Identification of Two New Bacteriophage T4 Genes That May Have Roles in Transcription and DNA Replication

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We have identified two bacteriophage T4 genes, 45.1 and 45.2, that map in the intergenic space between phage replication genes 46 (which encodes a recombination initiation protein) and 45 (which encodes a bifunctional protein required in replication and transcription). The existence of genes 45.1 and 45.2 had not been previously recognized by mutational analysis of the T4 genome. We cloned the T4 gene 45.1/45.2 segment, determined its nucleotide sequence, and expressed its two reading frames at high levels in bacterial plasmids. The results predicted molecular weights of 11,400 (100 amino acids) for gp45.1 and 7,500 (62 amino acids) for gp45.2. We also determined that in T4-infected Escherichia coli, genes 45.1 and 45.2 are cotranscribed with their distal neighbor, gene 45, by at least one mode of transcription. In an accompanying report (K. P. Williams, G. A. Kassavetis, F. S. Esch, and E. P. Geiduschek, J. Virol. 61:600–603, 1987), it is shown that the product of gene 45.1 is the so-called T4-induced 15K protein, an RNA polymerase-binding protein of unknown role in phage development. Possibly, T4 genes 45.2, 45.1, and 45 constitute an operon for host RNA polymerase-binding phage proteins. Jointly with Williams et al., we propose the term rpB (RNA polymerase-binding) to refer to T4 genes whose products bind to the host RNA polymerase and have adopted the name rpB for T4 gene 45.1.

In bacteriophage T4, the genetic cluster consisting of genes 45, 44, and 62 encodes DNA polymerase accessory proteins that enhance DNA synthesis on replicating phage DNA templates (25). T4 genes 44 and 62 specify the subunits of a DNA-dependent ATPase, and the T4 gene 45 protein interacts with and stimulates the activity of this ATPase. All three gene functions are essential for phage DNA replication (7, 21) and are components of the multienzyme complex involved in genome duplication, repair, and recombination. In addition, the gene 45 protein is a component of the T4-modified host RNA polymerase enzyme complex that transcribes phage genes during the late stages of T4 development in the infected Escherichia coli host (26, 38, 39). The ability of the gene 45 protein to participate in different multienzyme complexes may require that it be regulated not only with T4-induced DNA replication proteins, but with transcription proteins as well. In the course of analyzing transcriptional and translational regulation of the T4 gene 45-44-62 cluster, we became aware of the existence of a sizable DNA segment that maps upstream of gene 45, between the protein-coding sequences of this gene and its nearest known upstream neighbor, gene 46, which encodes a protein required for recombination-mediated initiation of phage DNA replication (16). We report here on the structure and coding properties of this intergenic region.

We have determined the nucleotide sequence of the DNA segment that maps between T4 genes 45 and 46, cloned it in expression plasmids, and demonstrated that it specifies two proteins, which we term the gene 45.1 (about 11.4 kilodaltons [kDa]) and gene 45.2 (about 7.5 kDa) proteins. The two genes are cotranscribed with T4 gene 45 and may have roles in controlling phage-induced transcription and replication. Williams et al. (35) have mapped the gene coding for an RNA polymerase-binding protein (the T4-induced 15K protein) to the location of the 45.1 cistron. These investigators also observed perfect homology between the amino-terminal sequence (23 residues) of purified T4 15K protein and the amino-terminal sequence predicted for the gene 45.1 product from our DNA sequencing studies. Possibly genes 45, 45.1, and 45.2 constitute an operon for RNA polymerase-binding proteins.

MATERIALS AND METHODS

Bacterial and T4 phage strains. The properties of most of the E. coli strains used in this study have been described in previous reports and are summarized in Table 1. Strains K802 and NapIV were both used as hosts for plasmids, although most of our measurements of expression of plasmid-encoded gene products were conducted with the NapIV clones. E. coli K802 was also used as the host for preparing lysates of the lambdoid phages, and the UV-sensitive K-12 strain 159 (LS1166) served as the host in experiments that measured levels of expression of the T4 genes carried by the lambdoid clones (see Results and Fig. 3).

