JOURNAL OF VIROLOGY, July 1983, p. 77–88
0022-538X/83/070077-12$02.00/0

Vol. 47, No. 1

Site-Specific Cleavage of Bacteriophage T4 DNA Associated with the Absence of Gene 46 Product Function

LISA M. ALBRIGHT AND E. PETER GEIDUSCHEK*

Department of Biology, University of California, San Diego, La Jolla, California 92093

Received 27 January 1983/Accepted 11 April 1983

A plasmid containing a copy of the late gene 23 was cleaved at two specific locations after bacteriophage T4 infection. Cleavage at the major site, which is at the 3′ end of gene 23, was detected only in the absence of gene 46 product function and was independent of the state of modification of cytosine residues. Cutting of plasmid (cytosine-containing) DNA at this site was independent of phage DNA replication and late transcription functions. A second cleavage site, in vector DNA, was also mapped. The minor extent of cutting at this site was independent of gene 46 function. Gene 46 codes for, or controls, an exonuclease involved in T4 DNA recombination and in degradation of cytosine-containing DNA.

The coupling of transcription of bacteriophage T4 late genes to replication of the viral DNA remains poorly understood, but some "activation" of the DNA template is thought to be provided by replication (37, 50). We have thought that some progress in defining the nature of DNA competence for late transcription could be made if one examined the effect of T4 infection on the structure of a plasmid containing a T4 late gene. We chose gene 23, which codes for the major capsid protein, for this purpose. Previous experiments have established that the plasmid-borne gene 23 is expressed late in infection and have defined some of the relevant parameters (16, 17). Analysis of deproteinized plasmid DNA uncovered an unexpected effect: a site-specific cleavage of T4 DNA occurred in the absence of gene 46 function. The gene 46 product is involved both in T4 recombination and in degradation of DNA. This report is concerned primarily with the site-specific cleavage.

MATERIALS AND METHODS

Media. M9S and tryptone broth have been described previously (6). H-2 (low phosphate) medium is medium B-2 of Studier (44), without phosphate, and with 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid–NaOH (pH 7.5) in place of bis-Tris (G. Kassavetis, personal communicaion.).

Bacteria. *Escherichia coli* CR63 and B40suI were the standard *supD* (amber-suppressing) hosts. B834SR is a *recA56* derivative (JC5088 [13] from J. Clark via D. Smith) of B834S (16) constructed by K. Jacobs. B834 *galU56* has been described previously (39) and was obtained from L. Snyder. The plasmid, pLA4, which contains a 2.6-kilobase-pair (kbp) segment of T4 genome that includes all of gene 23, and its derivatives, pLA3Δ2 and pLA5, have been described previously (16).

Bacteriophage. All of the mutants used in this work have been described previously (17) or were constructed from previously described mutants by standard crosses in *E. coli* CR63 or B40suI. In the text, the designation *amX* refers to an amber mutation in T4 gene *X*.

Infected bacteria. An overnight culture of *E. coli* B834SR(pLA4) was diluted to an absorbance at 650 nm (A_{650}) of 0.05 and grown in M9S with 20 μg of ampicillin per ml at 37°C to an A_{650} of 0.4 to 0.5. For the experiments described in Fig. 2, 3, and 4, the cells were labeled with [*methyl*-^{3}H]thymidine (TdR; 25 Ci/mmol, 1 μCi/ml) and uridine (1.5 mM) during the last two generations. Radioactive precursor was removed by centrifugation of the culture and resuspension of the cells in M9S. Aliquots of the culture were shifted to 30°C 10 min before infection at a multiplicity of infection of 5. We assumed that an A_{650} of 0.4 corresponded to 2×10^8 cells per ml. (B834SR gave a much lower viable titer than its parent, B834S, for an equivalent A_{650}. Cell killing as a function of the number of infecting phage indicated that the number of target cells per milliliter was much higher than the viable titer, so we used the A_{650} under the growing conditions described above as our measure of cell number.) Surviving bacteria and unadsorbed phage were measured 10 min after infection. The progress of infection was stopped by adding the culture to 0.5 to 2 volumes of ice-cold M9S containing NaN_3 (final concentration 10 mM).

For the experiment shown in Fig. 5 and 6, *E. coli* B834SR(pLA4) was grown at 37°C to an A_{650} of 0.4 in H-2 medium supplemented with 0.1% Casamino Acids, 20 μg each of methionine and thymine per ml, and 5 μg of ampicillin per ml. [^{32}P]phosphate (carrier-free, 50 μCi/ml) was added to 40 ml of the culture during the last generation of growth. The phosphate concentration in this medium (from Casamino Acids) was determined by T. Elliott to be 340 μM (12) and allowed exponential growth to an A_{650} of 0.8. The cells were sedimented, resuspended in 20 ml of M9S (1% Casamino Acids), and shaken at 30°C for 10 min before infection (multiplicity of infection = 3). Surviving

bacteria and unadsorbed phage were measured 10 min after infection. The progress of infection was stopped by sedimenting the cells.

Preparation of intracellular DNA. For the experiments shown in Fig. 2, 3, and 4, cell pellets (from 0.5 to 1.0 ml of culture) were suspended in 20 μl of TES (39 mM Tris-chloride [pH 7.5]–27.5 mM EDTA–11.8% sucrose–120 μg of lysozyme per ml) and incubated on ice for 20 min. A mixture of 40 μl of TTE (0.1% Triton X-100–50 mM Tris-chloride [pH 8]–10 mM EDTA) with 2 μl of 10% sodium dodecyl sulfate (SDS) and 16 μl of proteinase K (5 mg/ml) was added, and the lysed cells were incubated at 37°C for 1 to 2 h. The deproteinized DNA was sheared by pipetting the solution through the plastic tip of an adjustable micropipette. Then 16 μl of loading solution (15% Ficoll–0.1% bromophenol blue–0.1% xylene cyanol–1 mM EDTA) was added, and volumes containing ^3H counts corresponding to 0.01 to 0.02 A_{650} unit of the original culture were subjected to agarose gel electrophoresis as described below.

For the experiment shown in Fig. 5 and 6, the cell pellet (from 20 ml of culture) was resuspended in 200 μl of TES and incubated on ice as above. Then 400 μl of TTE was added, and the mixture was incubated on ice for 20 min and centrifuged (12,800 × *g* for 12 min). The supernatant fluid was made 0.25% in SDS and 1 mg/ml in proteinase K and was incubated at 37°C for 45 min. SDS was precipitated by the addition of KCl to 20 mM and incubation on ice for 5 min and was removed by centrifugation. The supernatant fluid was extracted with phenol (equilibrated with 0.1 M Tris-chloride [pH 8.0]–0.1 M NaCl–0.1% 8-hydroxyquinoline) and treated with pancreatic RNase (40 μg/ml) at 37°C for 25 min. After phenol extraction, the solution (volume = 425 μl) was applied to a 6-ml Sephadex G-50 column equilibrated with 10 mM Tris-chloride (pH 7.5)–0.1 mM EDTA–100 mM NaCl. The flow-through fractions were pooled, precipitated with ethanol, suspended in 150 μl of 10 mM Tris-chloride (pH 7.5)–50 mM NaCl–0.1 mM EDTA–10 mM $MgCl_2$–0.5 mM $CaCl_2$, and incubated with pancreatic DNase (1 μg/ml) for 10 min at 37°C and then with pancreatic RNase (20 μg/ml) in the presence of 20 mM EDTA (to inhibit DNase) for 10 min at 37°C. (The DNase treatment was an attempt to reduce contamination of linear with supercoiled plasmid DNA during separation by gel electrophoresis [see below], the RNase removed residual RNA in the DNA). NaCl was added to 100 mM, and the mixture was heated at 60°C for 10 min. One-sixth volume of loading solution was added, and the sample was applied to a 0.8% agarose gel as described below.

For the experiment shown in Fig. 7 and 8, cell pellets (from 5 ml of culture) were suspended in 100 μl of 50 mM Tris-chloride (pH 8)–10 mM EDTA–15% sucrose. Then 300 μl of 1.33% SDS–20 mM EDTA–proteinase K (0.5 mg/ml) was added, and the lysed cells were incubated at 37°C for 1 to 2 h. The DNA was sheared, SDS was removed by precipitation, and the protein was extracted with phenol as described above. The nucleic acid was precipitated three times with isopropanol and then was resuspended in 10 mM Tris-chloride (pH 7.5)–0.1 mM EDTA and treated with pancreatic RNase A (5 μg/ml).

