JOURNAL OF VIROLOGY, Oct. 1981, p. 65-77
0022-538X/81/100065-13$02.00/0

Vol. 40, No. 1

Membrane-Associated DNase Activity Controlled by Genes 46 and 47 of Bacteriophage T4D and Elevated DNase Activity Associated with the T4 *das* Mutation†

CLAUDIA MICKELSON[1]‡ AND JOHN S. WIBERG[2]*

Department of Biology, University of Rochester, Rochester, New York 14620,[1] and Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642[2]

Received 3 November 1980/Accepted 1 June 1981

Lethal, amber mutations in T4 genes 46 and 47 cause incomplete degradation of host DNA, premature arrest of phage DNA synthesis, accumulation of abnormal DNA replication intermediates, and defective recombination. These phenotypes can be explained by the hypothesis that genes 46 and 47 control a DNA exonuclease, but in vitro demonstration of such a nuclease has not yet been reported. Membrane and supernatant fractions from 46^- and 47^- mutant-infected and 46^+ 47^+ control-infected cells were assayed for the presence of the protein products of these genes (i.e., gp46 and gp47) and for the ability to degrade various DNA substrates to acid-soluble products in vitro. The two proteins were found only on membranes. The membrane fraction from 46^- 47^- mutant-infected cells digested native or heavily nicked *Escherichia coli* DNA to acid-soluble products three to four times slower than the membrane fraction from control-infected cells. No such effect was found in the cytoplasmic fractions. The effect on nuclease activity in membranes was the same whether 46^- and 47^- mutations were present singly or together. $NaClO_4$, a chaotropic agent, released both gp46 and gp47 from 46^+ 47^+ membranes, as well as the DNase activity controlled by genes 46 and 47. DNA cellulose chromatography of proteins released from membranes by $NaClO_4$ showed that gp46 and gp47 bound to the native DNAs of both *E. coli* and T4. Thus, the overall enrichment of gp46 and gp47 relative to total T4 protein was 600-fold (10-fold in membranes, 2-fold more upon release from membranes by $NaClO_4$, and 30-fold more upon elution from DNA cellulose). T4 *das* mutations, which partially suppress the defective phenotype of 46^- and 47^- mutants, caused a considerable increase in in vitro DNase activity in both membrane and cytoplasmic fractions. We obtained evidence that the das^+ gene does not function to inhibit *E. coli* exonuclease I or V, endonuclease I, or the UV endonuclease of gene *uvrA* or to decrease the activity of T4 exonuclease A or the T4 gene 43 exonuclease.

T4 bacteriophage genes 46 and 47 are involved in host DNA breakdown (38, 62), phage DNA replication (17, 28, 38, 62), and recombination (4, 5, 8, 9, 21, 26). Mutants with mutations in either one or both of these genes are not able to convert host DNA to acid-soluble products (i.e., "solubilize" the DNA) (62). The host DNA is fragmented but remains in pieces having an average M_r of 10^6 (38). Phage DNA replication begins normally but ceases at about 15 min postinfection (17, 62). During this early time, a rapidly sedimenting form of DNA is synthesized, but this DNA is not stable (28). The phage DNA prematurely dissociates from the cell membrane (15), and replication ceases with the accumulation of endonucleolytic damage to the released DNA (50, 53). Mutants in these genes also show reduced genetic recombination (4, 5, 21). At the molecular level, this is reflected in the failure to form single-stranded gaps on the infecting, parental phage DNA, which are necessary for the formation of branched, recombinant DNA structures (8, 9, 26, 47).

The observation by Wiberg (62) that gene 46 and 47 mutants are blocked in the in vivo conversion of host DNA to acid-soluble products led to the suggestion that these genes control a DNase activity. Subsequent in vivo studies are consistent with this hypothesis, although no in

† U.S. Department of Energy report UR-3490-1902.

‡ Present address: c/o Roberts, 27601 Northline Rd., Romulus, MI 48174.

vitro enzymatic activity has been attributed to these genes yet. In this paper we report the first in vitro evidence for absence of a DNase activity in gene 46 and 47 mutant-infected cells. This DNase activity was membrane associated and cofractionated with gp46 and gp47 during extraction from membranes by the chaotropic agent $NaClO_4$. We confirmed the work of others showing that gp46 binds to membranes and to DNA, and we demonstrated for the first time that gp47 also binds to membranes and to DNA.

Suppressor mutations designated *das* (for DNA arrest suppressor), which map in the gene 33–34 region, were isolated by Hercules and Wiberg on the basis that they increase phage production by 46^- or 47^- mutants (24). The *das* mutations also partially suppress the effects of 46^- and 47^- mutations on host DNA breakdown, phage DNA replication, and recombination (8, 24, 51). Krylov and Plotnikova (35) isolated T4B mutations that they designated suα, which also suppress defects in genes 46 and 47; these authors indicated that suα mapped between genes 49 and 55, far from the position of the *das* mutations. However, later it was determined that suα mutations in fact map in the same region as *das* mutations (V. N. Krylov, personal communication) and thus probably are in the same gene. We show here that *das* mutations cause a considerable increase in both membrane and cytoplasmic DNase activities measured in vitro.

(Most of the results were taken from a thesis presented by C.M. to the University of Rochester. A preliminary report of some of the results was presented at the 1974 Phage Meeting, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.)

MATERIALS AND METHODS

Phage and bacterial strains. *Escherichia coli* B, which is nonpermissive for T4 amber (*am*) mutants, was used as a host for all of our experiments. *E. coli* CR63 was used as a host to grow stocks of all phage amber mutants. The wild-type bacteriophage T4D ($T4^+$) and T4D *am* mutants that were not backcrossed were obtained from R. Epstein, R. Edgar, and W. Wood. Except for the *das* mutants, all of the phage mutants used in this study were backcrossed to $T4^+$ to eliminate possible secondary or extraneous mutations; suffixes consisting of a multiplication sign followed by a number (e.g., -X5) indicate the number of times a mutant was backcrossed with $T4^+$ for genetic purification (62). Multiple mutants were constructed as described by Wiberg et al. (65), except for those containing *das* mutations, whose construction was described by Hercules and Wiberg (24). General phage and bacterial protocols have been described previously (65). The DNA^- gene 42 amber mutation N55 × 5 was present in most of the phage mutants used in the early stages of this study in order to extend the synthesis of early proteins (64) and to decrease greatly the synthesis of T4 late proteins (27). To designate certain mutants, we used a previously introduced shorthand (39), in which only the gene number or name is enclosed in brackets. Thus, we use the following abbreviations in this paper: [33] for *am*N134×5; [42] for *am*N55×5; [43] for *am*E4322×3; [45] for *am*E10×3; [46] for *am*B14×5; [47] for *am*A456×5, except in Fig. 5, where [47] is an abbreviation for *am*NG163×3, and in Table 2, where [47] is an abbreviation for *am*H3×5; [*das*] for *das*13; and [*dexA*] for del(39-56)1×1, which is an rII^+ version of del(39-56)1. To produce [*dexA*], we crossed the latter mutant (which also contains the rII deletion 1589) (25) with $T4^+$ and isolated an rII^+ recombinant that retained the (39-56) deletion, as evidenced by its inability to produce several proteins in a gel electrophoretic analysis (43).

Chemicals. Bovine pancreatic RNase, RNase T_1, and pancreatic DNase I were purchased from Worthington Diagnostics. L-[4,5-^{3}H]leucine (0.5 Ci/mmol), L-[*U*-^{14}C]leucine (0.33 Ci/mmol), and Liquifluor scintillation fluid were obtained from New England Nuclear Corp. Bovine serum albumin fraction V was from Pentex Corp. [*methyl*-^{3}H]thymidine (3 Ci/mol) was obtained from Schwarz/Mann Bioresearch. NCS solubilizer was from Nuclear Chicago Corp. A molecular weight marker kit (Schwarz/Mann Bioresearch) was used for protein molecular weight estimations. Munktell cotton cellulose (chromatography grade 410; Bio-Rad Laboratories) was used as the DNA cellulose chromatography matrix. All other chemicals were reagent grade unless otherwise specified. All Tris buffers were made with Trizma base (Sigma Chemical Co.), and pH adjustments were made with HCl.