The T4 phage strains used included a large number of amber (am) mutants from the California Institute of Technology collection, which is maintained by W. B. Wood, University of Colorado. In addition, we used the regA mutants regAR9 and regAH12 (11), a T4D wild-type stock maintained in this laboratory, and a multiple mutant phage strain that we constructed and that carried lesions in T4 genes 32 (ama453), 42 (amN122), rifB (rUV375 ochre), and regA (R9). A regA* counterpart of this strain was also used. The genotypes of these strains were verified by recombinantion tests and by sodium dodecyl sulfate (SDS)-gel electrophoretic assays such as those described previously (11). In the gel assays used here, E. coli NapIV served as the host for T4 infections, and the growth medium was M9 supplemented with a mixture of 19 amino acids (methionine omitted) at 5 mM each, except tyrosine, which was added at 2.5 mM. This medium (termed M9-19) was particularly suitable for labeling T4-induced proteins with added [35S]methionine. The [35S]methionine was purchased from New England Nuclear Corp., Boston, Mass. (catalog no. NEG009A).

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TABLE 1. Bacterial strains used

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Description</th>
<th>Reference</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>NapIV</td>
<td>B* strain; hsdR, hsdM; thi</td>
<td>23</td>
<td>L. Gold</td>
</tr>
<tr>
<td>K802</td>
<td>K-12 strain; hsdR, hsdM, gal met supE</td>
<td>37</td>
<td>N. Murray</td>
</tr>
<tr>
<td>159</td>
<td>K-12 strain; F',,uvrA Str r sup (serine-inserting am suppressor)</td>
<td>5</td>
<td>L. Soll</td>
</tr>
<tr>
<td>(LS116)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR63</td>
<td>K-12 strain; supD60 λ'</td>
<td>1</td>
<td>R. S. Edgar</td>
</tr>
<tr>
<td>S/6 Strα</td>
<td>B strain; sup B Strα derivative of E. coli S/6</td>
<td>6, 13</td>
<td>This laboratory</td>
</tr>
</tbody>
</table>

α Strα, Streptomycin resistant.

E. coli plasmid and lambdoid phage vectors. Table 2 lists the plasmids and lambdoid phage strains we used to study expression and to determine the primary sequences of genes within the T4 genome 46-43 cluster. All the plasmids listed are derivatives of pBR322, a widely used cloning vector (2). Phages NM761, NM761-4, B9, and B36 were obtained from the libraries of Noreen Murray and John Abelson. They were grown in E. coli K802 and purified by polyethylene glycol precipitation by the methods of Williams and Blattner (34). The final, purified phage stocks were suspended in a buffer containing 10 mM MgCl2, 1% gelatin, and 10 mM Tris chloride, pH 7.5. In experiments involving the labeling of proteins specified by the lambdoid-T4 recombinants, we used the phosphate-based synthetic medium RM described by Murialdo and Siminovitch (22). This medium contains glycerol (as the carbon source), maltose, and 10 mM MgCl2, which provide optimal conditions for infections with lambdoid phages. Identities of the T4 genetic segments in phages NM761-4, B9, and B36 were initially determined by marker rescue tests. In these tests, small samples of E. coli K802 cells preinfected with the lambdoid recombinant under study were superinfected with tester T4 am mutants of genes 46, 45, 44, 42, and 43, and the mixed infections were subsequently spotted onto lawns of E. coli S/6 Strα, a host that does not support the growth of either the lambdoid phages or the T4 am mutants. Appearance of plaques in the spots after overnight incubation was indicative of recombination between the mutant T4 strains and corresponding wild-type sequences in the chimeric lambdoid phage. K802 cells infected with only the T4 tester phages served as negative controls.