Incorporation of radioactivity into nucleic acid was measured by precipitation of aliquots with ice-cold 10% Cl_3CCOOH, filtration onto glass fiber (for [^3H]TdR) or cellulose nitrate (for ^{32}P) filters, and counting in toluene-based scintillation fluid.

Preparation of plasmid markers. Linear pLA4 or pBR322 DNA was prepared by digestion of CsCl-purified supercoiled plasmid DNA with the restriction enzyme *Eco*RI or *Bam*HI. Covalently closed relaxed plasmid was made by treatment with crude topoisomerase I (9) in 0.1 M Tris-chloride (pH 8)–0.1 M NaCl for 30 min at 30°C. The reaction was stopped by the addition of SDS and extraction with phenol.

Gel electrophoresis. Agarose gels (2 mm vertical) were run in 40 mM Tris base–20 mM sodium acetate (pH 7.9)–1 mM EDTA (30). Ethidium bromide (EtBr; 0.5 μg/ml; Sigma Chemical Co., St. Louis, Mo.) was added to gels and running buffer as stated in the figure legends. Polyacrylamide (30:1 acrylamide:bisacrylamide) gels (1 mm) were run in 50 mM Tris base–50 mM boric acid–5 mM EDTA (pH 8.3 [35]).

Purification of DNA from gels. DNA was extracted from agarose gel slices by several cycles of freezing and thawing (47), applied to benzoylated napthoylated DEAE-cellulose (22) in gel buffer, and washed with 10 mM Tris-chloride (pH 7.5)–1 mM EDTA–0.1 M LiCl. The DNA was eluted with 10 mM Tris-chloride (pH 7.5)–1 mM EDTA–1 M LiCl–20% ethanol (18), precipitated with ethanol, and suspended in 10 mM Tris-chloride (pH 7.5)–0.1 mM EDTA. DNA was eluted from acrylamide gels essentially according to Knapp et al. (23), precipitated with ethanol, and resuspended as described above.

Southern transfer and hybridization. DNA from agarose gels was nicked in situ (48) and transferred for 16 h to nitrocellulose filter according to Southern (42). The filters were baked for 2 to 4 h at 80°C in vacuo. Prehybridization, hybridization, and washing were done according to Alwine et al. (2), using 2×10^4 to 2×10^5 cpm of nick translated probe per ml of hybridization fluid and approximately 50 μl of hybridization fluid per cm^2 of filter. After air drying, the moist filters were wrapped in plastic and exposed to DuPont Cronex X-ray film at −70°C, using an intensifying screen.

Nick translation. Plasmid DNA or purified restriction fragment (0.1 to 0.5 μg) was incubated with 2 ng of DNase I for 10 min at 37°C and then with 4.5 U of DNA polymerase I for 2 h at 15°C in 10 μl of 50 mM Tris-chloride (pH 7.5)–5 mM $MgCl_2$–10 mM β-mercaptoethanol–gelatin (100 μg/ml) plus one to four [α-^{32}P]deoxyribonucleoside triphosphates (200 to 3,000 Ci/mmol) at 5 μM and the remaining unlabeled deoxyribonucleoside triphosphates at 10 μM (29). The reaction was stopped by heating at 65°C for 10 min, and the mixture was diluted with 40 μl of 10 mM Tris-chloride (pH 7.5)–100 mM NaCl–0.1 mM EDTA and applied to a 1-ml Bio-Gel P-30 column (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with the same buffer. The flow-through fractions were pooled, precipitated with ethanol, and resuspended in 10 mM Tris-chloride (pH 7.5)–0.1 mM EDTA. The specific activity of the labeled DNA was, typically, 1×10^7 to 6×10^7 cpm/μg.

Materials. DNA polymerase I (EC 2.7.7.7) and pancreatic DNase I (EC 3.1.21.1) were from Boehringer Mannheim Corp. Proteinase K (E. Merck, Elmsford, N.Y.) was incubated at 5 mg/ml for 1 h at 37°C in 10 mM Tris-chloride (pH 7.5)–1 mM EDTA

and stored at −70°C before use. [^{3}H]TdR was from New England Nuclear Corp., Boston, Mass., and [α-^{32}P]deoxyribonucleoside triphosphates were from Amersham Corp., Arlington Heights, Ill. [^{32}P]phosphate in HCl was from International Chemical and Nuclear Corp., Irvine, Calif. Formamide (MCB Reagents, Cincinnati, Ohio) was routinely deionized before use with Bio-Rad AG501-X8(D) mixed bed resin and stored at −20°C. Nitrocellulose filters were from Schleicher & Schuell Co., Keene, N.H. or Millipore Corp., Bedford, Mass. Benzoylated naphthoylated DEAE-cellulose and ampicillin were from Sigma. Restriction enzymes *Hin*dIII and *Bam*HI were generously provided by F. Keppel, *Bgl*II was provided by J. Pène, and *Eco*RI was provided by T. Elliott. *Hinc*II was purchased from Bethesda Research Laboratories, Gaithersburg, Md., and *Eco*RV was from New England Biolabs, Beverly, Mass. Restriction enzymes were usually used according to commercial specifications, except that digestion with *Eco*RV was in 10 mM Tris-chloride (pH 8.0)–50 mM NaCl–20 mM $MgCl_2$–1 mM dithiothreitol–10 mM β-mercaptoethanol–100 μg of Pentex bovine serum albumin (Miles Laboratories, Inc., Elkhart, Ind.) per ml, using approximately 100 hour U per μg of DNA overnight at 37°C. In our hands, the enzyme partially restricted glucosylated hmC-containing T4 DNA under these conditions. Purified glucosylated hmC-containing DNA from T4 e^- virions and intracellular cytosine-containing phage DNA from T4 *denB*:*am*42:*am*56 phage-infected *E. coli* were the generous gifts of T. Elliott.

RESULTS

pLA4 (Fig. 1) contains a 2.6-kbp segment of T4 DNA, bounded by a *Hin*dIII site within the coding region of gene 22 and an *Eco*RI site between genes 23 and 24 (16), cloned into pBR322. Gene 23 is the only known T4 gene which remains intact in this clone. We wished to examine the physical structure of pLA4 in *E. coli* after infection by T4 under conditions in which the plasmid-borne gene 23 is actively transcribed by the phage-modified RNA polymerase. The genotype of the infecting phage was *denA*:*denB*:*am*56:*unf* because pLA4 efficiently complements gene 23 mutations in phage of this genetic background (16). The *denA* and *denB* (endonucleases II and IV) mutations eliminate degradation of cytosine-containing DNA after phage infection, the gene 56 (dCTPase-dUTPase) mutation allows dCMP-containing DNA to be synthesized during infection (24), and the *alc*/*unf* mutation allows late T4 transcription of cytosine-containing DNA (41). Thus, these mutations bypass the pathways for inhibiting transcription from, and for degrading, cytosine-containing DNA. Additional amber mutations in genes 12 and 23 were superfluous but were included in the genetic background for historical reasons. Henceforth, the genetic background *denA*:*denB*:*am*56:*unf*:*am*12:*am*23 will be referred to as **unf**. Additional genetic designations will follow this designation, e.g., **unf**:*am*46. To allow us to examine a fairly stable population of monomeric plasmid, *E. coli* B834SR, a *recA strA* derivative of B834 was transformed with each monomeric plasmid of interest and used as the host for infecting phage.

