complete by the 7 to 9 min labeling interval in the experiment shown in Fig. 6, then there was reasonably good qualitative agreement between in vivo and in vitro RNAs (lane D, Fig. 6; lane F, Fig. 7), although there were differences in the intensities of some of the bands. Thus, fragments 5, 11, and 21 were clearly hybridized by in vivo RNA whereas hybridization by in vitro RNA was barely detectable. Possibly, these differences in the relative efficiencies of transcription arise from more restrictive conditions for in vivo synthesis. On the other hand, fragment 22 was apparently transcribed efficiently in vitro but hybridization of in vivo RNA to this fragment was barely detected. However, studies of hybridization of RNAs synthesized throughout in-

![Fig. 7. Hybridization of RNAs synthesized in vitro to fragments of SP82 DNA.](image1)

![Fig. 8. Electrophoretic analysis of RNAs synthesized in vitro.](image2)

fection (36) showed that RNAs produced during the 9 to 11 min labeling interval hybridized strongly to fragment 22.

Comparison of patterns of hybridization by RNAs synthesized by B. subtilis holoenzyme (Lanes A and B) and E. coli core supplemented with B. subtilis α (Lane C) indicates that the latter transcripts are similar to those synthesized by B. subtilis holoenzyme in the presence of a less restrictive concentration of NaCl (lane B, Fig. 7). In contrast, the population of RNAs produced by E. coli holoenzyme (lane D, Fig. 7) contains a relatively greater proportion of transcripts hybridizing to fragments 1, 2, 6, 13, and 25 (i.e., the group of fragments which formed weak complexes with B. subtilis holoenzyme). These observations suggest, as in the preceding studies on complex formation, that replacement of the E. coli α subunit by B. subtilis α may permit greater discrimination between the two groups of early promoters. In general, there was good agreement between the patterns of RNA hybridized and the formation of binary complexes. In a few instances, fragments hybridized with RNA but were not bound in binary complexes: e.g., fragments 5, 24, 39, and 40 for the B. subtilis holoenzyme and fragments 24, 37, and 38 for the 8-min enzyme. Read-through from promoters located on transcribed fragments could account for these results.

Electrophoretic Separation of in Vitro Transcripts—The data presented in Figs. 4 and 7 indicate that addition of the 28K peptide to the E. coli core leads to the formation of stable complexes with promoters recognized by the 8-min enzyme.
and transcription from these promoters. It could be argued, however, that such transcription is incomplete or that proper termination does not occur. This possibility was examined by an electrophoretic comparison of transcripts synthesized by the holoenzymes and by the supplemented E. coli core.

As seen from lanes E and F of Fig. 8, there was good agreement in the sizes of the major transcripts and in the relative intensities of some of the RNAs produced by the 28K-supplemented E. coli core and the 8 min enzyme; these patterns differ from the pattern obtained by electrophoresis of RNAs synthesized by the E. coli holoenzyme (lane D, Fig. 8). Specifically, scans of radioautograms showed that there were 8 major and approximately 16 minor transcripts of matching mobilities in lanes E and F of Fig. 8; 2 or 3 minor bands found in scans of lane F were not present in scans of lane E. Although these observations must be viewed with caution, one interpretation of these data is that the 8 min enzyme and the 28K-supplemented E. coli core may produce the same RNA molecules, i.e. that both enzyme preparations recognize the same initiation and termination sequences. Additional experiments, especially sequencing of individual RNAs, would be required to determine if the RNAs produced by the two enzyme preparations are indeed identical.

Comparisons of transcripts produced by the B. subtilis (lanes A and B, Fig. 8) and E. coli (lane D, Fig. 8) holoenzymes disclosed differences in the relative intensities of individual bands. For example, one or two large molecular weight RNAs (>3000 bases) can be seen in lane D which are barely detectable in lane B and a larger relative amount of an RNA of ~1300 bases is seen in lane D than in lane B. The same qualitative differences were found by electrophoresis of RNAs produced at molar ratios of E. coli polymerase:DNA ranging from 20 to 8000 (data not shown). Electrophoresis and radioautography of products synthesized by the E. coli core supplemented with the B. subtilis α subunit yielded a pattern (lane C, Fig. 8) which may be more similar in array and relative intensities of the bands to the pattern seen in lane B, possibly indicating some specificity attributable to the α subunit.

DISCUSSION

The contribution of RNA polymerase to the specificity of transcription has recently been investigated in a detailed examination of the ability of polymerases of sulfur-requiring bacteria to interact with four well characterized T7 promoters (37). Most of the polymerases recognized the same initiation and termination sequences, as determined by electrophoretic analysis of the transcripts, suggesting that the different enzymes may possess similar structures or may assume similar conformations, thus permitting interaction with T7 promoters. The efficiency of utilizing individual promoters differed depending on the polymerase, indicating that the nature of the polymerase is one of the factors which determines "promoter strength."

