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Control of Phage P22 Tail Protein Expression by Transcription Termination

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The structural gene for *Salmonella* bacteriophage P22 tail protein, gene *9*, is separated from the remainder of the P22 late operon genes by the *imm*I region. Early transcription of *imm*I gene *ant*, which is immediately promoter proximal to gene *9*, occurs in the same direction as late gene transcription but does not enter gene *9* coding sequences (Susskind & Youderian, 1982). We have cloned gene *9* and surrounding sequences into pBR322 and subsequently positioned *lac* UV5 promoters at varying distances before the start of gene *9* by DNA manipulations *in vitro*. Using an *in vitro* phage assembly assay to measure *in vivo* expression of tail protein from these plasmids and *in vitro* transcription reactions to measure transcriptional template activity of DNA fragments isolated from these plasmids, we have identified a region of DNA between gene *ant* and gene *9* that behaves as a transcription termination signal. The DNA sequence of this region shows hyphenated dyad symmetry followed by a run of seven thymine residues on the coding strand. This sequence can be drawn in a potential stem-and-loop secondary structure similar to known rho-independent transcription termination signal sequences. We discuss the role of this transcriptional terminator sequence in gene *9* expression and the early to late transcriptional switch in the P22 infection cycle.

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1. Introduction

The lysis, head and tail genes of the *Salmonella* phage P22 appear to constitute a single operon (Weinstock *et al.*, 1980). These genes are all expressed late in the infection cycle, and their expression depends on the activator function encoded by P22 gene *23*. Gene *9*, which encodes the tail protein of P22, is located at the promoter distal end of the late operon. It is, however, separated from other morphogenetic and late operon genes by a region (*imm*I) whose genes, including *ant*, are expressed early in infection (Fig. 1; Susskind & Botstein, 1978). Early *imm*I transcription, initiated at the P_{ant} promoter, proceeds towards gene *9* and could potentially read into gene *9* (Fig. 1). Nevertheless, tail protein is not

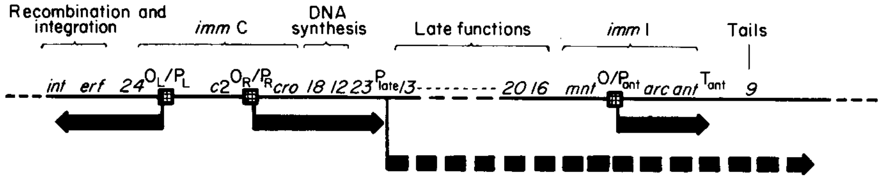

FIG. 1. Transcriptional patterns of P22 gene expression. Some of the genes and their functions are indicated on this schematic diagram of the P22 prophage map. Filled arrows represent early transcription. The broken arrow represents the extent of transcription initiated at the late promoter. (Adapted from Fig. 1 of Susskind & Youderian, 1982.)

synthesized early in infection (Susskind, 1980), and it has been suggested that a site (T_{ant}) terminates *imm*I transcription before it reaches gene *9* (Susskind & Botstein, 1978). Recent studies of *imm*I transcription *in vitro* have provided experimental support for this model (Susskind & Youderian, 1982).

We have recently completed DNA sequence studies of the P22 tail protein gene (Sauer *et al.*, 1982). To continue our studies of structure–function relationships in the tail protein, we sought to construct plasmids that direct the overproduction of the P22 tail protein. In the course of these studies, which are presented here, we have identified a DNA sequence between genes *ant* and *9* that prevents gene *9* expression *in vivo* and terminates transcription *in vitro*. We propose that this sequence is the site (T_{ant}) involved in regulation of tail protein synthesis during P22 infection.

2. Materials and Methods

(a) *Strains*

P22 *c*1-7, 13$^-$*am*H101 grown in *Salmonella typhimurium* DB7000 (*leu*A$^-$*am*414, *sup*^{0}) was the source of phage DNA used in the plasmid constructions described below. This phage was propagated on strain DB7004 (*leu*A$^-$*am*414, *sup*E). *Escherichia coli* strain MM294 was used as the recipient of all transformations. Plasmids pBR322 and pLJ3 have been described (Bolivar *et al.*, 1977; Johnsrud, 1978).