Differential labeling of phage proteins. Log-phase cultures of *E. coli* B (100 ml, 5×10^8 cells per ml, M9 medium) (32) were chilled, pelleted, and resuspended in the original volume of cold, fresh M9 medium. Samples of the resuspended cultures were warmed at 37°C for 3 min and then infected (zero time) with the appropriate phage at a multiplicity of infection of 10. At 2 min postinfection, 1 μCi of [^{14}C]leucine per ml was added to one culture (e.g., [42,46,47]-infected cells), and 3 μCi of [^{3}H]leucine per ml was added to another (e.g., [42]-infected cells). Unlabeled L-leucine was added to each culture at a final concentration of 2 μg/ml; this concentration gave a constant rate of incorporation for at least 20 min. At 20 min postinfection, the cultures were chilled, and equal volumes of the differentially labeled cultures were mixed and centrifuged at $5{,}000 \times g$ for 15 min at 5°C. The pellets were fractionated as described below. The incorporation of label into acid-precipitable material was determined during the 18-min labeling period by the method of Bollum (6). The terminology used for T4 proteins was that of Casjens and King (13), where the prefix gp- stands for protein gene product; thus, gp46 is the protein product of wild-type gene 46.

Cell fractionation. We used the cell fractionation procedure of Fox et al. (19) and Inouye and Guthrie (31), with minor modifications. A cell pellet was suspended in 1/20 the original culture volume of Tris sonication buffer (40 mM Tris-chloride, pH 8.0, 1 mM

EDTA [disodium salt], 1 mM 2-mercaptoethanol, 10 mM $MgCl_2$). The suspended cells, which were on ice throughout, were sonicated as described previously (62) for 4 min in 30-s bursts with 30-s rests between bursts. The completeness of disruption was always confirmed turbidimetrically with appropriate dilutions. For the experiment shown in Fig. 1 only, pancreatic DNase I (final concentration, 20 μg/ml) was then added, and this was followed by a 1-h incubation at 12°C. Control experiments showed that this DNase treatment had no effect on the membrane attachment of gp46 and gp47. The suspension was then centrifuged at 3,000 × *g* for 10 min in the cold to remove cell debris. Approximately 9% of the total acid-insoluble label was lost to the resulting low-speed pellet. The supernatant was centrifuged at 100,000 × *g* (maximum) for 40 min at 4°C in a Beckman Spinco SW50.1 swinging bucket rotor. The 100,000-×-*g* supernatant was dialyzed overnight at 4°C against Tris dialysis buffer (40 mM Tris-chloride, pH 8.0, 1 mM EDTA [disodium salt], 1 mM 2-mercaptoethanol, 50 mM NaCl); this dialyzed preparation was designated the cytoplasmic fraction. The 100,000-×-*g* pellet was blotted dry and was suspended in a volume of Tris dialysis buffer equal to 1/60 the original culture volume. A sample was removed, diluted, and assayed for protein as described below. This suspended pellet was designated the membrane fraction. Of the total acid-precipitable label incorporated, 75 to 80% remained in the supernatant and 10 to 15% remained in the membrane fraction. For cells grown in M9 medium, this procedure resulted in average protein concentrations of approximately 2.4 and 1.6 mg/ml in the supernatant and membrane suspensions, respectively. In the preparation of the cell extracts that were to be assayed for DNase activity, the DNase I treatment and all dialysis steps were omitted, and the fractions were assayed immediately.

$NaClO_4$ extraction of membranes. We used the method of Brooks et al. (10) to extract proteins from membranes with chaotropic agents. Each membrane suspension was incubated for 40 min at 12 to 15°C after the addition of 0.2 M (final concentration) $NaClO_4$. The suspension was then centrifuged at 48,000 × *g* (maximum) for 30 min at 5°C in a Sorvall SS-34 angle rotor, and the pellet was discarded. The 48,000-×-*g* supernatant was dialyzed overnight at 4°C against Tris dialysis buffer and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). The supernatants used in the in vitro assay for DNase activity were not dialyzed but were used directly. The 48,000-×-*g* supernatant contained 50% of the acid-precipitable label originally present in the untreated membranes. In contrast, only 5 to 7% was found in the 48,000-×-*g* supernatant if $NaClO_4$ was not added during the 40-min incubation.

Chloroform-methanol extraction of membranes. The procedure used was a modification of the lipid purification procedure of Folch et al. (18). A total of 50 volumes of cold chloroform-methanol (2:1, vol/vol) was added to a membrane suspension. This mixture was rotated for 15 min at 4°C. The insoluble material was sedimented by centrifugation at 5,000 × *g* for 15 min at 5°C. The supernatant was discarded, and the residual chloroform and methanol were evaporated at room temperature under a vacuum. The 5,000-×-*g* pellet was suspended in a small volume of cold Tris dialysis buffer and dialyzed overnight against the same buffer at 4°C. Approximately 83% of the label that sedimented at 100,000 × *g* was found in the pellet that was not soluble in chloroform-methanol.

SDS-PAGE. Tube gels (0.5 by 6 cm) containing 0.1% SDS, 7% acrylamide, 0.375 M Tris-chloride (pH 8.0), 0.03% *N,N,N',N'*-tetraethylmethylenediamine, 0.07% ammonium persulfate, and 0.183% *N,N'*-methylenebisacrylamide were made according to instructions supplied by Canalco, Inc., and were run at 2 mA/tube and room temperature. The dialyzed, double-labeled samples used for electrophoresis were incubated for 40 min at 40°C in sample buffer (50 mM Tris-chloride, pH 7.8, 1% SDS, 10% glycerol, 3% 2-mercaptoethanol, 0.005% bromophenol blue) to ensure complete disruption of membrane samples (54).

After electrophoresis, the tube gels were frozen on dry ice and cut into slices (thickness, 0.77 ± 0.07 mm) by being forced through a series of razor blades separated by thin washers. The individual gel slices were distributed to scintillation vials, and 2 ml of an NCS solubilizer-Liquifluor mixture (1:10, vol/vol) was added to each vial. The capped vials were incubated at 42°C overnight. More Liquifluor (10 ml) was added, and the radioactivity was measured with a model 3375 Packard Tri-Carb scintillation spectrometer. Double-label channel settings were used; the ^{14}C overlap was 18%, and the counting efficiencies for ^{14}C and ^{3}H were 54 and 20%, respectively. Purified proteins of known molecular weight were used to determine a calibration curve of log of M_r versus migration distance.

Labeling of bacterial and phage DNAs. The procedures of Kutter and Wiberg (38) were used to label bacterial and phage DNAs with [^{3}H]thymidine.

Isolation and purification of DNAs. (i) Native *E. coli* DNA. Native *E. coli* DNA was isolated from *E. coli* B by the method of Thomas et al. (58). RNA was digested by incubation for 4 h at 37°C with 20 μg of pancreatic RNase per ml and 20 μg of RNase T_1 per ml. The DNA solution was then subjected to three additional phenol extractions and dialyzed versus Tris dialysis buffer at 4°C.

(ii) T4 phage DNA. T4 phage DNA was isolated from intact $T4^+$ by the method of Thomas and Abelson (57). The purified DNA was dialyzed against Tris dialysis buffer at 4°C.

(iii) *E. coli* 10^6-dalton DNA. *E. coli* B in glycerol Casamino Acids medium (20) was infected at 37°C with [42,46,47] at a multiplicity of infection of 5. Total DNA was isolated from the infected cells by the method of Thomas et al. (58). Infection by this mutant degraded the host DNA to an average double-stranded molecular weight of 10^6 (38). It should be noted that about 10% of this preparation was T4 DNA from the phage used to infect the cells; however, this phage DNA was not labeled under the conditions used to label the bacterial DNA. The 10^6-dalton *E. coli* DNA substrate used for the experiment shown in Fig. 5 was isolated from [42,46]-infected cells.

(iv) Nicked *E. coli* DNA. The method used was a modification of the procedure of Oleson and Koerner (44). Pancreatic DNase (0.01 μg/ml) and 10 mM $MgCl_2$ were added to purified native DNA, and the solution

was incubated for 30 min at 37°C. This procedure rendered 16 ± 9% of the bacterial DNA acid soluble. The reaction was stopped by adding an equal volume of phosphate-buffered phenol. The DNA was extracted with phenol three times and dialyzed versus Tris dialysis buffer. No residual DNase I activity was detected in the final DNA preparations. DNase I introduces nicks (as opposed to double-strand breaks) when Mg^{2+} is the activating cation (40).

