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A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes

(T7 DNA polymerase/T7 gene *5* protein/proteolysis/β-lactamase/rifampicin)

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ABSTRACT **The RNA polymerase gene of bacteriophage T7 has been cloned into the plasmid pBR322 under the inducible control of the λ P_L promoter. After induction, T7 RNA polymerase constitutes 20% of the soluble protein of *Escherichia coli*, a 200-fold increase over levels found in T7-infected cells. The overproduced enzyme has been purified to homogeneity. During extraction the enzyme is sensitive to a specific proteolysis, a reaction that can be prevented by a modification of lysis conditions. The specificity of T7 RNA polymerase for its own promoters, combined with the ability to inhibit selectively the host RNA polymerase with rifampicin, permits the exclusive expression of genes under the control of a T7 RNA polymerase promoter. We describe such a coupled system and its use to express high levels of phage T7 gene *5* protein, a subunit of T7 DNA polymerase.**

During bacteriophage T7 infection, the right-most 80% of the genome is transcribed by a phage-encoded RNA polymerase, the product of gene *1* (Fig. 1) (1). In contrast to the multisubunit RNA polymerases of bacteria and eukaryotes, T7 RNA polymerase is a single polypeptide of molecular weight 98,800 (2, 3). The enzyme is specific for its own promoters, a conserved 23-base-pair (bp) sequence (4–6).

T7 RNA polymerase is present in relatively low amounts in T7-infected cells, constituting 0.1% of the cellular protein. To facilitate studies on its role in the initiation of T7 DNA replication (7), we have placed its gene on a plasmid under the control of the λ P_L promoter. When induced, T7 RNA polymerase constitutes 20% of the soluble protein, permitting a simple purification of the enzyme to homogeneity. Davanloo *et al.* (8) have also described the purification of T7 RNA polymerase from cells overexpressing the cloned T7 gene *1*.

A logical extension of these studies is to exploit the specificity of T7 RNA polymerase for its promoters to express other cloned genes. Transcription by *Escherichia coli* RNA polymerase can be inhibited selectively by the addition of rifampicin. Here, we use the T7 RNA polymerase/promoter system to overproduce bacteriophage T7 gene *5* protein, a subunit of the T7 DNA polymerase (Fig. 1).

MATERIALS AND METHODS

Strains. *E. coli* HMS262 (*thr⁻ leu⁻ lacY⁻ thi⁻ supE hsdR⁻ tonA⁻ trxA⁻*) is *E. coli* C600 (9) transduced with *hsdR* and *trxA⁻*. *E. coli* B/7004 (10) was the donor of *trxA⁻*. *E. coli* HMS273 [lac^{am} trp^{am} pho^{am} mal^{am} $supC^{ts}$ *rpsL tsx*::Tn*10* *lon*(Δ)100 $htpR^{am}$] is SG935 from S. Goff (Harvard Medical School). pACYC177 and pACYC184 have been described (11). pJL23, provided by J. Lodge and T. Roberts (Harvard Medical School), is a derivative of pACYC184 that contains

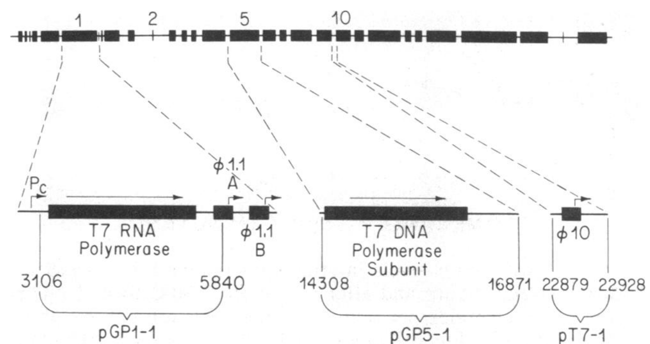

FIG. 1. The genetic map of bacteriophage T7 with inserts showing the cloned DNA fragments used in this study. T7 base pairs are numbered as described (6). Gene sizes reflect molecular weights of protein products. φ1.1A, φ1.1B, and φ10 correspond to the T7 RNA polymerase promoters located before genes *1.1* and *10*, respectively.

the λ repressor gene *cI857*. HMS273/pJL23 is *E. coli* HMS273 containing the plasmid pJL23. pKB280–*cI857*, a pBR322 derivative, contains the λ repressor gene under the control of the lactose promoter (12). pKC30 is a pBR322 derivative that contains the λ P_L promoter (13).

