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Defining a bacteriophage T4 late promoter: Bacteriophage T4 gene *55* protein suffices for directing late promoter recognition

(bacteriophage T4 late transcription/RNA polymerase reconstitution/gene regulation/gp55/RNA polymerase σ-factor independence)

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ABSTRACT **The RNA polymerase from bacteriophage T4-infected *Escherichia coli*, which specifically initiates transcription at phage T4 late promoters, is extensively modified by ADP-ribosylation of core subunits and by binding several virus-encoded subunits. We show here that one of these subunits, the phage T4 gene *55* protein, designated gp55, alone endows unmodified RNA polymerase core enzyme from uninfected *E. coli* with the ability to selectively initiate transcription at the phage T4 late promoters, without participation by *E. coli* RNA polymerase σ subunit.**

Bacteriophage T4 gene expression is temporally regulated, predominantly at the level of transcription. Three distinct promoter types, termed early, middle, and late, are known to be involved in this regulation (1, 2), and more might yet remain to be identified (3). Early transcription is initiated by the at-first-unmodified *Escherichia coli* RNA polymerase holoenzyme at T4 promoters that are indistinguishable from strong bacterial promoters. Middle transcription, which requires the T4 *motA*, *motB*, and *motC* gene products (4–7), utilizes T4 promoters that are thought to differ from T4 early promoters only in the so-called "−35" region. Recent work on the DNA-binding motA protein should make it possible to better define the consensus sequences of middle promoters precisely (refs. 8 and 9; E. N. Brody, personal communication). Late promoters share a characteristic sequence, T-A-T-A-A-A-T-A, in place of the canonical bacterial "−10" sequence but lack any homology in the −35 region (3, 10, 11).

The bacterial transcription apparatus undergoes a strikingly elaborate set of modifications after phage T4 infection. Five phage-encoded proteins bind to the RNA polymerase core (which is also modified by ADP ribosylation after infection). Three of these proteins, encoded by T4 genes *33*, *45*, and *55*, are required for late transcription *in vivo*. The phage genes coding for the other two proteins (M_rs *ca.* 15,000 and 10,000) have not been identified. In addition, a protein encoded by the T4 *alc/unf* gene restricts transcription to DNA containing hydroxymethylcytosine instead of cytosine (12, 13). Furthermore, T4 chromosomes (but not plasmids carrying T4 late genes) require an activation of the T4 DNA template *in vivo*, which is normally provided by concurrent T4 DNA replication.

It has been shown that two phage T4 late promoters, P_{23} and P_{24} (located just upstream of T4 genes *23* and *24*, which code for two proteins of the phage head), are selectively utilized *in vitro* by purified RNA polymerase isolated from T4-infected cells but not by RNA polymerase isolated from T4 gene *55* mutant-infected cells (3). This selective transcription operates equally on cytosine-containing recombinant plasmid DNA and on hydroxymethylcytosine-containing phage DNA. The *in vitro* relaxation of *in vivo* requirements regarding DNA replication and modification makes certain lines of inquiry into the positively regulated late gene transcription system readily accessible. In this paper we show that *E. coli* RNA polymerase σ subunit (factor) is not required for initiation at T4 late promoters and that the T4 gene *55* product, designated gp55, alone suffices to confer late-promoter specificity upon unmodified RNA polymerase core from uninfected bacteria.

MATERIALS AND METHODS

Materials. Poly[d(A-T)], unlabeled ribonucleoside triphosphates, and the diribonucleoside monophosphate GpA were from Pharmacia P-L Biochemicals; [α-^{32}P]UTP (410 Ci/mmol; 1 Ci = 37 GBq) was from Amersham Radiochemicals; rifampicin and protein A-Sepharose CL-4B were from Sigma; and phosphocellulose P-11 was from Whatman. Heparin-agarose was prepared by H. Choy as described (14). Polymin P from BASF Wyandotte (Parsippany, NJ) and Bio-Rex 70 from Bio-Rad were further prepared as described (15). Bovine serum albumin (crystallized fraction V) from Miles was acetylated (16) to remove trace ribonuclease activity. RNasin (30 units/μl) was from Biotec (Madison, WI). Plasmid pTE110 DNA was prepared as described (3). pTE110, a derivative of the cloning vector pUC8, contains a *Bgl* II–*Hpa* I segment of phage T4 with the gene *23* late promoter, P_{23}, inserted upstream of the bacteriophage T7 early terminator, T_{T7}, so oriented that initiation at P_{23} gives rise to a 420-nucleotide transcript ending at T_{T7} (11).