DNA cellulose chromatography. Approximately 1.5 g (dry weight) of DNA cellulose containing at least 2 mg of DNA per g of cellulose was used for each column. The procedures of Alberts et al. (1) were used to make DNA cellulose and column buffers. We made no modifications in the chromatography protocol described by Alberts et al. (1), except that the intermediate wash with 0.1 M NaCl buffer was omitted. Native DNA from either *E. coli* or T4 was used.

Enzyme, protein, and RNA determinations. The cytochrome b_1 contents of the various cell fractions were estimated by determining the increases in absorbance at 557.5 μm after excess sodium dithionite was added (14). The cell fractions were isolated in buffers without 2-mercaptoethanol. The difference spectrum (reduced/oxidized) was determined from 400 to 600 μm, and only two peaks were observed, at 427.5 and 557.5 μm.

β-Galactosidase (β-D-galactoside galactohydrolase; EC 3.2.3.23) activity was measured by the spectrophotometric method of Wallenfels; 10^{-5} M manganese acetate was added to optimize enzyme activity (59). Dilutions of the various cell fractions were assayed to ensure a linear relationship between enzyme activity and cell fraction concentration.

Succinic dehydrogenase (succinate oxidoreductase; EC 1.3.99.1) activity was measured by the method of King (34). The reduction of dichlorophenol indophenol was followed spectrophotometrically as a loss of absorbance at 600 nm between 30 and 90 s after 50 μl of the cell fraction was added. The specific activity of each cell fraction is expressed as micromoles of succinate oxidized per milligram of protein.

For RNA determinations, we used the procedure of Schneider (49).

For protein determination, the method of Bramhall et al. (7) was used, with bovine serum albumin as the standard.

DNase assay. *E. coli* B was grown to a concentration of 5×10^8 cells per ml at 37°C in M9 medium for all experiments except those shown in Fig. 4 and 5, where glycerol Casamino Acids medium was used. Portions of these were infected at a multiplicity of infection of 10 with different phage mutants. The infected cells were harvested after 20 min at 37°C, sonicated, and fractionated as described above. The standard DNase assay reaction mixture contained 40 mM Tris-chloride (pH 8.0), 1 mM EDTA (disodium salt), 1 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 25 mM NaCl, 6.6 μg of [^{3}H]thymidine-labeled DNA substrate per ml (except for the experiments shown in Fig. 4 and 5, where 3.6 μg/ml was used), and 0.17 volume of a cell fraction (total volume, 1.0 ml). The reaction was begun by adding the cell fraction to the rest of the assay mixture at 37°C, and incubation was for 30 min. The DNA substrate is noted for each experiment described below. The amount of DNA remaining acid insoluble in cold 5% trichloroacetic acid was determined at different times with duplicate 50-μl samples by using the paper disk method of Bollum (6).

No modifications were made in this protocol except where noted below. The DNase activities in the membrane fractions from cells grown in M9 medium were unstable during storage at 0 or −20°C. Thus, the in vitro assays were performed immediately after infection and cell fractionation. Growth and infection of cells in glycerol Casamino Acids medium (20) alleviated this problem for later experiments (see Fig. 4 and 5); such samples could be stored at −60°C for at least 2 weeks with negligible losses of activity.

Control experiments were performed to determine the effects of pH and $MgCl_2$ concentration during isolation of the cell fractions on in vitro DNase activity. Both [42,46,47]- and [42]-infected cells were tested. The pH was varied from 7 to 9, and the $MgCl_2$ concentration added was varied from 0 to 10 mM. The final conditions used were optimal for maximum activity in fractions from both control and mutant-infected cells.

RESULTS

Identification and location of gp46 and gp47. The first published identifications of gp46 and gp47 were by Cascino et al. (12) and O'Farrell et al. (43), respectively. Both of these groups of workers examined whole-cell extracts. In this study, we used fractionation of mutant- and control-infected cells to locate and identify gp46 and gp47 as membrane-associated proteins.

The 100,000-×-*g* supernatants and pellets from uninfected and [42]-infected cells were characterized by assaying for the following compounds: two membrane marker enzymes, succinic dehydrogenase and cytochrome b_1 (14, 48); β-galactosidase, a soluble cytoplasmic enzyme (19); RNA; and protein (Table 1). We found that the 100,000-×-*g* pellet was enriched for succinic dehydrogenase activity, containing 20 times the number of enzyme units per milligram of protein found in the supernatant. Cytochrome b_1 was detected in the 100,000-×-*g* pellet but not in the supernatant. In contrast, β-galactosidase activity was in the 100,000-×-*g* supernatant only. There were significant losses of β-galactosidase and succinic dehydrogenase activities during the fractionation procedure (Table 1). The RNA assay showed that a maximum of 7% of the total cellular RNA (largely ribosomes) pelleted during centrifugation at 100,000 × *g*. A comparison of the results for the [42]-infected and uninfected cells showed that the distribution of the bacterial components was not altered by phage infection. The cell fractionation procedure resulted in a membrane-enriched, 100,000-×-*g* pellet fraction that was reasonably free of soluble proteins and ribosomes. Thus, we referred to the 100,000-×-*g* pellet and the 100,000-×-*g* supernatant as

TABLE 1. *Distribution of cell components*[a]

Prepn	% Protein	% RNA	Succinic dehydrogenase		β-Galactosidase		Cytochrome b_1 ($\Delta A_{557.5}$/mg of protein)
			Sp Act	% Activity	Units	% Activity	
Uninfected cells							
Sonicated cells	100	100	0.019	100	800	100	ND[b]
5,000-×-*g* pellet	20.9	11	0.008	14	7.3	1	ND
100,000-×-*g* supernatant	68.0	84	0.002	8.6	496	62	—[c]
100,000-×-*g* pellet	10.6	5.3	0.061	34	0.6	0.1	0.0085
Phage-infected cells							
Sonicated cells	100	100	0.015	100	951	100	ND
5,000-×-*g* pellet	19.0	7	0.016	20	28.5	3	ND
100,000-×-*g* supernatant	61.0	86	0.002	6.6	637	67	—
100,000-×-*g* pellet	12.4	7	0.040	32	—	—	0.012

[a] A part of a culture of *E. coli* B in M9 medium was infected with [42] at a multiplicity of infection of 10 at 37°C. At 20 min postinfection the uninfected and phage-infected cells were collected by centrifugation. The subsequent sonication and centrifugation procedures are described in the text. The β-galactosidase assay was performed on *E. coli* B cells grown in glycerol Casamino Acids medium rather than M9 medium, primarily to avoid glucose-induced catabolite repression of synthesis of β-galactosidase. Absorbance measurements for the cytochrome b_1 assay were not done on the sonicated cell or 3,000-×-*g* pellet samples due to extremely high scattering. $\Delta A_{557.5}$, Increase in absorbance at 557.5 μm upon reduction by excess dithionite. The increase in absorbance was specific for cytochrome b_1 (14).

[b] ND, Not determined.

[c] —, Not detected.

the membrane and cytoplasmic fractions, respectively.

The products of genes 46 and 47 were identified by an SDS-PAGE analysis of the labeled proteins from [42]- and [42,46,47]-infected cell fractions. The [42]-infected cells were labeled with [^{3}H]leucine, and the [42,46,47]-infected cells were labeled with [^{14}C]leucine. These differentially labeled cells were mixed after the 8-min labeling period, and cytoplasmic and membrane fractions were isolated as described above. SDS-PAGE analyses of the cytoplasmic and $NaClO_4$-solubilized membrane proteins are shown in Fig. 1. Two differences (Fig. 1B, arrows) were observed in the membrane fraction only. The individual gene products were identified by comparing the band differences in [42, 46]-, [42,47]-, and [42]-infected cell fractions (data not shown). These identifications were confirmed on slab gels (instead of tube gels) in the accompanying paper (63).

