Translesion Synthesis is the Main Component of SOS Repair in Bacteriophage Lambda DNA

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Agents that interfere with DNA replication in *Escherichia coli* induce physiological adaptations that increase the probability of survival after DNA damage and the frequency of mutants among the survivors (the SOS response). Such agents also increase the survival rate and mutation frequency of irradiated bacteriophage after infection of treated bacteria, a phenomenon known as Weigle reactivation. In UV-irradiated single-stranded DNA phage, Weigle reactivation is thought to occur via induced, error-prone replication through template lesions (translesion synthesis [P. Caillet-Fauquet, M. Defais, and M. Radman, *J. Mol. Biol.* 117:95–112, 1977]). Weigle reactivation occurs with higher efficiency in double-stranded DNA phages such as λ , and we therefore asked if another process, recombination between partially replicated daughter molecules, plays a major role in this case. To distinguish between translesion synthesis and recombinational repair, we studied the early replication of UV-irradiated bacteriophage λ in SOS-induced and uninduced bacteria. To avoid complications arising from excision of UV lesions, we used bacterial *uvrA* mutants, in which such excision does not occur. Our evidence suggests that translesion synthesis is the primary component of Weigle reactivation of λ phage in the absence of excision repair. The greater efficiency in Weigle reactivation of double-stranded DNA phage could thus be attributed to some inducible excision repair unable to occur on single-stranded DNA. In addition, after irradiation, λ phage replication seems to switch prematurely from the theta mode to the rolling circle mode.

Exposure of *Escherichia coli* to agents that interfere with DNA replication leads to RecA-mediated cleavage of the LexA repressor, which induces a number of diverse physiological functions called the SOS response (32, 45). Among the multiple functions expressed during this response is a transient error-prone DNA processing event that seems to be responsible for most of the radiation- or chemical-induced mutagenesis (42). This phenomenon requires RecA-mediated cleavage of the UmuD protein and formation of a complex between the COOH fragment and the UmuC protein (3, 31, 37). The UmuDC complex may act by facilitating replication with a high level of misincorporation past noninstructive lesions in the DNA (2). Mutagenesis is observed not only on the bacterial chromosome but also on extra-chromosomal DNA, and it can easily be studied using viruses as probes (14). Induction of the host SOS response results in an increased survival of UV-irradiated bacteriophage (Weigle reactivation), which is accompanied by an increased mutation frequency in the progeny (13, 43). Genetic and biochemical evidence indicates that in single-stranded DNA phage, Weigle reactivation occurs via induced replication through the UV lesions (translesion synthesis) (6). Moreover in vitro studies of the extent of DNA synthesis on UV-irradiated ϕ X174 DNA have shown a large increase in the turnover of nucleoside triphosphates to free monophosphates through "idling" of DNA polymerases in front of lesions due to the associated 3'→5' exonuclease activity (38, 40). It has thus been proposed that the induced capacity to replicate the UV-irradiated template is made possible by an inhibition of the 3'→5' exonuclease (40). This

inhibition could be due to an action of RecA protein itself (16, 28).

Weigle reactivation and mutagenesis occur in double-stranded DNA phage with a higher efficiency than in a single-stranded DNA (12). We thus investigated the nature of the mechanism responsible for this difference. At least three mechanisms might account for Weigle reactivation of double-stranded DNA. (i) One mechanism might be translesion synthesis, which would proceed without any significant block of DNA replication on the damaged template and would depend upon functional *umuDC* genes, as for single-stranded DNA phage reactivation (12). (ii) Another possibility is recombinational repair of daughter strand gap following replication of the damaged DNA. This would convert the low-molecular-weight DNA obtained immediately after UV irradiation into high-molecular-weight DNA (18, 33). (iii) A third possibility is inducible excision repair, such as long patch repair (9, 10) depending on the *uvrAB* genes; mutants carrying a *uvrA* or *uvrB* mutation together with *umuDC* mutations are unable to reactivate lambda phage (20, 34).

In an attempt to distinguish the part played by the first two mechanisms, we have studied the early replication of UV-irradiated bacteriophage lambda in SOS-induced and uninduced *uvrA* bacteria. We present evidence that replication proceeds despite the presence of persisting lesions in the phage DNA, which suggests that translesion synthesis is the primary component of Weigle reactivation of lambda in the absence of excision repair. The greater efficiency of Weigle reactivation in double-stranded DNA phage may then be due to inducible excision repair which cannot repair single-stranded DNA (9, 10). In addition, after irradiation, the mode of lambda replication seems to switch prematurely from the theta type to the rolling circle type.