Construction of recombinant plasmids for overexpression of T4 genes. Standard techniques (19) were used to prepare plasmid and phage vector DNAs for restriction enzyme analyses and for subcloning the T4 genetic inserts of phages B36 and NM761-4. A chart showing the T4 genes encompassed within B36 and NM761-4 is shown in Fig. 1. The entire T4 DNA insert of phage B36 (a 5.5-kilobase [kb] EcoRI fragment) was ligated at the EcoRI site of the lac promoter-operator vector pH20. This yielded clones carrying both orientations of the T4 DNA, but neither recombinant type expressed the T4 genes to any detectable levels,
FIG. 2. Structures of the expression plasmid pGW7 and the T4 DNA segments that were cloned under control of the heat-inducible \( p_l \) promoter element of the vector. Construction of the plasmid clones and verification of their restriction maps were carried out as described in Materials and Methods and the legend to Fig. 1. All the pGW7 clones of the DNA segments diagrammed in panel B were derived from the JM1283 fragment (panel A). Fragments JM1168 and JM1283 (A) were cloned in the BamHI-Aval interval of pGW7: the other DNA fragments (B) were cloned in the BamHI-Sphl interval of this vector. Except for TH4553, the fragments diagrammed in panel B were first cloned in pUC18 within the multicloning site region bounded by the BamHI and Sphl sites. The parentheses indicate restriction enzyme sites on the T4 DNA that were destroyed in the pUC18 cloning (see text). In the drawing of pGW7, the boxes represent DNA from lambda phage and the line represents pBR322 DNA. Lambda transcription signals in pGW7 (A): \( \Phi_x \), right-hand promoter; \( \Phi_{rm} \), repressor maintenance promoters; \( p_l \), leftward promoter; \( t_l \), transcription termination signal. In the other drawings, the open boxes represent the protein-coding segments of T4 genes, the solid and dashed boxes represent intergenic domains.

that are not known to encode protein, and the stippled box (JM1168, panel A) is a segment from pBR322 DNA. The symbol \( \blacktriangle \) means that the designated T4 gene carries a terminal deletion located to the same side as the symbol. Abbreviations: B, BamHI; Sal→B, SalI site changed to BamHI site; also see legend to Fig. 1.
postinfection, respectively. Other conditions for the analysis of 35S-labeled proteins were similar to those described earlier for 14C-labeled samples (12).

Isolation of T4-induced mRNAs. The advantages of using rifampin-treated T4 regA infections for the isolation of stable transcripts of the T4 gene 45-44-62 cluster have been described (8). In the studies reported here, 40-ml E. coli NapIV cultures were grown at 30°C in M9-19 medium to 3 × 10^8 cells per ml, concentrated 10-fold in the same medium, warmed to 42°C, and then infected with an equal volume of the T4 regA or regA + phage strain under study. A temperature-sensitive regA mutation (regAH12 [11]) was used in the experiment described in this report (see Results and Fig. 6). The multiplicity of infection was 10. Rifampin (final concentration, 200 \( \mu \)g/ml) was added at 7 min postinfection, and the cultures were aerated at 42°C for 25 min before being mixed with 1 ml of a hot solution (in a boiling waterbath) of 10% SDS in 500 mM Tris chloride (pH 6.8)-20 mM disodium EDTA. Three minutes later each lysate was cooled to room temperature, 0.1 volume of 2 M sodium acetate, pH 5.2, was added, and the mixture was extracted three times with a 1:1 solution of phenol and chloroform. The phenol was presaturated with HCl. The nucleic acid was then ethanol precipitated in acidic solution (0.1 volume of 2 M sodium acetate, pH 5.2, 3 volumes of 95% ethanol at −20°C, and 0.25 volume of glacial acetic acid). After overnight incubation at −20°C, the precipitates were harvested by centrifugation (10,000 × g for 25 min) and dried under vacuum, and each was dissolved in 1 ml of 50 mM Tris chloride, pH 6.8, containing 10 mM MgSO_4_ and 2 mM dithiothreitol. Human placental RNase inhibitor (50 U; Promega Biotech) and pancreatic DNase I (40 \( \mu \)g; Boehringer Mannheim) were added, and the mixtures were incubated at room temperature for 30 min. Following two cycles of phenol-chloroform extraction, these nucleic acids were ethanol precipitated in the presence of 0.2 M sodium acetate, pH 5.2, and the harvested pellets (mostly RNA) were redissolved in water at a concentration of about 500 \( \mu \)g/ml. Portions were stored at −80°C in 70% ethanol containing 0.2 M sodium acetate, pH 5.2. RNA pellets in the portions were collected by centrifugation, dried, and redissolved before use in gel electrophoresis and Northern blotting experiments.

