Highly Asymmetric Transcription by RNA Polymerase Containing Phage-SP01-Induced Polypeptides and a New Host Protein

(heavy and light strands of Bacillus subtilis phage SP01 DNA)

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ABSTRACT An RNA polymerase (nucleosidetriphosphatase:RNA nucleotidyltransferase, EC 2.7.7.6) has been purified from phage-SP01-infected Bacillus subtilis that copies RNA almost exclusively from the heavy strand of native SP01 DNA, the DNA strand from which "middle" and "late" classes of RNA are copied in vivo. Hybridization-competition established that this RNA polymerase, termed enzyme A, preferentially synthesizes middle RNA in vitro. Enzyme A contains β', β, α, and two newly identified host polypeptides, δ (21,500 daltons) and ω (11,000 daltons). All of these polypeptides are associated with highly purified RNA polymerase from uninfected bacteria. In addition, enzyme A contains phage-induced subunits of 26,000, 24,000, and 13,500 daltons. Enzyme A lacks σ polypeptide, and strand-selective transcription by this enzyme is resistant to anti-σ antibody. A reconstitution experiment strongly suggests that the host δ protein is required in addition to a phage-induced subunit(s) (or an unidentified phage-induced modification) for strand-selective transcription of SP01 middle genes in vitro.

The Bacillus subtilis DNA phage SP01 directs the synthesis of six different classes of phage-specified transcripts in a temporally defined sequence (1). Two classes of early RNA (e and em) appear immediately after infection and are copied from both the heavy (H) and light (L) strands of SP01 DNA. This transcription is not inhibited by chloramphenicol and presumably does not require phage-specified protein synthesis. About 4-5 min after infection two classes of middle transcripts (m and m1) are first synthesized largely from strand H (1). This gene transcription requires the product of regulatory gene 28 (2, 3). Finally, late RNA transcripts (mL and l) do not appear until 8-10 min into the lytic cycle and are transcribed almost exclusively from strand H. Mutations in regulatory genes 33 and 34 block the synthesis of late RNA (1-3).

RNA polymerase (nucleosidetriphosphatase:RNA nucleotidyltransferase, EC 2.7.7.6) isolated from SP01-infected cells is associated with several new phage-induced polypeptides (4). It seemed possible that at least some of these proteins could be regulatory elements that direct the transcription of middle and late genes. In this paper, we report on the purification of an RNA polymerase from infected cells containing phage-induced subunits and a newly identified host polypeptide of 21,500 daltons. This enzyme (enzyme A) selectively transcribes middle genes from the H strand of SP01 DNA, whereas, RNA polymerase from uninfected cells transcribes early genes from both strands of SP01 DNA. As will be discussed, enzyme A is apparently different from other RNA polymerases isolated from phage-infected B. subtilis that have been reported to synthesize middle RNA in vitro (5-7).

METHODS

Preparation of SP01 DNA. Wild-type SP01 (obtained from D. Shub) was grown and partially purified as described (8) except that phage were initially precipitated with polyethylene glycol. Phage were further purified on a CsCl step gradient followed by equilibrium banding in CsCl. Template DNA was extracted with sodium dodecyl sulfate (NaDodSO4) at 65° (9). Strands of SP01 DNA were separated by the method of Sheldrick and Szybalski (10).

Purification of RNA Polymerase. B. subtilis strain NCTC 3610 was grown at 37° in 121A medium (8), infected at a multiplicity of 5 to 10 by SP01 during exponential growth, and harvested 12-14 min after infection. Frozen cells (50 g) were disrupted in 300 ml of buffer G (4) by sonication for 20 min at 0-4°. RNA polymerase was first partially purified through the DNase treatment step as described previously (4) except that the dialysis time after RNase (10 mg) treatment was reduced to 4 hr. After DNase (5 mg) treatment (3 hr at 0°) insoluble material was removed by centrifugation at 100,000 × g for 1 hr and the supernatant fluid was then directly applied to a column (4 × 80 cm) of Bio-Gel A-1.5 agarose internally supported by 6 mm glass beads. RNA polymerase was eluted from the column as a single peak of activity with buffer A (4) containing 0.3 M KCl and 10% glycerol. The enzyme was then dialyzed against buffer C (4) containing 20% glycerol and 0.1 M KCl and applied to a DEAE-cellulose column (4 × 7 cm). More than 90% of the enzyme was eluted step-wise in buffer C between 0.1 M and 0.24 M KCl. After dialysis against buffer C containing 0.1 M KCl, the enzyme was applied to a phosphocellulose column (2.3 × 4 cm). The bulk of the protein flowed through the column and about 30% of the enzyme eluted with the trailing edge of the protein in the flow-through. An additional 25% of the enzyme was eluted step-wise by buffer containing 0.2 M KCl. The remaining enzyme was then eluted with a linear gradient of 0.2-0.6 M KCl. About 12% of the RNA polymerase eluted at 0.25 M KCl and about 10% eluted at 0.30 M KCl. (Occasionally an additional small peak of enzyme activity eluted at 0.35 M KCl.) Together these fractions accounted for about 80% of the enzyme applied to the column. Enzyme eluting at 0.1 M KCl was almost in-