Enzymes and Chemicals. Restriction enzymes were from New England Biolabs. Phage T4 DNA ligase (14), T7 DNA polymerase (15), *E. coli* thioredoxin (16), T7 DNA (17), and plasmid DNAs (14) were prepared as described. pUC12 DNA was from P-L Biochemicals.

Enzyme Assays. The assay (100 μl) for T7 RNA polymerase contained 40 mM Tris·HCl, pH 8.0/20 mM $MgCl_2$/5 mM dithiothreitol/0.4 mM rNTPs ([^{3}H]rUTP, 30 cpm/pm)/60 μM T7 DNA/50 μg of bovine serum albumin per ml and the indicated amount of T7 RNA polymerase. Enzyme was diluted in 10 mM Tris·HCl, pH 7.5/10 mM 2-mercaptoethanol/1 mg of bovine serum albumin per ml. After incubation at 37°C for 15 min, acid-insoluble radioactivity was determined (2). One unit of activity is the amount that catalyzes the incorporation of 1 nmol of [^{3}H]rUTP into an acid-insoluble form in 60 min.

T7 gene *5* protein was determined by complementation with thioredoxin, the other subunit of T7 DNA polymerase (18), to restore activity (16). One unit of activity catalyzes the incorporation of 10 nmol of total nucleotide into an acid-insoluble form in 30 min.

Purification of T7 RNA Polymerase. Ten liters of cells (*E. coli* HMS273/pJL23/pGP1) were grown with aeration in a New Brunswick fermentor at 30°C in 2% tryptone/1% yeast extract/0.5% NaCl/0.2% glucose, pH 7.4. At OD_{590} = 3.0, the temperature was raised to 42°C. After 30 min, the temperature was lowered to 40°C for 120 min. The cells (95 g) were harvested, washed with 2 liters of 10% (wt/vol) sucrose/20 mM Tris·HCl, pH 8.0/25 mM EDTA, pH 8.0, at 0°C, resuspended in 250 ml of 10% sucrose/20 mM Tris·HCl,

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Abbreviation: bp, base pair(s).

pH 8.0/1 mM EDTA, and frozen in liquid N_2.

Cells (280 ml) were thawed at 0°C. Lysozyme was added to 0.2 mg/ml. After 45 min at 0°C, the cells were twice frozen in liquid N_2 and thawed at 0°C. The lysate was centrifuged and the supernatant was collected (fraction I, 265 ml).

Ammonium sulfate (93 g) was added to fraction I at 0°C over 60 min. The precipitate was collected and redissolved in 10 mM Tris·HCl, pH 7.5/0.1 mM EDTA/0.5 mM dithiothreitol/10% (vol/vol) glycerol (buffer A) to a conductivity equal to that of buffer A containing 50 mM NH_4Cl (fraction II, 480 ml).

A column of Whatman DE52 DEAE-cellulose (12.6 cm^2 × 23 cm) was equilibrated with buffer A containing 50 mM NH_4Cl. Fraction II was applied to the column and the column was washed with 600 ml of buffer A containing 50 mM NH_4Cl. Proteins were eluted with a 2.5-liter linear gradient from 50 to 300 mM NH_4Cl in buffer A. RNA polymerase activity eluted at 130 mM NH_4Cl (fraction III, 230 ml).

A column of Whatman P11 phosphocellulose (4.9 cm^2 × 20 cm) was equilibrated with 10 mM potassium phosphate buffer, pH 7.5/0.1 mM dithiothreitol/0.1 mM EDTA (buffer B). Fraction III was diluted with buffer B to 100 mM NH_4Cl and applied to the column. The column was washed with 250 ml of buffer B containing 200 mM KCl. Proteins were eluted with a 1-liter linear gradient from 200 to 400 mM KCl in buffer B. RNA polymerase, eluting at 300 mM KCl, was dialyzed against 20 mM potassium phosphate buffer, pH 7.5/50% (vol/vol) glycerol/0.1 mM EDTA/0.1 mM dithiothreitol (fraction IV, 130 ml). The enzyme was stored at −18°C.