RNA electrophoresis and Northern blotting. T4-induced RNA prepared as described above was suspended in 1 M freshly deionized glyoxal–10 mM sodium phosphate, pH 6.5 to 7.0, and then heated at 50°C for 1 h. Samples (usually 20 \( \mu \)g of nucleic acid) were then electrophoresed at room temperature in 1.5% agarose prepared in 10 mM sodium phosphate buffer at pH 6.5 to 7.0. Following electrophoresis, a strip of the gel was stained with ethidium bromide (1 \( \mu \)g/ml for 30 min), destained in electrophoresis buffer for 30 min, and then visualized on a 306-nm UV-transilluminator to assess the quality of resolution of RNA species (mostly 16S and 23S host rRNAs). The resolved RNA population was subsequently transferred to nitrocellulose paper (type BA85; Schleicher & Schuell) by salt diffusion and then hybridized with 32P-labeled DNA probes by established methods (19). The probes consisted of T4 DNA fragments that were excised from plasmid clones, separated by electrophoresis in low-melting-point agarose (Sea Plaque; Marine Colloids), and eluted from melted agarose sections by dilution and phenol extraction according to instructions from the manufacturer. The purified fragments were then nick-translated with E. coli DNA polymerase I in the presence of \( \alpha \)-32P-labeled dCTP and dATP (at 150 \( \mu \)Ci [45 to 50 \( \mu \)mol] each), and the products of DNA synthesis were separated from remaining nucleotides on Sephadex G-50 spum columns before being used in hybridizations with nitrocellulose-immobilized RNA.

RESULTS

Expression of T4 replication genes in lambdoid vectors. Three types of analyses were used to characterize the lambdoid phages NM761-4 and B36 for their content of T4 genetic sequences: (i) restriction enzyme mapping of isolated phage DNAs, (ii) marker rescue tests in coinfections of E. coli hosts with T4 am mutants and the recombinant phages, and (iii) gel electrophoretic analyses of proteins from 35S-labeled infections of E. coli with the lambdoid strains. Representative results of our analyses are summarized in Fig. 1 and 3. In addition to providing a physical map of the T4 DNA inserts, the restriction enzyme analyses (Fig. 1) also established the orientations of these inserts in the two phage vectors. The gel electrophoretic assays (Fig. 3) detected most of the proteins we expected to be specified by the cloned T4 DNA segments, despite the high background from vector-specific gene products. In other, similar experiments, NM761-4 and B36 failed to synthesize the T4 proteins when they were used to infect hosts bearing immunity for phages 21 and lambda, respectively (results not shown).
That is, expression of the T4 genes by the two recombinants appears to be primarily dependent on vector promoters, namely p\textsubscript{L}\textsuperscript{21} in NM761-4 and p\textsubscript{L} in B36, rather than on T4 promoters in the cloned DNA. We should emphasize that the experiment shown in Fig. 3 compared the expression of T4 genes in recombinant lambdoid phages with two types of conditions: standard T4 infections (T4 \textit{regA}\textsuperscript{+} and T4 \textit{regA} lanes) and heat inductions of cells bearing recombinant plasmids (pJM1168 and pJM1283 lanes). The T4 \textit{regA}\textsuperscript{+} and T4 \textit{regA} controls were included for identification of T4-specific proteins. The recombinant-plasmid controls were included for comparisons with results to be presented later (see Fig. 5). We note here that UV-irradiation of the UV-sensitive \textit{E. coli} strain 159 (LSI66) host used for the lambdoid phages diminished background bacterial protein synthesis considerably (Fig. 3) and allowed adequate visualization of phage-derived proteins (NM761, NM761-4, B9, and B36 lanes, Fig. 3). To further characterize the expression potential of the cloned T4 genes, we subcloned the respective inserts of NM761-4 and B36 in a variety of plasmid vectors and used these to derive DNA sequence information about protein-coding segments and intergenic domains. Some results of those studies are described elsewhere (32; Miller et al., in press: T. Hsu and J. D. Karam, manuscript in preparation). In this report, we mostly emphasize our findings about the segments designated g45.1 and g45.2 in Fig. 1. The products of these two putative T4 cistrons could not be unambiguously detected in the infections with NM761-4 and B36 shown in Fig. 3.