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MATERIALS AND METHODS

Bacterial and phage strains. Bacterial strains were MD177 *dnaA177 uvrA6 thi-1 thyA6 deoC lacY1 strA67 tonA21 supE44*, which was obtained by conjugating AB2437, HfrJ2 *uvrA6*, and E177 *dnaA177*; QD5003 *supF*, allowing plating of λ cI857 Sam7; and AG7, which is a thymine-requiring W3110 lysogen for λ cI857 Sam7. Phage were λ cI857 Sam7.

Media. To produce density-labeled phage stocks, M9 minimal medium (29) was supplemented with 1 mM MgSO_4 , 1 μg of thiamine per ml, 1 μM FeCl_3 , 1 mg of 98% substituted $^{15}\text{NH}_4\text{Cl}$ per ml, and 2 mg of 85% substituted $[^{13}\text{C}]\text{glucose}$ per ml. Both radioactive products were purchased from Centre d'Energie Atomique, Saclay, France. To produce light phage, M9 medium was supplemented with 0.2% Casamino Acids (Difco Laboratories, Detroit, Mich.) and 2 mg of glucose per ml. Tris-buffered salt solution containing 5 μg of thymidine per ml, 0.2% maltose, 0.4% Casamino Acids, 2.5 mM MgSO_4 , 1 μg of thiamine per ml, and 1 μg of FeSO_4 per ml was used for the phage replication experiment.

Radioactive phage stocks. Strain AG7 was grown overnight in supplemented heavy M9 medium at 30°C. It was then diluted 50-fold in fresh heavy medium. Exponential culture (about 5×10^8 bacteria per ml) was induced at 42°C for 15 min. [*methyl*- ^{14}C]thymidine (Centre d'Energie Atomique; 1 $\mu\text{Ci}/\text{ml}$; 50 mCi/mmol) was added, and the culture was held for 3 h at 37°C. Bacteria were concentrated 10-fold and kept overnight at 4°C in the presence of chloroform to allow lysis. DNase (Sigma Chemical Co., St. Louis, Mo.) (10 $\mu\text{g}/\text{ml}$) and RNase (Sigma) (50 $\mu\text{g}/\text{ml}$) treatments were performed at 37°C. Cell debris was centrifuged, and phage are titrated on QD5003 *supF* bacteria. Specific activity was routinely found to be approximately of 7.5×10^{-6} cpm per phage.

When phage were labeled with [*methyl*- ^3H]thymidine (Centre d'Energie Atomique; 2 $\mu\text{Ci}/\text{ml}$; 45 Ci/mmol), the same procedure was used except that bacteria were grown in light M9 medium. Phage used for DNA extraction were purified on a cesium chloride gradient in a SW50.1 rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 35,000 rpm. DNA was phenol (Friedel de Haen) extracted.

UV irradiation. Phage were irradiated in Tris-10 mM MgSO_4 with a UV germicide lamp at a dose of 40 J/m², producing on the average 20 pyrimidine dimers per phage DNA. This irradiation resulted in 1% phage survival which increased 10- to 20-fold under SOS-inducing conditions (data not shown). Bacteria at a concentration of $5 \times 10^8/\text{ml}$ were irradiated in 10 mM MgSO_4 with 7 J/m² to induce the SOS response.

Experimental procedure for isolation of replicative intermediates. An exponentially growing culture of MD177 *dnaA uvrA* was grown at 30°C in Tris-buffered medium to 1×10^8 to 2×10^8 bacteria per ml and then shifted for 90 min at 42°C to allow termination of the replication cycle and to prevent initiation of a new one. Bacteria were pelleted and suspended in 10 mM MgSO_4 . After irradiation, they were concentrated in 1 ml of Tris medium. Irradiated and intact [^{13}C]-density-labeled phage were allowed to adsorb on irradiated or unirradiated bacteria at 0°C for 30 min with a multiplicity of infection of 0.4 in order to prevent multiplicity reactivation. At this time, designated time zero, infected bacteria were diluted in 19 ml of prewarmed Tris-buffered medium, and phage were allowed to replicate at 42°C in the presence of 4 μl of [*methyl*- ^3H]thymidine per ml. Some 75 to 80% of the phage were adsorbed to the bacteria in either case as measured by radioactivity. Among these absorbed phage, 50% injected their DNA into the bacteria in each case (P.

