Evidence that the UV Endonuclease Activity Induced by Bacteriophage T4 Contains Both Pyrimidine Dimer-DNA Glycosylase and Apyrimidinic/Apurinic Endonuclease Activities in the Enzyme Molecule†

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We performed experiments to determine whether the phage T4-induced UV endonuclease activity is a single protein containing both pyrimidine dimer-DNA glycosylase and apyrimidinic endonuclease activities. The UV endonuclease activity is induced by the *denV* gene and codes for the glycosylase activity. We obtained several kinds of evidence that the protein containing the glycosylase activity also contains the apyrimidinic endonuclease activity. After chromatography on DEAE-cellulose, the two activities copurified during phosphocellulose chromatography and Sephadex G-100 chromatography, with a constant ratio of activities across the activity peaks. On Sephadex G-100 columns the molecular weights of the two activities agreed within 2,500 or less. When an extract of cells infected with the T4 V1 mutant was purified in exactly the same way as an extract of cells infected with T4 V1⁺, neither glycosylase nor apyrimidinic endonuclease activity was detected in the normal elution position of the T4 UV endonuclease activity. The glycosylase and apyrimidinic endonuclease activities were induced with similar kinetics, which were characteristic of immediate early rather than delayed early enzymes. This correlated well with the presumed major role of these activities in repairing thymine dimers in parental DNA before DNA replication begins. Finally, glycosylase and apyrimidinic endonuclease activities were lost in parallel during incubation of the enzyme at 46°C. Our results indicated that both of these enzyme activities are contained in the same enzyme molecule and, probably, in the same polypeptide.

Recently, it has become clear that the incision of lightly irradiated DNA by the UV-specific endonuclease activity induced by bacteriophage T4 occurs in two steps (2, 14, 16, 20). The first step is cleavage of the glycosylic bond between the pyrimidine and the deoxyribose in the 5' half of the pyrimidine dimer to produce an apyrimidinic (AP) site (Fig. 1). The second step is nicking of the dimer-containing strand on the 3' side of the AP site generated by the glycosylase. The product of the *denV* gene of T4 is essential for induction of this UV endonuclease activity (4), and Seawell et al. (16) have demonstrated that the *denV* gene codes for the glycosylase activity. However, the data of these workers did not show whether the *denV* gene product also contains the AP endonuclease activity, and, in fact, Seawell et al. stated that the simplest interpretation of their data is that the *denV* gene codes for the

pyrimidine dimer-DNA glycosylase but not for the AP endonuclease.

In this paper we present several kinds of evidence suggesting that the glycosylase and the AP endonuclease activities both reside in the same protein and, probably, in the same polypeptide and that this polypeptide is the product of the *denV* gene.

MATERIALS AND METHODS

Bacteria and bacteriophage. Bacteriophage T4*amN82* contains an amber mutation in gene 44 and is not able to synthesize phage DNA in the nonpermissive host *Escherichia coli* B/5 (22). We used this mutant because it overproduces early enzymes (22), and the UV endonuclease was thought to be an early enzyme (5). The *amN82* mutation also prevented premature lysis of infected cells during harvesting. The T4V1 mutant is defective in the *denV* gene and was obtained from Navin Sinha (Rutgers University), who obtained it from Harris Bernstein (University of Arizona). This mutant was isolated as a UV-sensitive

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FIG. 1. Mechanism of incision of irradiated DNA by T4 UV endonuclease preparations.

mutant after treatment with nitrous acid (8). The *amN82* and *V1* mutations were recombined in the permissive host *E. coli* CR63, as described previously (21), and the progeny were screened for sensitivity to UV light to detect the *T4amN82-V1* double mutant used here.

Preparation of PM2 DNA. Unlabeled bacteriophage PM2 DNA and PM2 DNA labeled with [*methyl*-³H]thymidine were prepared as described previously (10), except that phage were produced in *thy*⁺ cells grown in nutrient broth rather than minimal medium. The labeled PM2 DNA prepared in this way usually had a specific activity of 20 to 50 cpm/fmol of duplex circle.

