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In Vitro Packaging of UV Radiation-Damaged DNA from Bacteriophage T7

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When DNA from bacteriophage T7 is irradiated with UV light, the efficiency with which this DNA can be packaged in vitro to form viable phage particles is reduced. A comparison between irradiated DNA packaged in vitro and irradiated intact phage particles shows almost identical survival as a function of UV dose when *Escherichia coli* wild type or *polA* or *uvrA* mutants are used as the host. Although *uvrA* mutants perform less host cell reactivation, the *polA* strains are identical with wild type in their ability to support the growth of irradiated T7 phage or irradiated T7 DNA packaged in vitro into complete phage. An examination of in vitro repair performed by extracts of T7-infected *E. coli* suggests that T7 DNA polymerase may substitute for *E. coli* DNA polymerase I in the resynthesis step of excision repair. Also tested was the ability of a similar in vitro repair system that used extracts from uninfected cells to restore biological activity of irradiated DNA. When T7 DNA damaged by UV irradiation was treated with an endonuclease from *Micrococcus luteus* that is specific for pyrimidine dimers and then was incubated with an extract of uninfected *E. coli* capable of removing pyrimidine dimers and restoring the DNA of its original (whole genome size) molecular weight, this DNA showed a higher packaging efficiency than untreated DNA, thus demonstrating that the in vitro repair system partially restored the biological activity of UV-damaged DNA.

Living organisms maintain repair mechanisms to correct damage caused to their DNA by deleterious agents such as radiation or foreign chemicals. Although much of our knowledge concerning repair processes is derived from genetic investigations, more recent efforts have focused on a biochemical approach with purified enzymes thought to be important to repair processes (8, 9, 11, 14) or with more complete quasi-in vitro systems (24, 33). Also, it has been possible to monitor steps in the excision repair process with in vitro systems using crude extracts prepared from *Escherichia coli* and exogenous substrates that contain a known number of damaged sites (23, 32). With one of these systems, UV-irradiated DNA from bacteriophage T7 was treated with a damage-specific endonuclease from *Micrococcus luteus* to introduce incisions at the site of pyrimidine dimers. Subsequent incubation with an extract from *E. coli* repaired the DNA, as indicated by a number of criteria, including removal of pyrimidine dimers, UV-stimulated DNA synthesis, and restoration of the DNA to its original molecular weight. Although these biophysical measurements suggest that this repair is complete, it was of interest to determine whether DNA repaired in vitro carried the potential for forming viable T7 phage.

Because DNA from bacteriophage T7 can transfect *E. coli* with only low efficiency (1, 5, 21), it is difficult to test the biological activity of T7 DNA by transfection. However, Kerr and Sadowski (13) have described an in vitro system capable of packaging T7 DNA. It was also shown that, under conditions where recombination could occur between exogenous DNA and endogenous DNA present in the extracts used for packaging, it was possible to encapsulate purified T7 DNA into phage heads with high (5%) efficiency (31). We have modified this system to encapsulate T7 DNA under conditions such that the amount of endogenous DNA present was minimized, and an effort was made to reduce recombination. This system, which is dependent upon exogenous DNA, shows good efficiency and a linear relationship between DNA concentration and phage produced. The system was used to measure the "survival" of T7 DNA irradiated and then packaged in vitro. We have also used this technique to measure the ability of an in vitro repair system to restore the biological activity of damaged DNA.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. The strains of *E. coli* K-12 used in this study included W3110, *thy*; D110, *thy end polA1*; DR110 (a *polA*$^+$ revertant

of D110); HMS146, *recB21*; JG138, *thy rha lacZ polA1*; TN207, *thy uvrA*, derived from W3110; HMS83, *polA1 polB100 thy rha lacZ lys* (3); HMS85, *polB100 thy lacZ lys* (3); and 011', *thy sup*$^+$.

Bacteriophage T7 and amber mutants, thereof, from F. W. Studier, were grown as described (34). The amber mutations used in this study were: gene 3, *am*29; gene 4, *am*20; gene 5, *am*28; and gene 6, *am*147. In the text, T7 mutants are designated by subscript notation indicating gene only; e.g., $T7_{3,6}$ refers to the double amber mutant *am*29 and *am*147.