Our strategy was to isolate total intracellular DNA from *E. coli* B834SR(pLA4) late after infection with T4 **unf**, to resolve this DNA by agarose gel electrophoresis, and to probe a Southern blot of the gel specifically for plasmid DNA species by hybridization with nick translated pBR322 DNA. In uninfected cells, both pLA4 and pBR322 were found predominantly as supercoiled monomers (Fig. 2a, lanes 1 and 4, respectively). A minor band, comigrating with covalently closed relaxed monomeric marker, probably represents nicked monomeric plasmid. Supercoiled dimer pLA4 has approximately the same mobility as relaxed monomer under these electrophoresis conditions; two weak bands could be resolved in that region of other gels, indicating that our *E. coli* B834SR(pLA4) contained a minor population of dimer plasmid species (data not shown). Other minor bands probably represent various oligomeric plasmid species, but we have made no effort to identify them. (The autoradiographs in Fig. 2 were over-

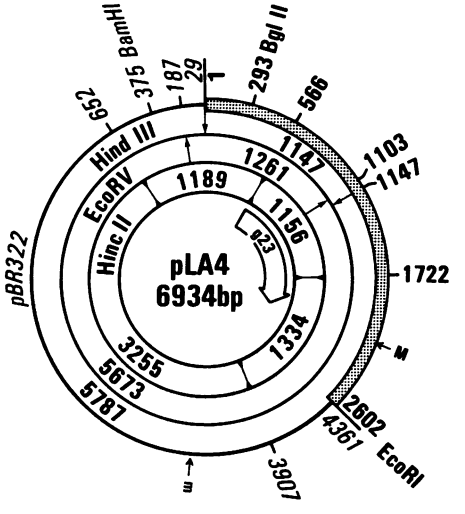

FIG. 1. Physical map of pLA4. pLA4 contains a segment of T4 DNA (dotted region) spanning gene 23, cloned between the *Hin*dIII and the *Eco*RI sites of pBR322. The positions of restriction sites in pBR322 (light italics) are given in terms of the coordinate system of Sutcliffe (46). Restriction sites in the T4 segment (bold letters) are from our own data and from extensive sequence data generously provided by A. Christensen and M. Parker. The locations of all sites except the *Eco*RI site are exact. Sites *M* and *m*, which are discussed in the text, are also shown here. The large arrow denotes the coding region and direction of transcription of gene 23.

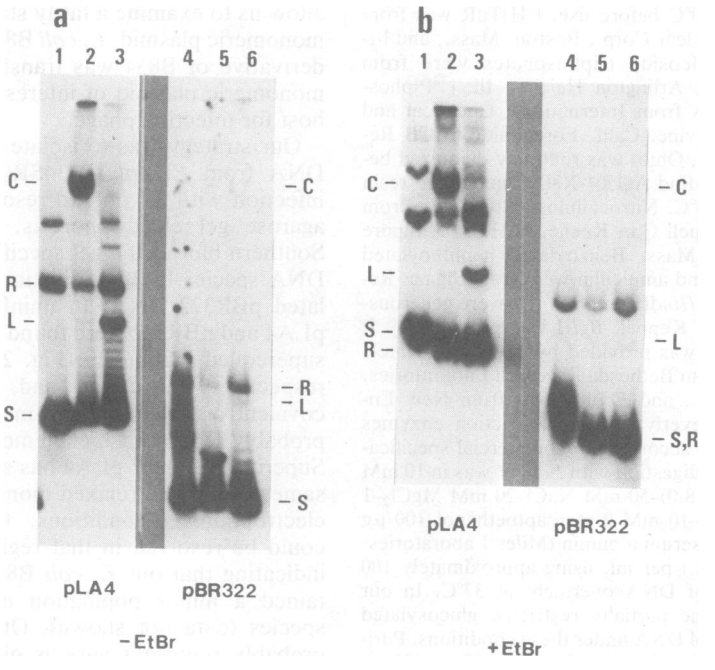

FIG. 2. Changes in plasmid structure after T4 infection. Southern hybridization of nick translated pBR322 to total intracellular DNA from B834SR(pLA4) (lanes 1 to 3) or B834SR(pBR322) (lanes 4 to 6). DNA was isolated from uninfected cells (lanes 1 and 4) or 35 min after infection with T4 **unf** (lanes 2 and 5) or with T4 **unf**:*am*46 (lanes 3 and 6). The mobility of marker DNA species is indicated as follows: C, sheared chromosomal DNA (both bacterial and viral); R, L, and S, covalently closed relaxed, linearized, and supercoiled pLA4 DNA, respectively, prepared as described in the text. Each lane contained the equivalent of 0.02 A_{650} unit of the original cell culture of B834SR(pLA4), or 0.06 A_{650} unit of B834SR(pBR322). (a) An 0.8% agarose gel. All DNA shown was prepared in the same experiment, but lanes 1 to 3 were run on a separate gel from lanes 4 to 6. (b) A 1.0% agarose gel containing 0.5 μg of EtBr per ml. Lanes 4 to 6 were autoradiographed three times longer than lanes 1 to 3.

exposed for the supercoiled monomer band to show minor species of plasmid DNA.)

Late after infection with T4 **unf**, a "ladder" of less supertwisted molecules was seen (Fig. 2a, lanes 2 and 5). Some hybridization was seen in the region of the gel containing sheared T4 and *E. coli* chromosomal DNA (Fig. 2a, lane 2; host DNA is not degraded after infection by *denA*:*denB* phage). Hybridization in that region might indicate recombination between the T4 chromosome and homologous segments of pLA4. It could also be due to physical trapping of plasmid DNA in the high-molecular-weight DNA during electrophoresis, because a hybridization signal was sometimes seen when purified pLA4 DNA was mixed with chromosomal DNA made from plasmid-less cells. This artifact was not reliably excluded from the experiments shown, and this aspect will not be discussed further.

After infection with **unf**:*am*46 phage, a new plasmid species, comigrating with linear pLA4 DNA, was seen over the ladder background (Fig. 2a, lane 3). Gene 46 codes for, or controls, part of an exonuclease function which plays a major role in recombination of phage DNA and degradation of host DNA (4, 49).

To allow us to look at the linearized plasmid without the ladder background of supercoils, electrophoresis was done in the presence of 0.5 μg of EtBr per ml. Under these conditions, covalently closed circular plasmid DNA is positively supercoiled and migrates faster than linear DNA of the same size. Linear pLA4 could now be seen not only after **unf**:*am*46 infection but also after **unf** (*am*46$^+$) infection (Fig. 2b, lanes 3 and 2, respectively). Linear pBR322 DNA was detected after **unf**:*am*46 infection (Fig. 2b, lane 6), but the proportion to total plasmid DNA was clearly not as high as for pLA4 (Fig. 2a, compare lanes 3 and 6). A small amount of linear plasmid was sometimes detected in uninfected cells, but not reproducibly (it can barely be seen in the original of Fig. 2b, lane 4). The high proportion of linear pLA4 DNA isolated after **unf**:*am*46 infection appeared to be the result of its accumulation, beginning 10 to 15 min after infection

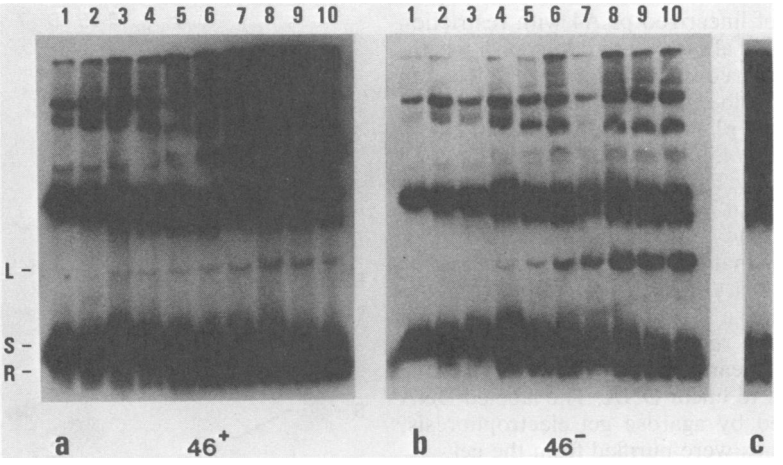

FIG. 3. Accumulation of linear pLA4 in the absence of gene 46 function. Southern hybridization (as in Fig. 2) was carried out to total intracellular DNA from B834SR(pLA4) separated on 1.0% agarose containing 0.5 μg of EtBr per ml. Each lane contains the equivalent of 0.01 A_{650} unit of original culture. (a) Lane 1, DNA from uninfected cells; lanes 2 to 10, DNA isolated 0, 5, 10, 15, 20, 25, 35, 45, and 55 min, respectively, after infection by T4 **unf**. (b) Same as in (a), except that the infecting phage were T4 **unf**:*am*46. The autoradiographic exposure of (a) was for 10 days; that of (b) was for 46 h. (c) A 46-h exposure of lane 9 in (a). L, S, and R are described in the legend to Fig. 2.

(Fig. 3b). In contrast, very little accumulation of linear pLA4 DNA occurred during an **unf** infection, and only at very late times, although a low level of linear DNA was detected throughout (Fig. 3a).