Other Methods. Polyacrylamide gel electrophoresis in the presence of 0.1% $NaDodSO_4$ was as described (6). Gels were either stained with Coomassie blue (19) or analyzed by autoradiography. Protein was determined by the method of Lowry *et al.* (20).

RESULTS

Cloning of Gene *1* of Phage T7. Cloned DNA fragments that contain gene *1* and adjacent T7 sequences are lethal to *E. coli* (ref. 8; unpublished results). The nucleotide sequence of this region (3, 6) reveals the presence of several potentially deleterious elements (Fig. 1). Immediately 5′ proximal to gene *1* is a weak *E. coli* RNA polymerase promoter, promoter C (21), while 3′ proximal are two T7 RNA polymerase promoters, ϕ1.1A and ϕ1.1B (6). If a T7 RNA polymerase promoter is located in the same orientation as gene *1* on a plasmid, then even a single T7 RNA polymerase molecule will result in lethal runaway amplification of the gene *1* product and plasmid transcription.

To circumvent this problem, we isolated a DNA fragment containing all of gene *1* but lacking the *E. coli* RNA polymerase promoter C and the two T7 RNA polymerase promoters. Since these elements are extremely close to gene *1* in T7, we constructed the T7 mutant ST9. This mutant has a deletion of the sequences 5840–5916, which includes the T7 RNA polymerase promoters ϕ1.1A and ϕ1.1B, and inserted at that site is the linker C-C-G-G-A-T-C-C-G-G-G-G-A-A-T, which creates a unique *Bam*HI restriction site. The *E. coli* promoter C was removed by limited digestion (3150 bp) with BAL-31 nuclease. The DNA was digested with *Bam*HI, and fragments 2700 bp long were isolated by gel electrophoresis. These fragments, having a flush end 5′ to gene *1* and a *Bam*HI-generated end 3′ to gene *1*, were ligated to the *Hpa* I and *Bam*HI ends of pKC30. The orientation of gene *1* is such that it is under the control of the λ P_L promoter (Fig. 2). The ligation mixture was used to transform HMS273/pJL23, a strain containing the temperature-sensitive λ repressor, c*I857*. After selecting recombinant plasmids that contain inserts of a size compatible with gene *1*, extracts were prepared from induced cells and assayed for T7 RNA polymerase activity.

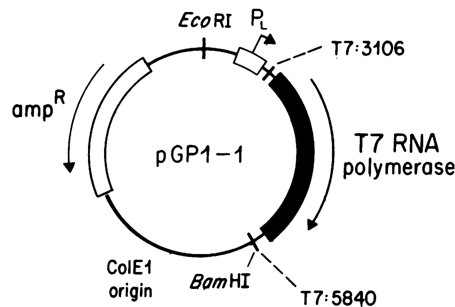

FIG. 2. Structure of pGP1-1. The T7 mutant ST9 was used to isolate the gene *1* fragment. It has a deletion of sequences 5840–5916 in T7, and inserted into that site is the *Bam*HI linker C-C-G-G-A-T-C-C-G-G-G-G-A-A-T. The right end of the cloned fragment is this *Bam*HI restriction site. The left end, at nucleotide 3106, was generated by digestion with BAL-31 nuclease. The gene *1* fragment was inserted between the *Hpa* I and *Bam*HI site of pKC30 (13); only the *Bam*HI site is regenerated.

One recombinant plasmid, pGP1-1, gives rise to high levels of T7 RNA polymerase activity after induction. DNA sequence analysis reveals that the insert begins at nucleotide 3106 in T7, 65 bp before the start of gene *1*. The insert ends at T7 sequence 5840, 18 bp past the termination codon of gene *1*.