Media and chemicals. L-broth, T-agar, and M9 media are described by Miller (26); L-broth was routinely supplemented with 10 μg of thymine per ml. Low-salt T7 diluent consists of 20 mM Tris-hydrochloride (pH 7.5), 10 mM $MgSO_4$, 10 μg of gelatin per ml, and 0.1 M NaCl (13).

Deoxyribonucleoside triphosphates were purchased from Schwarz/Mann Bioresearch. Spermidine was obtained from Sigma; bovine serum albumin (BSA) was purchased from Calbiochem.

DNA. Unlabeled and ^{3}H-labeled T7 DNA were prepared from wild-type T7, $T7_4$, or $T7_5$ phage grown on strain 011' as described by Richardson (30). All DNA concentrations are expressed as nucleotide equivalents.

Enzymes. An endonuclease from *M. luteus* (4), which specifically attacks pyrimidine dimers formed after UV irradiation, was generously provided by W. L. Carrier. Lysozyme was purchased from Calbiochem.

Preparation of phage-infected cell extracts for packaging. Extracts were prepared by a modification of the method described by Kerr and Sadowski (13). A 100-fold dilution of an overnight culture of suppressor-free *E. coli* was made into L-broth, and the cells were grown, with shaking, at 30°C to a density of approximately 5×10^8/ml (absorbance at 590 nm = 1.0). The cells were then infected with $T7_{3,5,6}$ at a multiplicity of infection of 3. (This multiplicity resulted in the killing of over 90% of the cells.) Infection was allowed to proceed for 18.5 min, at which time the culture was poured into a cold flask, chilled, and then centrifuged at 0°C for 5 min at $1,500 \times g$ in a Sorvall GSA rotor. The pellet was resuspended in low-salt T7 diluent (1/250 the original cell culture volume) and stored in a polycarbonate tube under liquid nitrogen. Cells could be stored in this way for months with no apparent loss of activity.

Immediately before use, the preparation of phage-infected cells was thawed rapidly at 30°C, incubated at 30°C for 2 min in the presence of 0.02 volume of lysozyme solution (1 mg/ml in 15 mM Tris-hydrochloride [pH 7.5]–7.5 mM $MgSO_4$–0.25 mM EDTA), and chilled to 0°C on ice. The extract was distributed in 0.020-ml portions into prechilled tubes.

Reaction conditions for packaging of DNA. DNA was diluted in 18 mM Tris-hydrochloride (pH 7.5), 22 mM $MgCl_2$, 9 mM spermidine, 10 mM 2-mercaptoethanol, 13 mg of BSA per ml, and 2% (wt/vol) sucrose. Ten-microliter samples of this reaction mixture, containing 1 pmol to 1 nmol of DNA per sample, were added to 20-μl portions of packaging extract and incubated at 30°C for 1 h. The reaction was terminated by the addition of 50 volumes of cold T7 diluent. After the appropriate dilutions were made in T7 diluent, 0.1-ml samples were mixed with 0.3 ml of suppressor-free indicator bacteria and 2.5 ml of soft T-agar, plated on T-agar plates, and incubated overnight at 30°C.

Preparation of cell extracts for repair of UV radiation-damaged DNA. The preparation of extracts from T7-infected bacteria and from uninfected bacteria has been previously described (12, 23, 36).

UV irradiation. DNA was irradiated in deep-well microscope slides with constant stirring at room temperature. DNA to be used in packaging reactions was irradiated in 135-μl quantities at a concentration of 0.1 mM. DNA to be used in the experiments on repair of UV-damaged DNA was irradiated, 50 to 100 μl at a time, at a concentration of 0.5 mM. A pair of germicidal lamps with an incident dose rate of 1.04 J/m^2 per s provided the UV source.

Reaction conditions for in vitro repair of UV radiation-damaged DNA. DNA (7.4 nmol) damaged by 0 to 25 J of UV radiation per m^2 was incubated at 30°C for 15 min, first in the presence of *M. luteus* endonuclease and then with extracts of uninfected HMS146, $T7_{3,6}$-infected D110, or $T7_{3,5,6}$-infected D110 under assay conditons previously described (23). The molecular weight of the repaired DNA was examined by alkaline sucrose gradient analysis (22) or, in some experiments, this DNA was added to packaging extracts to test for biological activity. Before addition to the packaging extract, the reaction mixtures were diluted 10-fold in a dilution buffer to give 22 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 9 mM spermidine, 18 mM Tris-hydrochloride (pH 7.5), 13 mg of BSA per ml, and 2% sucrose (final concentrations); 10-μl portions of this diluted reaction mixture were added to 20-μl samples of packaging extract. For gradient analysis, reactions were terminated by addition of EDTA to a concentration of 50 mM, and the samples were chilled before layering onto 5 to 20% alkaline sucrose gradients.