RNA polymerase holoenzyme prepared by D. P. Rabussay (17) had a specific activity of 165 milliunits/mg on poly[d(A-T)] in the assay described below (1 milliunit yields 1 nmol of UMP incorporated per min of RNA synthesis in that assay). Purified anti-*E. coli* RNA polymerase σ subunit rabbit IgG (anti-σ antibody) had been prepared by M. Filip and D. P. Rabussay. *E. coli* RNA polymerase core, the gift of M. Chamberlin (16) {specific activity, 249 milliunits/mg on poly[d(A-T)]}, contained significant residual σ activity (preparation PC, σ-contaminated). It was further depleted of σ by chromatography on Bio-Rex 70 (adapted from ref. 15). This σ-depleted core (preparation BR) had a specific activity of 240 milliunits/mg on poly[d(A-T)]. Traces of σ could be discerned when 5 μg of total enzyme protein was analyzed on an extensively developed silver-stained gel. Holoenzyme activity was measured on pTE110 in terms of the P_4 promoter-initiated, 120-nucleotide-long transcript from the region of the replicative origin (18). This is the most abundant RNA made by holoenzyme on pBR322 (18) and, apparently, on pTE110 DNA (see Fig. 1, lane a). The BR core enzyme showed a 2.4-fold reduction of σ-subunit activity relative to the PC core starting material.

Purification of Late Phage T4-Modified RNA Polymerase. RNA polymerase was prepared from *E. coli* DG156 (RNase I$^-$) infected with T4D e$^-$ phage at 37°C for 18 min. The purification procedure outlined below was optimized for the re-

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Abbreviation: anti-σ antibody, anti-*E. coli* RNA polymerase σ subunit IgG.

covery of β and β' subunits (15) from phage T4-infected cell extracts up to the step of chromatography on heparin-agarose. Aliquots of infected cells were lysed, and the bulk nucleic acids were removed by precipitation with polymin P as described by Burgess and Jendrisak (15), with minor modifications to ensure efficient shearing of the especially viscous, DNA-rich lysate of phage-infected bacteria. The polymin P precipitate was washed twice with buffer A containing 0.5 M NaCl [buffer A is 10 mM Tris chloride, pH 8.0/7.5% (vol/vol) glycerol/0.1 mM Na_3 EDTA/0.2 mM dithiothreitol]. RNA polymerase was then eluted with 4 ml of buffer A containing 1.0 M NaCl per gm of infected cells and was precipitated with ammonium sulfate (0.36 g/ml of solution). The ammonium sulfate precipitate was resuspended in sufficient buffer B (buffer A containing 10 mM $MgCl_2$) to give a final conductivity equal to that of buffer B containing 0.3 M NaCl (*ca.* 3-ml final volume per gm of cells). The suspension was added to heparin-agarose in buffer B (2 ml of agarose per gm of cells), allowed to adsorb to the matrix for 90 min at 2°C, and poured into a column (ref. 19, M. Gilman, personal communication), and then washed with buffer B containing 0.3 M NaCl to $A_{280} < 0.05$. Polymerase was eluted with buffer A containing 0.6 M NaCl and dialyzed into storage buffer [10 mM Tris chloride, pH 8.0/0.1 mM Na_3 EDTA/0.2 mM dithiothreitol/10 mM $MgCl_2$/100 mM NaCl/50% (vol/vol) glycerol (16)]. Total polymerase activity, assayed on poly[d(A-T)] and on pTE110, increased after each step of the purification, presumably due to removal of inhibitors and nucleases. This protein (225 mg) was loaded onto a 2.5 × 7 cm phosphocellulose P-11 column and eluted with a 300-ml 0–0.6 M NaCl linear gradient in T50 buffer (50 mM Tris chloride, pH 8.0/0.1 mM EDTA/0.2 mM dithiothreitol/50% glycerol). RNA polymerase was eluted as a single peak at 0.19 M NaCl. As T4 late-transcribing activity and gp55 subunit content were somewhat enriched in the leading shoulder of the RNA polymerase peak, appropriate fractions representing one-sixth of the total RNA polymerase were pooled (F43–46; 16 ml, 0.93 mg/ml, 56 milliunits/mg on pTE110; estimated to be *ca.* 90% pure). Protein concentrations (20) were determined after acid precipitation.