Reversing the labels did not alter the banding patterns. The band differences were not artifacts of $NaClO_4$ treatment of the membranes; extraction of lipids and lipoproteins from membrane fractions by chloroform-methanol followed by an SDS-PAGE anlaysis of the chloroform-methanol-insoluble membrane proteins revealed the same two protein differences that were observed in the $NaClO_4$-extracted samples (data not shown). The attachment of gp46 and gp47 to membranes was not affected by extensive treatment of membrane fractions with pancreatic DNase I (Fig. 1) or by isolation in low-ionic-strength buffers at different pH's (data not shown). We estimated that the molecular weights of gp46 and gp47 were 68,000 and 37,000, respectively. These values were in close agreement with those found by O'Farrell et al. (43) (71,000 and 37,000, respectively).

DNA binding of gp46 and gp47. The binding of gp46 and gp47 to DNA was monitored by SDS-PAGE analyses of DNA cellulose column eluates. [42,46,47]- and [42]-infected cells were labeled with [^{14}C]leucine and [^{3}H]leucine, respectively. These cultures were mixed and fractionated as described above, and the $NaClO_4$-solubilized membrane proteins were applied to DNA cellulose columns containing either T4 phage DNA or 10^6-dalton *E. coli* DNA. The columns were washed with 0.05 M NaCl column buffer until the levels of eluted radioactivity decreased to background values. All of the proteins retained on the column were then eluted in 2 M NaCl column buffer; the resulting eluates contained approximately 3 to 6% of the labeled proteins applied to the columns. The proteins in the column eluates were then analyzed by SDS-PAGE. Figure 2 shows the data for the 10^6-dalton *E. coli* DNA column. Both gp46 and gp47 bound to the DNA (Fig. 2B) and did not wash through in the 0.05 M NaCl buffer (Fig. 2A). Control experiments indicated that gp46 and gp47 were retained by interaction with the DNA or with other DNA-recognizing proteins, since chromatography of parallel samples on cellulose

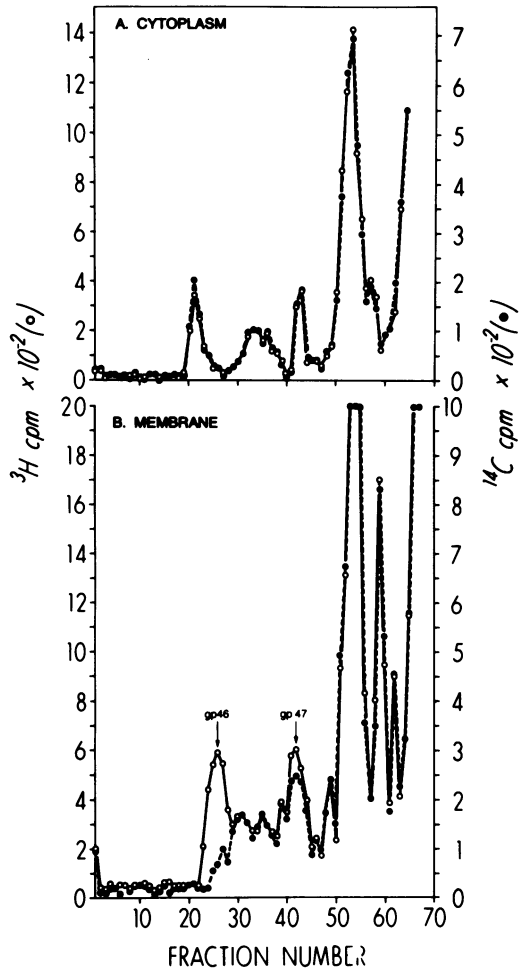

FIG. 1. *SDS-PAGE analysis of labeled cytoplasmic and $NaClO_4$-solubilized membrane proteins from [^{3}H]leucine-labeled [42]-infected cells* (○) *and [^{14}C]leucine-labeled [42,46,47]-infected cells* (●). *Cytoplasmic and membrane fractions were prepared from a mixture of differentially labeled cells as described in the text. The crude extract was treated with pancreatic DNase. Portions of the membrane fractions were treated with $NaClO_4$. The cytoplasmic (A) and $NaClO_4$-extracted membrane (B) proteins were analyzed on 0.1% SDS–7% polyacrylamide tube gels. Electrophoresis was from left to right. The fraction farthest to the right represents the upper edge of the bromophenol blue dye front.*

columns lacking DNA showed that 99.6% of the labeled proteins applied washed through in the 0.05 M NaCl buffer. Alberts et al. (1) also observed negligible binding of T4 proteins to DNA-free cellulose.

DNA cellulose chromatography resulted in 20- to 30-fold purification of gp46 and gp47 relative to the total labeled T4 proteins applied.

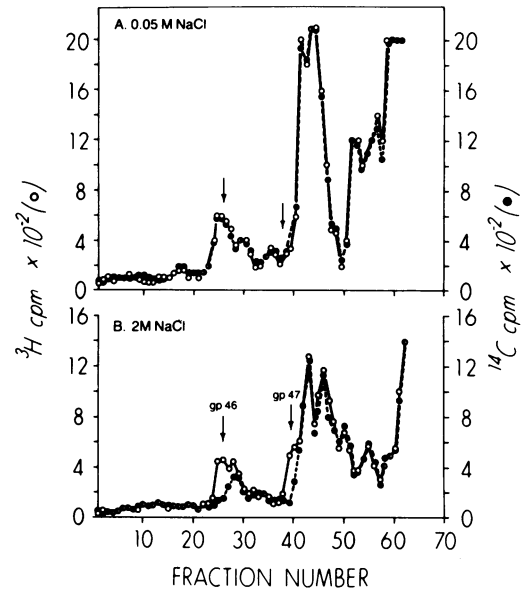

FIG. 2. *DNA cellulose chromatography of $NaClO_4$-extracted membrane proteins from [^{3}H]leucine-labeled [42]-infected cells* (○) *and [^{14}C]leucine-labeled [42,46,47]-infected cells* (●). *Extraction with $NaClO_4$ was as described in the text. This figure shows SDS-PAGE analyses of the proteins in 0.05 M NaCl column wash buffer (A) and the proteins retained on the DNA cellulose and eluted with 2.0 M NaCl buffer (B). The DNA column was prepared with 10^6-dalton E. coli DNA. Electrophoresis was from left to right.*

The same results were observed upon chromatography of comparable samples on T4 phage DNA cellulose, and the electrophoretic patterns of the proteins retained by the T4 and *E. coli* DNA columns were quite similar (data not shown). Thus, the overall enrichment of gp46 and gp47 relative to the total labeled T4 protein was about 600-fold (10-fold in membranes, 2-fold more upon release from membranes by $NaClO_4$, and 30-fold more upon elution from DNA cellulose).

Membrane DNase activity. Figure 3 shows the time course of solubilization of *E. coli* nicked DNA by the membrane and cytoplasmic fractions of [42,46]-, [42,47]-, and [42]-infected cells. The initial rate of solubilization by the [42]-infected membrane fraction was three- to four-fold higher than the rates observed with the [42,46]- and [42,47]-infected membrane fractions (Fig. 3B). This difference in the rates was still apparent after the proteins were released from membranes by $NaClO_4$ (Fig. 3C). We found no significant effect of the gene 46 and 47 mutations in the cytoplasmic fractions (Fig. 3A), which is consistent with the absence of gp46 and gp47 from cytoplasm (Fig. 1). We observed no differ-