Expression of T7 RNA Polymerase. When cultures of *E. coli* HMS273/pJL23/pGP1-1 are induced, T7 RNA polymerase accumulates over a 3-hr period (Fig. 3, lane B). The overproduced T7 RNA polymerase is soluble in extracts, repre-

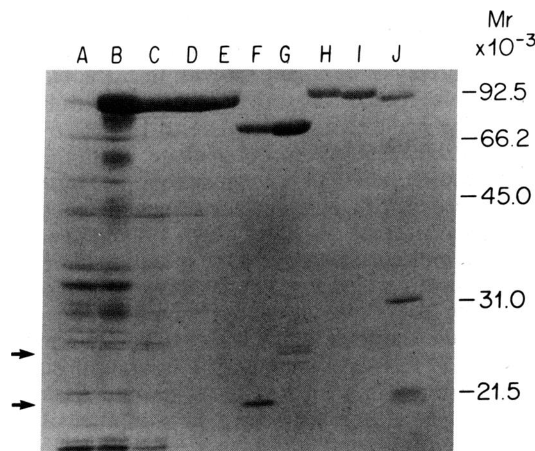

FIG. 3. Expression and purification of T7 RNA polymerase. Conditions for cell growth and enzyme purification are described in the text. A 9%–23% gradient polyacrylamide gel was stained with Coomassie blue after electrophoresis in the presence of $NaDodSO_4$. Lane A contains uninduced *E. coli* HMS273/pJL23/pGP1-1. Lane B contains induced cells. Lanes C, D, and E show fractions I, III, and IV of purified T7 RNA polymerase. Lane F contains the proteolytically cleaved T7 RNA polymerase (fraction IV). Quantitative cleavage was obtained by incubating extracts (*E. coli* HMS262/pJL23/pGP1-1) at 37°C in 10% sucrose/50 mM Tris·HCl, pH 8.0/100 mM NaCl for 60 min. Cleaved gene *1* fusion protein (fraction IV) and intact gene *1* fusion protein (fraction IV) are shown in lanes G and H. The fusion protein is produced by a derivative of pGP1-1, in which the first 6 codons of T7 gene *1* are replaced by the first 33 codons of the λ *N* gene (see text). Lane I contains T7 RNA polymerase purified from T7 phage-infected cells. Lane J contains protein markers (phosphorylase B, ovalbumin, bovine serum albumin, carbonic anhydrase, and trypsin inhibitor). Arrows indicate position of the smaller peptide resulting from proteolytic cleavage of T7 RNA polymerase (lane F) and gene *1* fusion protein (lane G).

senting 20% of the cellular protein (Fig. 3, lane C). Transcription from the P_L promoter also results in the synthesis of a M_r 6000 polypeptide containing the first 33 amino acids of the N protein of λ fused to 22 amino acids encoded by the region before gene *1*, and terminating 2 bp before the start codon for gene *1*. This polypeptide has run off the gel shown in Fig. 3.

Purification of T7 RNA Polymerase from Induced Cells. Induced *E. coli* HMS273/pJL23/pGP1-1 provides a source for the isolation of T7 RNA polymerase. We have designed a rapid purification procedure (Table 1) consisting of fractionation by ammonium sulfate, followed by chromatography on DEAE and phosphocellulose. The purity of each fraction is shown in Fig. 3 (lanes C, D, and E). Approximately 570 mg of homogeneous T7 RNA polymerase was obtained from 95 g of cells. The purified enzyme has 3 times the specific activity (190,000 units/mg) of homogeneous T7 RNA polymerase previously purified by us from phage-infected cells. Presumably, the high concentration of T7 RNA polymerase at the outset, as well as the rapid purification procedure, leads to greater stability of the enzyme.

Proteolytic Cleavage of T7 RNA Polymerase. During initial attempts to purify the overproduced T7 RNA polymerase, the enzyme was proteolytically cleaved at a specific region, a phenomenon also observed by Davanloo *et al.* (8). Under optimal conditions for proteolysis (a lon^+ *E. coli* host and incubation of cell extract in 100 mM NaCl at 37°C for 60 min) all of the gene *1* protein is cleaved at a site 25% of the distance from one end of the molecule. The two peptides (M_r, 75,000 and 23,000) remain associated throughout purification (Fig. 3, lane F). The cleaved form of T7 RNA polymerase has one-eighth the activity (23,000 units/mg) of the intact enzyme.