Other methods. Zone sedimentation in alkaline sucrose and determination of radioactivity have previously been described (22).

RESULTS

Characterization of in vitro packaging system. The in vitro system for encapsulating exogenous T7 DNA described by Sadowski and coworkers (13, 31) has been used to study in vitro recombination (31). However, the large amount of unirradiated endogenous DNA present in the extracts used in this packaging system, together with the possibility of in vitro recombination events, could complicate measurements of the efficiency with which irradiated DNA is packaged. In an effort to reduce the level of endogenous DNA present in the extracts used for packaging as well as the amount of molecular recombination, we attempted to perform in vitro packaging of wild-type T7 DNA using ex-

tracts of suppressor-free *E. coli* infected with T7 that carried amber mutations in genes 3, 5, and 6, three genes important in in vivo DNA replication and recombination (7, 15, 16, 27, 28, 32, 34). Previous studies (31) have shown that the products of genes 5 and 6 are also important for in vitro recombination. Use of this phage together with suppressor-free indicator bacteria reduced to zero the number of plaques found on control plates without exogenous DNA. To estimate the optimum conditions for packaging, relative efficiency was measured as a function of concentrations of magnesium, spermidine, and BSA. The results of these experiments are shown in Table 1. Magnesium was essential to in vitro packaging, and both BSA and spermidine improved the efficiency. Once standard conditions were adopted (as indicated in Materials and Methods), the reproducibility of the system was tested by comparing results obtained with extracts prepared under identical conditions and by comparing several assays performed with a single extract. Results from 140 assays derived from 29 extract preparations gave an average efficiency of 3×10^{-4} plaques per T7 genome equivalent; the standard deviation of these measurements was $\pm 2 \times 10^{-4}$. We encountered approximately the same variability between extracts as between assays using the same extract. At the lowest dilution tested, no plaques were found on the controls without exogenous DNA or without extract, indicating a background value at least four orders of magnitude less than the efficiencies found with complete reactions.

TABLE 1. *Effect of BSA, Mg^{2+}, and spermidine concentrations on packaging efficiency*[a]

Concn	Relative efficiency[b]
BSA (mg/ml)[c]	
0	1.0
3.2	12.3
6.5	12.3
13	16.9
19.5	6.5
26	1.4
Mg^{2+} (mM)[d]	
0	1.0
11	9.2
22	53.0
44	31.0
Spermidine (mM)[e]	
0	1.0
0.9	1.4
9.0	1.6
18.0	0.6
27.0	0.3

[a] Extracts from $T7_{3,5,6}$-infected W3110 were used to package T7 DNA present at 0.1 nmol in each reaction mixture. The amounts of added BSA, Mg^{2+} and spermidine-HCl in the dilution buffer were varied in each case from zero to the indicated concentration, with the other two ingredients held at the values shown in the footnotes. The number of phage produced was determined using strain W3110 as an indicator.

[b] The number of plaques produced at the indicated concentration relative to the phage produced with none of that ingredient added.

[c] At Mg^{2+} = 22 mM, spermidine = 9 mM.

[d] At BSA = 13 mg/ml, spermidine = 0.

[e] At BSA = 13 mg/ml, Mg^{2+} = 22 mM.