**Primary structure of the T4 gene 46-45 intercistronic segment.** The nucleotide sequences of T4 genes 45 and 46 have been determined (9, 29). Detailed restriction mapping of DNA encompassing the span between these two genes (Fig. 1) suggested to us that the region was sufficiently large (about 700 base pairs [bp]) either to be a protein-encoding segment of the T4 genome or to harbor regulatory signals that are important for expression of its neighboring phase DNA replication cistrons. We approached analysis of this region by determining its primary structure and analyzing its expression under control of plasmid-borne promoters.

Figure 4 outlines the strategy we used to determine the nucleotide sequence of the gene 46-45 intercistronic domain.
The fusion product consisting of 30°C includes M9-19 to a T4 infections clones plasmid both and shows this sequence with its deduced protein-encoding potential as well as with certain landmarks that may be relevant to its expression. The results of the primary sequence determinations suggested the existence of two open reading frames within this region, 45.1 and 45.2, the predicted protein products of which were 100 and 62 amino acid residues long, respectively. Based on the deduced amino acid sequences of the two gene products, gp45.1 is 11.4 kDa and gp45.2 is 7.5 kDa. Both genes appeared to possess strong ribosome initiation signals, and as will be shown below, both genes could be expressed at high levels in plasmid clones of the T4 gene 46-43 cluster; a protein having the same size as gp45.1 was detectably expressed in standard T4 infections (Fig. 3).

Overexpression of T4 genes 45.2, 45.1, 45, 44, 62, and regA in plasmid vectors. Because of differences in host range, phage adsorption, and growth requirements between T4 and lambdoid phages, it is difficult to compare the efficiencies of expression of the T4 genes in NM761-4 and B36 infections relative to standard T4 infections (Fig. 3). We estimate that synthesis of the detectable T4-derived proteins in E. coli 139 cells infected with lambda-T4 recombinants (Fig. 3) occurred at about 10% of the levels usually observed in infections of E. coli B with wild-type T4. By contrast, much higher levels of the T4 gene products could be generated when subfragments of the T4 inserts of NM761-4 and B36 were cloned in the expression plasmid pGW7. In the experiment for Fig. 5, we examined the clones diagrammed in Fig. 2 for synthesis of T4 gene products at 42°C. We observed little or no synthesis of T4-specific proteins when the pGW7 clones were grown at 30°C. (Only one example, pJM1168, is presented in Fig. 5 to demonstrate the differences in growth at 30 versus 42°C.) As can be seen (especially lanes TH4558, TH4663, and TH4665, Fig. 5), the intercistronic region between T4 genes 46 and 45 did indeed encode at least two proteins whose approximate molecular sizes are similar to those predicted from the DNA sequencing studies (Fig. 4). gp45.1 (11.4 kDa) migrated slightly faster than wild-type regA protein (14.6 kDa [32]). Other observations from the experiment shown in Fig. 5 are also worth noting. The pJM1168 (regA) clone overproduced the T4-specific proteins to a much higher extent than the pJM1283 (regA) clone. This result was in part expected because T4 genes 45, 44, and 62 are known to be regulated at the translational level by regA protein (11, 12). It appeared from these results and from the patterns observed with regA phage-infected cells (Fig. 5; also Fig. 3) that gp45.1 is also regulated by the regA gene function. The results with gp45.2 were not as clear. We were unable to detect this protein in either regA or regA phage infections, and its synthesis in heat-induced pJM1168 was considerably lower than that of the other proteins encoded by the T4 DNA segment of this clone (Fig. 5). Its synthesis, however, could be increased by removal of surrounding T4 DNA (i.e., lanes TH4556, TH4558, and TH4665, Fig. 5). In two-plasmid assays, gp45.2 was relatively insensitive to regA-mediated repression.