(b) *DNA isolation*

P22 phage DNA was isolated by extraction with sodium dodecyl sulfate (Susskind & Botstein, 1975). Large-scale plasmid DNA isolations were performed by the method of

Davis *et al.* (1980). Small-scale plasmid preparations were made as follows: colonies were inoculated into 10 ml of LB broth (10 g tryptone, 5 g yeast extract, 5 g NaCl, 1 ml of 1 M-NaOH, per liter) containing the appropriate selective drug and were grown overnight to saturation. The cells were harvested by centrifugation and resuspended in 0·5 ml of 50 mM-Tris · HCl (pH 8·2). Fifty microliters of a fresh solution of 10 mg lysozyme/ml in 0·25 M-Tris · HCl (pH 8·1) was added to the cell suspension. Following a 30 min incubation at room temperature, the cells were lysed by adding an equal volume of phenol saturated with 0·1 M-Tris · HCl (pH 7·1). The tubes were inverted 5 times and then centrifuged for 15 min at room temperature in an Eppendorf microcentrifuge. The aqueous layer was removed from the cell debris and vigorously extracted with 0·3 ml of phenol. The resulting aqueous layer was removed after centrifugation and extracted twice with ether, brought to 0·4 M-ammonium acetate by the addition of 8 M-ammonium acetate, and precipitated with 3 vol. ethanol. The resulting nucleic acid preparation was resuspended in 100 μl of 10 mM-Tris · HCl (pH 8·1), 1 mM-EDTA (Tris/EDTA buffer) and was incubated with 0·1 μg RNase for 30 min at room temperature. The resulting plasmid preparation was usually adequate for 5 to 10 restriction enzyme analyses.

(c) *Tail protein assays*

Rapid tail protein assays were performed by spot complementation of P22 heads for plaque formation as described by Berget & Poteete (1980). Quantitative assays were performed by measuring plaque-forming units following complementation (Israel *et al.*, 1967). In these experiments, harvested cells were resuspended in an equal weight of B_{25} buffer (Berget & Poteete, 1980) and subjected to 1 cycle of freezing and thawing. This cell suspension was sonicated for 1 min and clarified by centrifugation at 20,000 revs/min in a Sorval SS34 rotor for 30 min. The supernatants of these cell extracts were serially diluted in phage dilution buffer and these dilutions were assayed for tail protein. Protein concentration was determined using BioRad Protein Reagent and bovine serum albumin as a standard.

(d) *Transcription* in vitro

Restriction fragments used as template DNA in transcription experiments were purified by electrophoresis in polyacrylamide gels and were extracted from the gel matrix (Maxam & Gilbert, 1980). Portions of the eluted fragments were rerun on analytical gels along with DNA standards of known concentration. DNA concentrations were estimated by visual comparison of band intensities following staining with ethidium bromide. Transcription reactions were carried out in transcription buffer containing 40 mM-Tris · HCl (pH 7·8), 100 mM-KCl, 10 mM-$MgCl_2$. The final concentration of DNA fragments was approximately 2·5 nM. In the first stage of the reaction, RNA polymerase holoenzyme (purified from *E. coli* and generously provided by W. McClure) was added to give a final concentration of 40 nM, and was incubated with the DNA fragments for 25 min at 37°C in a volume of 18 μl. Transcription was initiated by the addition of 2 μl of a mixture containing 1 mM-ATP, 0·5 mM-GTP, 0·5 mM-CTP, 0·5 mM-[^{32}P]UTP (9 Ci/mmol), and 1 mg heparin sulfate/ml in transcription buffer. After 10 min at 37°C, the reactions were terminated by the addition of 20 μl of deionized formamide and 2 μl of 0·1% Bromphenol Blue, 0·1% xylene cyanol. These samples were applied to a 7·5% acrylamide (29 : 1, acrylamide : bisacrylamide) gel in 8 M-urea, Tris/borate/EDTA buffer (Maxam & Gilbert, 1980) and were subjected to electrophoresis and autoradiography.

(e) *DNA sequencing*

DNA sequencing was performed by the chemical method of Maxam & Gilbert (1980) on singly end-labeled fragments of DNA isolated from preparative polyacrylamide gels.

(f) *Materials*

Restriction enzymes were obtained from New England Biolabs or Bethesda Research Laboratories. BAL31 exonuclease, polynucleotide kinase, and Klenow fragment of *E. coli* DNA polymerase I were obtained from New England Biolabs. 5-Bromo-4-chloro-3-indolyl-beta-D-galactoside (XG) was obtained from Sigma. Alkaline phosphatase was obtained from Boeringer Mannheim GmbH.