T7 RNA polymerase is not cleaved in intact cells. *In vivo*, induced cells containing T7 RNA polymerase radioactively labeled with $[^{35}S]$methionine can be chased for 2 hr without detectable proteolysis (data not shown). In extracts, the protease activity fractionates together with the outer membrane of *E. coli*. The protease is insensitive to EDTA, *o*-phenanthroline, and diisopropyl fluorophosphate; it is inhibited by *N*-ethylmaleimide, low salt concentration, low temperature (0°C), the presence of DNA, and a lon^- genetic background. The decreased proteolysis of T7 RNA polymerase in lon^- mutants is an indirect effect of the *lon* mutation (22), because the cellular location of the protease and its sensitivity to inhibitors differ from those of the *lon* protease (23).

To determine the location of the cleavage, we purified a derivative of T7 RNA polymerase that is larger in size by M_r 3000. This protein is produced by a plasmid analogous to pGP1-1, except that the T7 insert begins at nucleotide 3189 (Fig. 1). As a consequence, the first six amino acids of T7 RNA polymerase are replaced by the first 33 amino acids of the λ N protein. This M_r 102,000 fusion protein is also susceptible to proteolysis, although the cleavage is less specific, occurring at four sites over a region of 20 amino acids (Fig. 3, lane G). The large proteolytic fragment is the same size as that obtained with gene *1* protein, while the small fragment is larger than the small polypeptide produced by cleavage of gene *1* protein by M_r 3000. The region cleaved therefore lies 25% of the distance from the amino end of T7 RNA polymerase. The specific activity of fraction IV of cleaved fusion protein (19,000 units/mg) is one-sixth that of intact fusion protein (120,000 units/mg).

Expression of Specific Genes with the Use of Cloned T7 Gene *1* and a T7 RNA Polymerase Promoter. The expression system shown in Fig. 4 consists of two compatible plasmids, pGP1-2 and pT7-1. pGP1-2, a derivative of pACYC177, provides for expression of T7 RNA polymerase. It consists of gene *1* of phage T7 under the control of the inducible λ P_L promoter, and the gene for the heat-sensitive λ repressor, *cI857*.

To express a given gene, the gene is inserted into the second plasmid, pT7-1. pT7-1 contains a T7 RNA polymerase promoter, ϕ10, isolated from a 40-bp T7 fragment (Fig. 1). A polylinker containing eight different restriction sites lies adjacent to the promoter to facilitate the insertion of DNA fragments. Transcription from the Ø10 promoter results in expression of the cloned gene and the β-lactamase gene. Exclusive expression of these genes is achieved, after heat induction of T7 RNA polymerase, by the addition of rifampicin to shut off *E. coli* RNA polymerase transcription; the addition of $[^{35}S]$methionine results in specific labeling of these plasmid-encoded proteins.

Expression and Overproduction of T7 Gene *5* Protein. With this expression system, we have directed the synthesis of T7 gene *5* protein. T7 DNA polymerase consists of two subunits, a M_r 84,000 protein encoded by T7 gene *5* and the M_r 12,000 thioredoxin of the host (16, 18). A fragment of T7 DNA containing gene *5* was inserted into the polylinker of pT7-1 to create pGP5-1 (Fig. 4). After heat induction and addition of rifampicin, the plasmid proteins were labeled with $[^{35}S]$methionine. The autoradiogram of the gel electrophoresis in the presence of $NaDodSO_4$ (Fig. 5, lanes G–I) shows the profile of labeled proteins. After induction, the M_r 82,000 gene *5* protein is the predominant labeled protein synthesized (lane I), even in the absence of rifampicin (lane H). β-Lactamase and the M_r 14,000 T7 gene *5.3* protein are also synthesized. Approximately 5×10^6 cpm can be incorporated into these proteins from 10 μCi of $[^{35}S]$methionine (1 Ci = 37 GBq). Lanes G–I are shown stained with Coomassie blue in lanes G′–I′, demonstrating the amount of gene *5* protein present prior to and after induction of *E. coli* HMS262/pGP1-2/pGP5-1. To maximize the amount of expressed protein, induced cells are incubated in enriched medium with rifampicin for several hours; under these conditions the gene *5* protein is the predominant protein in the cell (lane J). Although up to 30% of the cellular protein is gene *5* protein, only approximately one-third is soluble in the absence of $NaDodSO_4$.