The effect of variation in DNA concentration was also measured under the standard conditions. In these experiments, extracts prepared from strain W3110 infected with $T7_{3,5,6}$ were used to package wild-type T7 DNA varying in concentration from 1 pmol to 1 nmol/reaction, and the infectivity was determined with strain W3110 as indicator bacteria. As seen from the 45° slope of the straight line in Fig. 1, the number of plaques found was directly propor-

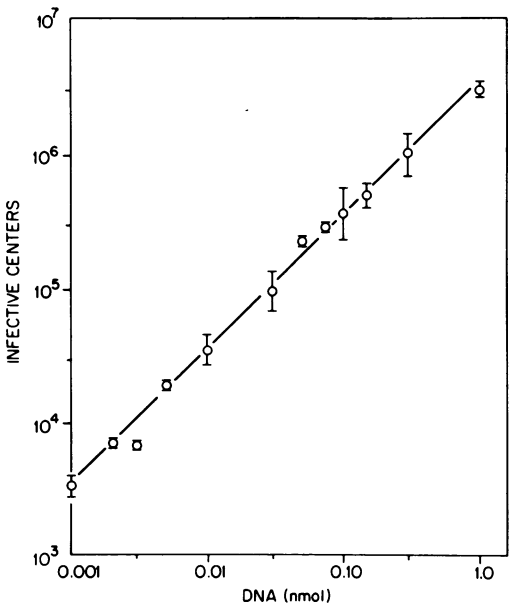

FIG. 1. *In vitro encapsulation of T7 DNA as a function of DNA concentration. Wild-type T7 DNA was diluted in 10 mM Tris-hydrochloride (pH 7.5) and then incubated with extracts prepared from strain W3110 infected with $T7_{3,5,6}$ before dilution and plating (in duplicate) on strain W3110. The number of infective centers produced was compared with the amount of DNA added to the reaction mixture. Average values are shown from two experiments with five separate determinations at each DNA concentration. Error bars reflect standard deviations.*

tional to the DNA concentration over a range of three orders of magnitude. Similar results were obtained with strains D110 and TN207 (data not shown).

Although in vitro recombination due to T7 gene products was expected to be low under the conditions described here, it was of interest to measure the level of molecular recombination directly. To do this, DNA was purified from T7 with amber mutations in gene 4 or 5. Extracts were prepared from strain W3110 infected with $T7_{3,5,6}$ and used to package these DNA preparations. Measurement of packaging efficiency by use of a suppressor-free indicator strain showed that the DNA with an amber mutation in gene 5 was expressed with an efficiency over four orders of magnitude lower than that found with wild-type DNA (Table 2). The DNA with an amber mutation in gene 4 was expressed about 100 times more efficiently than the DNA with a mutation in gene 5, possibly due to recombination with the endogenous DNA, which contains a wild-type copy of gene 4 (31). However, the mutant gene 4 DNA was expressed at about 1/300 the efficiency of wild-type DNA.

Effect of UV irradiation. To compare the effect of UV irradiation of intact T7 phage with irradiation of T7 DNA subsequently encapsulated into phage heads, three strains of *E. coli* were used. These included a wild type (strain W3110), a mutant deficient in the DNA synthetic activity of DNA polymerase I (strain D110), and a repair-deficient mutant (strain TN207) reported to lack an endonuclease responsible for incision at the sites of pyrimidine dimers (2, 32). First, the effects of UV irradiation in vivo were measured. Figure 2A shows

TABLE 2. *Efficiencies of packaging mutant DNAs*[a]

T7 DNA	DNA (nmol)	PFU	Efficiency	
			%	Relative to wild type
WT	0.03	5.74×10^4	2.55×10^{-2}	1.00
WT	0.10	1.73×10^5	2.31×10^{-2}	1.00
4^-	0.10	4.84×10^2	6.45×10^{-5}	2.65×10^{-3}
4^-	0.30	2.34×10^3	1.04×10^{-4}	4.28×10^{-3}
4^-	0.50	4.80×10^3	1.28×10^{-4}	5.27×10^{-3}
5^-	0.50	3.00×10^1	8.00×10^{-7}	3.29×10^{-5}

[a] DNA extracted from T7 wild type (WT), $T7_4$, or $T7_5$ was diluted in 10 mM Tris-hydrochloride (pH 7.5); the amounts of DNA shown were packaged using identical reaction mixtures and an extract prepared from strain W3110 infected with $T7_{3,5,6}$. The resulting phage were plated using strain W3110 as an indicator to give the indicated value of PFU. The ratio of wild type to $T7_4$ phage in the preparation used for the $T7_4$ DNA was 5×10^{-6}. This ratio for the $T7_5$ DNA was 4×10^{-5}.

