Gene D5 Product of Bacteriophage T5: DNA-Binding Protein Affecting DNA Replication and Late Gene Expression

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Gene D5 is not only necessary for replication of bacteriophage T5 DNA and for shutoff of expression of some early genes, but has been found to be necessary also for the expression of late T5 genes. The polypeptide product of gene D5 has been identified, an intragenic map of gene D5 has been constructed, and the direction of transcription of gene D5 has been established. The polypeptide coded by gene D5 has been shown to be a DNA-binding protein with affinity for both double- and single-stranded DNA.

The product of gene D5 has previously been shown to have a dual role in the infectious process of bacteriophage T5. On the one hand it is required for T5 DNA synthesis (8), and on the other hand it appears necessary for the shutoff of synthesis of several early proteins (8). The products of other genes required for T5 DNA synthesis (e.g., T5 DNA polymerase) do not significantly affect the temporal sequence of expression of T5 genes as do similar gene products in the T4 system (16). Cells infected with T5 mutants defective in gene D5 have previously been reported to synthesize all early proteins for an extended period of time but to be unable to synthesize phage DNA (8). We report here that the polypeptide product of gene D5 (gpD5) is a DNA-binding protein and is required for the expression of late genes. In addition, gpD5 is identified, an intragenic map of gene D5 is presented, and the direction of transcription of gene D5 is established.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. *Escherichia coli* F and B are nonpermissive strains, and F_{sup} is a permissive strain for amber mutants of T5. T5_{am12a}, T5_{am23a}, T5_{am105d}, T5_{am106e}, T5_{am107a}, and T5_{am128a} all have amber codons in gene D5, whereas T5_{am11}, T5_{am40c}, and T5_{am18a} have amber codons in genes D4, D7, and D9, respectively. All amber mutants were isolated in this laboratory (D.J.M.).

Growth and infection of bacteria and preparation of radioactively labeled extracts for polyacrylamide gel electrophoresis. *E. coli* was grown in morpholinopropane sulfonate (MOPS)-glucose-salts medium (15) at 37°C to 2 × 10⁸ cells per ml, harvested by centrifugation, and resuspended at 0°C in MOPS-salts at 5 × 10⁹ cells per ml (14). The

concentrated cells were infected with T5⁺ or various amber mutants of T5 at an average multiplicity of infection of 7 (range = 4.4 to 9.0). Adsorption of phage to bacterial cells was accomplished by incubation for 15 min at 0°C followed by 10 min at 37°C, after which 1 volume of phage bacterium complexes was poured into 24 volumes of MOPS-glucose-salts (zero time) and aeration was begun.

Samples of bacteria were taken before addition of phage to determine the concentration of input bacteria and at 10 min after commencement of aeration to determine the concentration of uninfected cells and free phage. Uninfected cells were less than 1% (range = 0.013 to 0.22%) except for infection with T5_{am12a} where 1.3% of the cells remained uninfected, and free phage was less than 1% except for T5_{am105d} (4.9%), T5_{am106e} (7.3%), and T5_{am12a} (24.2%).

At desired times after aeration of the infected culture was begun, 5 ml was withdrawn, quickly mixed with 0.5 ml of ¹⁴C-labeled amino acids (1 μCi) in a test tube, and aerated for 5 min at 37°C. At the end of each labeling period, the radioactive samples were cooled quickly to 0°C and centrifuged at 10,000 rpm for 15 min in an SS34 rotor of a Sorvall RC2B centrifuge at 4°C. The resulting pellets were resuspended in 0.5 ml of sample preparation buffer (12), solubilized, and subjected to electrophoresis in 15% polyacrylamide gels containing sodium dodecyl sulfate by using the buffer system of Laemmli (12). After electrophoresis, the gels were fixed, stained, dried onto Whatman 3MM paper, and subjected to autoradiography as previously described (14).

Recombinational analysis. *E. coli* F_{sup} was grown in nutrient broth (NB) (1) to a concentration of about 10⁸ cells per ml, harvested by centrifugation, and resuspended at 0°C in NB containing 1 mM CaCl₂ (NB-Ca) to a concentration of 10¹⁰ cells per ml. Stocks of phage mutants were declumped by heating for 15 min at 46°C, and 6 × 10⁹ PFU of each of the two mutants used for a genetic cross was placed in a test tube in a total volume of 0.9 ml. A 0.1-ml volume of

su^+ cells at 10^{10} /ml was rapidly mixed into the 0.9-ml suspension of phage mutants, and adsorption was permitted for 10 min at 0°C followed by 2 min at 30°C . A portion of the phage bacterium complexes was then diluted 10^4 -fold in NB-Ca and aerated at 30°C for 90 min. Five to 10 drops of chloroform were then added to ensure lysis of all infected cells and to kill any uninfected bacteria.