FIG. 5. Overexpression of T4 genes 45.2, 45.1, 45, 44, 62, and regA by pGW7 recombinants. E. coli NapIV strains carrying the plasmids diagrammed in Fig. 2 were grown at 30°C in M9-19 medium to about 3 x 10^8 cells per ml. The cultures were then diluted in M9-19 to a concentration of 2 x 10^8 cells per ml, and 1-ml portions were aerated at 42°C. After 30 min at this temperature, 10 μCi of [35S]methionine was added, and aeration was continued for another 30 min before the cells were harvested and processed for SDS-gel electrophoresis and autoradiography. The autoradiogram shown includes 30°C controls for the cloning vector (pGW7) and for pJM1168. The T4 infection control was prepared as described in the legend to Fig. 3. The band labeled 44* is assumed to be a protein fusion product consisting of an N-terminal gene 44-encoded segment and a C-terminal segment encoded by pGW7 DNA. Identification of other autoradiogram bands was based on comparisons with the T4 infection control lane and on results of marker rescue experiments. The positions of specific gene products within each lane are marked.

FIG. 6. Northern blot analysis of RNA from the T4 gene 45.2-45.1-45.4 cluster. RNAs were prepared from rifampin-treated T4 wild-type (WT) and T4 regAH12 infections of E. coli NapIV cultures and were analyzed as described in Materials and Methods. A, B, and C refer to the three RNA bands used in the analyses; the probes are diagrammed below. The numbers 1 through 5 refer to 32P-labeled bands (DNA-RNA hybrids) detected with the probes. Probe A gave a weak signal for an RNA species (band 5) that hybridized strongly with internal gene 44 probes in other assays not shown here. The x marks hybridization with 23S host rRNA, which occurred in some of our assays. The figure also summarizes our interpretations of the blots (wavy arrows). See the legend to Fig. 1 for restriction site abbreviations.
whereas gp45.1 was very sensitive to the regA repressor (results not shown).

Expression of genes 45.1 and 45.2 in T4-infected cells. Because of their close linkage to DNA replication genes, we considered the possibility that T4 genes 45.1 and 45.2 are coregulated with neighboring cistrons. We used RNA purified from T4-infected cells and DNA probes internal to the T4 gene 46-45.2-45.1-45 region in a number of Northern blotting assays to look for transcriptional overlaps between the two newly discovered T4 genes and their neighbors on the phage chromosome. We detected such overlaps for the gene 45.2-45.1-45 subcluster (Fig. 6). Two gene 45 DNA probes that did not extend into the 45.1 and 45.2 regions (probes A and B, Fig. 6) detected two RNA bands (bands 3 and 4, about 1 and 1.3 kb long, respectively) that were also seen with a probe from the 45.1-45.2 region (probe C, Fig. 6). The larger of these two bands (band 4) was estimated to include the coding segments of all three genes in the subcluster. This result led us to conclude that T4 genes 45.2, 45.1, and 45.4 are cotranscribed in phage-infected cells by at least one mode of transcription. We also noted that probe A (Fig. 6) detected a band (band 5) that extended 60 bp into the T4 gene 44 coding region, detected a large-sized RNA population (band 5, about 1.9 kb long) that we presume to be the T4 gene 44-62-regA transcript that was alluded to by Trojanowska et al. (32). A heterogeneous band of similar size yielded strong hybridization signals with probes from internal segments of T4 genes 44, 62, and regA (T. Hsu and J. D. Karam, manuscript in preparation). As depicted in Fig. 6, RNA band 5 was not detected by probes B and C, which mapped to regions upstream of the junction between genes 45 and 44.

We should emphasize that at least some of the RNA bands depicted in Fig. 6 may represent processed, rather than primary, transcripts since the phage-induced RNA used in the experiment was isolated from infected cells that had been incubated with rifampin for several minutes prior to extraction (see Materials and Methods). These experimental conditions were used to enrich for gp45 and gp45.1 translational activities in the regA infection (8) and to facilitate detection of the mRNAs by Northern blotting assays. When the analyzed T4-induced RNA was isolated early after infection and without rifampin treatment, the Northern blots yielded high levels of background hybridization with the 32P-labeled probes, and we were unable to distinguish RNA banding patterns, especially in the region corresponding to bands 3 and 4 in Fig. 6 (results not shown). The possibility of mRNA processing for this T4 genetic region and its relevance to the relative abundance of the RNA bands seen in Fig. 6, particularly bands 1 and 2, will be discussed further in the next section.