that wild-type T7 phage show significantly lower survival (host cell reactivation) when plated on the excision-deficient host strain TN207 compared with wild-type strain W3110. The discontinuity in the survival curve at 7 J/m² has been observed by others (6, 20) and has also been seen with bacteriophage T1 (10), but its explanation is obscure. The *polA1* strain was identical to the wild type in its ability to support the growth of UV-irradiated T7. This result does not agree with an earlier report by other workers (20). However, this experiment was repeated several times with strains D110 (*polA1*), JG138 (*polA1*), HMS83 (*polA1 polB100*), and HMS85 (*polB100*), and in no case have we measured any difference in host cell reactivation when comparing wild-type strains with mutants deficient in DNA polymerase I or II. Figure 2B shows a similar experiment, except that in this case the T7 DNA was irradiated before being packaged into phage heads. The effects of this in vitro UV irradiation mimic the in vivo situation almost perfectly (including the discontinuity seen with a wild-type host). Thus, the in vitro packaging assay described here allows a sensitive and efficient determination of damage remaining in purified irradiated DNA, and the biological consequences of the damage closely parallel the in vivo situation. The data in Fig. 2 do not determine whether the packaging extract or the indicator strain is defective in UV repair. However, an experiment was performed in which unirradiated and irradiated DNA was packaged by extracts of both strains W3110 (wild type) and TN207 (*uvrA*), and the resulting phage was plated using each strain as an indicator. The results (Table 3) show that in all cases the UV sensitivity depended upon the indicator strain rather than the strain used to prepare the packaging extract. These data favor the argument that irradiated DNA is packaged into phage heads, but that these show reduced survival upon infection of a repair-deficient host.

One interesting aspect of the data in Fig. 2 is the lack of involvement of DNA polymerase I. Since the *uvrA* mutant, blocked in the first step of excision repair (2, 8), is clearly deficient in host cell reactivation, it appears that the excision repair pathway must contribute to the ability of bacteriophage T7 to survivie UV damage. Yet DNA polymerase I or II, either of which might be responsible for the resynthesis step (25, 35), does not seem to be involved. In addition to the possibility that residual DNA polymerase levels (17) in the bacterial mutants used here might suffice for the resynthesis step

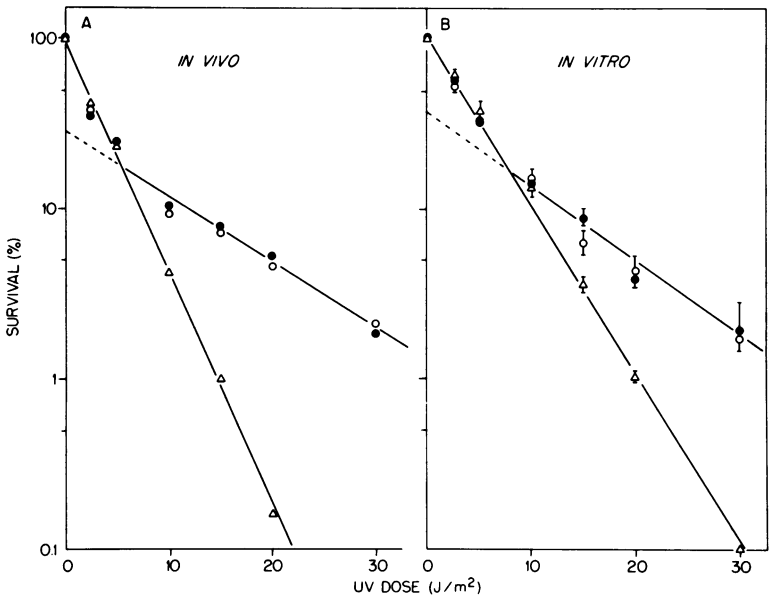

FIG. 2. *Comparison of survival of UV-irradiated T7 phage and T7 DNA packaged in vitro. (A) Suspensions of T7 phage diluted to 5 × 10⁸ PFU/ml in low-salt T7 diluent were irradiated at room temperature, 1.0 ml at a time, in 7-cm-diameter watch glasses with constant stirring at an incident dose rate of 1.04 J/m² per s. Phage were diluted in L-broth and plated on the indicated strain: (○) strain W3110 (wild type); (●) strain D110 (polA1); (△) strain TN207 (uvrA). (B) T7 DNA at 0.1 mM was irradiated with UV at the doses shown. The DNA was packaged in vitro by use of extracts prepared from T7 $_{3,5,6}$-infected cultures of the indicated strain. The resulting phage were plated on the same strain used to prepare the extracts for packaging. The number of phage resulting from irradiated DNA was compared with the unirradiated control to give the percent survival. Average values for several determinations are shown. Error bars indicate standard deviations; points without error bars had standard deviations smaller than the symbol. Symbols are the same as in (A).*