(g) *Plasmid constructions*

(i) *pTP28*

A $4 \cdot 5 \times 10^3$ base-pair DNA fragment containing gene *9* was generated by complete digestion of P22 phage DNA with *Eco*RI and partial digestion with *Bam*HI (see Fig. 2). The fragment was purified by agarose gel electrophoresis and the DNA was eluted from an excised piece of the gel. It was ligated with the large fragment generated by digesting plasmid pBR322 to completion with *Eco*RI and *Bam*HI. Cells transformed by the resulting hybrid plasmids were selected for ampicillin resistance and screened for tetracycline sensitivity. Plasmid DNA was prepared from single transformants and was analyzed by gel electrophoresis following digestion with *Eco*RI and *Bam*HI. pTP28 was found to have the structure shown in Fig. 2 (data not shown).

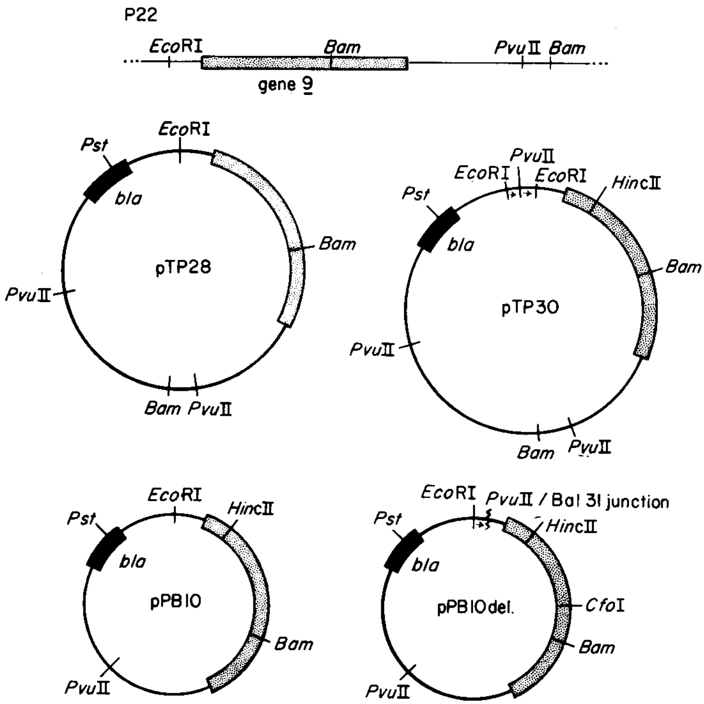

FIG. 2. Derivation of plasmids used in this study. The 4·5 kb ($4 \cdot 5 \times 10^3$ base) segment of P22 chromosomal DNA which contains gene *9* is shown at the top. The distance between the *Eco*RI and first *Bam*HI site is approx. 1·9 kb, the distance between the two *Bam*HI sites is approx. 2·6 kb, and the distance between the second *Bam*HI site and the *Pvu*II site is approx. 0·3 kb. The construction of plasmids pTP28, pTP30 and pPB10 is described in Materials and Methods. The derivation of deletion plasmids from pPB10 is described in Results. Restriction sites used for sequencing, plasmid reductions or *in vitro* transcription experiments are indicated on the appropriate diagrams.

(ii) *pPB10*

DNA sequences between the two *Pvu*II sites of pTP28 were deleted by digestion with *Pvu*II followed by ligation at low DNA concentration. The structure of the resulting plasmid, pPB10, was determined to be that shown in Fig. 2 (data not shown).

(iii) *pTP30*

Plasmid pTP30 was generated by mixing plasmids pTP28 and pLJ3, digesting with *Eco*RI and ligating the resulting mixture of fragments. pLJ3 was used as a source of 2 tandem *lac* UV5 promoters, which are carried on a small *Eco*RI fragment. Cells were transformed with the ligation mixture and were selected for ampicillin resistance and screened for tetracycline resistance. Tetracycline-resistant colonies were assumed to have incorporated unwanted regions of pLJ3 and were discarded. Ampicillin-resistant cells containing the *lac* operator (and promoter) on multicopy plasmids were identified by their dark blue colony color on XG indicator plates. Colonies were tested for tail protein production and both producers and non-producers were obtained. One of the producers, pTP30, was characterized further and found to contain the tandem *lac* promoter fragment with the promoters directed towards gene *9* as shown in Fig. 2 (data not shown).