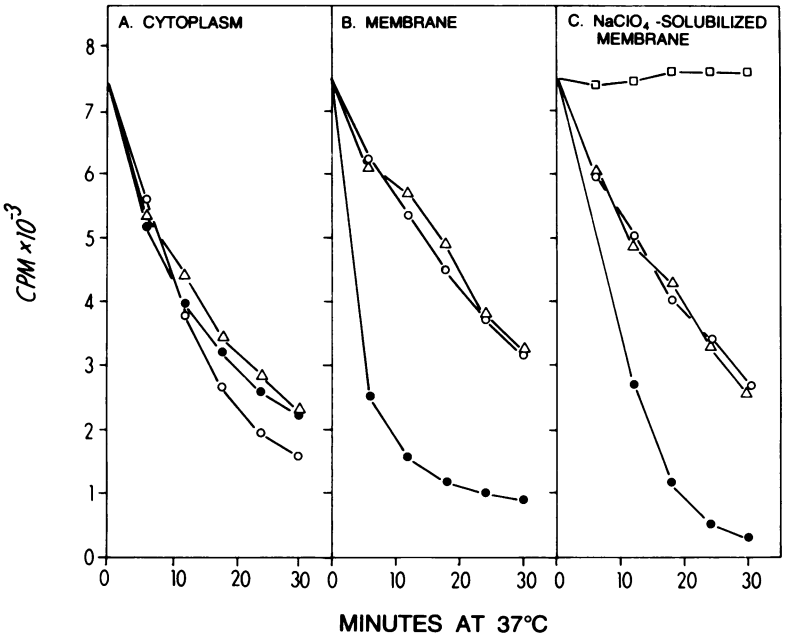

FIG. 3. *Time course of digestion of nicked E. coli DNA by cytoplasmic (A), membrane (B), and $NaClO_4$-extracted membrane (C) proteins from parallel cultures infected with [42]* (●), *[42,46]* (△), *and [42,47]* (○). *Membrane and cytoplasmic fractions were isolated as described in the text. For (C), one-half of the membrane fraction from each culture was treated with $NaClO_4$. All fractions were assayed for DNase activity as described in the text. In the control* (□), *an equivalent volume of sonication buffer was substituted for cell extract.*

ences in DNase activity among mutants lacking the function of gene 46 or gene 47 or both. The substitution of native *E. coli* DNA for the heavily nicked *E. coli* DNA substrate had little effect on the patterns of DNase activity (data not shown). Also, the difference in substrate solubilization was not dependent on the presence of the gene 42 mutation; this difference was apparent even between $T4^+$- and [46,47]-infected cell fractions (data not shown).

The reduced activity in the 46^- and 47^- fractions was not due to the presence of an inhibitor that was normally neutralized in 46^+ 47^+ infections; equal mixtures of [42]- and [42,46,47]-infected cell membrane fractions gave DNase activity that was about the average of the two fractions assayed separately (data not shown).

After these observations it became clear that mutations in most genes required for phage DNA replication, including mutations in gene 42, allowed low-level synthesis of late T4 proteins (66). At least two T4-induced DNases, both endonucleases, have been shown to be late proteins (2, 33). Gene 46 and 47 mutations decrease transcription of late T4 genes compared with transcription of early genes (21, 52). We considered the possibility that the higher rate of DNA solubilization observed in [42]-infected cell fractions was due to synthesis of one or more late nucleases and that the loss of activity observed in the 46^- and 47^- mutant infections might be indirect (i.e., due to a further reduction in synthesis of late proteins). A gene 45 mutation which virtually eliminated synthesis of all late proteins (11, 66) was tested for membrane DNase activity. A comparison of the nuclease activities in the membrane fractions from [45]- and [42]-infected cells showed that the gene 45 mutant gave only a slightly decreased level of DNase activity (Fig. 4) compared with the gene 42 mutant. The addition of the gene 43 mutation *am*E4322×5, which eliminated both T4 DNA polymerase and 3′,5′-exonuclease activities (42), to the gene 45 mutant resulted in a further reduction in membrane activity (Fig. 5). The effects of gene 46 and 47 mutations on the membrane-associated nuclease activity from [43,45]-infected cells are shown in Fig. 5. The loss of functional gp46 and gp47 was associated with a considerable loss of membrane-associated DNase activity. Thus, it is clear that the 46^--47^- effect was not due to decreased synthesis of some late nuclease(s), but that genes 46 and 47 controlled a membrane-associated DNase activity that was demonstrable in vitro.

Basis of residual phage production by

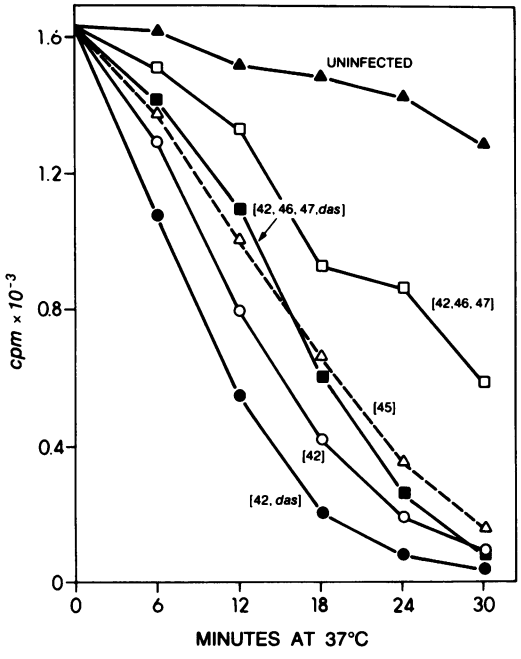

FIG. 4. *Time course of digestion of 10^6-dalton E. coli DNA by membrane fractions from cells infected with [42, das13]* (●), *[42]* (○), *[45]* (△), *[42,46,47, das13]* (■), *and [42,46,47]* (□) *and from uninfected cells* (▲). *Membrane fractions were prepared as described in the text.*

46⁻ and 47⁻ mutants. Amber mutants in genes 46 and 47 produce a significant but low burst of phage in an Su^- host such as *E. coli* B (17, 62), and all infected cells yield phage (62). Minner and Bernstein suggested that this limited growth may be due to partial compensation for the gene 46 and 47 functions by other bacterial or phage gene products (41). These authors presented evidence that loss of the host *recBC* function or the phage rII and *denB* (endonuclease IV) functions still allowed limited phage production by 46^- or 47^- mutants. In experiments described below, in which the suppressive effect of the *das*13 mutation was tested in a series of *E. coli* mutants, phage production by [46,47] was also measured as a necessary control. In all cases, [46,47] yielded a typical burst of 5 to 10 phage per cell, which confirms the conclusion of Minner and Bernstein that the *recBC* function is not necessary for this residual phage production (41) and extends the conclusion to include the *sbcB*, *endA*, and *uvrA* genes.

The virtual abolition of [46,47] phage production by the addition of a *dexA* deletion mutation (Table 2) suggested that T4 exonuclease A may be the function that substitutes weakly for the function of genes 46 and 47. Alternatively, one or more of the other genes deleted by this mutation could be responsible for the effect. However, the addition of the *dexA* deletion to [45,46]

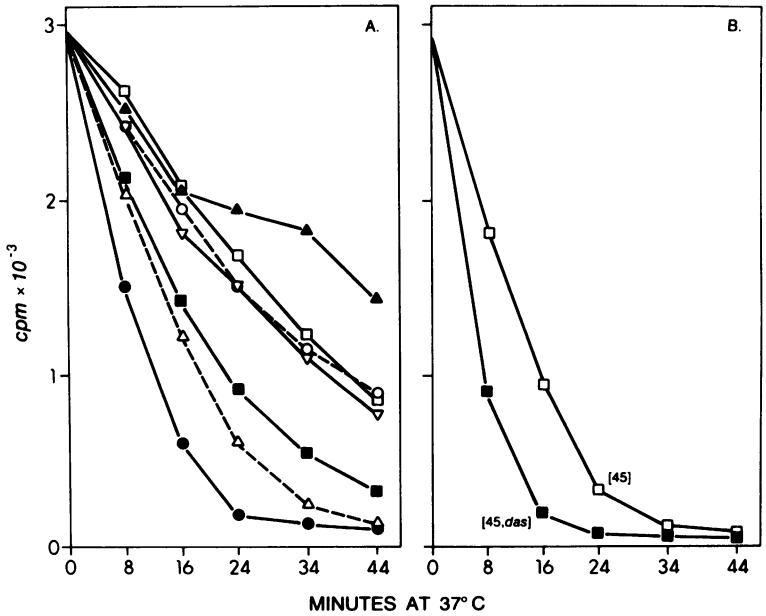

FIG. 5. *(A) Time course of digestion of 10^6-dalton E. coli DNA by membrane fractions from cells infected with [43,45]* (■), *[43,45,47]* (□), *[43,45,46]* (▽), *[43,45,46,47]* (○), *[43,45, das11]* (△), *and [43,45, das13]* (●) *and from uninfected cells* (▲). *(B) Digestion by membrane fractions from [45]* (□) *and [45, das13]* (■) *phage-infected cells. (A) and (B) are from the same experiment.*

TABLE 2. *Effect of the T4 dexA deletion on the suppressor activity of the das mutation*[a]

Phage	No. of phage produced per cell at:	
	22 min	50 min
T4$^+$	51 (100)[b]	227 (100)
[*das*]	64 (125)	226 (100)
[*dexA*]	40 (78)	237 (104)
[*das, dexA*]	24 (47)	187 (82)
[46, 47]	4.3 (8.4)	8.3 (3.7)
[46, 47, *das*]	11.5 (23)	99 (44)
[46, 47, *dexA*]	0.8 (1.6)	1.9 (0.84)
[46, 47, *das, dexA*]	2.1 (4.1)	26 (11)

[a] *E. coli* B was grown in glycerol Casamino Acids medium at 37°C to a concentration of 5×10^8 cells per ml. The cells were then infected at 37°C at a multiplicity of infection of 7 and sampled into chloroform-saturated dilution broth at 22 and 50 min for total phage, which were plated onto *E. coli* CR63 at 30°C. The *dexA* mutation is the deletion del(39-56)1×1 (see text).