of excision repair, it was possible that *E. coli* DNA polymerase III (18) or T7 DNA polymerase (7) might be involved. An in vitro experiment was performed to test the involvement of T7 DNA polymerase in DNA repair. Strains D110 and DR110 were each infected with $T7_{3,6}$ or $T7_{3,5,6}$ and extracts were prepared (12). T7 DNA was UV irradiated and treated with a UV damage-specific endonuclease from *M. luteus*. The incised DNA was incubated with extracts from the phage-infected cells under conditions similar to those described for extracts prepared from uninfected *E. coli* (23), and the reaction mixtures were subjected to zone sedimentation on alkaline sucrose gradients. The addition of the damage-specific endonuclease was necessary since earlier studies (23, 32) had indicated that extracts of the type used here are essentially unable to incise UV-irradiated DNA. It was found (Fig. 3A), as expected, that extracts prepared from DR110 (*polA*⁺) infected with $T7_{3,6}$ or $T7_{3,5,6}$ could restore incised DNA to the same molecular weight as the unirradiated control. Extracts prepared from D110 (*polA1*) infected with $T7_{3,5,6}$ could not increase the molecular weight of incised DNA (Fig. 3B). However, a partial restoration of UV-damaged DNA was seen when extracts prepared from D110 infected with $T7_{3,6}$ were used. Thus, gene 5 mu-

TABLE 3. *Effect of indicator bacteria on UV survival of DNA packaged in vitro*[a]

Extract	Indicator	Survival (%)
W3110	W3110	4.2
W3110	TN207	0.66
TN207	W3110	3.2
TN207	TN207	0.53

[a] DNA, either unirradiated or irradiated with 20 J of UV per m², was packaged in vitro at the standard assay conditions described in the text, and packaging extracts were prepared from $T7_{3,5,6}$-infected strain W3110 (wild type) or TN207 (*uvrA*). The resulting phage were plated using both W3110 and TN207 as indicator bacteria. The percent survival represents a comparison of irradiated and unirradiated DNA packaged with the indicated extract and plated on the indicated plating bacteria.