RNA Synthesis. RNA polymerase activity was routinely assayed in 50 μl of reaction medium (40 mM Tris chloride, pH 8.0/10 mM $MgCl_2$/10 mM 2-mercaptoethanol/50 mM NaCl/100 μg of acetylated bovine serum albumin per ml) with either 40 μg of poly[d(A-T)] per ml, 400 μM ATP, and 100 μM [α-^{32}P]UTP (50–200 cpm/pmol) or 20 μg of pTE110 per ml, 400 μM each ATP, GTP, and CTP, and 100 μM [α-^{32}P]UTP (500–2000 cpm/pmol) for 10 min at 37°C. Reaction mixtures were assembled on ice and transferred to 37°C to start transcription. When the reaction products were destined for electrophoretic analysis, RNA synthesis was stopped with 200 μl of 10 mM Tris chloride, pH 8/3 mM Na_3EDTA. An aliquot was assayed for incorporation of [^{32}P]UMP into RNA, and the remainder was extracted with phenol, precipitated with ethanol, resuspended in 20 μl of 98% formamide containing tracking dyes, boiled 3 min, and loaded on a 4% polyacrylamide gel containing 7 M urea (21).

Anti-σ IgG–Protein A-Sepharose Treatment of RNA Polymerases. One microgram of RNA polymerase holoenzyme, RNA polymerase core (preparation PC), or late phage T4-modified RNA polymerase (F43–46) was incubated in 15 μl of the above-defined reaction medium also containing 150 mM NaCl and 0.05% Nonidet P-40 with or without 50 μg of anti-σ IgG at 0°C for 5 min. The same buffer (7.5 μl) or 7.5 μl of a 67% slurry of protein A-Sepharose in that buffer was then added, mixed, incubated 5 min at 0°C, and centrifuged to remove Sepharose-bound material. A 15-μl aliquot of supernatant solution was removed and assayed on pTE110 in the presence of 150 mM NaCl and 15 units of RNasin. Duplicate samples were examined for σ content by gel electrophoresis (22), followed by silver-staining (23). Although the protein A-Sepharose was insufficient to complex all of the IgG present, σ subunit was preferentially removed from the supernatant [since antigen–IgG complexes bind much more rapidly to protein A than free IgG does (24)], and only minor quantities of σ remained in the supernatant (data not shown).

Isolation of Phage T4 gp55 and Reconstruction of Late Transcription Specificity with Unmodified *E. coli* RNA Polymerase Core. Late phage T4-modified RNA polymerase (186 μg of F43–46) was electrophoresed on a 2-mm-thick 12.5% polyacrylamide gel with 1-cm-wide wells. Protein bands were made visible by KCl precipitation of $NaDodSO_4$–protein complexes (25). Gel slices containing protein with apparent M_rs of 10,000, 15,000, 23,000 (gp55) and 26,000 were excised. Protein was eluted from the gel matrix and renatured as described by Hager and Burgess (25).