Phage-encoded replication functions are not necessary for the formation of linear pLA4 DNA. Mutations in genes 43 (DNA polymerase), 52 (topoisomerase II subunit: DNA-delay phenotype), or 32 (single-stranded nucleic acid binding protein) in the **unf** background did not prevent linearization of pLA4 (Fig. 4, lanes 5, 7, and 11). Mutations in genes 43 or 52 also did not prevent the accumulation of linear pLA4 when coupled with a gene 46 mutation in the same genetic background (Fig. 4, lanes 6 and 8). A mutation in gene 55 (coding for a late RNA polymerase subunit) suppresses the DNA arrest phenotype of gene 46 mutants (5), but it did not prevent the formation or accumulation of linear pLA4 DNA (Fig. 4, lanes 9 and 10). Because mutations in genes 32, 43, and 55 prevent late gene expression (5, 38), linearization of pLA4 must be independent of late functions, despite the late accumulation of linear DNA.

The quantitative difference between linear pLA4 and pBR322 (Fig. 2) suggested that pLA4 might be cut primarily in the T4 segment. Examination of other plasmids supported that hypothesis. pLA5 (16), which contains the *Hin*dIII-*Eco*RI fragment of pLA4 extending from 1,147 to 2,602 on the T4 part of the map (Fig. 1), behaved in the same way as pLA4. pLA3Δ2 (16), which has a deletion extending from 2,602 to between 1,368 and 1,147 in T4 coordinates, behaved like pBR322 (data not shown). Thus, the linearization of pLA4 appeared to be localized to the *Hin*dIII-*Eco*RI fragment spanning the 3′ end of gene 23.

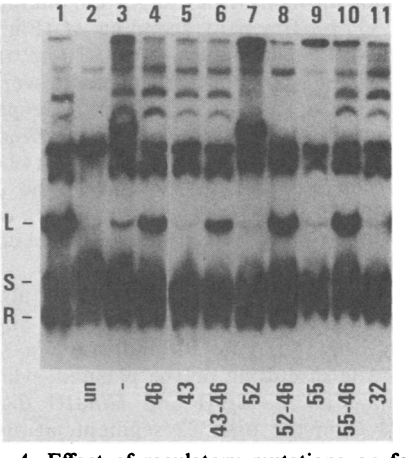

FIG. 4. Effect of regulatory mutations on formation of linear pLA4. Southern hybridization (as in Fig. 2) was carried out to total intracellular DNA from B834SR(pLA4) separated on 1.0% agarose containing 0.5 μg of EtBr per ml. Each lane contains the equivalent of 0.01 A_{650} unit of original culture. Lane 1, mixture of linear, supercoiled, and relaxed marker pLA4 DNA, prepared as described in the text. Lane 2, DNA isolated from uninfected cells. Lanes 3 to 11, DNA isolated 35 min after infection by T4 **unf** (lane 3) with additional amber mutations in genes 46 (lane 4), 43 (lane 5), 43 and 46 (lane 6), 52 (lane 7), 52 and 46 (lane 8), 55 (lane 9), 55 and 46 (lane 10), and 32 (lane 11). L, S, and R are described in the legend to Fig. 2.

Digestion of linearized pLA4 with restriction enzymes should allow one to distinguish whether the cut occurred in vivo at a specific location or in some distribution over the T4 segment. In vivo ^{32}P-labeled pLA4 was prepared from *E. coli* B834SR(pLA4) 45 min after infection by T4 **unf**:*am*52:*am*46. The gene 52 mutation was included because it lowered the ratio of the "ladder" background to linear plasmid. To further reduce contamination of linear with supercoiled plasmid, the DNA was briefly treated with DNase to introduce a small number of single-strand breaks, thereby reducing the proportion of supercoiled to linear plasmid without converting circular DNA to linear DNA. The labeled DNA was separated by agarose gel electrophoresis, and five species were purified from the gel.

Digestion of linear monomer pLA4 DNA (Fig. 5b, lane 4) with *Bam*HI yielded two major fragments whose sizes of 4.5 and 2.4 kbp add up to the size of pLA4 (Fig. 5b, lane 9). Thus, linear pLA4 had been generated in vivo by cleavage primarily at one specific location, which we designate as *M*. *Eco*RI cleavage of linear monomer at 2,602 yielded a major 465- to 490-base pair (bp) fragment (Fig. 6, lane 3, band D), unambiguously mapping *M* to 2,112 to 2,137 in T4 coordinates. (The corresponding 6.5-kbp fragment was not resolved from linear pLA4 in this acrylamide gel but was seen in agarose gels [data not shown]). *Hin*cII digestion did not yield the 1,334-bp fragment spanning *M* (Fig. 1) as expected; instead, new 415- to 420-bp and approximately 1,000-bp-long fragments were generated (Fig. 6, lane 7, bands E and A, respectively). Thus, *M* is 415 to 420 bp from the *Hin*cII site at 1,722 in T4 coordinates, at 2,137-2,142 on the T4 map, very near the 3′ end of the coding sequence of gene 23. (The ochre codon of gene 23 begins at 2,139 [M. Parker, personal communication]).

*Bam*HI digestion of linear monomer also generated smaller amounts of 3.0- and 4.0-kbp fragments (Fig. 5b, lane 9), indicating a second minor site of cutting on the plasmid, which we designate as *m*. *Bgl*II and *Hin*dIII digestion placed *m* in the pBR322 segment, at approximately 3,350 (data not shown). The 990-bp fragment generated by *Eco*RI digestion of linear monomer (Fig. 6, lane 3, band B) mapped *m* to 3,370 on the pBR322 map. *Hin*cII digestion at 3,907 in the pBR322 segment yielded a 480-bp fragment (figure 6, lane 7, band C) mapping *m* to 3,427 in pBR322. This region (3,370 to 3,427) of the plasmid is near the 3′ end of the β-lactamase structural gene (45).

DNA was also purified from a band which migrated more slowly than either linear or relaxed pLA4 DNA (Fig. 5b, lane 2). *Bam*HI digestion yielded linear pLA4 in addition to the major and minor digestion products of linear monomer (Fig. 5b, lane 7). The simplest explanation is that this DNA was a head-to-tail dimer of pLA4 which had been linearized in vivo at *M*

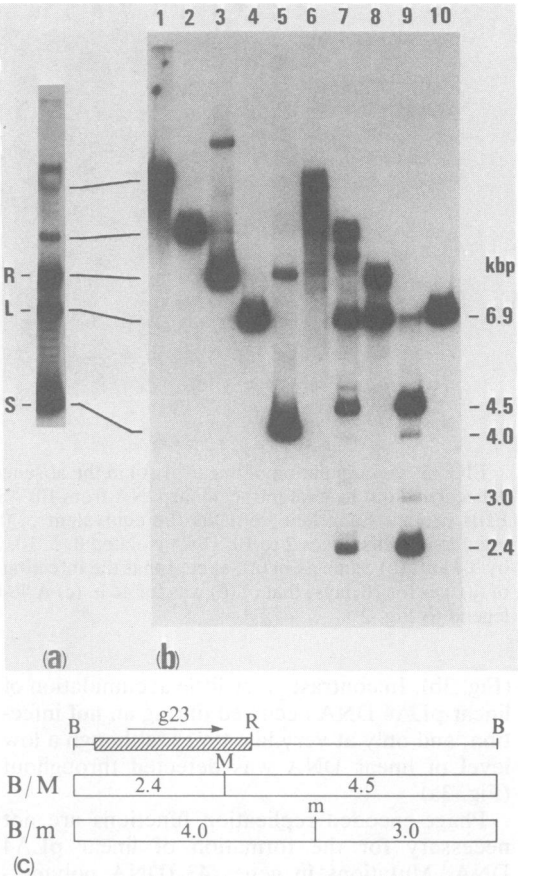

FIG. 5. Isolation of ^{32}P-labeled pLA4 after infection and mapping of the site of linearization. (a) Southern hybridization to total intracellular DNA, as described for Fig. 4, lane 8, except that the separating gel was 0.8% agarose without EtBr. (b) Individual species of ^{32}P-labeled DNA isolated 45 min after infection of B834SR(pLA4) by **unf**:*am*52:*am*46 phage, purified by two rounds of agarose gel electrophoresis and extraction from the gels. The connecting lines to (a) show the corresponding (unlabeled) species detected by hybridization to a Southern blot. For example, lane 4 contains in vivo labeled linear monomer pLA4 DNA, corresponding to that shown by Southern hybridization in (a). Lanes 1 to 5: DNA species without further digestion; lanes 6 to 10: DNA from lanes 1 to 5, respectively, after digestion with *Bam*HI. Each lane contains approximately equal counts of DNA and does not represent the proportions seen in vivo. This autoradiograph is overexposed to show the minor bands in lanes 7 and 9. (c) Explanatory diagram showing origins of various fragments. Restriction sites are abbreviated: B, *Bam*HI; R, *Eco*RI. T4 DNA is shown as the hatched area.