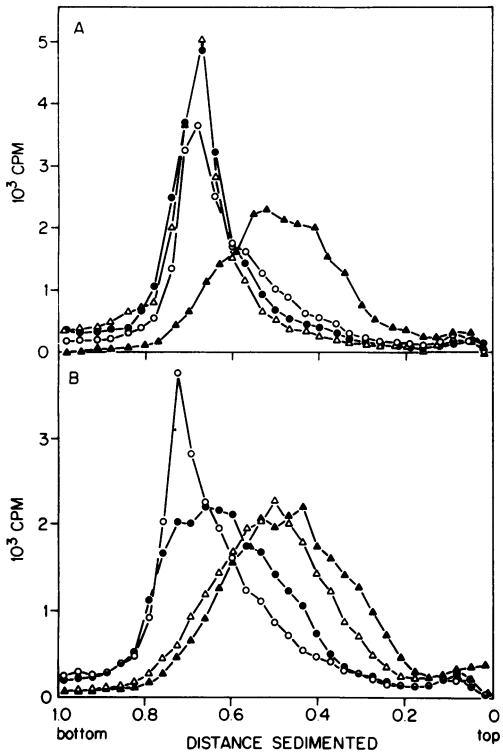

FIG. 3. *Repair of UV-damaged DNA in vitro.* ^{3}H-*labeled T7 DNA was irradiated with 25 J/m² and then incubated for 15 min at 30°C with a damage-specific endonuclease from M. luteus to produce single-strand breaks at the sites of pyrimidine dimers (4, 23). Portions of this DNA were incubated at 30°C for 15 min in 100-μl reaction mixtures containing 3 nmol of incised DNA, 30 mM Tris-hydrochloride (pH 7.5), 25 mM $MgCl_2$, 80 μM each of the four deoxyribonucleoside triphosphates, 0.25 mM NAD, 12 mM 2-mercaptoethanol, and 40 μl of gently lysed extracts prepared from strain DR110 or D110 (polA) that had been infected with $T7_{3,6}$ or $T7_{3,5,6}$ as previously described (12, 23). After the addition of 50 mM EDTA (final concentration), the reaction mixture and appropriate controls were sedimented through alkaline sucrose gradients in a Spinco SW56 rotor at 49,000 rpm for 120 min at 20°C. Profiles of acid-insoluble radioactivity recovered from these gradients are shown. (A) Results from strain DR110; (B) results from strain D110. Symbols:* (○) *unirradiated control;* (▲) *irradiated and treated with UV-endonuclease only;* (●) *reincubated with extract from $T7_{3,6}$-infected cells;* (△) *reincubated with $T7_{3,5,6}$-infected cells.*

tants, which have reduced levels of T7 DNA polymerase (7), are unable to perform in vitro DNA repair when *E. coli* DNA polymerase I levels are also low.

Resurrection of UV-damaged DNA. Strain D110 was used to measure the efficiency of packaging DNA repaired in vitro. DNA from wild-type T7 was irradiated with incident doses of UV from 0 to 20 J/m². Half of each sample was retained as an untreated control. The remainder was incubated first with a damage-specific endonuclease from *M. luteus* and then with an extract prepared from uninfected strain HMS146 (*recB21*), as previously described (23). An extract prepared from strain D110 infected with $T7_{3,5,6}$ was used to package the DNA in vitro, and infectivity was measured with strain D110 as indicator bacteria. Figure 4 shows the results of this experiment. It is apparent that removal of pyrimidine dimers and restoration of the DNA to its original molecular weight by in vitro repair (23) also results in a significant increase in the ability of this damaged DNA to be packaged into viable phage particles.

DISCUSSION

Restoration of biological activity is a demanding test for the fidelity of any in vitro DNA repair or DNA replication system. A difficulty in testing product T7 DNA from these systems arises from the poor infectivity of linear duplex T7 DNA in transfection assays (1, 5, 18). Therefore, to determine the extent to which UV-irradiated DNA could be correctly repaired in vitro, an in vitro packaging system (13, 31)

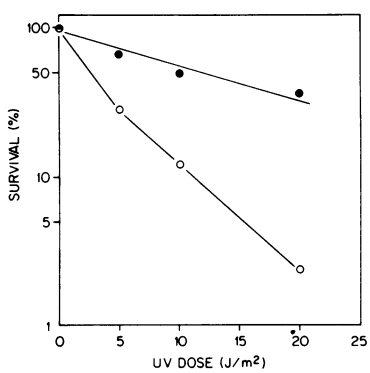

FIG. 4. *Repair of UV radiation-damaged DNA. T7 DNA was irradiated with the indicated doses of UV, and a portion was retained as an untreated control. The remainder was treated with a damage-specific endonuclease from M. luteus and then with a gently lysed extract from uninfected strain HMS146, as described in the legend to Fig. 3. The reaction mixtures were diluted with cold T7 diluent, 0.01 ml (containing 0.03 nmol of DNA) was packaged in vitro by the use of extracts prepared from D110 (polA) infected with $T7_{3,5,6}$, and the resulting phage were plated on strain D110. Comparison of the number of phage produced by the irradiated and unirradiated DNA gave percent survival as shown in the figure. Symbols:* (●) *repaired DNA;* (○) *untreated control.*