Abbreviations: 2 × SSC, 0.3 M NaCl/0.03 M sodium citrate (pH 7.4); NaDodSO4, sodium dodecyl sulfate; H and L, heavy and light strands of SP01 DNA; CAM, chloramphenicol.
eluting at 0.25 M KCl (enzyme B) and 0.30 M KCl (enzyme C) from phosphocellulose were obtained in pure form by zone centrifugation.

**In Vitro RNA Synthesis and Hybridization Reactions.** The synthesis of [H]RNA in vitro was as described previously (14) except that the reaction mixture contained SP01 DNA (24 μg/ml) as template and the incubation was for 10 min at 37°. Hybridization reactions were incubated at 68° for 3–4 hr and then treated as described (14).

**Preparation of RNA Pulse-Labeled In Vivo and the Purification of Unlabeled RNAs.** One milliliter of cells (2.4 × 10⁶) grown in 121A medium (8) was labeled with 100 μCi of [3H]-uridine (28 Ci/mol) for 2 min at the indicated times after infection (multiplicity of infection = 5). The cells were rapidly chilled, brought to 0.01 M in sodium azide, and RNA was extracted as described previously (14). Unlabeled RNA was purified (14) from cells grown in 121 A medium, infected with SP01 (multiplicity of infection = 5–10), chilled at the indicated times after infection by pouring cells over frozen 121 medium containing sodium azide, and harvested by centrifugation.

**Anti-σ Antiserum.** Anti-σ antibody was prepared by immunizing a rabbit with σ purified by NaDodSO₄ polyacrylamide gel electrophoresis. The anti-σ gamma globulin inhibited σ polypeptide, but did not affect core polymerase (R. Tjian, D. Stinchcomb, and R. Losick, manuscript in preparation).

**RESULTS**

**Polypeptide composition of enzymes A, B, and C**

RNA polymerase was purified from SP01-infected B. subtilis as described in the Methods. Chromatography on phosphocellulose resolved at least three different forms of RNA polymerase, which are referred to as enzymes A, B, and C. After further purification, the subunit structure of each enzyme was analyzed by electrophoresis on an NaDodSO₄ polyacrylamide slab gel. First, for comparison, Fig. 2 shows the subunit structure of RNA polymerase from uninfected B. subtilis. Holoenzyme (sample 2, Fig. 2), purified to apparent homogeneity (11), contained β′, β, σ, ε, and two additional polypeptides of approximately 21,500 and 11,000 daltons that were not previously described. These proteins have been named δ and ω, respectively. [A possible reason that δ and ω were not detected earlier is that electrophoresis on NaDodSO₄ gels of 5% polyacrylamide in phosphate buffer as employed previously (11) is not as sensitive as the slab gel procedure of the present study (13).] Core polymerase, purified by phosphocellulose chromatography of holoenzyme, lacked both the σ and δ subunits (sample 1, Fig. 2). (“Holoenzyme” and “core polymerase” will hereafter refer only to RNA polymerase from uninfected cells. These forms of the enzyme contain the polypeptides illustrated for them in Fig. 2.)

Enzyme A contained polypeptides corresponding in mobility to β′, β, σ, δ, and ω as well as three other proteins of molecular weights 26,000, 24,000, and 13,500 (sample 3, Fig. 2). All of the polypeptides coseparated with RNA polymerase during zone centrifugation, the last step in the purification of enzyme A (Fig. 1). (On heavily loaded gels trace amounts of three other polypeptides were occasionally observed.) The polypeptide of enzyme A with the mobility of δ coelectro-