Enzyme assays. The fluorescence procedure described by Fitcher and Morgan (6) was adapted for assaying pyrimidine dimer-DNA glycosylase activity. A solution containing 0.01 M Tris-chloride (pH 7.5), 0.02 M NaCl, and about 20 fmol of PM2 DNA molecules per μ l was irradiated for 30 s at a distance of 20 cm from two General Electric 15-W germicidal lamps to produce pyrimidine dimers in the DNA (20). The final assay mixture contained 0.13 M NaCl, 0.1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate buffer (pH 7.3), 0.01 M EDTA, 0.002 M Tris-chloride (pH 7.5), 3.25 μ g of bovine serum albumin, and 200 fmol of irradiated PM2 in a total volume of 50 μ l. Usually, 1 or 2 μ l of enzyme was added, and the assay mixtures were incubated for 10 min at 37°C. Each reaction was terminated by adding 2 ml of 0.02 M potassium phosphate (pH 11.8), containing 0.2 mM EDTA and 0.5 μ g of ethidium bromide per ml. The resulting samples were heated for 8 min at about 95°C to denature the DNA and to separate the strands of nicked circles. The samples were cooled and then assayed for fluorescence at 600 nm during excitation at 525 nm with a Perkin-Elmer PM2 fluorescence

spectrophotometer. Only unnicked DNA molecules renatured after the 95°C treatment and subsequently fluoresced in the spectrophotometer. This assay determined not only the number of nicked DNA molecules produced during the 37°C incubation, but also the number of molecules containing AP sites, because AP sites led to strand breakage during the 95°C treatment in alkali. Therefore, this assay measured the total glycosylase activity, whether the product contained an AP site or was subsequently nicked by AP endonuclease activity.

We used the AP endonuclease assay described previously (10, 20), except that 0.01 M MgCl₂ was replaced by 0.01 M EDTA for our experiments. This assay measured only the number of nicked DNA molecules present after the 37°C incubation.

For both of the above-described assays we calculated the absolute number of AP sites and nicks, or nicks only, introduced into the DNA population by using the first term of the Poisson distribution (20).

The deoxycytidine-5'-triphosphate nucleotidohydrolase (dCTPase) activity was assayed as described previously (19), and proteins were determined by the method of Lowry et al. (11), using trypsin as the standard.

Enzyme purification. Extracts were prepared from *E. coli* B/5 cells infected with wild-type T4, *T4amN82*, or *T4amN82-V1* and were shaken for 30 min at 37°C. Purification of the T4-induced UV endonuclease was performed essentially as described by Friedberg and King (5). Details of the experiments are given in the figure legends. Wild-type T4 was used only for the induction experiments shown in Fig. 7.

RESULTS

An extract of *E. coli* cells infected with phage *T4amN82* was prepared and fractionated as described by Friedberg and King (5). The purification steps used included two-phase separation and sequential chromatography on DEAE-cellulose, phosphocellulose, and Sephadex G-100. The T4-induced UV endonuclease activity did not bind to DEAE-cellulose but was eluted in the column wash. The fractions containing pyrimidine dimer-DNA glycosylase activity were combined and fractionated on phosphocellulose (Fig. 2). When these fractions were assayed for both pyrimidine dimer-DNA glycosylase and AP endonuclease activities, the two activities eluted in exactly the same place (at about 0.35 M potassium phosphate, pH 7.0). Both assays were performed in the absence of divalent metal ions and in the presence of 0.01 M EDTA to suppress other endonuclease activities which might be present and interfere with the assays. When untreated PM2 DNA (i.e., not irradiated or depurinated) was used as a substrate, no nicking activity was detected in fractions 55 through 75 in either assay (data not shown). Within the limits of error of the two assays, the ratio of activities was essentially constant across the ac-