Analysis of progeny phage was carried out by plating on su^+ and su^- cells as previously described (11). Percent recombination was calculated as titer on $su^- \times 2 \times 100$ /titer on su^+ .

Preparation of cell extracts for affinity chromatography on DNA-cellulose. *E. coli* B was grown in MOPS-glucose-salts medium to 5×10^8 cells per ml, and the culture was divided into two equal parts of 200 ml each. Phage was added to one 200-ml portion at an input ratio of six per bacterial cell, and [^3H]leucine was then added to a concentration of $5 \mu\text{Ci/ml}$. To the other 200-ml portion, [^{14}C]leucine was added to a concentration of $1 \mu\text{Ci/ml}$. Both infected and uninfected cultures were incubated for 20 min at 37°C , the cells were harvested by centrifugation, and the pellets were combined by resuspension in 5.0 ml of extract buffer (1.7 M NaCl–1 mM $\text{Na}_3\text{-EDTA}$ –1 mM β -mercaptoethanol–20 mM Tris-hydrochloride, pH 7.4). The cells were broken by sonic oscillation, the cell-free extract was treated with DNase, the debris was removed by low-speed centrifugation, and the ribosomes were removed by high-speed centrifugation (3). The DNA-free extracts were dialyzed against chromatography buffer (50 mM NaCl–1 mM β -mercaptoethanol–1 mM $\text{Na}_3\text{-EDTA}$ –20 mM Tris-hydrochloride, pH 7.4). Any precipitate formed during dialysis

was removed by centrifugation, and the final clear extract was made 10% (wt/wt) in glycerol (3).

Affinity chromatography on DNA-cellulose. DNA cellulose was prepared by the method of Alberts and Herrick (3) with either native or heat-denatured calf thymus DNA. The columns of DNA-cellulose were 0.5 cm in diameter and 5.1 cm high, with a void volume of 0.45 ml at 0.05 M NaCl. A 5-ml quantity of DNA-free extract was loaded onto a DNA-cellulose column, and the column was washed with chromatography buffer until no further radioactivity was eluted. Most of the radioactivity eluted during this wash with buffered 0.05 M NaCl. The bound proteins were eluted in a stepwise elution program indicated for each column in Fig. 4.

Reagents. The ^{14}C -amino acid mixture, [^{14}C]leucine, and [^3H]leucine used for labeling cells was supplied by Schwarz/Mann. All common chemicals were reagent grade.

RESULTS

The pattern of polypeptide synthesis induced by a T5 mutant (*am106e*) with an amber mutation in gene D5 is shown in Fig. 1. This sodium dodecyl sulfate-gel pattern of crude extracts from infected cells confirmed the earlier observation (8) that synthesis of early proteins continues for an extended period of time. However, the more striking observation from this pattern was the virtual absence of late proteins. Each of the mutants with an amber mutation in gene D5 was defective in the expression of late genes, but

FIG. 1. Temporal classes of polypeptides synthesized by *T5am106e* as revealed by electrophoresis in polyacrylamide gels. The number below each pattern represents the time in minutes after aeration of infected cultures was begun in growth medium at which a 5-min labeling period was begun. Pre-early polypeptides are designated PE1 to PE9. Prominent late polypeptides synthesized after infection with $T5^+$ are indicated by white arrowheads. Late polypeptides do not appear after infection with *T5am106e*. Black arrowheads identify gpD5. U, Uninfected pattern; am, Amber fragment that appears after infection with *T5am106e*.

each mutant induced a barely detectable level of some late proteins (Fig. 2). T5-*am128a* induced the least amount of late polypeptides that can be detected by our autoradiographic technique, whereas T5-*am12a* induced more late polypeptides than any other mutant, but nevertheless much less than T5⁺.