The ability of each excised protein to confer late transcription specificity on RNA polymerase core was measured by transcription on pTE110 by using dinucleotide-dependent transcription initiation (26) in order to select initiation at P_{23} and minimize core transcription on pTE110. The 5′ end of the P_{23} transcript maps within the sequence T-G-A-T. Accordingly, 100 μM GpA was tested and found to allow efficient initiation at P_{23} when the four ribonucleoside triphosphates were present at 2 μM. [However, it should be noted that the principal findings of this paper could also be derived from experiments (not shown) in which dinucleotide priming was not used.] The effect of each renatured protein on transcription of pTE110 by *E. coli* RNA polymerase core was examined as follows: 20 μl of each renatured protein (2% of the total) or 20 μl of corresponding buffer (50 mM Tris chloride, pH 8.0/0.1 mM Na_3EDTA/1 mM dithiothreitol/20% glycerol/100 μg of acetylated bovine serum albumin per ml) was incubated with 0.9 μg of RNA polymerase core (PC preparation) for 5 min at 0°C in 40 μl of solution adjusted to the composition of reaction medium containing 140 mM NaCl. pTE110 (1 μg in 2 μl) was added next and incubated for 5 min at 37°C. Transcription was initiated with 10 μl of a GpA/nucleotide mixture (500 μM GpA, 12.5 μM each ATP, GTP, CTP, and [α-^{32}P]UTP, the last-named at 21,000 cpm/pmol) in reaction medium containing 140 mM NaCl. After 5 min of synthesis, 5 μl of rifampicin at 1 mg/ml was added to prevent further initiation. After a further 5 min, RNA chains were rapidly elongated to completion by "chasing" with 5 μl of a 5 mM mixture of each unlabeled ribonucleoside triphosphate. RNA chains averaged *ca.* 150 nucleotides and did not exceed 300 nucleotides in length at the onset of the chase (data not shown).

RESULTS

Dispensability of σ Subunit for Late Transcription *in Vitro*. We first examined the possibility that *E. coli* σ factor might participate in late promoter recognition, since a proposal to that effect had been made based on measurements of total RNA synthesis during infection of *E. coli* harboring a temperature-sensitive mutation in *rpoD*, the σ structural gene (27). The effect of anti-σ antibody on transcription of pTE110 by holoenzyme, core, and late phage T4-modified RNA polymerase is shown in Fig. 1. A transcript initiated at the T4 late promoter P_{23} and terminated at the T_{T7} terminator would be 420 nucleotides long (11). The 120-nucleotide transcript, initiated at the holoenzyme promoter P_4 (18), was used to monitor σ activity (Fig. 1, lane a). Addition of 50 μg of anti-σ IgG virtually eliminated its synthesis (Fig. 1, lane b); the residual transcription in lane b resembled the product generated by RNA polymerase core (lane k). The most prominent core-specific transcript in this exposure of the autoradiogram is designated CT. In lighter exposures, the patterns of bands contained within the overexposed high molecular weight portions of lanes b and k were also similar