[b] The numbers in parentheses are percentages of the T4$^+$ values.

had little or no effect on membrane DNase activity against 10^6-dalton *E. coli* DNA (data not shown).

Effect of *das* mutations on DNase activity. The addition of the *das*13 mutation to [42] or [42,46,47] increased both membrane nuclease activity (Fig. 4) and cytoplasmic nuclease activity (data not shown) about twofold. This increase was observed whether genes 46 and 47 were functional or not. Similar increases were produced by adding the *das*13 mutation to [43,45] or [45] (Fig. 5). The *das*11 mutation, which maps at a different site than *das*13 in the *das* gene (C. S. Barker and J. S. Wiberg, unpublished data), produced a definite but somewhat lower increase. Since we do not know what types of mutations (nonsense, missense, etc.) are represented among the *das* mutants, we suggest that the *das*11 mutant is less deficient in the *das* gene function than is *das*13.

The work of Hercules and Wiberg (24) suggested that *das* is an early gene, since it produces an effect in the absence of phage DNA synthesis. These authors showed that host DNA degradation in vivo was faster and more extensive in a [42,*das*13] infection than in a [42] infection. Our results prove the suggestion that *das* is an early function, since a *das* effect was observed clearly in a 45$^-$ infection (Fig. 5), where no significant late proteins were made (66).

Does suppression by *das* mutations involve known DNase genes? Two possible explanations for the excess DNase activity and the suppressive effect associated with *das* mutations are (i) that *das* mutations cause overproduction of a known DNase and (ii) that the *das*$^+$ gene produces an inhibitor of a known DNase. We tested both of these possibilities. The fact that the *das*13-induced increase in DNase activity occurred in a 43$^-$ 45$^-$ background (Fig. 5A), in which the 43$^-$ mutation inactivated the gene 43 exonuclease, indicated that the gene 43 exonuclease was not involved in the *das* effect.

We asked whether a *das* mutation suppresses by causing overproduction of T4 exonuclease A, which is controlled by the *dexA* gene (60); the *dexA* gene has been mapped between genes 39 and 56 (60), far from the *das* region (24). If this model is correct, a deletion that removes the *dexA* gene should abolish the suppressor capacity of a *das* mutation. Table 2 shows that the *das* mutation exhibited much of its suppressor activity at 50 min despite total loss of the *dexA* gene. That the *dexA* mutation appeared to reduce the suppressor activity of the *das* mutation somewhat may simply have been a reflection of the depressant effect of the *dexA* deletion itself on phage production in a 46$^-$ 47$^-$ background (Table 2). This *dexA* deletion is also known to block the synthesis of two T4 proteins (M_r, 12,000 and 50,000) (25, 43), whose functions have not been identified. Thus, we can conclude that the *das* mutation does not act simply by causing overproduction of T4 exonuclease A; if such overproduction does occur, it cannot be the whole explanation of the *das* phenotype.

Another possibility is that *das* mutants may be defective in a T4 inhibitor of a host DNase. Tanner and Oishi (56) and Yamazaki (67) have shown that one such inhibitor inhibits the *E. coli recBC* nuclease; thus, a defective *das* gene may leave the *recBC* nuclease (exonuclease V) active after T4 infection. Such a model predicts that a *das* mutation would not suppress a 46$^-$ or 47$^-$ mutation in a *recBC*-defective host. We tested this with *E. coli* DM183 (from E. Witkin), a B/r strain that contains the mutation *recB*21; at 37°C, [*das*, 46,47] produced 115 phage per cell, whereas [46,47] produced 9. We obtained similar results with *E. coli* JC5519 (from A. J. Clark), which contains the mutations *recB*21 and *recC*22. These results indicated strongly that the *das*$^+$ gene was not responsible for inhibiting exonuclease V, the *recBC* nuclease.

The functioning of several other *E. coli* genes (3) apparently is not required for suppression by the *das*13 mutation; these include *sbcB* (exonuclease I), *endA* (endonuclease I), and *uvrA* (UV endonuclease). The evidence for this is that the *das*13 mutation increased phage production by [46,47] by at least a factor of 9.3 at 37°C on the following *E. coli* strains: JC7623 (from A. J.

Clark), which contains mutations *sbcB*15 (less than 1.5% of the normal level of exonuclease I activity) (36), *recB*21, and *recC*22; ER22 (from J. Eigner), which produces less than 0.1% of the normal level of endonuclease I (J. Eigner, personal communication); and DM203 (from E. Witkin), which contains the *recB*21 mutation and a *uvrA* mutation.

DISCUSSION

Genes 46 and 47. Under the conditions developed by Alberts et al. (1) for DNA cellulose chromatography, we found that gp46 and gp47 bound to *E. coli* and T4 phage DNAs. We cannot say whether this binding is direct or indirect (through other DNA-recognizing proteins in the membrane fraction) or whether gp46 and gp47 bind to DNA independently of each other. Huang and Buchanan (30) also observed binding of gp46 to T4 DNA cellulose, but, in contrast to our results, these authors found that gp47 washed through their DNA column (i.e., it did not bind). There are a number of differences between their study and ours in the isolation of extracts, types of DNA columns, and methods used that might account for the different conclusions.

Hamilton and Pettijohn (23) found that gp46 bound to isolated, condensed, replicating T4 DNA, but they did not examine gp47. In their DNA preparations they also found gp39, gp52, and gprIIB, which have been identified as the T4 proteins (along with gprIIA) that are bound most tightly to membranes (29, 46, 55, 61). The condensed DNA preparation of these workers apparently was bound to small pieces of bacterial membrane, so that the specificity of the protein binding was not certain. Our results indicate that gp46 and gp47 could be bound to both membranes and DNA in such a preparation.

Our identification of gp46 and gp47 as membrane-associated proteins is based on their cofractionation with membrane marker enzymes. The membrane attachment does not appear to be due to random adsorption since the two proteins remained membrane associated when fractions were isolated in low-ionic-strength buffers at different pH's. Treatment of the membrane fractions with pancreatic DNase I did not release gp46 and gp47 into cytoplasm (Fig. 1), suggesting that the association was not via DNA or that the DNA at the membrane was not accessible to DNase I digestion.

Two studies on the modification of host cell membranes by phage-induced proteins published since our work was done also described a quantitative association of gp46 with the host cell membranes and spheroplast envelopes (16, 55). gp47 was not identified in either of these studies. Takacs and Rosenbusch (55) did not classify gp46 as an "integral" membrane protein, since it could be released from membranes by incubation with a chaotropic agent. By this criterion, although we found that gp47 was membrane associated, it also is not an integral membrane protein since incubation with $NaClO_4$ released it from membranes. However, solubilization of a protein by chaotropic agents does not rule out the possibility that the protein has a functional interaction with the membrane (10, 16, 34, 45), but it does show that the binding interaction is probably ionic.