FIG. 2. Elution of T4 UV endonuclease on phosphocellulose. An extract was prepared from 3.8 g of *E. coli* B/5 infected with T4amN82 as described by Friedberg and King (5). After two-phase separation and chromatography on DEAE-cellulose, the fractions containing pyrimidine dimer-DNA glycosylase activity were pooled and applied to a 12-ml column of phosphocellulose equilibrated with 0.05 M potassium phosphate buffer (pH 7.0) containing 3% polyethylene glycol. The column was eluted with a linear gradient of 0.05 to 0.5 M potassium phosphate (pH 7.0) containing 3% polyethylene glycol (100 ml of each buffer) starting at fraction 16. The fractions were then assayed for pyrimidine dimer-DNA glycosylase activity (●), AP endonuclease activity (○), conductivity (Δ), and absorbance at 280 nm (A_{280}) (■). The enzyme activities are expressed as femtomoles of nicks produced per minute per microliter of enzyme, and the conductivity is expressed as micromhos per centimeter.

tivity peak. In various experiments this ratio varied between 5 and 7.

A portion of fraction 62 was then chromatographed on Sephadex G-100 to determine whether the two activities could be separated by a difference in their apparent molecular weights (Fig. 3). Once again, the glycosylase and AP endonuclease activities coincided exactly; both activities eluted at an apparent molecular weight based on its Stokes radius of about 26,000, using horse heart cytochrome *c* and ovalbumin as standards. A molecular weight difference of about 2,500 would have been sufficient to shift one of the enzyme peaks by one fraction from the peak observed.

The results described above strongly suggested that the two enzyme activities resided in the same protein molecule. However, it was possible that the UV endonuclease activity consisted of two separate proteins which eluted together on the columns described above. To investigate this possibility, we prepared extracts of cells infected with T4amN82 and cells infected with the T4amN82-V1 double mutant and performed fractionations in parallel with small amounts of the two extracts. Table 1 shows that

the cells were indeed infected and that at least one other early enzyme (the dCTPase activity) was induced by T4amN82-V1 in these cells. The protein elution profiles on DEAE-cellulose appeared to be fairly similar for the two extracts, with the exception of the complete absence of pyrimidine dimer-DNA glycosylase activity in the V1 extract (Fig. 4).

The active fractions from the T4amN82 column were then combined and chromatographed on phosphocellulose, and the comparable fractions from the T4amN82-V1 column were also combined and chromatographed. These columns were eluted with step gradients instead of continuous gradients to define better where the enzyme proteins eluted (Fig. 5). Whereas both the glycosylase and the AP endonuclease activities eluted in the expected fractions of the T4amN82 column, neither activity was apparent in the T4amN82-V1 column. This indicated that the AP endonuclease activity which copurified with the pyrimidine dimer-DNA glycosylase activity on phosphocellulose was not a separate polypeptide which coincidentally eluted in the same fractions as the glycosylase.

To test whether the glycosylase and AP en-

FIG. 3. Elution of T4 UV endonuclease on Sephadex G-100. A 0.75-ml portion of fraction 62 from the phosphocellulose column described in the legend to Fig. 2 was chromatographed on a 38-ml Sephadex G-100 column equilibrated with 0.5 M potassium phosphate buffer (pH 7.0) containing 3% polyethylene glycol. Fractions (1.3 ml) were collected and assayed for pyrimidine dimer-DNA glycosylase (●) and AP endonuclease (○) activities. The enzyme activities are expressed as femtomoles of nicks produced per minute per microliter of enzyme. The positions of the elution peaks of ovalbumin (OVAL) and horse heart cytochrome c (CYTO) are indicated by the arrows; the molecular weights of these standards were about 45,000 and 13,400, respectively.