3. Results

Plasmid pTP28 (Fig. 2), constructed from pBR322 and a $4{\cdot}5\times10^3$ base-pair fragment of P22 DNA, contains the entire structural gene for the P22 tail protein, as well as 5′ and 3′-flanking sequences (Sauer *et al.*, 1982). The tail protein gene is approximately 2×10^3 base-pairs long and starts 364 base-pairs into the P22 DNA from the *Eco*RI site. During cloning the gene is separated from its natural promoter P_{late}, and thus no known promoter for gene *9* transcription remains in the P22 DNA of pTP28. Plasmid pTP30 was constructed as a potential tail protein-producing plasmid by inserting a small DNA fragment containing tandem *lac* promoters into the *Eco*RI site of pTP28. Comparison of tail protein levels in cells carrying pTP30 and pPB10 (a smaller plasmid derived from pTP28)

TABLE 1

Quantitation of P22 tail protein produced by various gene 9 *containing plasmids*

Plasmid	Tail protein (phage equivalents/mg protein in crude extract)
pPB10	4×10^7
pPT30	4×10^8
pPB10Δ6-1	7×10^6
pPB10Δ5-3	1×10^8
pPB10Δ8-6	1×10^8
pPB10Δ8-7	4×10^8
pPB10Δ7-8	6×10^{12}
pPB10Δ8-1	6×10^{12}
pPB10Δ8-2	4×10^{13}

Extracts were prepared from *E. coli* MM294 cells grown to midlog phase in the presence of 100 μg ampicillin/ml.

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                                                      30
5'-GAATTCTCGT   ACATCATTGG   AATAGCGAGA
   CTTAAGAGCA   TGTAGTAACC   TTATCGCTCT
                                 Δ6-2

                                                      60
   GACGTTTTAA   TTAATCACAC   GCGAGATGTT
   CTGCAAAATT   AATTAGTGTG   CGCTCTACAA
                             Δ6-4
                      Δ7-1
                                                      90
   GATATTAATG   AACCTGACGG   GCCAACGAAT
   CTATAATTAC   TTGGACTGCC   CGGTTGCTTA
                        Δ6-1

                                                     120
   CTTTCCGCAT   GGATGAGACT   TAAGGATAAA
   GAAAGGCGTA   CCTACTCTGA   ATTCCTATTT
       Δ5-3

                                                     150
   GAGCTTCCAC   CTTCATTACA   TCGCTACTGA
   CTCGAAGGTG   GAAGTAATGT   AGCGATGACT

                                                     180
   CAGATAACCA   ACGCAACGAC   CCAGCTTCGG
   GTCTATTGGT   TGCGTTGCTG   GGTCGAAGCC
         Δ8-6   Δ8-7

                                                     210
   CTGGGTTTTT   TTATGCCCAA   AATTCACCGT
   GACCCAAAAA   AATACGGGTT   TTAAGTGGCA
   Δ7-8

                                                     240
   AGCCACGCTT   AGGTAATGAG   CTTGAAGGAG
   TCGGTGCGAA   TCCATTACTC   GAACTTCCTC
                      Δ8-1

                                                     270
   AGACCTACAA   AAAAATTGTA   GGTCGAAAAG
   TCTGGATGTT   TTTTTAACAT   CCAGCTTTTC
                             Δ8-2
                                                     300
   CGAACAAAAT   AACTTCCGAA   AAAGTTGTTT
   GCTTGTTTTA   TTGAAGGCTT   TTTCAACAAA

                                                     330
   TATCACAAAA   AATTCACCGT   AGCCATGCTG
   ATAGTGTTTT   TTAAGTGGCA   TCGGTACGAC

                                                     360
   CGGCAATTCC   TTGCATCTGG   AGCAAATTAA
   GCCGTTAAGG   AACGTAGACC   TCGTTTAATT

   ▽                                                 390
   ATGACAGACA   TCACTGCAAA   CGTAGTTGTT
   TACTGTCTGT   AGTGACGTTT   GCATCAACAA
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FIG. 3. The sequence of DNA preceding P22 gene *9*. The sequence in the 5'-flanking region of gene *9* is shown with the numbering starting at the first base of the *Eco*RI recognition sequence 5' to gene *9*. The points of fusion of the *lac* UV5 promoter to gene *9* in the pPB10Δ plasmids are indicated by filled arrowheads. DNA sequence between each of these arrows and the *Eco*RI site is missing in each of the deletion plasmids described in Results. The symmetrical sequences in the region of hyphenated dyad symmetry between base-pairs 168 and 186 are overlined. The open arrowhead pointing to base-pair 361 indicates the first base of the methionine initiation codon of gene *9* (Sauer *et al.*, 1982). Hyphens have been omitted from sequences for clarity.

indicated that, although tail protein was made in increased amounts in pTP30 transformed cells, the overall levels of tail protein in such cells were still remarkably low (see Table 1).