DISCUSSION

It is clear that the intercistronic region bounded by T4 DNA replication genes 45 and 46 is capable of specifying two proteins, gp45.1 and gp45.2, at least when expressed under control of a plasmid-borne leftward promoter (pL) from phage lambda (Fig. 5). Our identification of these two proteins in extracts of T4-infected cells remains somewhat uncertain. A protein having the same size as plasmid-encoded gp45.1 was detected in gel electrophoretic assays of 35S-labeled extracts from T4 infections (Fig. 5), but we have not proven that the two species are identical. With gp45.2, the level of synthesis of the protein in T4-infected cells may be too low to be detectable by the types of assays used here. During the preparation of this report, we learned (Williams et al. [35]) that our predicted amino acid sequence for gp45.1 (Fig. 4) included a complete homology with all of the 23 amino acid residues determined for purified T4-induced 15K protein, a polypeptide that coisolates with RNA polymerase from phage-infected cells (30). Williams et al. also showed that a mixed synthetic oligonucleotide based on the determined amino-terminal sequence of the 15K protein hybridized to T4 DNA in the gene 45.1 region. Therefore, it appears that the 15K protein and gp45.1 are identical. We should note in this regard that the molecular weight of gp45.1, as predicted from the amino acid sequence shown in Fig. 4, was about 11,400, rather than 15,000; however, gp45.1 from our clones (Fig. 5) comigrated with purified 15K protein on SDS gels (K. Williams and E. P. Geiduschek, personal communication), and it appears that the size of the so-called 15K protein is overestimated on standard SDS-polyacrylamide gels. It will be important to purify the plasmid-generated gp45.1 and to assay for possible association with T4-modified and unmodified RNA polymerase.

Our primary motivation in examining the structure and expression of the T4 gene 45-45.2 region was its very close linkage to phage genes that are known to be essential for DNA replication and transcription. Also, a strong signal for initiation of replication (an origin) has been mapped to this general area of the T4 chromosome (15, 21). The discovery that gp45.1 is probably the T4 RNA polymerase-binding 15K protein raises the possibility that both this protein and gp45.2 become involved in replication-dependent transcriptional events during T4 growth in its E. coli host. This possibility is particularly attractive in view of the results shown here (Fig. 6) that genes 45.1 and 45.2 were cotranscribed with gene 45, which encodes a protein that is essential for both phage DNA replication and transcription (25, 39). The association of 15K protein to RNA polymerase is one of several noncovalent modifications that the host enzyme undergoes after phage infection. Four additional phase-induced proteins, termed the 22K (T4 gp55), 12K (T4 gp33), 10K (T4 gene unknown), and T4 gene 45 (DNA polymerase accessory) protein, all exhibit RNA polymerase-binding properties (26, 27, 30). T4 gp55 and gp33 coisolate with the polymerase in substoichiometric quantities and are known to be required for phage late-gene expression in vivo (3, 7); gp55 has been shown to be required in the utilization of late promoters in vitro as well (14). Nonsense and temperature-sensitive mutations in T4 genes 55 and 33 are conditionally lethal, and infections with the mutants under nonpermissive conditions result in little or no appearance of late mRNA (3). The binding of T4 gp45 to RNA polymerase has been examined by affinity column chromatography with enzymes from uninfected and infected cells. It interacts only with enzyme from infected cells, i.e., with polymerase that has already undergone some phage-induced modification (26).