TABLE 1. Induction of dCTPase by T4amN82 and T4amN82-V1

Phage	Concn of protein (mg/ml)	dCTPase activity ^a	% of cells infected
T4amN82	4.1	5.5	95
T4amN82-V1	4.3	3.0	73

^a Expressed as nanomoles of dCMP produced per minute at 30°C per microliter of extract used. No activity was detected in uninfected cells.

donuclease activities were equally sensitive to denaturation at a high temperature, a portion of phosphocellulose fraction 62 (Fig. 2) was incubated at 46°C for varying lengths of time and then assayed for both glycosylase and AP endonuclease activities (Fig. 6). The results indicated that both activities were lost in parallel during incubation for up to 17 min at 46°C.

The results of Friedberg and King (5) suggested that the T4 UV endonuclease is induced beginning between 2 and 5 min after infection and that induction continues linearly for as long as 20 min after infection. This conclusion appears to be inconsistent with the later observation of Pawl et al. (13) that excision of thymine dimers from parental phage begins immediately

after infection and continues for only 5 to 7 min. Because the kinetics of induction of this enzyme were determined originally with crude extracts and a relatively nonspecific assay (5), we reinvestigated this question. We prepared extracts from cells infected with wild-type phage T4 for varying lengths of time and fractionated these extracts on small DEAE-cellulose and phosphocellulose columns. The glycosylase and AP endonuclease activities were calculated from the total activity of each enzyme present in the appropriate phosphocellulose fractions. These fractions were free of contaminating nuclease activities, which interfered with assays in cruder preparations. Our results indicated that both activities were induced starting as early as 40 s after infection and reached maximum levels by about 4 to 6 min after infection (Fig. 7). In contrast, the activity of the "delayed early" enzyme dCTPase did not begin to appear until 4 min after infection. Thus, both activities of the UV endonuclease were induced much earlier than the dCTPase activity, and UV endonuclease is presumably an "immediate early" rather than a delayed early enzyme (15).

DISCUSSION

Our results strongly support the hypothesis that the T4-induced UV endonuclease activity

FIG. 4. Chromatography of extracts on DEAE-cellulose. Extracts were prepared from cells infected with T4amN82 (0.56 g) and cells infected with T4amN82-V1 (0.58 g). After two-phase separation, the upper layers were applied to 2.5-ml columns of DEAE-cellulose equilibrated with 0.05 M Tris-chloride (pH 8.0) containing 3% polyethylene glycol. The columns were washed with this same buffer, and the fractions were assayed for pyrimidine dimer-DNA glycosylase activity (●) and absorbance at 280 nm (A_{280}) (□). The enzyme activity is expressed as femtomoles of nicks produced per minute per microliter.

FIG. 5. Chromatography of DEAE-cellulose fractions on phosphocellulose. The fractions indicated by the brackets in Fig. 4 were combined and applied to 2.5-ml phosphocellulose columns equilibrated with 0.05 M potassium phosphate (pH 7.0) containing 3% polyethylene glycol. These columns were then washed successively with 10-ml portions of 0.05, 0.2, and 0.425 M potassium phosphate (pH 7.0) containing 3% polyethylene glycol. The fractions were assayed for pyrimidine dimer-DNA glycosylase activity (●), AP endonuclease activity (○), and conductivity (Δ). The enzyme activities are expressed as femtomoles of nicks produced per minute per microliter of enzyme, and the conductivity is expressed as micromhos per centimeter.

FIG. 6. Enzyme inactivation at 46°C. A portion of fraction 62 from the phosphocellulose column described in the legend to Fig. 2. was incubated at 46°C, and at varying times 1-μl portions were removed and assayed immediately for pyrimidine dimer-DNA glycosylase (●) and AP endonuclease (○) activities.

resides in a single enzyme molecule that possesses both pyrimidine dimer-DNA glycosylase activity and AP endonuclease activity. It is not unusual for the T-even phages to induce multifunctional enzymes, perhaps in the interest of making more efficient use of their genetic material. Some examples of this include phage-induced deoxyribonucleoside-5'-monophosphate