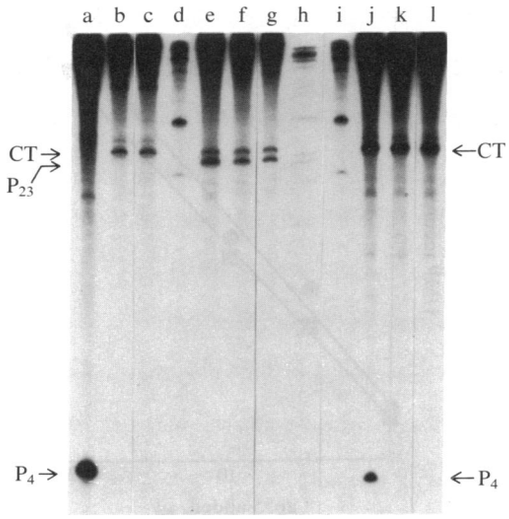

FIG. 1. Effect of antibody to *E. coli* σ subunit on transcription *in vitro*. One microgram of RNA polymerase holoenzyme (lanes a–c), late phage T4-modified RNA polymerase (lanes e–g), or RNA polymerase core (preparation PC; lanes j–l) was incubated with (lanes b, c, f, g, k, and l) or without (lanes a, e, and j) 50 μg of anti-σ IgG. Protein A-Sepharose (lanes c, g, and l) or buffer (lanes a, b, e, f, j, and k) was subsequently added, followed by centrifugation. The supernatants were added to transcription mixtures containing pTE110 DNA, and the synthesized RNA was analyzed as described. The picomoles of UMP incorporated into RNA during the labeling stage, for the samples shown in the respective lanes, were as follows: a, 367; b, 124; c, 116; e, 164; f, 126; g, 90; j, 272; k, 224; l, 220. Electrophoresis was carried out on a 4% polyacrylamide gel containing 7 M urea. Control lanes showing that protein A-Sepharose alone had essentially no effect on RNA synthesis were left out of this figure. An RNase III digest of phase T7 early RNA (28) (lanes d and i), and a labeled *Hpa* I digest of T7 DNA (29) (lane h) served as size standards. The 420- and 120-nucleotide-long transcripts initiated at P_{23} and P_4 are indicated.

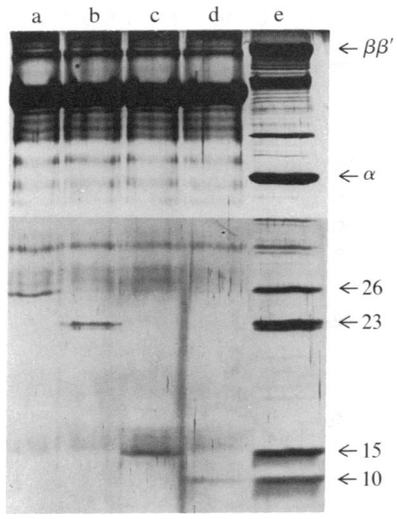

FIG. 2. Polyacrylamide gel electrophoretic analysis of subunits isolated from late phage T4-modified RNA polymerase. Two percent of the M_r 26,000 (lane a), gp55 (M_r 23,000) (lane b), M_r 15,000 (lane c), and M_r 10,000 (lane d) protein preparations were electrophoresed on a 12.5% polyacrylamide gel (22) and silver-stained (23); M_rs are shown $\times 10^{-3}$. Lane e: nine micrograms of the late T4-modified RNA polymerase which was used to prepare the subunits. The abundant proteins common to lanes a–d are contributed by the acetylated bovine serum albumin in the renaturation buffer. The top of the figure has been underexposed relative to the bottom.

(data not shown). Evidently, *E. coli* RNA polymerase core alone generates specific transcripts on this DNA. The transcribing activity of the core enzyme on pTE110 was salt sensitive (150 mM NaCl was used here to diminish core transcription) and dependent on superhelicity of the DNA (ref. 3; data not shown).

In contrast to the situation at the holoenzyme promoter P_4, the synthesis of the P_{23}-initiated transcript (identified in Fig. 1 at the left of lane a) by late phage T4-modified RNA polymerase was insensitive to the presence of anti-σ IgG (compare lanes e and f in Fig. 1). In order to address the possibility that late enzyme-attached σ subunit bound to antibody could still function, most of the σ–IgG complex was removed by binding to protein A-Sepharose prior to transcription, without effect on P_{23} utilization (compare lanes f and g). The remaining possibility, that σ might be shielded from antibody in T4-modified RNA polymerase, is addressed below.

Conversion of RNA Polymerase Core from Uninfected *E. coli* to Late Transcription Specificity by Phage T4 gp55. The effect of phage T4-induced RNA polymerase-binding proteins on late transcription *in vitro* was examined next. Late T4-modified RNA polymerase was denatured and electrophoresed through 12.5% polyacrylamide containing NaDodSO$_4$. Individual polypeptides were eluted and renatured as described. Their degree of purification is shown in Fig. 2. The M_r 26,000 protein did not coelute precisely with RNA polymerase on phosphocellulose but was examined further because its size is similar to that of gp45. The T4-modified RNA polymerase contained only a trace, if any, of the M_r 12,000 gp33 (Fig. 2, lane e).