Although many factors could conceivably have contributed to the limited expression of gene *9* in cells carrying pTP30, the most likely possibility seemed to be that not all transcription initiated at the *lac* promoters reached the structural gene for tail protein. To investigate this question, we sequenced 400 base-pairs of DNA extending towards gene *9* from the *Eco*RI site of pPB10. This sequence (Fig. 3) includes the 3′ end of the *ant* gene, the entire *ant–9* intercistronic region and the 5′ end of gene *9* (Sauer *et al.*, 1982; R. T. Sauer, W. Krovatin, J. DeAnda, P. Youderian & M. M. Susskind, unpublished data). The sequencing strategy and the extent of reliable sequence information obtained from each set of sequencing reactions are shown in Figure 4. Examination of this sequence revealed a region of

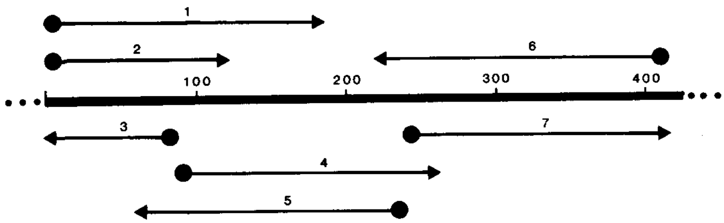

FIG. 4. DNA sequencing strategy. The bold line indicates the region of sequenced DNA that precedes and includes the coding sequences for the amino terminus of the P22 tail protein gene (gene *9*). Co-ordinates of restriction enzyme recognition sites given below are in base-pairs (bp) from the first nucleotide of the *Eco*RI site promoter proximal to gene *9*. The filled circles indicate the position of the label introduced into each fragment, and the arrowheads indicate the extent of sequences read from the sequencing gels. Fragment 1: *-*Eco*RI (bp 1) secondarily cut at *Bam*HI (bp 1887). Fragment 2: *-*Eco*RI (bp 1) secondarily cut at *Taq*I (bp 263). Fragment 3: *-*Hin*fI (bp 87) secondarily cut at *Eco*RI (bp 1). Fragment 4: *-*Hin*fI (bp 87) to *Hin*fI (bp 415) strand separated. Fragment 5: *-*Taq*I (bp 263) secondarily cut at *Msp*I (bp −462 in pBR322 sequences). Fragment 6: *-*Hin*fI (bp 415) to *Hin*fI (bp 87) strand separated. Fragment 7: *-*Taq*I (bp 263) secondarily cut at *Msp*I (bp 478). * Indicates labeled end of fragment. Fragment 2 was 5′-labeled with [γ-^{32}P]ATP and polynucleotide kinase following treatment with alkaline phosphatase. All other fragments were 3′-labeled using Klenow fragment of *E. coli* DNA polymerase I and the appropriate [α-^{32}P]dNTP.

hyphenated dyad symmetry capable of forming a seven-base-pair stem and a four-base loop followed by seven T residues. This sequence, which extends from base-pair 169 to 186 (see Fig. 3), is drawn in a potential stem-and-loop structure in Figure 5. This structure resembles known transcriptional terminators (Rosenberg & Court, 1979). To test whether this sequence regulated gene *9* expression, we constructed a series of pPB10-derived plasmids in which none, part or all of this sequence was deleted.

DNA sequences beginning at the *Eco*RI site and extending toward the beginning of gene *9* were deleted by digestion with nuclease *in vitro*. Plasmid pPB10 was opened by cleavage at its single *Eco*RI site, and samples were digested for varying lengths of time with the double-stranded exonuclease BAL31. Each sample was then treated with *Pst*I, resulting in cleavage within the plasmid beta-lactamase gene and separation of the exonuclease-treated portions of pBR322 and

FIG. 5. Potential stem-and-loop conformation of the proposed *ant* terminator. The filled arrows indicate the positions of fusions with the *E. coli lac* promoter sequences in plasmids pPB10Δ8-6, pPB10Δ8-7 and pPB10Δ7-8. Of these 3 fusions, only pPB10Δ7-8 directs synthesis of significant quantities of P22 tail protein. The open arrowhead indicates the 3′ end of the P22 *ant* gene (Sauer *et al.*, unpublished data).