Our finding that both gp46 and gp47 are membrane associated is consistent with the observation that T4 and *E. coli* DNAs prematurely dissociate from membranes in 46^- and 47^- infections (15, 50, 53) and implies a role for these proteins in membranes. Our in vitro assays demonstrated membrane-associated DNase activity in 46^+- and 47^+-infected cells that was not present in 46^- and 47^- infections. This activity, like gp46 and gp47, was released from membranes by the chaotropic agent $NaClO_4$. This membrane-associated DNase activity was observed primarily on three forms of *E. coli* DNA (native, nicked, and 10^6-dalton DNAs). This lack of size specificity did not change after gp46 and gp47 were released from membranes. Native and denatured T4 phage DNAs (which contained 5-hydroxymethylcytosine in place of cytosine) were tried as substrates in the in vitro assay; in addition to being very poor substrates compared with *E. coli* DNA, these DNAs exhibited no differences in activity between 46^- 47^- and control (46^+ 47^+) fractions. A slight and nonreproducible effect of genes 46 and 47 on membrane DNase activity with T4 replicative DNA was observed but not studied further, as the results with the *E. coli* substrates were far clearer and were highly reproducible. Since T4 DNA appears to be a substrate for the gene 46-47 nuclease in vivo, this in vitro resistance to digestion warrants further study.

The most convincing evidence that the DNase controlled by these genes is an exonuclease which is capable of attacking DNA at nicks to create gaps is that of Prashad and Hosoda (47). These workers showed that in a DNA^-, $DNA\ ligase^-$ infection, parental T4 DNA accumulated gaps in vivo, the repair of which required the in vitro action of both DNA polymerase and DNA ligase; in contrast, when the infecting phage was also 46^- or 47^-, only DNA ligase was required in vitro to close the discontinuities in the parental DNA. Along with our results, this finding con-

stitutes strong evidence that genes 46 and 47 together control a single exonuclease; we observed no differences among the single 46^- and 47^- and double 46^- 47^- mutant-infected cell fractions in our in vitro assay, and the loss of either function in vivo left the same DNA substrate, a double-stranded molecule containing nicks and having a 3'-OH, 5'-PO_4 configuration (47).

***das* mutations.** We showed that the *das* mutations were associated with a considerable increase in DNase activity on nicked or 10^6-dalton *E. coli* DNA. These results supported the model suggested by Hercules and Wiberg (24), in which *das* mutations suppress the 46^- and 47^- phenotype by causing higher than normal levels of a nuclease able to substitute partially for the nuclease activity controlled by genes 46 and 47. Such a role for *das* mutations is analogous to that shown for *sbcA* mutations of *E. coli*, which phenotypically suppress a deficiency of *E. coli recBC* exonuclease (exonuclease V) by derepressing the synthesis of *E. coli* exonuclease VIII (37); *recE* is the structural gene for the latter enzyme (37). The possible analogy between *das* and *sbcA* mutations was noted by Shah and Berger (51); however, these authors misquoted work from the laboratory of Clark as showing that *sbcA* mutations cause overproduction of exonuclease I. We show here that the *das*13 mutation retains its suppressive ability when *E. coli* genes *recB*, *recC*, *sbcB*, *endA*, and *uvrA* are defective; these results indicate that the das^+ gene does not act by inhibiting the nuclease function controlled by any of these genes.

Another possibility is that *das* mutations are "up-promoter" mutations in or near the structural gene for a T4 DNase. Alternatively, the das^+ gene may repress partially the expression of a T4 DNase gene located elsewhere on the T4 genome. We eliminated two such possibilities by showing that deletion of the T4 *dexA* gene (exonuclease A) did not prevent suppression by the *das*13 mutation (Table 2) and that an exonuclease-deficient gene 43 mutation did not prevent the overproduction of DNase activity caused by a *das* mutation (Fig. 5A).

ACKNOWLEDGMENTS

We thank Bruce Alberts for his hospitality and guidance to C.M. in the technique of DNA cellulose chromatography. We are indebted to Cynthia Aldrich, Mark Camel, and Janice Van Knapp for excellent technical assistance.

This paper was based in part on work performed under a contract with the U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics. C.M. was supported by a Public Health Service predoctoral traineeship from the National Institutes of Health to the Department of Biology, University of Rochester.

LITERATURE CITED

1. **Alberts, B. M., F. J. Amodio, M. Jenkins, E. D. Gutman, and F. L. Ferris.** 1968. Studies with DNA-cellulose chromatography. I. DNA-binding proteins from *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **33**:289–305.
2. **Altman, S., and M. Meselson.** 1970. A T4-induced endonuclease which attacks T4 DNA. Proc. Natl. Acad. Sci. U.S.A. **66**:716–721.
3. **Bachmann, B.J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
4. **Berger, H., A. J. Warren, and K. E. Fry.** 1969. Variations in genetic recombination due to amber mutations in T4D bacteriophage. J. Virol. **3**:171–175.
5. **Bernstein, H.** 1968. Repair and recombination. Cold Spring Harbor Symp. Quant. Biol. **33**:325–331.
6. **Bollum, F. J.** 1959. Thermal conversion of nonpriming deoxyribonucleic acid to primer. J. Biol. Chem. **234**: 2733–2734.
7. **Bramhall, S., N. Noack, M. Wu, and J. R. Loewenberg.** 1969. A simple colorimetric method for the determination of protein. Anal. Biochem. **31**:146–148.
8. **Broker, T. R.** 1973. An electron microscopic analysis of pathways for bacteriophage T4 recombination. J. Mol. Biol. **81**:1–16.
9. **Broker, T. R., and I. R. Lehman.** 1971. Branched DNA molecules: intermediates in T4 recombination. J. Mol. Biol. **60**:131–149.
10. **Brooks, D., L. L. Mays, Y. Hatefi, and F. E. Young.** 1971. Glucosylation of teichoic acid: solubilization and partial characterization of the uridine diphosphoglucose:polyglycerolteichoic acid glucosyl transferase from membranes of *Bacillus subtilis*. J. Bacteriol. **107**:223–229.
11. **Cardillo, T. S., E. F. Landry, and J. S. Wiberg.** 1979. *regA* protein of bacteriophage T4D: identification, schedule of synthesis, and autogenous regulation. J. Virol. **32**:905–916.
12. **Cascino, A., E. P. Geiduschek, R. L. Cafferata, and R. Haselkorn.** 1971. T4 DNA replication and viral gene expression. J. Mol. Biol. **61**:357–367.
13. **Casjens, S., and J. King.** 1975. Virus assembly. Annu. Rev. Biochem. **44**:555–611.
14. **Deeb, S. S., and L. P. Hager.** 1964. Crystalline cytochrome b_1 from *Escherichia coli*. J. Biol. Chem. **239**: 1024–1031.
15. **Earhart, C. F.** 1970. The association of host and phage DNA with the membrane of *Escherichia coli*. Virology **42**:429–436.
16. **Ennis, H. L., and K. D. Kievitt.** 1977. Alteration of the *Escherichia coli* membrane by addition of bacteriophage T4 proteins synthesized after infection. J. Virol. **22**:553–560.
17. **Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis.** 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28**:375–394.
18. **Folch, J., I. Ascoli, M. Lees, J. A. Meath, and F. N. LeBaron.** 1951. Preparation of lipid extracts from brain tissue. J. Biol. Chem. **191**:833–841.
19. **Fox, C. F., J. R. Carter, and E. P. Kennedy.** 1967. Genetic control of the membrane protein component of the lactose transport system of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **57**:698–705.
20. **Fraser, D., and E. A. Jerrel.** 1953. Amino acid composition of T3 bacteriophage. J. Biol. Chem. **205**:291–295.
21. **Fry, S. E.** 1979. Stimulation of recombination in phage T4 by nitrous acid-induced lesions. J. Gen. Virol. **43**: 719–722.