The effect of each of these proteins on transcription of pTE110 DNA by *E. coli* RNA polymerase core is shown in Fig. 3. gp55 (apparent M_r 23,000) generated the P_{23} late transcript (Fig. 3, lane e), but addition of the M_r 10,000, 15,000, or 26,000 proteins (lanes c, d, and f, respectively) had no such effect. We attribute the low level of the P_{23} transcript present in lane f to slight trailing of the gp55 band on the original isolation gel. Transcripts made by RNA polymerase core (lane b) and late phage T4-modified RNA polymerase (lane g) are shown for reference. The functional state of the denatured and renatured gp55 can be assessed by comparing lanes e and g. For lane e, an amount of gp55 representing the yield from 3.7 μg of T4-modified RNA polymerase was added to 0.9 μg of core enzyme. The yield of P_{23} transcript exceeded that of 0.9 μg of T4-modified polymerase (lane g). Thus, the renaturation must have been at least 30% effective. This result also argues strongly against the possibility that a minor and inapparent contamination by one of the other known T4-modified RNA polymerase-binding proteins, was responsible for conferring specific late promoter recognition.

All four protein preparations stimulated the residual σ subunit activity of this core enzyme as judged by the increased abundance of the P_4 transcript (compare lanes c–f with lane b in Fig. 3). We have not investigated this effect further. Being common to all samples, it might result from component(s) introduced during isolation and renaturation rather than from the individual phage T4 proteins. The apparently nonspecific ≈2-fold stimulation of total incorporation by proteins of M_rs 10,000, 15,000, and 26,000 may have a related cause. The τ transcript (Fig. 3), made by late T4-modified RNA polymerase (lane g) and by RNA polymerase core with gp55 (lane e), appears to have been generated by low nucleotide concentration-dependent termination of transcription because it was detected only when elongation of RNA chains at low concentrations of ribonucleoside triphosphates lasted >6 min (data not shown). A comparison of lanes b and e in Fig. 3 indicates that gp55, which greatly stimulated total RNA synthesis (see Fig. 3 legend and Fig. 4) also stimulated specific transcription by RNA polymerase core (but proteins of M_rs 10,000, 15,000, or 26,000 did not), since many of the

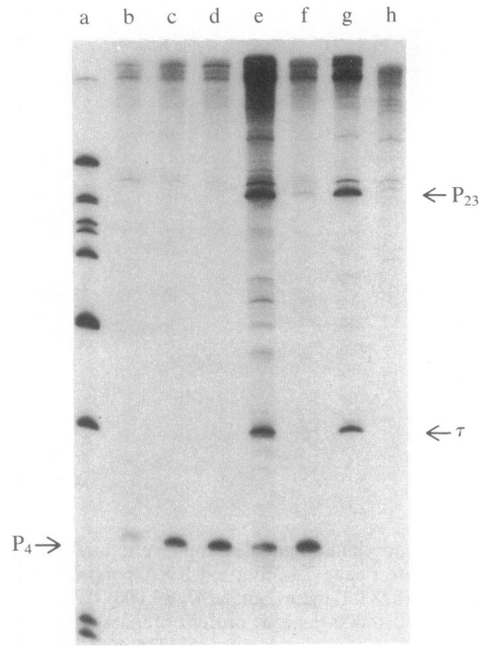

FIG. 3. Reconstruction of phage T4 late transcription specificity with unmodified *E. coli* RNA polymerase core and gp55. RNA polymerase core (0.9 μg of PC preparation) was incubated with buffer (lane b), M_r 10,000 protein (lane c), M_r 15,000 protein (lane d), M_r 23,000 protein (gp55) (lane e), or M_r 26,000 protein (lane f) and used to transcribe pTE110 DNA as described. Lane g shows the transcripts made by 0.9 μg of late T4-modified RNA polymerase. A labeled *Hin*fI digest of pBR322 (30) (lane a) and an *Hpa* I digest of phage T7 DNA (lane h) served as size standards. Electrophoresis was as in Fig. 1. The picomoles of UMP incorporated into RNA in 10 min at 37°C in the respective lanes were as follows: b, 1.0; c, 2.0; d, 2.1; e, 11.0; f, 2.5; g, 4.2. The transcripts initiated at P_4 and P_{23} are marked at the sides of the figure. The τ transcript is discussed in the text.

minor bands present in lane e also could be seen in much smaller amounts in lane b (see Fig. 5, also).