P22 DNA. This mixture was combined with a purified 850 base-pairs *Pst*I-*Pvu*II fragment from pTP30 and intact plasmids were reconstructed by ligation. The added pTP30 fragment contains a portion of the beta-lactamase gene and a *lac* UV5 promoter. Its ligation to the *Pst*I fragment carrying the nuclease-treated P22 DNA restores ampicillin resistance and fuses the *lac* promoter to the endpoint of nuclease digestion (see Fig. 2). Cells transformed by such plasmids were isolated following selection for ampicillin resistance and screening for the presence of *lac* promoter/operator sequences by dark blue colony color on XG indicator plates. Several candidates of this type were picked and assayed for P22 tail protein production. These fell into two classes: those that produce pTP30 levels of tail protein and those that produce 10,000-fold more (Table 1).

To determine the relationship between tail protein production and DNA removed during nuclease digestion, the appropriate *lac* promoter–P22 DNA junction fragments of several plasmids, including those listed in Table 1, were sequenced. In each case, the desired fragment was sequenced following labeling at the *Eco*RI site and secondary cutting at a *Cfo*I site within gene *9*. The sequences obtained extended through the 96 base-pair *lac* promoter sequence, across the *Pvu*II/BAL31 junction, and into P22 DNA (see Fig. 2). These sequences identify the fusion points between *lac* promoter and P22 DNA for each plasmid and thus define a series of deletion endpoints (Fig. 3). A simple relation exists between the extent of DNA deleted and tail protein production *in vivo*. Those deletion plasmids, which retain the sequence of hyphenated dyad symmetry preceding gene *9*, produce low levels of tail protein. Those in which some or all of this sequence has been removed synthesize relatively high levels of tail protein. In plasmid pPB10Δ8-7, the *lac* promoter is fused to P22 DNA at a point six bases before the beginning of the region of hyphenated dyad symmetry. This plasmid directs synthesis of 4×18^8 phage equivalents of tail protein per mg of crude extract protein (Table 1). In plasmid pPB10Δ7-8, the point of fusion between *lac* promoter sequences and P22 sequences occurs within the putative terminator site, and sequences that would be required for proper stem-and-loop formation are not present. This plasmid directs synthesis of 6×10^{12} phage equivalents of tail protein per mg of crude extract protein (Table 1). The deletions in pPB10Δ7-8 and pPB10Δ8-7 plasmids thus bracket a sequence of 21 bases, whose removal allows efficient expression of tail protein from the *lac* promoter. These experiments, by themselves, do not reveal whether the inhibition of gene *9* expression occurs at the translational or transcriptional level, nor do they speak to the mechanism of the inhibition.

To address these questions, we assayed transcription *in vitro* using fragments of DNA isolated from the deletion plasmids as template. An *Eco*RI-*Hinc*II DNA fragment containing the *lac* promoter fused to P22 DNA (except in the case of pPB10, which has no *lac* promoter) was isolated from each of the plasmids listed in Table 1 and was used for these experiments (see Fig. 2). A *Pvu*II-*Hinc*II fragment was used in the case of pTP30. Transcription reactions were analyzed by electrophoresis on denaturing gels. An autoradiogram of such a gel is shown in Figure 6, where the major transcripts from each reaction are indicated by filled circles. As would be predicted if the DNA sequence from base-pair 169 to 186 in Figure 3 was a transcription termination signal, the major transcription products become smaller as the *lac* promoter is positioned successively closer to this sequence. The estimated sizes of the transcripts do not allow precise placement of the 3′ termini of the transcripts with respect to the DNA sequence, but the sizes are consistent with termination occurring within or very close to the poly(T) region of the putative terminator site. When the sequence that comprises the putative terminator is completely removed, as in the case of pPB10Δ8-1 and pPB10Δ8-2, the major transcripts become much larger and correspond approximately in size to what would be expected if these represented run-off transcripts initiated at the *lac* promoter. Overall, the results of tail protein production *in vivo* directed by the deletion plasmids, and the transcription experiments *in vitro* are consistent with the hypothesis that DNA between base-pair 169 and 186 (Fig. 3) functions as a transcription terminator.