22. **Guha, A., W. Szybalski, W. Salser, A. Bolle, E. P. Geiduschek, and J. F. Pulitzer.** 1971. Controls and polarity of transcription during bacteriophage T4 development. J. Mol. Biol. **59**:329-349.
23. **Hamilton, S., and D. E. Pettijohn.** 1976. Properties of condensed bacteriophage T4 DNA isolated from *Escherichia coli* infected with bacteriophage T4. J. Virol. **19**:1012-1027.
24. **Hercules, K., and J. S. Wiberg.** 1971. Specific suppression of mutations in genes 46 and 47 by *das*, a new class of mutations in bacteriophage T4. J. Virol. **8**:603-612.
25. **Homyck, T., Jr., and J. Weil.** 1974. Deletion analysis of two nonessential regions of the T4 genome. Virology **61**:505-523.
26. **Hosoda, J.** 1976. Role of genes 46 and 47 in bacteriophage T4 reproduction. II. Formation of joint molecules in biparental recombination. J. Mol. Biol. **106**:277-284.
27. **Hosoda, J., and C. Levinthal.** 1968. Protein synthesis by *Escherichia coli* infected with bacteriophage T4D. Virology **34**:709-727.
28. **Hosoda, J., E. Mathews, and B. Jansen.** 1971. Role of genes 46 and 47 in bacteriophage T4 reproduction. I. In vivo deoxyribonucleic acid replication. J. Virol. **8**:372-387.
29. **Huang, W. M.** 1975. Membrane-associated proteins of T4-infected *Escherichia coli*. Virology **66**:508-521.
30. **Huang, W. M., and J. M. Buchanan.** 1974. Synergistic interactions of T4 early proteins concerned with their binding to DNA. Proc. Natl. Acad. Sci. U.S.A. **71**:2226-2230.
31. **Inouye, M., and J. P. Guthrie.** 1969. A mutation which changes a membrane protein of *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **64**:957-961.
32. **Kellenberger, E., and J. Séchaud.** 1957. Electron microscopical studies of phage multiplication. II. Production of phage-related structures during multiplication of phage T2 and T4. Virology **3**:256-274.
33. **Kemper, B., and J. Hurwitz.** 1973. Studies on T4-induced nucleases. Isolation and characterization of a manganese-actived T4-induced endonuclease. J. Biol. Chem. **248**:91-99.
34. **King, T. E.** 1967. Preparation of succinate dehydrogenase and reconstitution of succinate oxidase. Methods Enzymol. **10**:322-331.
35. **Krylov, V. N., and T. G. Plotnikova.** 1971. A suppressor in the genome of phage T4 inhibiting expression of mutation in genes 46 and 47. Genetics **67**:319-326.
36. **Kushner, S. R., H. Nagaishi, and A. J. Clark.** 1972. Indirect suppression of *recB* and *recC* mutations by exonuclease I deficiency. Proc. Natl. Acad. Sci. U.S.A. **69**:1366-1370.
37. **Kushner, S. R., H. Nagaishi, and A. J. Clark.** 1974. Isolation of exonuclease VIII: the enzyme associated with the *sbcA* indirect suppressor. Proc. Natl. Acad. Sci. U.S.A. **71**:3593-3597.
38. **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 mutants defective in genes 46, 47, and 56. J. Mol. Biol. **38**:395-411.
39. **Kutter, E. M., and J. S. Wiberg.** 1969. Biological effects of substituting cytosine for 5-hydroxymethylcytosine in the deoxyribonucleic acid of bacteriophage T4. J. Virol. **4**:439-453.
40. **Melgar, E., and D. Goldthwait.** 1968. Deoxyribonucleic acid nucleases. II. The effect of metals on the mechanism of action of deoxyribonuclease I. J. Biol. Chem. **243**:4409-4416.
41. **Minner, C. A., and H. Bernstein.** 1976. Genes 46 and 47 of phage T4: possible compensation for loss of their function. J. Gen. Virol. **31**:277-280.
42. **Nossal, N.** 1969. A T4 bacteriophage mutant which lacks deoxyribonucleic acid polymerase but retains the polymerase-associated nuclease. J. Biol. Chem. **244**:218-220.
43. **O'Farrell, P. L., L. M. Gold, and W. M. Huang.** 1973. The identification of prereplicative bacteriophage T4 proteins. J. Biol. Chem. **248**:5499-5501.
44. **Oleson, A. E., and J. F. Koerner.** 1964. A deoxyribonuclease induced by infection with bacteriophage T2. J. Biol. Chem. **239**:2935-2943.
45. **Patel, L., S. Shuldiner, and H. R. Kaback.** 1975. Reversible effects of chaotropic agents on the proton permeability of *Escherichia coli* membrane vesicles. Proc. Natl. Acad. Sci. U.S.A. **72**:3387-3391.
46. **Peterson, R. F., K. D. Kievitt, and H. L. Ennis.** 1972. Membrane protein synthesis after infection of *Escherichia coli* B with phage T4: the rIIB protein. Virology **50**:520-527.
47. **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.
48. **Salton, M. R.** 1972. Bacterial membranes. Crit. Rev. Microbiol. **1**:161-197.
49. **Schneider, W. C.** 1957. Determination of nucleic acid in tissues by pentose analysis. Methods Enzymol. **5**:680-684.
50. **Shah, D. B., and H. Berger.** 1971. Replication of gene 46-47 amber mutants of bacteriophage T4D. J. Mol. Biol. **57**:17-34.
51. **Shah, D. B., and H. Berger.** 1973. Effect of a gene-specific suppressor mutation (*das*) on DNA synthesis of gene 46-47 mutants of bacteriophage T4D. J. Virol. **12**:328-333.
52. **Shalitin, C., and S. Kahana.** 1970. Conversion of T4 gene 46 mutant deoxyribonucleic acid into nonviable bacteriophage particles. J. Virol. **6**:353-362.
53. **Shalitin, C., and Y. Naot.** 1971. Role of gene 46 in bacteriophage T4 deoxyribonucleic acid synthesis. J. Virol. **8**:142-153.
54. **Shapiro, B. M., A. G. Siccardi, Y. Hirota, and F. Jacob.** 1970. On the process of cellular division in *Escherichia coli*. II. Membrane protein alterations associated with mutations affecting the initiation of DNA synthesis. J. Mol. Biol. **52**:75-89.
55. **Takacs, B. J., and J. P. Rosenbusch.** 1975. Modification of *Escherichia coli* membranes in the prereplicative phase of phage T4 infection. J. Biol. Chem. **250**:2339-2350.
56. **Tanner, D., and M. Oishi.** 1971. The effect of bacteriophage T4 infection on an ATP-dependent deoxyribonuclease in *Escherichia coli*. Biochim. Biophys. Acta **228**:767-769.
57. **Thomas, C. A., Jr., and J. Abelson.** 1966. The isolation and characterization of DNA from bacteriophage, p. 553-561. *In* G. L. Cantoni and D. R. Davies (ed), Procedures in nucleic acid research. Harper & Row, Publishers, New York.
58. **Thomas, C. A., Jr., K. I. Berns, and T. J. Kelley, Jr.** 1966. Isolation of high molecular weight DNA from bacterial and cell nuclei, p. 535-540. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research. Harper & Row, Publishers, New York.
59. **Wallenfels, K.** 1963. β-Galactosidase (crystalline). Methods Enzymol. **5**:212-219.
60. **Warner, H. R., D. P. Snustad, J. F. Koerner, and J. D. Childs.** 1972. Identification and genetic characterization of mutants of bacteriophage T4 defective in the ability to induce exonuclease A. J. Virol. **9**:399-407.
61. **Weintraub, S. B., and F. R. Frankel.** 1972. Identification of the T4 rIIB gene product as a membrane protein. J. Mol. Biol. **79**:589-615.
62. **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.

63. **Wiberg, J. S.,T. S. Cardillo, and C. Mickelson.** 1981. Genetic and amber fragment maps of genes 46 and 47 of bacteriophage T4D. J. Virol. **40**:309–313.
64. **Wiberg, J. S., M. L. Dirksen, R. H. Epstein, S. E. Luria, and J. M. Buchanan.** 1962. Early enzyme synthesis and its control in *E. coli* infected with some amber mutants of bacteriophage T4. Proc. Natl. Acad. Sci. U.S.A. **48**:293–302.
65. **Wiberg, J. S., S. Mendelsohn, V. Warner, K. Hercules, C. Aldrich, and J. L. Munro.** 1973. SP62, a viable mutant of bacteriophage T4D defective in regulation of phage enzyme synthesis. J. Virol. **12**:775–792.
66. **Wu, R., and E. P. Geiduschek.** 1975. The role of replication proteins in the regulation of bacteriophage T4 transcription. I. Genes 45 and hydroxymethyl-C-containing DNA. J. Mol. Biol. **96**:513–538.
67. **Yamazaki, Y.** 1971. A reduced activity of a deoxyribonuclease requiring ATP in *Escherichia coli* infected by bacteriophage T4. Biochim. Biophys. Acta **247**:535–541.