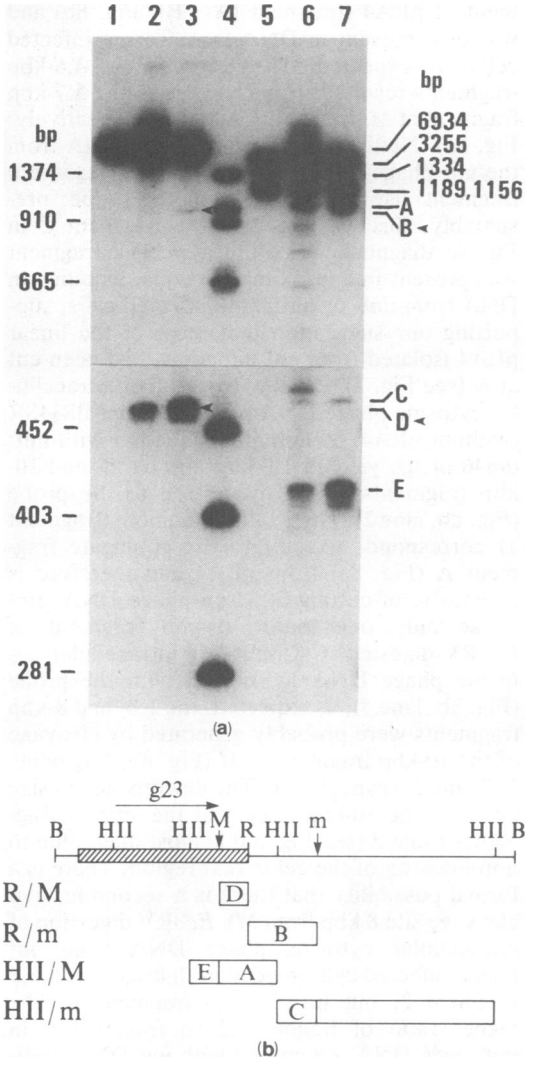

FIG. 6. Further mapping of sites *M* and *m* on pLA4. (a) Supercoiled monomer (lanes 1 and 5), linear dimer (lanes 2 and 6), and linear monomer (lanes 3 and 7) pLA4 DNA, described in the legend to Fig. 5 and the text, were digested with *Eco*RI (lanes 1 to 3) or *Hin*cII (lanes 5 to 7) and separated by electrophoresis through 5% acrylamide. Lane 4, molecular weight markers whose sizes are given to the left of the figure. Bands A, D, and E are due to cleavage at *M* in vivo, and bands B and C are due to cutting at *m*. The horizontal arrowheads in lane 3 identify bands B and D. The fragments whose sizes are shown to the right of the figure are those generated by complete digestion of pLA4 with either of the two enzymes (see Fig. 1). The top half of this composite figure is from a 24-h autoradiographic exposure; the bottom half is from a 4-day exposure of the same gel, replacing the corresponding part of the 24-h exposure. (b) Explanatory diagram showing map coordinates of fragments A to E. Restriction sites are abbreviated: B, *Bam*HI; R, *Eco*RI; HII, *Hin*cII. T4 DNA is shown as the hatched area.

or *m*. Whether the dimer was already present in uninfected cells or formed after T4 infection is not known. *Eco*RI or *Hin*cII digestion of this linear dimer DNA (Fig. 6, lanes 2 and 6, respectively) yielded major and minor fragments which appeared to be identical to those generated by digestion of linear monomer. Thus, linearization of dimer and monomer DNA had taken place at the same locations. Other minor bands whose origins we cannot explain were also generated by restriction enzyme digestion of linear dimer. It is likely that some of those minor bands did not represent pLA4 sequences, since they appeared not to hybridize to pLA4 on a Southern blot (data not shown).

Supercoiled monomer pLA4 DNA, purified in the same way, yielded only the expected fragments upon digestion with *Bam*HI, *Eco*RI, or *Hin*cII (Fig. 5b, lanes 5 and 10, Fig. 6, lanes 1 and 5, respectively). DNA which comigrated with relaxed monomer marker was also purified and yielded linear pLA4 upon (incomplete) digestion with *Bam*HI (Fig. 5b, lanes 5 and 10). This DNA was probably a mixture of supercoiled dimer and nicked monomer, which comigrated under these electrophoresis conditions, as discussed above. Just as supercoiled monomer experienced some nicking during purification from agarose, so did some of the DNA from this mixture (Fig. 5b, lanes 5 and 3, respectively).

As a control, the labeled *E. coli* DNA from infected cells was also purified. Digestion with *Bam*HI generated a smear, as expected (Fig. 5b, lanes 1 and 6, respectively).

To determine whether cleavage at sites *M* and *m* was affected by gene 46 function in an identical way, total intracellular DNA from *E. coli* B834SR(pLA4) was purified either before or 50 min after infection with T4 **unf** or **unf**:*am*46, digested with *Bam*HI, separated by agarose gel electrophoresis, and hybridized in a Southern blot to nick translated pBR322 DNA (Fig. 7). The 3.0-kbp fragment, indicative of in vivo cleavage at *m* (see Fig. 5b) was present in equivalent amounts in *Bam*HI-digested DNA prepared from either the **unf** or the **unf**:*am*46 infection. The corresponding 4.0-kbp fragment also appeared to be present above the high background hybridization in this region. The 2.4-kbp fragment, indicative of cleavage at *M*, was detected only in DNA prepared from **unf**:*am*46 infection. A disproportionate amount of nick translated pBR322 DNA was able to hybridize to the corresponding 4.5-kbp fragment rather than to the 2.4-kbp fragment, due to its much larger region of homology (see Fig. 1). It is difficult to say whether the 4.5-kbp fragment was present in **unf** DNA above the already mentioned high background; if it was, the level was

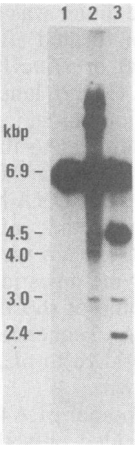

FIG. 7. Effect of gene 46 function on cleavage of pLA4 at *M* or *m*. Southern hybridization (as in Fig. 2) was carried out on *Bam*HI-digested intracellular DNA prepared from B834SR(pLA4), before (lane 1) or 50 min after infection by T4 **unf** (lane 2) or by phage with an additional mutation in gene 46 (lane 3). Each lane contains 0.125 A_{650} unit of original culture.

too low for detection of the 2.4-kbp fragment in this Southern blot. Clearly, the cleavage event at *m* seemed to be unaffected by gene 46 function, whereas events at *M* were greatly affected. It is possible that the linear pLA4 seen throughout **unf** infection (Fig. 3) was largely due to cleavage at *m*.

Is cleavage at *M* in pLA4 specific for plasmid DNA or for cytosine-containing DNA, or does it also occur in wild-type phage DNA? The 1,455-bp *Hin*dIII-*Eco*RI fragment (extending from 1,147 to 2,602 on the T4 map; Fig. 1) was used as a probe spanning *M* to examine intracellular phage DNA with various amounts of modification at cytosine residues. The extracted DNA was digested with EcoRV, which is able to cut glucosylated hmC-containing phage DNA (26). Because the probe contains no *Eco*RV sites, it should hybridize to one *Eco*RV fragment from the T4 genome, which would be split in two by cleavage at *M* (Fig. 8a, fragments 1 and 2). One of the two resulting fragments should be the same size as that generated by *Eco*RV digestion of pLA4 which had been linearized at *M* (Fig. 8a, fragments 1 and A).