FIG. 7. Induction kinetics of pyrimidine dimer-DNA glycosylase and AP endonuclease activities by wild-type T4. *E. coli* B/5 was infected with phage at 37°C, and at different times 300-ml portions were removed and poured onto ice. Extracts were prepared from the infected cells (0.5 to 0.6 g), and these extracts were fractionated sequentially on 1-ml DEAE-cellulose and phosphocellulose columns as described in legends to Fig. 4 and 5. The fractions eluted from the phosphocellulose column by 0.425 M potassium phosphate (pH 7.0) containing 3% polyethylene glycol were assayed for glycosylase and AP endonuclease activities, and the crude extracts were assayed for dCTPase activity. The results are expressed as total enzyme units per gram of infected cells; 1 enzyme unit equaled 1 pmol of nicks introduced per min at 37°C for the glycosylase (●) and AP endonuclease (○) activities, and 1 μmol of dCMP formed per min at 30°C for the dCTPase activity (■).

kinase (3), deoxycytidine-5'-triphosphate nucleotidohydrolase (19), polynucleotide kinase (1), and the product of gene 63 (17).

Other workers have also presented data which support this hypothesis. Demple and Linn (2) observed that their T4 UV endonuclease preparation lost UV and AP endonuclease activities at the same rate during storage. Seawell et al. (16) reported that the ratio of glycosylase activity to AP endonuclease activity was 5 in the leading edge, the center, and the trailing edge of their phosphocellulose peak and that the two activities sedimented together during sucrose gradient centrifugation. Although these authors also observed that the AP endonuclease activities in crude extracts of cells infected with heat-sensitive *denV* mutants were not heat labile, whereas the glycosylase activities were, the significance of this observation seems rather ambiguous because extracts of uninfected cells contained equivalent levels of AP endonuclease activity. Hence, the results of the assays for AP endonuclease activity in the unfractionated extracts of Seawell et al. do not seem to be meaningful.

The data which we obtained do not support the hypothesis that the *denV* gene product is a glycosylase which coincidentally is contaminated with a separate protein having AP endonuclease activity. However, our data do indicate that either (i) a single polypeptide codes for both the glycosylase and AP endonuclease activities and this polypeptide is the product of the *denV* gene, or (ii) the enzyme is a tight complex between the *denV* gene product and a separate polypeptide with AP endonuclease activity. If the latter is true, the AP endonuclease could be either a phage-induced polypeptide or a host polypeptide. It seems unlikely that the AP endonuclease is a host polypeptide because the only known *E. coli* AP endonuclease with the same nicking specificity as the AP endonuclease activity of T4 UV endonuclease is endonuclease III (20), and this enzyme is too large (7) to bind to another polypeptide to form a complex with a molecular weight of only 26,000. Furthermore, the AP endonuclease is induced by the phage with kinetics similar to those of glycosylase induction (Fig. 7). These results clearly indicate that the UV endonuclease activity is either a single, induced polypeptide containing both glycosylase and AP endonuclease activities or a tight complex between two simultaneously induced polypeptides. If the two activities are in separate polypeptides, their elution on phosphocellulose and stability at 46°C depend upon tight binding to each other.

In the accompanying paper (12), McMillan et al. show that DNA containing AP sites inhibits

the glycosylase activity. This indicates that the glycosylase and AP endonuclease activities may share an active site. Furthermore, these workers also show that *denV* mutants failed to induce an AP endonuclease activity which was detectable in crude extracts under their assay conditions. Combined with our data, these results constitute strong but not unequivocal evidence that both enzyme activities are found in a single polypeptide, which is the product of the *denV* gene.