![Fig. 1. Zone centrifugation of enzyme A. DEAE-Sephadex-purified enzyme A (1.2 mg) was concentrated against Ficoll to 0.6 ml and sedimented through a 12 ml linear gradient of 10–30% (v/v) glycerol in buffer A (4) containing 0.1 M KCl at 2° for 20 hr at 39,000 rpm in an International SB 283 rotor. Fractions (0.54 ml) were collected and assayed with 2.8 μg of SP01 DNA as template as previously described (12) except that KCl was omitted from the reaction mixtures (4). One unit of RNA polymerase activity is defined as the amount of enzyme that incorporates 1 nmol of [14C]AMP in 10 min at 37°. Protein concentration was determined from the absorbance at 280 nm (4). Beginning with fractions 1 and 2, 50 μl from adjacent fractions were pooled and subjected to electrophoresis on a 25 cm long slab gel containing NaDodSO₄ in a Tris–glycine buffer and a linear gradient of 7–15% polyacrylamide (13). Sedimentation standards, β-galactosidase (β-gal) and hemoglobin (Hb), were sedimented in parallel in a duplicate gradient.

![Fig. 2. NaDodSO₄ polyacrylamide gel electrophoresis of RNA polymerase from uninfected and SP01-infected B. subtilis.](image-url)
The RNA-RNA annealing reactions contained 30–40 ng of [3H]RNA (400 cpm/ng) synthesized in vitro by the indicated enzyme and 0.15–0.3 mg of the indicated unlabeled RNA (described in the legend to Fig. 4) in a total volume of 150 μl of 2× SSC. The reactions were incubated at 69°C for 3 hr, chilled, and incubated with RNase A (20 μg) and RNase T1 (20 units) in 1 ml of 2× SSC for 1 hr at 35°C. RNase-resistant material was precipitated with trichloroacetic acid in the presence of 20 μg of bovine serum albumin as carrier.

Phage SP01 induces several polypeptides that bind to RNA polymerase (4). The 26,000 and 24,000 dalton species of enzyme A coelectrophoresed with the radioactively labeled phage-induced polypeptides IV and V of our previous study (Fig. 4 of ref. 4). A “pulse-chase” experiment had shown that polypeptides IV and V are synthesized de novo following phage infection, whereas, host subunits β′, β, and α are synthesized before infection (4). An analogous “pulse-chase” experiment indicates that polypeptides δ and ω in enzyme A are synthesized before phage infection, while subunits IV and V and the 13,500 dalton species (hereafter referred to as subunit VI) are induced after SP01 infection.

**Strand-selective transcription by enzyme A**

To provide a stringent test of the specificity of in vitro transcription by enzymes A, B, and C, we have taken advantage of the reported strand selectivity of middle and late gene transcription in vitro (1). The experiment of Fig. 3a shows that RNA synthesized in vitro early after infection by phage SP01 hybridizes to both the heavy (H) and light (L) strands of SP01 DNA (an H/L hybridization ratio of 2.0), while RNA synthesized during the middle or late part of the lytic cycle hybridizes almost exclusively to H strand (H/L hybridization ratios of 10 (Fig. 3b), and 16 (data not shown), respectively). Therefore, RNA polymerase that directs early RNA synthesis (or initiates randomly in vitro) would be expected to copy RNA from both strands of native SP01 DNA, while enzyme that directs middle or late RNA synthesis should copy RNA predominantly from strand H. As expected, RNA synthesized in vitro by either holoenzyme or core polymerase hybridized to both strands of SP01 DNA with H to L ratios of 2.1 (Fig. 3c) and 1.4 (Fig. 3d), respectively. Similarly, RNA synthesized by enzymes B and C hybridized with only a slight preference to H strand DNA (H/L hybridization ratios of 2.1 (Fig. 3e) and 1.7 (data not shown), respectively). In striking contrast, however, enzyme A copied RNA almost exclusively from the H strand of native SP01 DNA (an H/L hybridization ratio of 11, Fig. 3f).

RNA-RNA annealing experiments provided an independent test of the asymmetry of transcription. [3H]RNA synthesized in vitro was annealed to nonradioactive RNA that had been purified from SP01-infected bacteria at various times after infection; the percentage of [3H]RNA that formed RNA-RNA duplexes with in vitro synthesized messenger RNA was taken as a measure of the proportion of in vitro synthesized RNA representing anti-messenger RNA. As expected, B. subtilis holoenzyme, an enzyme reported to specifically transcribe the early genes of SP01 DNA in vitro (16), synthesized only a low proportion of anti-messenger RNA (Table 1). On the other hand, a large percentage of the RNA synthesized by either core polymerase or enzyme C contained anti-messenger RNA sequences. Enzyme B synthesized a substantial proportion of anti-messenger RNA but less than that synthesized by core polymerase or enzyme C. However, enzyme A synthesized little anti-messenger RNA.