When total intracellular DNA was prepared from *E. coli* B834SR(pLA4) 50 min after infection with **unf**:*am*46 phage, digested with *Eco*RV, and hybridized in a Southern blot to the nick translated 1,455-bp *Hin*dIII-*Eco*RI fragment, three major fragments generated from plasmid DNA were detected (Fig. 8b, lane 4; compare with lane 2, DNA made from plasmid-less cells). The 5.7-kbp fragment is the large *Eco*RV fragment of pLA4 (fragment [A +B], Fig. 8b) and was also present in DNA from T4 **unf**-infected cells, as expected. The 1.0- and ca. 4.6-kbp fragments resulted from cleavage of the 5.7-kbp fragment at *M* (fragments A and B, respectively; Fig. 8a). Neither was evident in the DNA from the **unf** phage infection. A minor (ca. 2.35-kbp) fragment also hybridized with the probe; presumably, this was the 2,460-bp fragment C in Fig. 8a, diagnostic of cutting at *m*. This fragment was present in approximately equal amounts in DNA from **unf**- or **unf**:*am*46-infected cells, supporting our suggestion that most of the linear pLA4 isolated from **unf** infections had been cut at *m* (see Fig. 7). *Eco*RV digestion of intracellular cytosine phage DNA prepared from B834SR (without pLA4) 50 min after infection with **unf**:*am*46 phage yielded 1.0-kbp and ca. 8- and 10-kbp fragments which hybridized to the probe (Fig. 8b, lane 2). The 1.0-kbp fragment (fragment 1) corresponds exactly to the conjugate fragment A (Fig. 8a) from pLA4 and therefore is diagnostic of cutting at *M* on phage DNA. Because only one major 10-kbp fragment of *Eco*RV-digested, CsCl-purified intracellular cytosine phage DNA hybridized with the probe (Fig. 8b, lane 5), as expected, the 1.0- and 8-kbp fragments were probably generated by cleavage of the 10-kbp fragment at *M* (Fig. 8b, fragments 1, 2, and 3, respectively. The discrepancy in size between the 10-kbp band and the sum of fragments 1 and 2 [see Fig. 8a] is most likely due to non-linearity of the gel in that region. There is a formal possibility that there is a second in vivo cleavage site 8 kbp from *M*). *Eco*RV digestion of intracellular cytosine phage DNA from **unf** phage-infected cells yielded both fragment 3 and fragment 2, but little or no fragment 1. The higher ratio of fragment 2 to fragment 3 in **unf**:*am*46 DNA, compared with **unf** DNA, indicates to us that fragment 2 also resulted from cutting at *M*.

To determine whether cleavage occurred in hmC-containing, partially glucosylated phage DNA, intracellular phage DNA was prepared from *E. coli* B834 *galU*56 50 min after infection with T4 *am*12:*am*23, *am*12:*am*23:*am*46, *denA*:*denB*:*am*12:*am*23, or *denA*:*denB*:*am*12:*am*23:*am*46. (The bacteria lack uridine diphosphoglucose pyrophorylase and cannot efficiently glucosylate hmC residues on phage DNA [39].) Digestion of each of these DNA preparations with *Eco*RV yielded fragments 1, 2, and 3, as detected by hybridization with the *Hin*dIII-*Eco*RI probe (Fig. 8c, lanes 4 to 7). In particular, the ratio of fragments 1 and 2 to fragment 3 was increased in DNA from those infections which lacked the function of gene 46. Other fragments were detected in this DNA, but not in cytosine-containing DNA. The function of the cytosine-

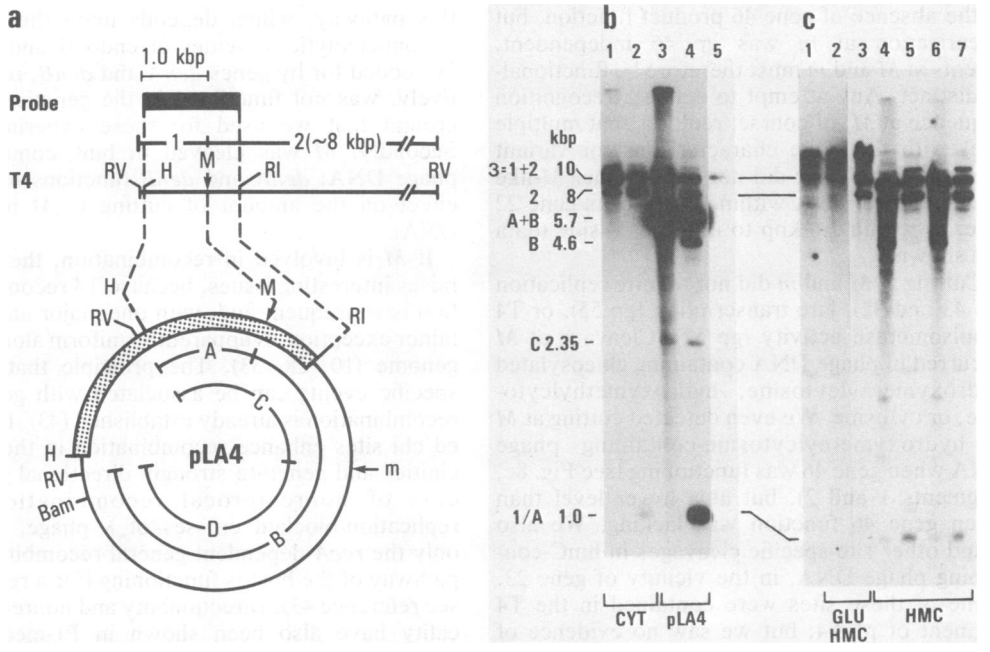

FIG. 8. Examination of phage DNA for the presence of *M*. (a) Schematic of the experiment. pLA4 is as in Fig. 1. A *Hin*dIII-*Eco*RI probe spanning *M* is hybridized to a Southern blot of *Eco*RV-digested phage and plasmid DNA. The probe should hybridize to one *Eco*RV fragment on the phage genome, as shown. When this DNA is cut in vivo at *M*, the probe should hybridize to fragments 1 and 2. Fragments A, B, and C on *Eco*RV-cut pLA4 should hybridize to the probe, as shown, according to whether the plasmid is also cut at *M* or *m*. Fragments 1 and A represent the same T4 DNA segment. (b) Southern hybridization of *Eco*RV-cut intracellular cytosine containing DNA prepared from B834SR (lanes 1 and 2) or B834SR(pLA4) (lanes 3 and 4) 50 min after infection with T4 **unf** (lanes 1 and 3) or **unf**:*am*46 (lanes 2 and 4). Lane 5, *Eco*RV-digested, CsCl-purified, cytosine-containing intracellular phage DNA prepared from *denB*:*am*56:*am*42 (dCMP hydroxymethylase)-infected bacteria. Lanes 1 to 4, each containing 0.125 A_{650} unit of original culture, are from a 24-h exposure, and lane 5 is from a 5-day exposure of the same Southern filter. (c) Southern hybridization, as in (b), of DNA from B834SR (lanes 2 and 3) or B834 *galU*56 (lanes 4 to 7) infected with *am*12:*am*23 (lanes 2 and 4), *am*12:*am*23:*am*46 (lanes 3 and 5), *denA*:*denB*:*am*12:*am*23 (lane 6), or *denA*:*denB*:*am*12:*am*23:*am*46 (lane 7). Lanes 2 to 5 contain 0.1 A_{650} unit of original culture, and lanes 6 and 7 each contain 0.125 A_{650} unit. Lane 1: *Eco*RV-digested phage DNA purified from T4 e^- virions. Lanes 1 and 2 are from a 2-week exposure, and lanes 3 to 7 are from a 20-h exposure of the same filter. Bands 1, 2, A, B, and C are described in (a).

DNA-specific endonucleases II and IV, coded for by genes *denA* and *denB*, respectively, did not appear to have an effect on cleavage at *M* (Fig. 8c; compare lanes 4 and 5 to 6 and 7).

In our hands, intracellular glucosylated hmC-containing phage DNA, prepared from B834SR 50 min after infection with T4 *am*12:*am*23:*am*46, was not cut nearly as well by *Eco*RV as was less-modified DNA. Partial cleavage yielded fragment 2 and a fragment with approximately the mobility of fragment 1 (Fig. 8c, lane 3). This smaller fragment most probably is fragment 1 with mobility slightly decreased due to glucosylation of the DNA. Under the same rationale as for *Eco*RV, *Taq*I digestion confirmed that cleavage at *M* occurred both in glucosylated-hmC and in cytosine-containing phage DNA when the function of gene 46 was lacking (data not shown). We conclude that cleavage at *M* occurred in phage DNA, regardless of the state of modification of cytosine residues.

DISCUSSION

T4 infection induced two types of change in the physical structure of pLA4: the linking number was increased, and it was cut in a site-specific manner. In this paper, we have focused on the cleavage event and will postpone discussion of the linking number changes until more is known about them. We found that site-specific cleavage of pLA4 occurred at two functionally different locations: *M*, the major site, is located very near the 3′ end of the coding region of gene 23, and *m*, the minor site, is in the β-lactamase gene of pBR322. Cutting at *M* was detected only

in the absence of gene 46 product function, but linearization at *m* was gp 46 independent. Events at *M* and *m* must therefore be functionally distinct. Any attempt to define a recognition sequence at *M*, of course, requires that multiple sites with the same characteristics, or variant sites, be found. We did not find another *M*-like site in cloned DNA within 6 kbp to its gene 22 side, or within 2.6 kbp to its gene 24 side (data not shown).