Caillet-Fauquet and M. Defais, unpublished results). At different times, the incubation was stopped by pouring the sample in 10 ml of 10 mM Tris-1 mM EDTA in an ice bath. Bacteria were washed and suspended in 2 ml of 10 mM Tris-1 mM EDTA. Lysozyme (Sigma) was added at a concentration of 2 mg/ml, and lysis took place for 30 min at 0°C. Proteinase K (E. Merck AG, Darmstadt, Federal Republic of Germany) was added at 400 $\mu\text{g}/\text{ml}$ along with 0.05 ml of 10% Sarkosyl (CIBA-GEIGY Corp., Summit, N.J.). The samples were then incubated for 1 h at 37°C and subjected to a neutral cesium chloride gradient after the addition of 4.5 g of lysate at 5.9 g of CsCl (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The samples were centrifuged to equilibrium in a 50 Ti Beckman rotor at 30,000 rpm for 40 h at 20°C. Seven-drop fractions were collected. The total radioactivity of 20- μl fractions was counted in an automatic liquid scintillation system (model MR300; Kontron Instruments, Everett, Mass.). Fractions corresponding to each peak (HH, HL, and LL) were pooled for further analysis.

Alkaline and neutral sucrose gradients. Neutral sucrose gradients (5 to 20%; Merck) containing 1 M NaCl, 20 mM Tris (pH 8), and 5 mM EDTA were centrifuged at 40,000 rpm for 120 min in the SW50.1 Beckman rotor at 20°C. Alkaline sucrose gradients (5 to 20%) containing 1 mM EDTA, 70 mM NaCl, and 0.3 M NaOH were centrifuged at 45,000 rpm for 150 min at 20°C in the same rotor. Markers were [^3H]-labeled DNA *Hind*III fragments purified on 10 to 40% sucrose gradients in 1 M NaCl, 20 mM Tris (pH 8), and 5 mM EDTA and centrifuged at 26,000 rpm for 24 h in a SW27.1 Beckman rotor at 15°C. Fractions were trichloroacetic acid precipitated, and radioactivity was counted.

T4 endonuclease V treatment. DNA samples in 10 mM Tris hydrochloride (pH 8.0)-100 mM NaCl-10 mM EDTA containing 1 mg of bovine serum albumin per ml were incubated for 15 min at 37°C with an amount of T4 endonuclease V capable of incising 10^{10} dimers per min. Samples were then analyzed on alkaline sucrose gradients as described by Seawell et al. (35).

Southern blot hybridization. Lambda DNA from each peak of the CsCl gradient was treated with *Hind*III restriction enzyme according to conditions recommended by the supplier (Bethesda Research Laboratories), subjected to gel electrophoresis, and transferred to nitrocellulose filters following the procedure of Whal et al. (41).

HPLC analysis of thymidine dimers. Analytical high-performance liquid chromatography (HPLC) analyses were performed on a chromatograph (model 6000A pump and model U6K injector; Waters Associates, Inc., Milford, Mass.) attached to a variable-wavelength UV detector (model 481) with $\mu\text{Bondapak}$ C18 and Nucleosil C18 columns eluted with 0.1% tetrahydrofuran (Merck) at 0.3 ml/min. Three-drop fractions were collected into liquid scintillation vials containing 4 ml of Ready Solv M.P. (Beckman), and radioactivity was determined. Authentic cyclobutane thymine dimer standards were prepared by photosensitization of a thymine solution in the presence of acetone, using a Rayonet RPR100 reactor emitting at wavelengths less than at 300 nm as previously described (21). Stereoisomers were separated by using a semipreparative HPLC $\mu\text{Bondapak}$ column (0.78 by 30 cm) protected by a guard column and identified by their mass spectra and chromatographic behavior as previously described (4, 27). The *cis-syn* thymine dimers were used as chromatographic markers.

Phage were irradiated at different UV doses, and their DNA was phenol extracted, dialyzed against 10 mM Tris-1

mM EDTA, and lyophilized. Hydrolysis by formic acid was for 2 h at 180°C. Hydrolysates were analyzed by HPLC, and radioactivity was counted. Increasing UV fluences up to 100 J/m² produced a linear increase in the amount of thymine dimers (data not shown).

Bacteria (MD177) were labeled with [³H]thymidine overnight (2 µCi/ml) and during exponential growth (4.5 µCi/ml) at 30°C. They were subsequently incubated at 42°C for 90 min. The culture was then irradiated (100 J/m²) and divided into two samples, one of which was immediately treated with 1.3 mg of lysozyme per ml and 100 µg of DNase-free RNase per ml and phenol extracted. The bacterial DNA was then hydrolyzed as described above. Specific activity was between 17×10^3 and 20×10^3 cpm/µg of DNA. The other half of the culture was incubated for 15 min at 42°C in the dark and then treated as described above.