### Table 1. Anti-messenger RNA synthesized in vitro

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>CAM RNA</th>
<th>10 min RNA</th>
<th>20 min RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holoenzyme</td>
<td>4</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Core polymerase</td>
<td>38</td>
<td>42</td>
<td>48</td>
</tr>
<tr>
<td>Enzyme A</td>
<td>7</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Enzyme B</td>
<td>26</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Enzyme C</td>
<td>42</td>
<td>46</td>
<td>48</td>
</tr>
</tbody>
</table>

Fig. 3. Hybridization of [3H]RNA pulse-labeled in vitro and [3H]RNA synthesized in vitro to the H and L strands of SP01 DNA. Hybridization reactions contained the indicated amounts of H strand (○) and L strand (□) DNA and the following [3H]-RNAs in 175 μl of 0.3 M NaCl/0.03 M sodium citrate, pH 7.4 (2 × SSC): (a) RNA extracted from cells pulse-labeled 1–5 min after infection [input = 26,900 cpm (120 cpm/ng); background = 70 cpm]; (b) RNA extracted from cells pulse-labeled 8–10 min after infection [input = 21,800 cpm (100 cpm/ng); background = 65 cpm]; (c) RNA synthesized in vitro by holoenzyme from uninfected B. subtilis [input = 30,000 cpm (400 cpm/ng); background = 250 cpm]; (d) RNA synthesized in vitro by core polymerase from uninfected B. subtilis [input = 17,000 cpm (400 cpm/ng); background = 475 cpm]; (e) RNA synthesized in vitro by enzyme B [input = 20,500 cpm (400 cpm/ng); background = 240 cpm]; (f) RNA synthesized in vitro by enzyme A [input = 41,900 cpm (400 cpm/ng); background = 445 cpm]. The backgrounds indicated above were subtracted from the radioactivity hybridized. The ordinate scale of panels (a) and (b) was expanded relative to the other panels of the figure since a large fraction of the input [3H]RNA synthesized in vitro represented B. subtilis RNA and hence did not hybridize to SP01 DNA.
Fig. 4. Competition of the hybridization of \([\text{H}]\text{RNA}\) to SP01 DNA by unlabelled RNA purified from SP01-infected \(B.\) subtilis at different times after phage infection. Hybridization reactions contained the \([\text{H}]\text{RNAs}\) described below; 2.5 \(\mu\)g of denatured SP01 DNA or 0.5 \(\mu\)g of \(H\) strand SP01 DNA, and the indicated amounts of unlabelled competitor RNA in a total volume of 150–200 \(\mu\)l of 2 \(\times\) SSC. Hybridization-competition of (a) early SP01 RNA pulse-labeled 1–3 min after phage infection; 10\% of the input RNA (22,600 cpm) hybridized in the absence of competitor, (b) middle SP01 RNA pulse-labeled 8–10 min after SP01 infection; 38\% of the input RNA (21,000 cpm) hybridized, (c) RNA synthesized \(in\) \(vitro\) by \(B.\) subtilis holoenzyme; 37\% of the input RNA (25,800 cpm) hybridized, (d) RNA synthesized \(in\) \(vitro\) by enzyme \(A\); 73\% of the input RNA (33,300 cpm) hybridized, (e) RNA synthesized \(in\) \(vitro\) by core polymerase from uninfected \(B.\) subtilis; 35\% of the input RNA (9100 cpm) hybridized, and (f) RNA synthesized \(in\) \(vitro\) by enzyme \(B\); 42\% of the input RNA (9100 cpm) hybridized. For each reaction a background of less than 3\% of the input was subtracted from the radioactivity that hybridized. RNA synthesized by enzyme \(A\) was hybridized to \(H\) strand DNA, since this allowed more quantitative hybridization; for purposes of comparison, \(H\) strand was also used to hybridize RNA synthesized by holoenzyme. All other hybridizations were to denatured SP01 DNA. Competitor RNA was purified as described in the Methods. CAM (C) RNA was prepared from bacteria treated with 150 \(\mu\)g/ml of chloramphenicol before phage infection; 10' (■) and 20' (△) RNAs were purified from cells 10 and 20 min after infection; and 22' \(\text{sus F4}\) (○) RNA was purified from cells 22 min after infection with the mutant phage \(\text{sus F4}\) (obtained from S. Okubo).