As controls, the comparable experiment was carried out using *E. coli* HMS262/pGP1-2 (cells containing T7 gene *1*, but no T7 promoter), and *E. coli* HMS262/pGP1-2/pT7-1 (cells containing T7 gene *1* and T7 ϕ10 expressing only β-lactamase) (Fig. 4). T7 RNA polymerase is present in all three strains after (Fig. 5, lanes B, E, and H) but not prior to (lanes A, D, and G) induction. If rifampicin is added after induction, no proteins are labeled when the cells lack a T7 RNA polymerase promoter (lane C). When pT7-1 is present,

Table 1. Purification of T7 RNA polymerase

Fraction	Step	Protein, mg	Total units, × 10^6	Specific activity, units/mg	% recovery
I	Extract	5200	220	42,000	100
II	Ammonium sulfate	2700	210	78,000	95
III	DEAE-cellulose	770	120	160,000	55
IV	Phosphocellulose	570	110	190,000	50

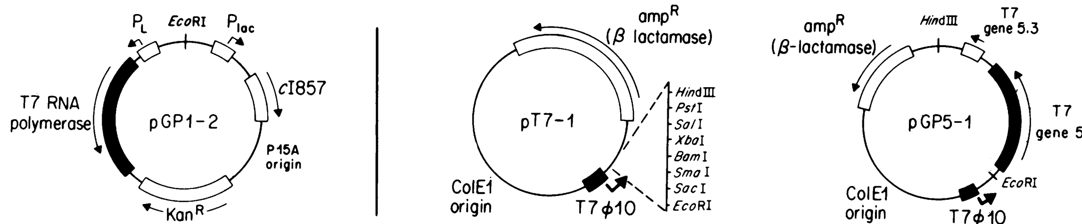

FIG. 4. Coupled T7 RNA polymerase/promoter system. All cells contain pGP1-2 (*Left*), which expresses T7 RNA polymerase. This plasmid, 7200 bp in size, contains the 3200-bp *Bam*HI/*Bgl* II fragment of pGP1-1 (T7 gene *1*), the 1100-bp *Pst* I/*Eco*RI fragment of pKB280-*cI857* (λ repressor gene), and the 2900-bp *Bam*HI/*Pst* I fragment of pACYC177 [the kanamycin-resistance gene (Kan^R) and the P15A origin]. Cells also contain either pT7-1 or pGP5-1. pT7-1, 2400 bp in size, contains the T7 *Taq* I/*Xba* I fragment from nucleotides 22879–22928 (Ø10), the 70-bp polylinker region of pUC12, and nucleotides 2065–4360 from pBR322 (the β-lactamase gene and the ColE1 origin; see ref. 24). pGP5-1, 5900 bp in size, contains the T7 *Nde* I/*Aha* III fragment from nucleotides 14308–16871 (T7 gene *5* and *5.3*), inserted with *Bam*HI linkers into the *Bam*HI site of pT7-1.

only β-lactamase is radioactively labeled (lane F). β-Lactamase appears as three bands: the top is the M_r 29,000 precursor (24), the middle band is the M_r 27,000 processed enzyme, while the lower band is a translated segment reading counterclockwise on pT7-1.

The gene *5* protein produced after induction has full activity as measured by its ability to complement thioredoxin to produce an active T7 DNA polymerase (Fig. 6). DNA synthesis in extracts prepared from induced *E. coli* HMS262/pGP1-2/pGP5-1 is stimulated 100-fold by purified thioredoxin. The specific activity of gene *5* protein in extracts is 1050 units per mg of protein, compared to 10,300 units/mg for homogeneous T7 DNA polymerase (15).

In the absence of thioredoxin (*E. coli trx*A$^-$), cells can tolerate high levels of gene *5* protein. When extracts are prepared from uninduced cells, the gene *5* protein represents 1% of the soluble protein (Fig. 6). This activity results from residual expression of gene *1* from the P_L promoter at 30°C. In *trx*$^+$ cells containing thioredoxin, the expression of gene *5* protein is lethal to some *E. coli* strains (e.g., HMS262 *trx*$^+$); pGP1-2 and pGP5-1 are not compatible in these strains. To express T7 gene *5* in these cells, T7 RNA polymerase must be more tightly repressed (see *Discussion*).