RESULTS

Kinetics of lambda replication during Weigle reactivation.

To analyze how the bacterial replication machinery deals with lesions in double-stranded phage DNA, we studied lambda early replication in SOS-induced *uvrA* bacteria infected with irradiated phage at a low multiplicity of infection (≈ 0.4). This precluded reactivation of phage by recombination between damaged lambda genomes, since at this multiplicity of infection, statistically, about 60% of the bacteria were uninfected and 35% contained only one phage genome. To distinguish between phage and bacterial replication, we used [¹⁴C]-prelabeled [¹³C]-density-labeled phage (see Materials and Methods), and to minimize bacterial replication, we used *E. coli dnaA uvrA* mutants at the nonpermissive temperature to prepare the lambda replication intermediates. The *dnaA* gene product is not necessary for lambda replication initiation (17). In addition, a shift to 42°C, preventing new initiation of bacterial replication, does not induce the SOS response (8, 30). The host cells were SOS induced prior to infection by exposure to an irradiation of 7 J/m². We compared lambda replication intermediates at different times after infection in (i) intact phage infecting an unirradiated host, (ii) irradiated phage infecting an unirradiated host, (iii) irradiated phage infecting an irradiated host. In each case, the cells were lysed and intracellular lambda DNA was analyzed in CsCl density gradients. We analyzed lambda intermediates at times from 5 to 15 min after infection. Typical profiles are shown in Fig. 1. The presence of lambda DNA in each peak was confirmed by Southern hybridization (Fig. 2). Phage replication proceeds bidirectionally (theta structure) from a unique origin during the early phase of the lytic growth. This phase ends 15 min after infection. At later times DNA is synthesized in a rolling circle mode, forming concatemers which represent multiple units of lambda DNA.

In the case of unirradiated phage after 5 min of infection, Fig. 1A shows a typical separation of parental unreplicated DNA (HH), replicated molecules from the first replication round (HL), and those molecules owing to later rounds of replication, including HL and LL molecules.

Figure 1B represents the replication of irradiated lambda in the nonirradiated host after 7 minutes of infection, while Fig. 1C shows the replication of irradiated lambda in irradiated bacteria after the same incubation time. When lambda phage were irradiated, the singly replicated molecules (HL) were not as well separated from the light peak (LL) as in the control; irradiation of the host did not change this result (compare Fig. 1B and C). One possible explanation for this finding could be that when phage are irradiated they change

FIG. 1. Analysis of replicating lambda DNA on neutral CsCl gradient. (A) Unirradiated phage replicating in unirradiated bacteria after a 5-min infection. (B) Irradiated phage (40 J/m²) replicating in unirradiated bacteria after a 7-min infection. (C) Irradiated phage (40 J/m²) replicating in irradiated bacteria (7 J/m²) after a 7-min infection. Symbols: ●, parental ¹⁴C-labeled DNA; ○, newly synthesized ³H-labeled DNA. Data represent the radioactivity incorporated in phage DNA alone, since a control gradient containing only uninfected bacterial lysate from a parallel culture was run at the same time and the resulting radioactivity (less than 10%) owing to residual bacteria replication was subtracted. The presence of lambda DNA in each peak was confirmed by Southern hybridization (Fig. 2).

from theta to rolling circle replication earlier than they usually do, thereby preventing a good separation between hybrid density (HL) and light (LL) molecules.

We analyzed lambda replication intermediates at different times after infection in the three cases described above (Fig. 3). After 5 min of infection, about 65% of the unirradiated viral DNA was undergoing replication. The same amount of irradiated DNA was replicated in 6 to 7 min. At early times, there was no significant difference between DNA replication in the induced and the noninduced host. However, after several rounds of replication, the SOS-induced bacteria replicated irradiated lambda DNA more efficiently than did the uninduced host. Thus, in the absence of excision, Weigle reactivation could be detected within 15 min of infection by the phage. We note that phage replication did not seem to be blocked or significantly delayed at early times after infection by the presence of 20 pyrimidine dimers per phage DNA molecule. This result contrasts with the block of replication observed in bacterial DNA containing 300 pyrimidine dimers, which represent about one-fifth to one-sixth the density of lesions in the irradiated phage genome (31).

Analysis of replicating DNA. We wished to determine the events occurring in double-stranded phage DNA containing lesions and undergoing replication. At least two possibilities could be envisaged. (i) As already shown in bacteria, gaps could be generated in daughter strands opposite the lesions, and these could then be ultimately repaired. (ii) Translesion synthesis could permit replication past lesions in an error-prone fashion.