Cutting at *M* and *m* did not require replication (gp 43 and 32), late transcription (gp 55), or T4 topoisomerase activity (gp 52). Cleavage at *M* occurred in phage DNA containing glucosylated hydroxymethylcytosine, hydroxymethylcytosine, or cytosine. We even detected cutting at *M* in hydroxymethylcytosine-containing phage DNA when gene 46 was functioning (see Fig. 8c, fragments 1 and 2), but at a lower level than when gene 46 function was lacking. We also found other site-specific cleavages in hmC-containing phage DNA, in the vicinity of gene 23. Some of these sites were contained in the T4 segment of pLA4, but we saw no evidence of cleavage at these sites in the plasmid or in cytosine-containing phage DNA (see Fig. 8b and c).

Our failure to detect cutting at *M* in pLA4 in a gene 46^+ infection means that either (i) pLA4 cut at *M* is turned over in a gp 46-dependent process, and so little accumulates in a gene 46^+ infection that it fails to be detected, or (ii) pLA4 is cut at *M* only in the absence of gene 46 function. The fact that we did detect cutting at *M* in the presence of gene 46 function, in phage DNA containing hmC but not cytosine, is compatible with the notion that turnover might occur much more rapidly in cytosine-containing DNA than in hmC-containing DNA.

Gene 46 (and 47) codes for, or controls, an exonuclease which participates in multiple processes during T4 infection (31, 36). The gene 46 product functions in the major pathway for degradation of cytosine-containing phage or host DNA which has already been cleaved by T4 endonuclease II or IV (24, 25). It probably also acts on hmC-containing DNA, since general phage recombination is dependent upon gene 46 infection (4, 7). Other processes, such as certain DNA repair pathways and the second stage of phage DNA replication, are thought to involve recombination because of their dependence on gene 46 function (3, 8, 36). It seems natural to associate the cleavage at *M* with one or more of these gp 46-associated pathways or with the consequence of the failure of gp 46 to function in one of these pathways.

We suggest that cleavage at *M* is not involved in the major degradation pathway of cytosine-containing DNA for the following reasons. First, this pathway, which depends upon the initial endonucleolytic activities of endo II and endo IV, coded for by genes *denA* and *denB*, respectively, was not functional in the genetic background that we used for these experiments. Secondly, *M* was cleaved in hmC-containing phage DNA; *denA* and *denB* functions had no effect on the amount of cutting at *M* in this DNA.

If *M* is involved in recombination, then this raises interesting issues, because T4 recombination is so frequent and, with one major and one minor exception, is apparently uniform along the genome (10, 28, 33). The principle that site-specific events can be associated with general recombination is already established (43). Isolated chi sites enhance recombination in their vicinities and generate strongly directional gradients of nonreciprocal recombination in replication-blocked crosses of λ phage, when only the *recA*-dependent general recombination pathway of the host is functioning (for a review, see reference 43). Directionality and nonreciprocality have also been shown in P1-mediated transduction of chi-containing λ prophage (14). There is no general enhancement of recombination in the gene 23 region (33, 52), and no detectable gradient of recombination frequency at the *M*-proximal end of gene 23 (10), although the entire region extending beyond gene 21 is apparently free of other *M*-like sites. However, the existence of additional gene 46 state-dependent cleavage sites in hmC-DNA (Fig. 8c) and the existence of multiple pathways of T4 recombination (15), both of which might obscure a gradient of recombination, weaken that objection. Knowing the frequency of *M*-like sites in the T4 genome would help in deciding whether the events at *M* relate to a pathway of T4 recombination.

Our interest in the site-specific cleavage at *M* was stimulated by a previous finding that expression of a plasmid-borne copy of gene 23 was differentially affected (when compared with expression of the same gene located on the T4 chromosome) by a gene 46 mutation (17; T. Mattson, G. van Houwe, A Bolle, and R. H. Epstein, unpublished data referred to in reference 17). Plasmid-dependent gp23 synthesis after T4 *denA*:*denB*:*am*12:*am*23 (*unf*$^+$) infection is totally dependent on gene 46 function; gp 23 synthesis after T4 **unf** infection is only partially dependent on gene 46 function. We were also very much intrigued by the work of Snyder and Champness on the *gol-lit* phenomenon. Briefly stated, *E. coli lit* mutants are nonpermissive for T4 wild-type phage at the level of late gene expression. T4 *gol* mutants compensate for the defect (and therefore grow on *lit*$^-$ bacteria). The intriguing property of *gol* mutations is that they

exert their effects on gene expression only in *cis* (in mixed infections) and that the *cis* effect extends over many kilobase pairs of DNA (11). The six *gol* mutants which have been examined thus far all map between bp 885 and 925, in T4 coordinates, near the 5′ end of gene 23 (W. Champness and L. Snyder, submitted for publication). A recent mapping of the gene 23 promoter (18) allowed us to assay directly late promoter activity on a plasmid from which *M* and the *gol*$^+$ region had been deleted. The gene 23 promoter was active on this plasmid after infection by T4 **unf**, in the presence and in the absence of gene 46 function (data not shown). Thus, the presence of *M* and consequent events at *M* are not absolutely required for transcription of the plasmid-borne gene 23 after infection by T4 phage with the **unf** genetic background. By the same token, the *gol*$^+$ region is not required for late gene expression from this plasmid in *lit*$^+$ bacteria.

The endonucleolytic activity responsible for cleavage at *M* must be capable of cutting both hmC- and cytosine-containing DNA and must be independent of late gene expression. Seven endonucleases which are induced by T4 infection have been described. Of these, endonucleases II and IV have already been eliminated from consideration. Endonuclease V, the product of gene *denV*, acts at pyrimidine dimers (51). Endonuclease VII, the product of gene 49, cuts single-stranded DNA and has the ability to cut at the bases of cruciform structures with long hairpin stems, analogous to Holliday structures (19, 20, 32, 34). Endonucleases I, III, and VI (whose structural genes have not been identified) attack both T4 and cytosine-containing DNA in vitro (1, 21, 40). Endonucleases I, VI, and VII are late functions (1, 20, 21) and can be excluded because cleavage occurs in the absence of late proteins. Endonuclease III might be an early or middle enzyme (40) and might be responsible for cleavage at *M*.

In closing, it is interesting to note that partly similar observations on site-specific cleavage of phage T7 DNA have recently been reported (27). Cleavage is seen only in the absence of the function of T7 gene 6 exonuclease, which is involved in recombination, host chromosomal degradation, and removal of RNA primers for DNA synthesis. The in vivo cleavages depend on the gene 3 endonuclease, gene 4 primase, and gene 5 DNA polymerase, and appear to occur near two origins of T7 genome replication. In contrast, we have seen no replication requirement for cleavage at *M*.

ACKNOWLEDGMENTS

We thank A. Christensen and M. Parker for generously providing us with sequence data before publication, and L. Snyder for a careful and critical reading of the text.