Thus, both holoenzyme and enzyme \(A\) catalyzed highly asymmetric transcription of native SP01 DNA \(in\) \(vitro\).

Enzyme \(A\) synthesizes middle RNA

Hybridization-competition established that enzyme \(A\) generated middle RNA \(in\) \(vitro\). \([\text{H}]\text{RNA}\) synthesized \(in\) \(vitro\) was hybridized to SP01 DNA in the presence of competitor RNAs isolated at early (CAM), middle (10 min) and late (20 min) times after SP01 infection of \(B.\) subtilis. (As a control, the effect of the competitors on the hybridization of RNA pulse-labeled \(in\) \(vitro\) at early and middle times in the lytic cycle is shown in Fig. 4a and b.) The hybridization of \([\text{H}]\text{RNA}\) synthesized by enzyme \(A\) was more effectively competed by middle RNA than by the early competitor (Fig. 4d). Although late RNA competed significantly (Fig. 4d), RNA isolated late in the lytic cycle contains middle RNA (Fig. 4b) as well as late sequences. As further evidence that enzyme

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\begin{array}{|c|c|c|c|}
\hline
\text{Enzyme} & \text{Factors} & \text{Anti-}\sigma & \text{Total [H]RNA} \\
\text{A} & - & - & 410 \times 10^{-4} \\
\text{A} & + & - & 280 \times 10^{-4} \\
\text{A} & - & \sigma & 550 \times 10^{-4} \\
\text{B} & - & - & 590 \times 10^{-4} \\
\text{B} & + & - & 640 \times 10^{-4} \\
\text{B} & - & \delta & 100 \times 10^{-4} \\
\text{B} & + & \delta & 130 \times 10^{-4} \\
\hline
\text{H/L ratio} & & & \\
7.5 & & & \\
8.0 & & & \\
4.5 & & & \\
1.9 & & & \\
1.7 & & & \\
1.4 & & & \\
1.7 & & & \\
\hline
\end{array}
\]

\([\text{H}]\text{RNA} was synthesized as described in the Methods in reaction mixtures (0.5 ml) containing enzymes \(A\) (5.6 \(\mu\)g) or \(B\) (3.7 \(\mu\)g) purified through the phosphocellulose chromatography step or core RNA polymerase (3.5 \(\mu\)g, ref. 11) from uninfected cells. Reaction mixtures also contained, where indicated, purified \(\sigma\) (3.5 \(\mu\)g, ref. 11), the \(\beta\)-containing fraction (0.8 \(\mu\)g) described in the text, or sufficient anti-\(\sigma\) antiserum to inactivate 5 \(\mu\)g of holoenzyme. Hybridization reactions contained 5% of the in \(vitro\) synthesized RNA plus increasing amounts of H and L strand DNA as illustrated in Fig. 3. The H/L hybridization ratios were calculated from the \([\text{H}]\text{RNA} hybridized to 0.65 \(\mu\)g of each DNA strand.

A generated largely middle sequences, RNA from bacteria infected with \(\text{sus F4}\), a mutant of SP01 blocked in late RNA synthesis (2), was almost as effective a competitor as the middle RNA and more effective than the late RNA (Fig. 4d). In contrast, the hybridization of RNA synthesized \(in\) \(vitro\) by holoenzyme was more effectively inhibited by the early RNA than by the middle or late competitors (Fig. 4e). Together with the experiments of Fig. 3 and Table 1, the above findings demonstrate that enzyme \(A\) preferentially and asymmetrically transcribed middle genes of SP01 DNA while holoenzyme selectively copied SP01 early genes.

It should be noted that hybridization-competition experiments, by themselves, are a relatively insensitive measure of the specificity of \(in\) \(vitro\) transcription. For example, random transcription of the SP01 genome by core polymerase generated sequences whose hybridization was inhibited by early, middle and late competitors (Fig. 4e). (Not all of the competitive RNA need \(represent\) \(in\) \(vitro\) sequences, however, since the hybridization to DNA of anti-messenger RNA would also be inhibited by the competitor RNA.) As another example, middle RNA inhibited the hybridization of a substantial portion of the RNA synthesized by enzyme \(B\) (Fig. 4f), even though this enzyme exhibited little strand selectivity of transcription (Fig. 3e).