We and Seawell et al. (16) have observed that the glycosylase is about five times more active than the AP endonuclease under the assay conditions which we used. Although it could be argued that under appropriate conditions these two activities would be equal, a plausible argument is that the AP sites generated by depurination are not the normal substrate (Fig. 1) for the AP endonuclease activity. It will be interesting to compare quantitatively the activity of the endonuclease on AP sites generated by depurination with the activity on AP sites generated by pyrimidine dimer-DNA glycosylase to determine whether the pyrimidine dimer attached to the adjacent deoxyribose influences either the rate of the reaction or the K_m of the substrate.

The UV endonuclease found in *Micrococcus luteus* has also been studied extensively (9, 18). At first glance, this activity appears to consist of glycosylase and AP endonuclease activities which can be separated by chromatography on phosphocellulose, Sephadex G-75, hydroxylapatite, and carboxymethyl cellulose (9). The final preparation had a ratio of pyrimidine dimer glycosylase activity to AP endonuclease activity of 94. Using a less highly purified *M. luteus* enzyme, Tomilin et al. (18) also demonstrated the presence of AP endonuclease activity in their preparation. In addition, they showed that the UV endonuclease and AP endonuclease activities were inactivated in parallel at 50°C and that depurinated DNA competitively inhibited the UV endonuclease activity and irradiated DNA inhibited the AP endonuclease activity. These latter results support the hypothesis that there is a single enzyme containing both activities.

The results of Tomilin et al. (18) with the *M. luteus* enzyme should be re-evaluated in light of recent information concerning the mechanism of incision of DNA containing pyrimidine dimers. Because incisions by T4 and *M. luteus* UV endonucleases generate an intermediate containing an AP site, the observation that irradiated DNA inhibits the AP endonuclease activity does not distinguish between the one-enzyme and two-enzyme hypotheses. We have also observed strong inhibition by irradiated DNA when the T4 enzyme was used (data not shown). In contrast, the inhibition of incision of irradiated

DNA by depurinated DNA is less ambiguous, and McMillan et al. have observed such inhibition with the T4 UV endonuclease (12). The observation that the *M. luteus* AP endonuclease and pyrimidine dimer-DNA glycosylase activities are inactivated in parallel (18) must also be reconsidered. The reaction products were analyzed by agarose gels, so if the glycosylase had acted to produce an AP site which could not be cleaved subsequently by the AP endonuclease because of thermal denaturation of the AP endonuclease, it would appear that the glycosylase had also been inactivated.

Based on the data described above, neither the *M. luteus* UV endonuclease nor the T4 UV endonuclease has been shown unequivocally to contain both pyrimidine dimer-DNA glycosylase and AP endonuclease activities in the same polypeptide. Our results with the T4 enzyme indicate that the two activities exist in the same enzyme molecule, and the small size and other properties of this molecule suggest that both activities exist in the same polypeptide.

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ADDENDUM IN PROOF

Y. Nakabeppu and M. Sekiguchi recently reported that the T4-induced pyrimidine dimer-DNA glycosylase and apurinic/aprimidinic endonuclease activities copurified and were found in a preparation containing only a 16,000-dalton polypeptide (Proc. Natl. Acad. Sci. U.S.A. 78:2742-2746, 1981). They also reported that *denV* mutants failed to induce either activity.