DISCUSSION

We have described the purification of T7 RNA polymerase from *E. coli* cells harboring gene *1* of phage T7 and overproducing the enzyme some 200-fold. Our own incentive for overproducing the enzyme was to have sufficient quantities of homogeneous T7 RNA polymerase to use in studies on its role in T7 DNA replication (7). In addition, the fact that T7 RNA polymerase is a monomeric protein and recognizes a delineated promoter sequence makes it an attractive enzyme to use as a model system for studying protein–DNA interactions.

E. coli DNA does not have promoters for T7 RNA polymerase (2). When >2% of the cellular protein is T7 RNA polymerase, we find no change in the growth rate of *E. coli*. However, when a single T7 RNA polymerase promoter is present, then even low levels of T7 RNA polymerase are lethal to the cell, presumably because its efficient transcription serves as a sink for ribonucleoside triphosphates. The induced T7 RNA polymerase complements T7 phage defective in gene *1*. We, as well as Davanloo *et al.* (8), have constructed T7 phage with deletions in gene *1* that propagate only in *E. coli* cells expressing T7 RNA polymerase.

To analyze the products of cloned genes using the T7 RNA polymerase/promoter system, it is necessary to clone a T7 promoter in conjunction with the gene to be expressed. This can be accomplished by inserting a DNA fragment containing the promoter into the plasmid, or alternatively, by recloning the gene into a parent vector that contains a T7 RNA polymerase promoter. The T7 RNA polymerase/promoter system provides an attractive alternative to the mini- (26) or maxicell (27) procedures for labeling plasmid-encoded proteins. All transcription by T7 RNA polymerase is directed from the unique T7 promoters. The host RNA polymerase is

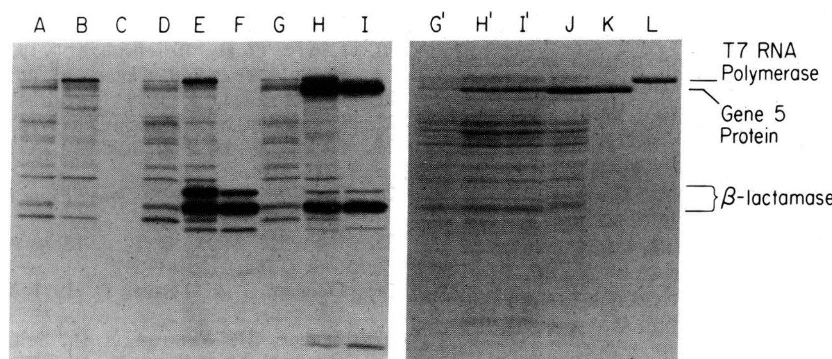

FIG. 5. Expression of T7 gene *5* by T7 RNA polymerase. *E. coli* HMS262/pGP1-2 alone (lanes A–C), containing pT7-1 (lanes D–F), or containing pGP5-1 (lanes G–J) were grown in M9 medium supplemented with thiamine (20 μg/ml) and 18 amino acids (0.1%, minus cysteine and methionine). Cells (1 ml) were grown at 30°C to OD_{590} = 0.4 (uninduced: lanes A, D, and G). Temperature was shifted to 42°C for 20 min (induced: lanes B, E, and H). Rifampicin (200 μg/ml) was added, and after 10 additional min at 42°C, the cells were grown for 20 min at 30°C (induced plus rifampicin: lanes C, F, and I). After pulse labeling with 10 μCi of [^{35}S]methionine, the cells were harvested, resuspended in 60 mM Tris·HCl, pH 6.8/1% $NaDodSO_4$/1% 2-mercaptoethanol/10% glycerol/0.01% bromophenol blue, heated to 95°C for 3 min and loaded onto a 14% polyacrylamide gel containing 0.1% $NaDodSO_4$. Lanes G′, H′, and I′ are identical to lanes G, H, and I except they were stained with Coomassie blue. In lane J, *E. coli* HMS262/pGP1-2/pGP5-1, after induction, was incubated with rifampicin for 2 hr. Lanes K and L contain purified T7 gene *5* and T7 RNA polymerase, respectively.