Asymmetric transcription by enzyme \(A\) is resistant to anti-\(\sigma\) antibody

Strand-selective transcription by enzyme \(A\) was not impaired by antibody directed against \(\sigma\) polypeptide (Table 2). In fact, the addition of \(\sigma\) itself to enzyme \(A\) actually caused partial inhibition of strand-selective transcription (Table 2). NaDODSO4 slab gel analysis had indicated that enzyme \(A\) contained no detectable \(\sigma\) polypeptide (Fig. 2), and the above experiments argue against the possibility that even trace amounts of \(\sigma\) could be acting catalytically to promote asymmetric transcription by enzyme \(A\).
The host $\delta$ polypeptide stimulates asymmetric transcription by enzyme B

A comparison of the subunit structure of enzyme A and B (Fig. 2) raises the possibility that the host $\delta$ polypeptide could be critical for strand-selective transcription. To test this, $\delta$ was isolated from uninfected B. subtilis by chromatography of purified holoenzyme on phosphocellulose. The $\delta$ preparation contained only $\delta$ and some contaminating $\sigma$ polypeptide that was inactivated with anti-$\sigma$ antiserum. Addition of $\delta$ to enzyme B stimulated the strand selectivity of transcription to nearly that characteristic of enzyme A (Table 2). However, the addition of $\delta$ to core polymerase from uninfected cells had little effect on the strand selectivity of transcription by this enzyme (Table 2). Thus, these experiments argue that in addition to a phage-induced polypeptide(s) (or some other undetected modification of polymerase) host $\delta$ protein is also apparently required for highly asymmetric transcription.

[More detailed account of the above experiment will be published elsewhere (J. Pero, J. Nelson, R. Tjian, D. Stinchcomb, and R. Losick, manuscript in preparation).]

DISCUSSION

The strand-selective transcription of SP01 middle genes by enzyme A provides a dramatic demonstration of a change in the transcriptional specificity of a bacterial RNA polymerase containing phage-induced polypeptides and lacking $\sigma$ factor. Do any of the three phage-induced subunits (IV, V, and VI) associated with enzyme A direct middle gene transcription? It is unlikely that subunit V accounts for the transcriptional specificity of enzyme A, since mutants that do not produce this polypeptide in association with RNA polymerase (4) apparently synthesize middle RNA normally in vivo (2, 17). On the other hand, either phage-induced subunit IV or VI (or both) could direct middle gene transcription in vitro. In fact, an earlier study (4) raised the possibility that subunit IV could be the protein product of gene 28, a regulatory gene whose product is essential for middle gene transcription in vivo (2, 3). However, it is not excluded that an unidentified phage-induced subunit or modification of RNA polymerase directs middle gene transcription by enzyme A.

Strand-selective transcription by enzyme A also appears to require a newly described host protein called $\delta$. Enzyme B contains phage-induced subunits IV and trace amounts of VI but exhibited little strand selectivity without the addition of a $\delta$-containing fraction from uninfected bacteria. However, the addition of $\delta$ to core polymerase from uninfected B. subtilis had little effect on strand selectivity of transcription. Thus $\delta$ could be a host subunit of RNA polymerase required in addition to a phage-induced polypeptide(s) (or some other unidentified modification of core enzyme) for highly asymmetric transcription of SP01 middle genes in vitro. It should be noted that $\delta$ could act either to promote middle gene transcription or to suppress incorrect transcription.

Duffy and Geiduschek (5) have previously reported asymmetric synthesis of middle RNA by RNA polymerase from SP01-infected B. subtilis. Recently, these workers (18) reported that their enzyme contains $\beta'$, $\beta$, $\alpha$, newly identified host polypeptides of 11,000 and 9500 daltons, and phage-induced polypeptides of 28,000 and 13,000 daltons. They do not report a protein equivalent to the host $\delta$ subunit and their enzyme could correspond to our enzyme B. Since these workers have not measured the strand selectivity of their polymerase and since their conditions of RNA synthesis differ from those employed here, we cannot compare confidently the transcriptional properties of their enzyme with those of the polymerases described here. However, their enzyme synthesizes more anti-messenger RNA than our enzyme A although less anti-messenger than most of our preparations of enzyme B.

RNA polymerase has also been purified from B. subtilis infected with phage SP82, a phage closely related to SP01 (6). Spiegelman and Whiteley (7) have reported that this enzyme synthesizes middle classes of SP82 RNA in vitro (7). This enzyme is reported to contain, in addition to $\beta'$, $\beta$, and $\alpha$, a 16,000 dalton polypeptide and small amounts of two other polypeptides not present in enzyme from uninfected cells. They do not report the presence of a polypeptide equivalent to the host $\delta$ subunit described here or the strand selectivity of their enzyme.

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