LITERATURE CITED

1. Cameron, V., and O. C. Uhlenbeck. 1977. 3'-Phosphatase activity in T4 polynucleotide kinase. *Biochemistry* 16:5120-5126.
2. Demple, B., and S. Linn. 1980. DNA N-glycosylases and UV repair. *Nature (London)* 287:203-208.
3. Duckworth, D. H., and M. J. Bessman. 1967. The enzymology of virus-infected bacteria. X. A biochemical-genetic study of the deoxynucleotide kinase induced by wild type and *amber* mutants of phage T4. *J. Biol. Chem.* 242:2877-2885.
4. Friedberg, E. C., and J. J. King. 1969. Endonucleolytic cleavage of UV-irradiated DNA controlled by the *V+* gene in phage T4. *Biochem. Biophys. Res. Commun.* 37:646-651.
5. Friedberg, E. C., and J. J. King. 1971. Dark repair of ultraviolet-irradiated deoxyribonucleic acid by bacteriophage T4: purification and characterization of a dimer-specific phage-induced endonuclease. *J. Bacteriol.* 106:500-507.
6. Futcher, A. B., and A. R. Morgan. 1979. A novel assay for endonucleases acting at apurinic sites and its use in measuring AP endonuclease activity in repair-deficient mutants of *Saccharomyces cerevisiae*. *Can. J. Biochem.* 57:932-937.
7. Gates, F. T., and S. Linn. 1977. Endonuclease from *Escherichia coli* that acts specifically upon duplex DNA damaged by ultraviolet light, osmium tetroxide, acid or x-rays. *J. Biol. Chem.* 252:2802-2807.
8. Harm, W. 1963. Mutants of phage T4 with increased sensitivity to ultraviolet. *Virology* 19:66-71.
9. Haseltine, W. A., L. K. Gordon, C. P. Lindan, R. H. Grafstrom, N. L. Shaper, and L. Grossman. 1980. Cleavage of pyrimidine dimers in specific DNA sequences by a pyrimidine dimer-DNA glycosylase of *M. luteus*. *Nature (London)* 285:634-641.
10. Kuhnlein, U., E. E. Penhoet, and S. Linn. 1976. An altered apurinic DNA endonuclease activity in group A and group D xeroderma pigmentosum fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.* 73:1169-1173.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
12. McMillan, S., H. J. Edenberg, E. H. Radany, R. C. Friedberg, and E. C. Friedberg. 1981. The *denV* gene of bacteriophage T4 codes for both pyrimidine dimer-DNA glycosylase and apyrimidinic endonuclease activities. *J. Virol.* 40:211-223.
13. Pawl, G., R. Taylor, K. Minton, and E. C. Friedberg. 1976. Enzymes involved in thymine dimer excision in bacteriophage T4-infected *Escherichia coli*. *J. Mol. Biol.* 108:99-109.
14. Radany, E. H., and E. C. Friedberg. 1980. A pyrimidine dimer-DNA glycosylase activity associated with the *V* gene product of bacteriophage T4. *Nature (London)* 286:182-185.
15. Salser, W., A. Bolle, and R. Epstein. 1970. Transcription during bacteriophage T4 development: a demonstration that distinct subclasses of the "early" RNA appear at different times and that some are turned off at late times. *J. Mol. Biol.* 49:271-295.
16. Seawell, P. C., C. A. Smith, and A. K. Ganesan. 1980. *denV* gene of bacteriophage T4 determines a DNA glycosylase specific for pyrimidine dimers in DNA. *J. Virol.* 35:790-797.
17. Snopek, T. J., W. B. Wood, M. P. Conley, P. Chen, and N. R. Cozzarelli. 1977. Bacteriophage T4 RNA ligase is gene 63 product, the protein that promotes tail fiber attachment to the baseplate. *Proc. Natl. Acad. Sci. U.S.A.* 74:3355-3359.
18. Tomilin, N. V., E. B. Paveltchuk, and T. V. Mosevitskaya. 1976. Substrate specificity of the ultraviolet-endonuclease from *Micrococcus luteus*. Endonucleolytic cleavage of depurinated DNA. *Eur. J. Biochem.* 69:265-272.
19. Warner, H. R., and J. E. Barnes. 1966. Evidence for a dual role for the bacteriophage T4-induced deoxycytidine triphosphate nucleotidohydrolase. *Proc. Natl. Acad. Sci. U.S.A.* 56:1233-1240.
20. Warner, H. R., B. F. Demple, W. A. Deutsch, C. M. Kane, and S. Linn. 1980. Apurinic/aprimidinic endonucleases in repair of pyrimidine dimers and other lesions in DNA. *Proc. Natl. Acad. Sci. U.S.A.* 77:4602-4606.
21. 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.
22. 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.