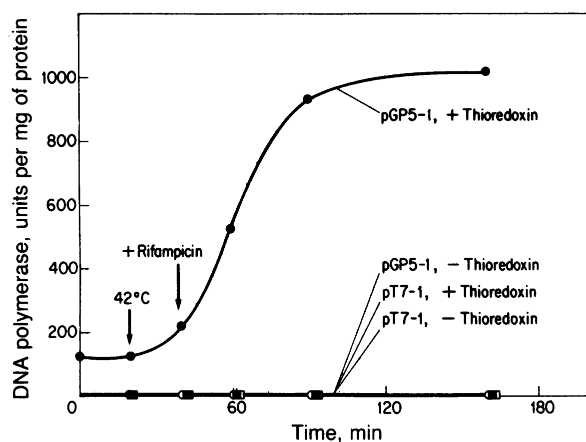

FIG. 6. Induction of gene *5* protein. Cells (*E. coli* HMS262/pGP1-2/pT7-1 and HMS262/pGP1-2/pGP5-1) were grown at 30°C in 2% tryptone/1% yeast extract/0.5% NaCl/ and 0.2% glucose (pH 7.4). At 20 min (A_{590} = 1.5), the temperature was shifted to 42°C. At 40 min, rifampicin was added (200 μg/ml), and the cultures were incubated with aeration at 30°C. At the intervals shown, 50-ml aliquots were removed, and fraction II was prepared from extracts as described (25), with the modification that the lysis buffer contained 1.2 M NaCl, which was necessary to solubilize the overproduced gene *5* protein. Fractions were assayed for DNA polymerase activity in the presence or absence of purified thioredoxin as described (16). □, HMS262/pGP1-2/pT7-1, minus thioredoxin; ■, HMS262/pGP1-2/pT7-1, plus thioredoxin; ○, HMS262/pGP1-2/pGP5-1, minus thioredoxin; ●, HMS262/pGP1-2/pGP5-1, plus thioredoxin.

inhibited specifically by the addition of rifampicin (28). Since *E. coli* mRNA decays rapidly, all mRNA in the cell is produced from the T7 RNA polymerase promoter. T7 RNA polymerase synthesizes RNA by a rapid and processive mechanism. In fact, *in vitro*, transcription by T7 RNA polymerase from a T7 promoter on a plasmid results in transcripts several times the plasmid length (29).

We have presented two examples of the production of high levels of specifically labeled proteins by the T7 RNA polymerase/promoter system. β-Lactamase and the phage T7 gene *5* protein are overproduced and represent the only radioactively labeled proteins. Since T7 RNA polymerase will circumvent a plasmid several times without terminating, the location of the promoter on the plasmid is unimportant; comparable levels of T7 gene *5* protein are obtained if the promoter is placed immediately before or after gene *5*. Under optimal conditions, 30% of the cellular protein is gene *5* protein, compared to only 0.01% in T7 phage-infected cells (25).

A problem can arise if the T7 RNA polymerase promoter is directing the expression of a gene product toxic to *E. coli*. For example, we have used this system to express T7 gene *2* protein, which, because it inhibits *E. coli* RNA polymerase, is lethal when expressed in the cell. Under uninduced conditions, the *cI857* λ repressor does not repress the P_L promoter tightly enough to completely inhibit synthesis of T7 RNA polymerase. To express T7 gene *2* protein, we have reduced the uninduced level of T7 RNA polymerase by placing a terminator for *E. coli* RNA polymerase into pGP1-2, such that expression of gene *1* from the P_L promoter is dependent on transcriptional readthrough.

In addition to the studies described here, the T7 RNA polymerase/promoter system should be useful *in vivo* to synthesize anti-sense RNA to probe specific gene functions and *in vitro* to generate specific RNA transcripts. Such transcripts are useful as RNA substrates and as single-stranded probes. The T7 RNA polymerase/promoter system should also be useful for directing gene expression in organisms other than *E. coli*.

Note Added in Proof. Amino acid sequence determination of the NH_2 terminus of the large proteolytic fragment of T7 RNA polymerase (Fig. 3, lane F) reveals that the cleavage site lies between amino acid 172 (lysine) and amino acid 173 (arginine) (Rodney M. Hewick, Genetics Institute, Cambridge, MA, unpublished results).

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