In the present investigation, we have approached the question of specificity by measuring the transcription of early and middle genes of phage SP82 by mixtures of polymerase components purified from two unrelated bacteria. In these experiments, E. coli core preparations were supplemented with the B. subtilis α subunit or with the 28K peptide derived from 8 min enzyme, a modified form of polymerase obtained from B. subtilis 8 min after infection with SP82. As documented elsewhere (13), these subunits are required for the recognition of early and middle SP82 gene promoters, respectively.

A number of observations (the ability to transcribe SP82 DNA but not ω29 or T7 DNAs, the specificity of formation of binary complexes with restriction fragments of SP82 DNA, and the specificity of transcription as detected by hybridization to restriction fragments) indicate that the 28K peptide can interact with the E. coli core subunits in a specific manner. The most striking result of the present studies was the finding that this interaction results in the synthesis of an array of RNA molecules which is very similar to the array produced by the 8 min enzyme. The similarities in the sizes of the major transcripts suggest that both preparations recognized the initiation and termination sequences defining some of the major middle genes. These observations, combined with earlier comparisons of RNAs synthesized in vivo and in vitro by the 8 min enzyme (3), provide additional evidence that the 28K peptide plays a major role in the transcription of SP82 middle genes.

The interaction of the 28K peptide with the E. coli core was not very efficient probably because these components were derived from polymerases having different requirements for optimum catalysis. For example, the maximum activity for the 28K-supplemented E. coli core was observed at a concentration of NaCl (0.2 M, Fig. 2) which is optimal for the E. coli holoenzyme (20, 22), but which inhibits the activity of the 8 min enzyme significantly (Fig. 2). The sensitivity of the latter enzyme to NaCl appears to be a property of the 28K peptide (or of the interaction of the 8 min enzyme with promoters) since NaCl did not inhibit the activity of the B. subtilis holoenzyme to the same extent. The E. coli and B. subtilis polymerases also differ in subunit composition, i.e. the B. subtilis enzymes contain the δ peptide and two ω peptides. It is not known what role these peptides play in the interaction of regulatory peptides with the core assembly and with promoter sequences. In addition to the relatively poor catalytic activity of the 28K-supplemented E. coli core, comparisons of this preparation with the 28K-supplemented B. subtilis core revealed quantitative differences in the abilities of the two preparations to interact with individual SP82 promoters, as judged by the formation of binary complexes, the hybridization to restriction fragments, and the electrophoretic analysis of RNAs. Such quantitative differences could be attributed to differences in the abilities of the two core preparations to interact with the 28K peptide and/or to utilize specific middle gene promoters. Additional information on the interaction of the 28K peptide with the homologous and heterologous core preparations may come from experiments using protein cross-linking reagents.

Parallel studies with E. coli core supplemented with B. subtilis α provided less convincing evidence for specificity attributable to the B. subtilis α subunit. Both the B. subtilis and E. coli holoenzymes formed binary complexes and transcribed the same DNA fragments under given conditions, and the only difference between the two enzymes appeared to be a lesser degree of specificity by the E. coli enzyme and a few differences in the arrays of transcripts detected electrophoretically. The observation that preparations of E. coli core supplemented with B. subtilis α more closely resembled B. subtilis holoenzyme with respect to the formation of binary complexes, hybridization, and the sizes of transcripts may indicate some specificity due to the B. subtilis α subunit.

The DNA fragments which were strongly bound by the B. subtilis holoenzyme have been shown by detailed mapping analysis to be located in the terminally redundant regions (hence, the SP82 genome contains two copies of the early promoters). These fragments are transcribed immediately after infection and are probably the source of the class H1 and L1 RNAs described earlier (38). Fragments which were bound more weakly by B. subtilis holoenzyme are transcribed slightly later in infection and some of these fragments are not located in the terminally redundant part of the genome. The
delay in transcription of this group of early promoters may be due to weaker promoter strengths, fewer early promoters/fragment and/or lower DNA copy number.

Measurements of total RNA synthesis indicate that the interactions of the B. subtilis specificity-determining peptides with the E. coli core are clearly not as efficient as their interactions with the B. subtilis core, but determinations of the concentration of active polymerase molecules in the mixed subunit preparations have not been made. A quantitative assay has been developed for the measurement of active molecules of E. coli RNA polymerase using known promoters on T7 DNA (17) but, as shown in Table I, T7 DNA is a poor template for the B. subtilis holoenzyme and is not transcribed by the SP82-modified polymerase. Isolation of fragments of SP82 DNA containing early and middle gene promoters will be required for the direct measurements of the effectiveness of the interaction of the B. subtilis subunits with the interactions with the B. subtilis core subunits preparations and will provide a more accurate assessment of the contribution of different core preparations to the specificity of transcription in the presence of B. subtilis peptides.

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