The ability of gp55 to confer late transcription specificity on RNA polymerase core allowed us to examine the role of σ subunit in a different way. Increasing concentrations of gp55 were added to two preparations of RNA polymerase core, PC (σ-contaminated) and BR (σ-depleted), and stimulated total transcription to approximately the same extent (Fig. 4). Increasing the amount of gp55 correspondingly increased the amount of the P_{23} transcript (Fig. 5). The level of P_{23} utilization was unaffected by the σ-depletion of the BR core enzyme, but holoenzyme promoter P_4 utilization was much reduced (compare lanes f–i with lanes b–e). To eliminate the possibility that gp55 and other phage T4-encoded proteins in T4-modified RNA polymerase might shield σ from antibody binding, we treated the σ-depleted core enzyme from uninfected *E. coli* with anti-σ IgG before adding gp55 without effect on the utilization of P_{23} (compare lane j with lane i).

DISCUSSION

The principal findings of this work are that the utilization of the phage T4 late promoter P_{23} does not depend on the function of the *E. coli* RNA polymerase initiation factor, σ, and that late promoter specificity can be conferred on the unmodified *E. coli* RNA polymerase core solely by the T4 gene *55* product, gp55 (M_r 23,000 protein). A. Goldfarb (personal communication), approaching this question in a different way, has come to apparently similar conclusions. It has been proposed that *E. coli* σ might be directly involved in late promoter recognition (27), but our experiments argue strongly against that. Previous experiments with a cellophane disk system (17) also showed that T4 early but not late transcription was inhibited by anti-σ antibody. Addition of σ to this cell-free system stimulated early but not late transcription (unpublished data). Altered promoter recognition of *B. subtilis* RNA polymerase also involves substitutions of the major vegetative σ subunit (σ^{55}) (31–36), and no instance in

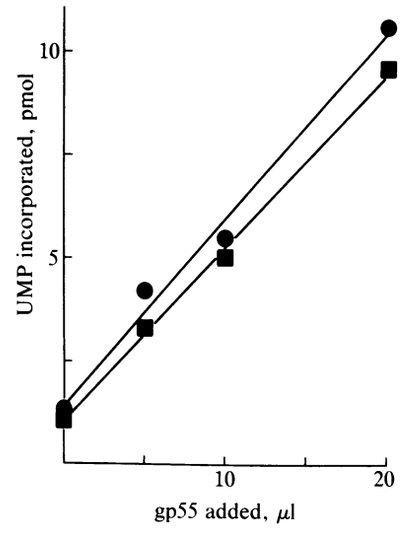

FIG. 4. Effect of added gp55 on total transcription of pTE110 DNA by σ-contaminated RNA polymerase core (PC preparation) and σ-depleted RNA polymerase core (BR preparation). The conditions for RNA synthesis were as described in *Methods* for Fig. 3. pTE110 DNA was transcribed by 0.9 μg of RNA polymerase core PC (●) or BR (■) after preincubation with gp55. Ten microliters of gp55 represents the yield from 1.9 μg of phage T4 late-modified RNA polymerase in the purification described in *Methods*, or 1% of the entire sample.