The product of gene D5 (gpD5) is a polypeptide with a molecular weight of 24,000 as determined by a comparison of its effective electrophoretic mobility with that of polypeptides of known molecular weight (Fig. 1; reference 14). N-terminal fragments of gpD5 can be detected after electrophoresis and autoradiography of extracts of cells infected with most of the D5 amber mutants used in this study (Fig. 2). The molecular weights of these fragments can be used to generate a physical map of gene D5 in which the positions of the amber codons are presumed to be the C-terminal end of each N-terminal fragment (Fig. 3A). This physical map agrees reasonably well with the intragenic map of gene D5 generated by recombinational analyses (Fig. 3B), except for the amber locus in *am107a*, which appears to be at or near the amber locus in *am128a* in the physical map, but further away from *am128a* in the genetic map. Also, *am12a* and *am23a* are very close together in the genetic map, but only *am12a* generated a detectable N-terminal fragment in su⁻ cells. We cannot account yet for these discrepancies.

The correlation of the genetic and physical maps of gene D5 allows a rough calculation of the recombinational frequency per base pair within gene D5. If we ignore recombination fre-

quencies between T5*am107a* and T5*am12a* or T5*am128a*, because they appear much higher than expected, we are left with recombination frequencies of 0.21, 0.52, 1.31, and 1.70% that correspond to physical distances of approximately 33, 412, 415, and 447 base pairs. These values yield a recombination frequency per base pair in these physical regions of 0.0064, 0.0013, 0.0032, and 0.0038, respectively, for an average of 0.0037 ± 0.0010 . This value is remarkably similar to 0.0037 reported for the recombination frequency per base pair within gene 23 of bacteriophage T4 (4; see also 9). Recombination therefore occurs at close to the same frequency in the T5 system as it does in the T4 system.

FIG. 3. Physical and genetic maps of gene D5. The physical map (A) is based upon the molecular weights of intact gpD5 (24,000), determined from Fig. 1, and of amber fragments, determined from Fig. 2. The genetic map is constructed relative to the physical map by equating positions 12a and 106e. Numbers between the arrows represent recombinational frequencies in percents.

FIG. 2. Early polypeptides synthesized by T5 amber mutants defective in gene D5 and by T5⁺. The amber mutants are designated below their respective patterns. The band representing gpD5 in the T5⁺ patterns is indicated by a black arrowhead, and the position of gpD5 in the amber mutant patterns is similarly indicated. am, Bands representing amber fragments. U, Uninfected pattern.

The physical map together with the intra- and intergenic maps of gene D5 (Fig. 3 and Table 1) establishes the direction of transcription of gene D5 as rightward, that is, from gene D4 toward gene D6 in the genetic and physical maps of T5 as they are usually written (5, 10, 13, 16). Such a direction of transcription dictates that the sense strand for gene D5 is the interrupted strand, a conclusion that is compatible with proposed transcriptional maps of T5 (5, 10).

The product of gene D5 is a DNA-binding protein. Fig. 4a and b show the elution pattern of extracts of uninfected and T5-infected cells from columns of both double-stranded DNA-cellulose and single-stranded DNA-cellulose. A major protein component eluted from both types of DNA-cellulose columns at 0.4 and 0.6 M NaCl. This protein represented about 10% of labeled soluble proteins found after infection with T5⁺ or with several DNA-negative mutants. However, the amount of this protein component was greatly reduced in extracts of cells infected with T5am12a, which has an amber codon in gene D5. The small amount of protein from such extracts (Fig. 4c) that eluted at 0.4 and 0.6 M NaCl may be due to N-terminal fragments of gpD5, to small amounts of intact protein due to a strong D5 promoter and a low level of suppression, and to some other T5-specific proteins made in small amounts. The amount of protein that elutes at 0.4 and 0.6 M NaCl was normal in

FIG. 4. DNA affinity chromatography of extracts from uninfected cells and from cells infected with T5(+), T5am12a (defective in gene D5), or T5am11 (defective in gene D4). Elution profiles a, c, and d were obtained with denatured calf thymus DNA, whereas profile b was obtained with native calf thymus DNA. The vertical arrows point to the first fraction collected at the concentration of NaCl indicated above or beside the arrows in the stepwise elution program. Fractions of 0.45 ml were collected. Symbols: ○, ¹⁴C in proteins from uninfected cells; ●, ³H in proteins from infected cells. Total counts per minute (100% values) per column varied from 10⁶ to 5 × 10⁶.