The biological roles of the 10K and 15K proteins remain largely unclear, although some insights have been derived from in vitro studies. The 10K protein exhibits salt-dependent inhibition of host sigma factor (31) and so may be a modulator of transcription initiation efficiency in vivo. The 15K protein was recently shown to be the most stably bound to the host polymerase of the T4-induced polypeptides (17) and to cause a temperature-dependent decrease in promoter utilization by sigma-saturated enzyme (18). Interpretation of these in vitro observations in physiological terms will benefit from the isolation and study of mutations in the genes that encode the T4 10K and 15K proteins. We should point out that although there is reason to believe that gene 45.1 encodes the 15K protein, there is as yet no evidence relating
the 10K protein to gp45.2 (7.5 kDa, as predicted from the nucleotide sequence for the gene; Fig. 4). We are attempting to generate mutations in genes 45.1 and 45.2 by in vitro manipulations in order to incorporate these into phage genomes and assess the biological consequences. It would be interesting to find out whether gp45 or gp45.2 can stimulate the activity of gp45.1 (15K protein) in vitro or whether gp45.1 or gp45.2 can mediate gp45 binding to RNA polymerase. Such interactions would be analogous to the interactions of gp45 with the T4 gp44/gp62 ATPase that stimulate the ATPase activity and T4 DNA polymerase processivity (25). Also, the low dosage of gp45.2 may have caused it to be missed in preparations of RNA polymerase purified from T4-infected cells, and it would be interesting to see whether plasmid-overproduced gp45.2 will coisolate with the host enzyme in significant amounts.

Finally, we want to emphasize that the results shown in Fig. 6 demonstrate the existence of T4-induced RNA species (band 4, Fig. 6) that encompass the combined sequences of genes 45, 45.1, and 45.2; however, the quantitative differences we observed between the four separable RNA populations from these three genes probably do not reflect differences in levels of transcriptional initiations at four different promoters. RNA-processing events cannot be ruled out. In preliminary attempts at characterizing the detectable RNA populations, we identified four sets of RNA 5' ends by using avian myeloblastosis virus reverse transcriptase in DNA primer extension assays (unpublished results). Although we recognize the susceptibility of such assays to artifacts, we have, for the sake of discussion, marked the positions of the determined ends on the sequence chart in Fig. 4. These positions are roughly corresponding to bands 1 and 4, respectively, in Fig. 6) mapped downstream of nucleotide sequence arrangements that resemble the sequence ATTGCTT (−35) . . . TATAAT (−10), which has been implicated in T4 mot-dependent initiations of transcription (see reference 4 for a review). These sequence arrangements are also drawn into Fig. 4. The T4 mot gene product is required for "middle-promoter" recognition in T4-infected cells, and gene 45 is known to be partially under mot control (4). N. Guild, M. Gayle, and L. Gold (personal communications) have also detected the 5' ends we ascribe to bands 1 and 4 (Fig. 4 and 6) and have further demonstrated that band 1 is not dependent, whereas band 4 is not. Interestingly, the 5' end of RNA band 4 coincided with the first nucleotide of the predicted Shine-Dalargino sequence for the gene 45.2 reading frame. This may explain why gp45.2 was synthesized at low levels in T4-infected cells (Fig. 5), despite a predicted strong translational initiation signal for gene 45.2. No obvious promoter-like sequences could be assigned to the other two RNA populations (bands 2 and 3) that were detected (Fig. 4), which may mean that these are processed species. We should perhaps also mention that in some experiments, and depending on growth temperature and medium composition, band 1 rather than band 2 was the most abundant of the four species depicted in Fig. 6. Probably, bands 1 and 2 (Fig. 6) represent the major gp45-synthesizing in vitro activity that was detected in the RNA fractions we described previously (8). In other work focusing on transcription termination and processing in the T4 gene 46-43 cluster (unpublished results), we observed polar effects on distal-gene expression by sequences in the junctions between genes 46 and 45.2 and between genes 45 and 44, but not between genes 45.1 and 45. Therefore of the two putative stem structures predicted from the nucleotide sequence of the gene 45.2 and 45.1 region (Fig. 4), only the one upstream of gene 45.2 (downstream of gene 46) seems to be physiologically relevant.

Together with the work of Williams et al. (35), this work has identified the structural gene for an awkwardly named T4-induced protein, the 15K protein, which is not 15,000 daltons in size. The designation 45.1 is also not descriptively informative. Therefore, jointly with Williams et al., we propose the term rpb for T4 genes that encode RNA polymerase-binding proteins and assign the name rpb A to T4 gene 45.1.

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