One of the templates examined by *in vitro* transcription exhibited partial retention of terminator function. Plasmid pPB10Δ7-8 bears the shortest deletion that results in substantial tail protein synthesis *in vivo*. This plasmid lacks the stem-and-loop sequences of the putative terminator (Fig. 5) but retains the string of seven T residues following the stem-and-loop. Approximately half of the *in vitro* transcription products from pPB10Δ7-8 correspond in size to the template DNA fragment, suggesting that deletion of the stem-and-loop sequences prevents efficient termination. However, the remaining transcripts are short, reflecting failure of RNA polymerase to transcribe past the partially deleted terminator. The relative production of the short and long transcripts from pPB10Δ7-8 DNA depends on the transcription reaction conditions. For example, at a lower concentration of UTP, most pPB10Δ7-8 transcriptions are terminated near the poly(T) sequence (data not shown); while in the experiment shown, approximately half of the transcripts terminate near this region. We have not distinguished in this experiment between termination and pausing of RNA polymerase (Chamberlin, 1976). Alternatively, the fusion of the *lac* promoter sequences to the remainder of the termination signal in pPB10Δ7-8 may create a sequence that functions, at least *in vitro*, as a termination signal. By examining

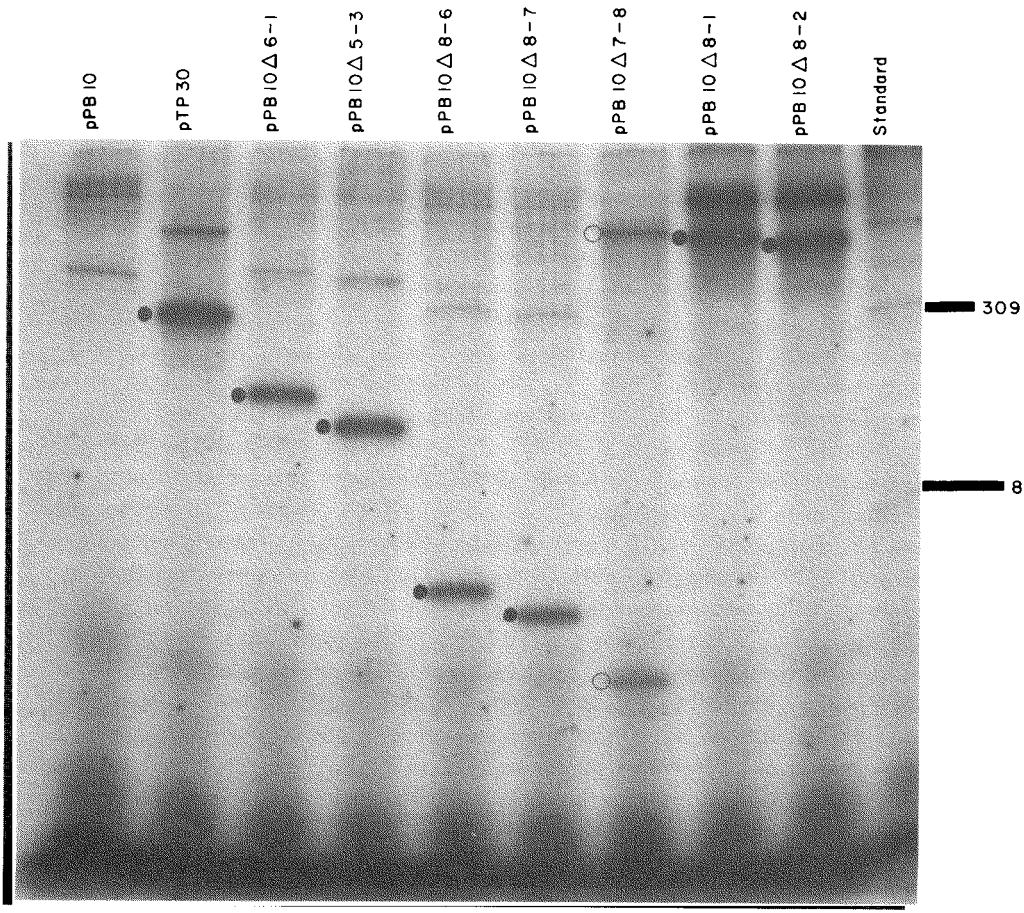

FIG. 6. Termination of transcription initiated at the *lac* UV5 promoter *in vitro*. Details of the procedure are described in Materials and Methods. The DNA fragments used as templates include: an *Eco*RI-*Hinc*II fragment from pPB10 (included as a control; it lacks the promoter, see Fig. 2); a *Pvu*II-*Hinc*II P_{lac}-containing fragment from pTP30; and *Eco*RI-*Hinc*II P_{lac}-containing fragments from the plasmids derived from pPB10 by deletion of P22 sequences and insertion of P_{lac}, as described in the text. The size markers are run-off transcripts initiated at P22 P_{RM} (upper band) and P_R (lower band) on an *Alu*I fragment template (Poteete & Ptashne, 1982) and are shown as numbers of nucleotides. The major transcription products are indicated by filled circles. The transcription products for pPB10Δ7-8 are indicated by open circles. Exposure was for 8 h.