TABLE 1. Three-factor crosses for orientation of gene D5 with respect to adjacent genes

Cross	% Recombinants		
	Experimental	Expected ^a if order is:	
		D4-12a-105d-(D6)-D7	D4-105d-12a-(D6)-D7
D4·am11-D5·am12a × D5·am105d	1.3	1.6	0.1
D4·am11-D5·am105d × D5am12a	0.3	0.1	1.6
D5·am12a-D7·am40c × D5am105d	0.3	0.2	1.5
D5·am105d-D7am40c × D5am12a	0.9	1.5	0.2

^a The expected percentage of recombinants is calculated from the following percentage of recombination obtained from two-factor crosses: D4·am11 × D5·am12a = 6.4%; D4·am11 × D5·am105d = 7.3%; D4·am11 × D7·am40c = 17.1%; D5·am12a × D5·am105d = 1.7%; D5·am12a × D7·am40c = 12.6%; D5·am105d × D7·am40c = 10.5%.

extracts of cells infected with mutants that have amber codons in genes other than D5, e.g., T5am11 (Fig. 4d) and T5am18a. The peak fraction that appeared during elution from a denatured DNA-cellulose column with 0.4 M NaCl of an extract from cells infected with T5am18a, which has an amber codon in gene D9, was subjected to electrophoresis on a polyacrylamide gel containing sodium dodecyl sulfate (Fig. 5). The bulk of the ³H that eluted at 0.4 M NaCl migrated to a position corresponding to a molecular weight of 24,000. The T5-specific polypeptide with this molecular weight is absent when cells are infected with amber mutants of T5 defective in gene D5 (Fig. 2).

DISCUSSION

The data reported in this paper reveal that gpD5 plays a principal role in the regulation of

FIG. 5. Electrophoresis of peak fractions of ^3H -protein eluting from denatured DNA-cellulose column at 0.4 M NaCl. The extract loaded on the DNA cellulose column was prepared from cells infected with T5am18a (defective in gene D9). The vertical arrows show the positions to which ovalbumin (Ov) and lysozyme (Ly) migrate relative to the ^3H -protein. Ovalbumin and lysozyme have molecular weights of 47,000 and 14,400, respectively.

expression of late T5 genes, in addition to its role in the replication of T5 DNA (8), and apparently in shutting off the expression of some early genes (8). The necessity of gpD5 in DNA replication and in the turn on of late genes appears similar to the role of gp45 in T4 DNA replication and in the turn on of late T4 genes (16). In the T4 system, gp45 binds to a modified RNA polymerase core of the host (17), thereby presumably altering its promoter recognition properties (16). Two other gene products, gp33 and gp55, are necessary for expression of late T4 genes. In the T5 system, two other gene products, gpD15 and gpC2, are also necessary for normal expression of late genes (6, 7). The product of gene D15 of T5 is a nuclease that may modify the T5 DNA so that it is a proper template for transcription of late genes (6), whereas the product of gene C2 of T5 is a 96,000-dalton polypeptide that may bind to host RNA polymerase in T5-infected cells (7). The product of gene C2 of T5 is required not only for expression of late genes, but also for continued expression of early genes. Hence, all gene expression ceases in cells infected with T5 mutants defective in gene C2. Neither gp33 nor gp55 of T4 is known to possess nuclease activity, and expression of early genes continues when either gp33 or gp55 is defective. Hence, gp33 and gp55 of T4 do not appear analogous to gpD15 and gpC2 of T5 even though both pairs of gene products are necessary for expression of late genes in their respective systems.

If gp45 of the T4 system is analogous to gpD5 of the T5 systems, we might expect the latter to bind to RNA polymerase. D. Ratner (personal communication) has detected a polypeptide with a molecular weight of about 23,000 in T5-infected cells that binds particularly well to host RNA polymerase core enzyme. Whether this polypeptide is gpD5 remains to be demon-

strated, but the similarity in their molecular weights and their probable involvement in the transcriptional process of the infected cell suggest that it may be. On the other hand, we have shown that gpD5 binds to both double- and single-stranded DNA. Because gpD5 is required for both DNA replication and the turn on of late genes, it would appear that its binding to DNA relates to its role in DNA replication, perhaps in a manner similar to gp32 of T4 (2), whereas its apparent binding to RNA polymerase relates to its role in late transcription. One protein could participate in two different processes if it were partitioned between modified and unmodified forms, each of which has a separate function.

We have presented an intragenic and intergenic map of gene D5 and correlated the intragenic map to a physical map of gene D5 to provide a firm basis for biochemical studies of its product. Such studies are in progress.

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