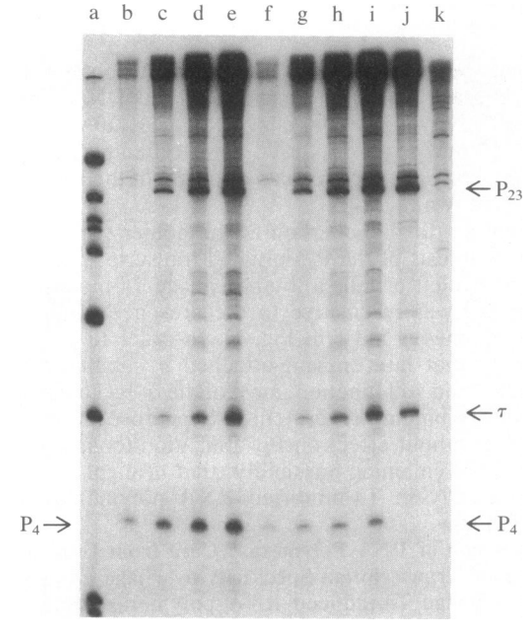

FIG. 5. Effect of added gp55 on the utilization of P_{23} by RNA polymerase core. pTE110 was transcribed by 0.9 μg of RNA polymerase core (PC preparation; lanes b–e) or σ-depleted core (BR preparation; lanes f–i) after prior incubation with none (lanes b and f), 0.5% (lanes c and g), 1.0% (lanes d and h), or 2.0% (lanes e and i) of the renatured gp55 preparation as specified in the legend to Fig. 4. The reaction mixture analyzed in lane j was the same as that shown in lane i except that 50 μg of anti-σ IgG was incubated with the core polymerase for 5 min at 0°C prior to the addition of gp55. Labeled *Hin*fI-digested pBR322 (lane a) and *Hpa* I-digested phage T7 DNA (lane k) served as size standards. Electrophoresis was as in Fig. 1.

which σ is functionally modified by another protein has yet been noted.

Could σ subunit modification, *in principle*, lead to altered promoter recognition or is the structure of RNA polymerase incompatible with that kind of mechanism? If the binding selectivity (though not necessarily the total affinity) of RNA polymerase for DNA were entirely contributed by separate, relatively small, binding domains of σ, like those of the phage λ cro and cI repressors (37, 38), held in place by the polymerase core acting as a kind of jig (36), then substitution might be the general rule, and σ modification might be incompatible with the structural constraints of the protein. Along the lines of that speculation, one might think of the phage T4 gp55 as substituting for only one of the two DNA-binding domains of *E. coli* σ because it directs selective binding to a sequence substituting only for the *E. coli* promoter −10 consensus sequence.

We have noted the ability of the core enzyme to generate specific transcripts on superhelical pTE110 DNA *in vitro* (Fig. 1, lanes k and l), particularly at low ionic strength (data not shown). Supercoiling may generate the structural singularities (39, 40) that favor this activity, which σ factor suppresses but the renatured gp55 stimulates. *E. coli* σ is known to suppress nonspecific binding by RNA polymerase core to DNA (41), but (native) gp55 may lack this discrimination-enhancing property.

If late promoter selection requires only gp55, then how does gp33 work? The amber mutants in gene *33* generate late transcription defects that are (only) slightly less extreme *in vivo* than those of gene *55* mutants, so that it has been assumed, in the past, that the phage T4 gp33 and gp55 together would turn out to be a σ factor substitute (e.g., ref. 42). It now seems that gp33 might prove to have some less direct mode of action, for example serving as an additional component blocking the interaction of *E. coli* σ with the RNA polymerase, as the M_r 10,000 protein and ADP-ribosylation of the α subunits apparently do (43, 44). While gp55 binds to agarose-immobilized RNA polymerase holoenzyme, core, and T4-modified core (45), gp33 binds to RNA polymerase unmodified core and T4-modified core but not to holoenzyme. Thus, it appears that gp33 competes with σ for the same sites on RNA polymerase core. It has been suggested that gp33 has a higher affinity for core when it contains gp55 (1); thus, gp33 should block interaction of σ with RNA polymerase core–gp55 complexes. Nevertheless, it is by no means certain that gp33 exerts its very strong influence on late transcription *in vivo* solely through an anti-σ mechanism. In fact, although gp55 may be the heart of this regulatory system, deciphering the roles of the other virus-encoded RNA polymerase-binding proteins remains a challenging and interesting problem.

The regulation of bacteriophage SPO1 late (class m_2l) transcription involves the products of two viral genes that code for RNA polymerase-binding proteins. The SPO1 gp34 corresponds roughly in size to phage T4 gp55, and the SPO1 gp33, to the T4 gp33 (46, 47). It will be interesting to know whether the analogy extends further, in the sense of only one of these two SPO1-encoded RNA polymerase-binding proteins sufficing for SPO1 m_2l-class promoter recognition.

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