the sequence created by this fusion we have been able to draw two potential stem-and-loop structures. One structure with remarkable potential stability involves the entire *lac* operator region and positions the poly(T) sequence 11 residues promoter distal to the stem. The other structure with marginal potential stability positions the poly(T) sequence three residues promoter distal to the stem. The relevance of the partial termination observed *in vitro* with pPB10Δ7-8 is uncertain. Cells carrying this deletion plasmid produce levels of tail protein similar to those found in cells carrying plasmids in which the entire terminator structure is deleted. However, it is possible some termination may occur *in vivo*, but its effect on the levels of tail protein production may not be dramatic enough to be detected in the tail assay.

4. Discussion

The evidence presented in this paper indicates that a transcription termination signal similar in structure to many rho-independent terminators is located between P22 genes *ant* and *9*. This terminator sequence negatively regulates gene *9* expression *in vivo* and terminates transcription directed towards gene *9* sequence *in vitro*. Deletion of this terminator sequence in plasmids carrying the tail gene and a properly oriented *lac* promoter allows a high level of tail protein production. In studies reported elsewhere, we have used cells bearing one of these plasmids (pPB10Δ8-2; renamed pPB13) as a source of tail protein for purification (Sauer *et al.*, 1982). Although tail protein can also be purified in large amounts from P22-infected *Salmonella typhimurium*, this protein is always contaminated with a low but significant quantity of viable phage particles (Berget & Poteete, 1980). The use of the engineered plasmids described here to direct tail protein synthesis avoids this problem, since tail protein can be synthesized in *E. coli* in the absence of other P22 gene products.

The higher molecular weight transcripts in Figure 6 probably represent transcripts that have initiated from the ends of the restriction enzyme fragments rather than from the *lac* promoter. As would be expected if this were the case, these transcripts vary in size proportionally with the distance from one end of the template to the transcription termination signal. Or as in the case of PB10Δ8-1 and 8-2 these transcripts are the length of the entire template. This demonstrates that this type of non-promoter-initiated transcription is sensitive to the same termination signals as promoter-initiated transcription. End initiation has been observed by others and has been shown to be inhibited by ρ-factor (Lau *et al.*, 1982; Wu *et al.*, 1981). The *in vitro* reactions performed in this study contained no ρ-factor.

We have formally shown that deletion of a specific DNA sequence increases transcription of the P22 tail protein gene initiated at a *lac* promoter. Several considerations suggest that this sequence is, in fact, the *ant* terminator. First, transcription initiated at P_{ant} *in vitro* terminates at or very close to the terminator sequence that we have identified in this work (Susskind & Youderian, 1982). Second, examination of the DNA sequence between the end of the *ant* gene and the beginning of gene *9* (Fig. 3) reveals no other sequences that resemble termination sequences, and a variety of genetic evidence suggests the presence of a terminator signal in this region (Susskind & Botstein, 1978). Third, in the absence of antiterminator proteins (like the lambda *N* protein), the function of transcriptional termination sequences is generally not promoter-specific (Ward & Gottesman, 1982). Thus, a sequence that terminates transcription initiated at a *lac* promoter should also terminate transcription initiated at P_{ant}.

If P_{ant}-initiated transcription is terminated at T_{ant} before reaching gene *9*, how then does transcription initiated at P_{late} some 20×10^3 bases upstream, reach gene *9*. The switch from early to late gene expression depends on the gene *23* activator function (for a review, see Susskind & Botstein, 1978). The gene *23* protein is thought to antiterminate transcription at a site close to the beginning of the late operon and thereby allow extension of the short, constitutively synthesized P22 gene *a* RNA into the late operon (Roberts *et al.*, 1976). Acting in a similar manner, gene *23* protein could allow RNA polymerase to override subsequent termination signals such as T_{ant}. Gene *23* protein could presumably act in two ways. It could modify RNA polymerase at the beginning of the late operon, rendering the modified transcriptional apparatus insensitive to downstream terminators. Alternatively, gene *23* protein could act independently at each termination (or termination plus utilization) site.

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