L.M.A. is a predoctoral trainee of the U.S. Public Health Service. This research was supported by a Public Health Service grant from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Altman, S., and M. Meselson.** 1970. A T4-induced endonuclease which attacks T4 DNA. Proc. Natl. Acad. Sci. U.S.A. **66**:716–721.
2. **Alwine, J. C., D. J. Kemp, B. A. Parker, J. Reiser, J. Renart, G. R. Stark, and G. M. Wahl.** 1979. Detection of specific RNAs or specific fragments of DNA by fractionation in gels and transfer to diazobenzyloxy-methyl paper. Methods Enzymol. **68**:220–242.
3. **Bernstein, C.** 1981. Deoxyribonucleic acid repair in bacteriophage. Microbiol. Rev. **45**:72–98.
4. **Bernstein, H.** 1968. Repair and recombination in phage T4. I. Genes affecting recombination. Cold Spring Harbor Symp. Quant. Biol. **33**:325–331.
5. **Bolle, A., R. H. Epstein, W. Salser, and E. P. Geiduschek.** 1968. Transcription during bacteriophage T4 development: requirements for late messenger synthesis. J. Mol. Biol. **33**:339–342.
6. **Bolle, A., R. H. Epstein, W. Salser, and E. P. Geiduschek.** 1968. Transcription during bacteriophage T4 development: synthesis and relative stability of early and late RNA. J. Mol. Biol. **31**:325–348.
7. **Broker, T. R.** 1973. An electron microscopic analysis of pathways for bacteriophage T4 recombination. J. Mol. Biol. **81**:1–16.
8. **Broker, T. R., and A. H. Doermann.** 1975. Molecular and genetic recombination of bacteriophage T4. Annu. Rev. Genet. **9**:213–244.
9. **Camerini-Otero, R. D., and G. Felsenfeld.** 1977. Supercoiling energy and nucleosome formation: the role of the arginine-rich histone kernel. Nucleic Acids Res. **4**:1159–1181.
10. **Celis, J. E., J. D. Smith, and S. Brenner.** 1973. Correlation between genetic and translational maps of gene 23 in bacteriophage T4. Nature (London) New Biol. **241**:130–132.
11. **Champness, W., and L. Snyder.** 1982. The *gol* site: a cis-acting bacteriophage T4 regulatory region that can affect expression of all the late genes. J. Mol. Biol. **151**:395–407.
12. **Chen, P. S., Jr., T. Y. Toribara, and H. Warner.** 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1758.
13. **Clark, A. J.** 1967. The beginning of a genetic analysis of recombination proficiency. J. Cell. Physiol. **70**(Suppl. 1):165–180.
14. **Dower, N. A., and F. W. Stahl.** 1981. Chi activity during transduction-associated recombination. Proc. Natl. Acad. Sci. U.S.A. **78**:7033–7037.
15. **Hamlett, N. V., and H. Berger.** 1975. Mutations altering genetic recombination and repair of DNA in bacteriophage T4. Virology **63**:539–567.
16. **Jacobs, K., L. Albright, D. Shibata, and E. P. Geiduschek.** 1981. Genetic complementation by cloned bacteriophage T4 late genes. J. Virol. **39**:31–45.
17. **Jacobs, K., and E. P. Geiduschek.** 1981. Regulation of expression of cloned bacteriophage T4 late gene 23. J. Virol. **39**:46–59.
18. **Kassavetis, G. K., and E. P. Geiduschek.** 1982. Bacteriophage T4 late promoters: mapping 5′ ends of T4 gene 23 mRNAs. EMBO J. **1**:107–114.
19. **Kemper, B., and M. Garabett.** 1981. Studies on T4-head maturation. 1. Purification and characterization of gene-49-controlled endonuclease. Eur. J. Biochem. **115**:123–131.
20. **Kemper, B., M. Garabett, and U. Courage.** 1981. Studies on T4-head maturation. 1. Substrate specificity of gene-49-controlled endonuclease. Eur. J. Biochem. **115**:133–141.
21. **Kemper, B., and J. Hurwitz.** 1973. Studies on T4-induced nucleases. Isolation and characterization of a manganese-

activated T4-induced endonuclease. J. Biol. Chem. **248**:91–99.
22. **Kiger, J. A., Jr., and R. L. Sinsheimer.** 1969. Vegetative lambda DNA. IV. Fractionation of replicating lambda DNA on benzoylated-naphthoylated DEAE cellulose. Proc. Natl. Acad. Sci. U.S.A. **40**:467–490.
23. **Knapp, G., J. S. Beckman, P. F. Johnson, S. A. Fuhrman, and J. Abelson.** 1978. Transcription and processing of intervening sequences in yeast tRNA genes. Cell **14**:221–236.
24. **Kutter, E., A. Beug, R. Sluss, L. Jensen, and D. Bradley.** 1975. The production of undergraded cytosine-containing DNA by bacteriophage T4 in the absence of dCTPase and endonucleases II and IV, and its effects on T4-directed protein synthesis. J. Mol. Biol. **99**:591–607.
25. **Kutter, E. M., and J. S. Wiberg.** 1968. Degradation of cytosine-containing bacterial and bacteriophage DNA after infection of *Escherichia coli* B with bacteriophage T4D wild type and with mutants defective in genes 46, 47 and 56. J. Mol. Biol. **38**:395–411.
26. **Kuzmin, N. P., V. I. Tanyashin, and A. A. Bayev.** 1982. Eco RV restriction of T-even phage DNA and cloning resulting fragments into pBR322. Dokl. Akad. Nauk SSSR **265**:737–739.
27. **Lee, D. D., and P. D. Sadowski.** 1982. Bacteriophage T7 defective in the gene 6 exonuclease promotes site-specific cleavages of T7 DNA in vivo and in vitro. J. Virol. **44**:235–240.
28. **Levy, J. N., and E. B. Goldberg.** 1980. Region-specific recombination in phage T4. I. A special glucosyl-dependent recombination system. Genetics **94**:519–530.
29. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage λ. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
30. **McDonell, M. W., M. N. Simon, and F. W. Studier.** 1977. Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. J. Mol. Biol. **110**:119–146.
31. **Mickelson, C., and J. S. Wiberg.** 1981. Membrane-associated DNase activity controlled by genes 46 and 47 of bacteriophage T4D and elevated DNase activity associated with the T4 *das* mutation. J. Virol. **40**:65–77.
32. **Mizuuchi, K., B. Kemper, J. Hays, and R. A. Weisberg.** 1982. T4 endonuclease VII cleaves Holliday structures. Cell **29**:357–365.
33. **Mosig, G.** 1968. A map of distances along the DNA molecule of phage T4. Genetics **59**:137–151.
34. **Nishimoto, H., M. Takayama, and T. Minagawa.** 1979. Purification and some properties of deoxyribonuclease whose synthesis is controlled by gene 49 of bacteriophage T4. Eur. J. Biochem. **100**:433–440.
35. **Peacock, A. C., and C. W. Dingman.** 1968. Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose-acrylamide composite gels. Biochemistry **7**:668–674.
36. **Prashad, N., and J. Hosoda.** 1972. Role of genes 46 and 47 in bacteriophage T4 reproduction. II. Formation of gaps on parental DNA of polynucleotide ligase defective mutants. J. Mol. Biol. **70**:617–635.
37. **Riva, S., A. Cascino, and E. P. Geiduschek.** 1970. Uncoupling of late transcription from DNA replication in bacteriophage development. J. Mol. Biol. **54**:103–119.
38. **Riva, S., A. Cascino, and E. P. Geiduschek.** 1970. Coupling of late transcription to viral replication in bacteriophage T4 development. J. Mol. Biol. **54**:85–102.
39. **Runnels, J., and L. Snyder.** 1978. Isolation of a bacterial host selective for bacteriophage T4 containing cytosine in its DNA. J. Virol. **27**:815–818.
40. **Sadowski, P. D., and I. Bakyta.** 1972. T4 endonuclease IV. Improved purification procedure and resolution from T4 endonuclease III. J. Biol. Chem. **247**:405–412.
41. **Snyder, L., L. Gold, and E. Kutter.** 1976. A gene of bacteriophage T4 whose product prevents true late transcription on cytosine-containing T4 DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3098–3102.
42. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
43. **Stahl, F. W.** 1979. Special sites in generalized recombination. Annu. Rev. Genet. **13**:7–24.
44. **Studier, F. W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124**:307–316.
45. **Sutcliffe, J. G.** 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. U.S.A. **75**:3737–3741.
46. **Sutcliffe, J. G.** 1978. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
47. **Thuring, R. W. J., J. P. M. Sanders, and P. Borst.** 1975. A freeze-squeeze method for recovering long DNA from agarose gels. Anal. Biochem. **66**:213–220.
48. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.
49. **Wiberg, J. S.** 1966. Mutants of bacteriophage T4 unable to cause breakdown of host DNA. Proc. Natl. Acad. Sci. U.S.A. **55**:614–621.
50. **Wu, R., E. P. Geiduschek, and A. Cascino.** 1975. The role of replication proteins in the regulation of bacteriophage T4 transcription. II. Gene 45 and late transcription uncoupled from replication. J. Mol. Biol. **96**:539–562.
51. **Yasuda, S., and M. Sekiguchi.** 1970. Mechanism of repair of DNA in bacteriophage. II. Inability of ultraviolet-sensitive strains of bacteriophage in inducing an enzyme activity to excise pyrimidine dimers. J. Mol. Biol. **47**:243–255.
52. **Yee, J. K., and R. Marsh.** 1981. Alignment of a restriction map with the genetic map of bacteriophage T4. J. Virol. **38**:115–124.