was used to monitor the biological activity of the damaged DNA. It was reported that exogenous DNA could be packaged most efficiently by extracts of T7-infected *E. coli* when molecular recombination occurred between exogenous DNA and the endogenous DNA present in the extracts (31). However, it was conceivable that recombination between endogenous DNA and the 5% or less of the exogenous DNA that is eventually expressed as phage particles might mask UV damage. In effect, a type of multiplicity reactivation (19) might occur in vitro. Also, it would be convenient to work with a packaging system that was strictly dependent upon exogenous DNA. It was found that by using T7 phage with DNA replication and recombination arrested by amber mutations in genes 3, 5, and 6, extracts of phage-infected, suppressor-free *E. coli* could be used to package exogenous wild-type T7 DNA into phage particles that could be expressed on suppressor-free indicator bacteria. The efficiency with which one genome equivalent of DNA could be made into a viable phage particle with these extracts was 0.03%, a value lower than that previously reported (31) but higher than the efficiencies found with transfection systems (5). The number of plaques produced was linear with DNA concentration up to 1 nmol of DNA per 0.03-ml reaction mixture (Fig. 1); no plaques were produced in the absence of exogenous DNA. A comparison of DNAs with amber mutations that could or could not be complemented by endogenous DNA suggested a low level of in vitro recombination. Since the experiment shown in Table 2 involved a double crossover between closely linked genes, this level of recombination may be significant. Thus, in spite of the multiple deficiencies in T7 genes important in recombination, our results do not rule out the possibility that recombination (perhaps due to the host enzymes) may play a role in in vitro packaging (31). The data in Fig. 2 indicate good agreement between the in vivo and in vitro survival of phage or DNA irradiated with doses of UV sufficient to introduce up to 15 pyrimidine dimers per T7 genome (23). This suggests that in vitro recombination does not reactivate the damaged DNA to any great extent and that in vitro packaging is an effective means of determining the amount of damage present in exogenous DNA.

The results shown in Fig. 2 indicate that wild-type levels of DNA polymerase I are not essential for host cell reactivation of UV-irradiated wild-type T7 phage. Figure 3 shows that extracts prepared from $T7_{3,6}$-infected strain DR110 or D110 can restore T7 DNA irradiated with low doses of UV and incised at each dimer site by a damage-specific endonuclease to about the same single-strand molecular weight as unirradiated DNA. However, similar extracts prepared from a *polA* mutant infected with $T7_{3,5,6}$ are unable to restore damaged DNA in vitro. The partial restoration of incised DNA performed in vitro by an extract from a $T7_{3,6}$-infected *polA* strain (Fig. 3B) does not guarantee that the gene 5 product can catalyze DNA repair in a biological sense. However, the in vitro repair deficiency associated with the gene 5 mutation offers a convenient explanation for the results obtained with the *polA* mutant in the experiment shown in Fig. 2 and invites speculation that the T7 DNA polymerase may be able to substitute for *E. coli* DNA polymerase I in executing repair resynthesis, perhaps in coordination with the remaining 5',3'-exonuclease of DNA polymerase I still present in this *polA* mutant (17). Also, our result offers assurance that there is little or no in vitro repair of incised DNA by extracts with reduced levels of both *E. coli* DNA polymerase I and T7 DNA polymerase.

An objective of the present study was to determine how accurately DNA could be repaired in vitro by incubation of UV-irradiated DNA, first with a damage-specific endonuclease from *M. luteus* to introduce incisions at the site of pyrimidine dimers and then with gently lysed extracts from uninfected *E. coli* to excise the dimers, resynthesize the damaged region, and restore the DNA to intact genome size by ligation. It had previously been shown by biophysical and biochemical criteria that DNA repair by this in vitro process was nearly complete, and that this repair was accompanied by repair resynthesis due to DNA polymerase I (23). However, restoration of biological activity could be accomplished only if all the damage was recognized by the *M. luteus* enzyme and if the resynthesis step was completed with high fidelity. To test this, irradiated T7 DNA was repaired in vitro and then encapsulated into phage particles. An extract from strain D110 infected with $T7_{3,5,6}$ was chosen to package the DNA in order to eliminate the possibility that some repair resynthesis might occur during packaging. Figure 4 shows that exposure of UV-irradiated DNA to the in vitro repair system increases the biological activity of the damaged DNA. The relative number of plaques produced by repaired DNA subjected to an incident dose of 20 J/m^2 corresponds to a dose of 4 J/m^2 in the untreated control, indicating removal of about 80% of the damage. It is not clear whether the remaining damage is due to the failure of the *M. luteus* endonuclease to recognize certain types of damage (29) or if a fraction

of single-strand breaks remains in the DNA after in vitro repair. Also, it is possible that in vitro reinsertion of incorrect bases during repair resynthesis might produce a small fraction of intact T7 genomes that cannot be expressed on a suppressor-free host.

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LITERATURE CITED

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