We reasoned that if daughter strand gap repair was involved in Weigle reactivation of lambda phage, this recom-

FIG. 2. Southern blot of lambda DNA contained in each peak of a CsCl gradient. Phage and bacteria were irradiated. Replication took place for 15 min. Fractions of each peak were pooled, and the same volume (60 μ l) was treated by *Hind*III restriction enzyme, run on a 1% agarose gel for 11 h at 50 V, transferred to nitrocellulose paper, and hybridized with 32 P-labeled lambda DNA (gift of J. Palmer). Lanes: A, lambda DNA control; B, lambda DNA from HH peak; C, lambda DNA from HL peak; D, lambda DNA from LL peak.

binational process should produce hybrid-density DNA molecules, detectable upon analysis performed under denaturing conditions. For this purpose we pooled fractions 9 to 12 of Fig. 1B and C corresponding to the well-defined hybrid

FIG. 3. Kinetics of lambda DNA synthesis in induced and non-induced bacteria. The amount of DNA recovered from CsCl gradients is expressed as the ratio of 3 H-labeled newly synthesized material to the 14 C-labeled phage DNA input. Each point is an average of 10 experiments. Symbols: $\odot$, unirradiated phage, unirradiated bacteria; $\bullet$, irradiated phage (40 J/m 2), irradiated bacteria (7 J/m 2); $\blacksquare$, irradiated phage (40 J/m 2), unirradiated bacteria.

FIG. 4. Average size (Kb, kilobases) of irradiated lambda DNA. Lambda phage were irradiated (40 J/m 2), and DNA was phenol extracted before treatment with T4 endonuclease V. The sample was loaded onto an alkaline sucrose gradient. The markers were 3 H-labeled lambda DNA *Hind*III restriction fragments.

density peak of unirradiated phage and analyzed their densities in alkaline cesium chloride gradients. We were able to detect radioactivity peaks corresponding only to heavy and light strands (data not shown), implying that if strand exchange did occur, it was not significant. An additional possibility is that DNA in the HL peak came from replicating undamaged DNA molecules. However, as can be seen in Fig. 4, when [3 H]-labeled irradiated phage DNA was treated with T4 endonuclease V and sedimented on an alkaline sucrose gradient, we did not detect any full-size, intact DNA, which would have sedimented at the bottom of the gradient. Instead, the observed size of the DNA fragments indicate that an average of 10 dimers per single-strand molecule was produced by UV irradiation of the DNA.

We then undertook an analysis of the size distribution of the newly synthesized strands sedimenting at the hybrid position in the neutral CsCl gradient (Fig. 1) at early times after infection, i.e., 7-min incubation for irradiated phage and 5-min incubation for intact phage. For this purpose, the same fractions 9 to 12 of Fig. 1A, B, and C were analyzed on alkaline sucrose gradients. Purified [3 H]-labeled lambda DNA *Hind*III fragments were run in a parallel gradient at the same time as markers. If discontinuities were present in daughter strands at each lesion, the mean size of the newly synthesized DNA should have been around 5 kilobases, corresponding to the interdimer distance measured in 40-J/m 2 -irradiated lambda DNA after digestion with the T4 endonuclease V (Fig. 4). Instead, we observed that the newly synthesized strand of irradiated phage undergoing replication in SOS-induced bacteria had a mean size of about 23 kilobases, which is approximately half the size of a

FIG. 5. Average size (Kb, kilobases) of newly synthesized lambda DNA in SOS-induced or control bacteria measured on alkaline sucrose gradients. a, Average size of lambda DNA; b, average size of newly synthesized lambda DNA; c, average size of newly synthesized lambda DNA when irradiated phage (40 J/m^2) infected irradiated bacteria (7 J/m^2); d, average size of newly synthesized lambda DNA when irradiated phage (40 J/m^2) infected unirradiated bacteria; e, interdimer distance in irradiated phage DNA (40 J/m^2) as determined by treatment with T4 endonuclease V. Each bar represents an average of six experiments. The mean size of each DNA molecule was calculated by comparing its migration to the migration of a marker.

replicated molecule (Fig. 5). This indicates that the induction of the SOS response allowed the replication machinery to synthesize DNA continuously on the damaged template. A more striking result came from the size analysis of new strands of irradiated phage infecting noninduced bacteria. In this case, newly synthesized DNA was composed of segments ranging around 19 kilobases, implying that even when bacteria were not SOS induced, the replication machinery did not seem to be blocked at every lesion, in contrast to bacterial replication (18, 33). Thus, when bacteria have to replicate damaged phage DNA, they apparently synthesize DNA continuously. The lesions in this case do not seem to represent a block in the progression of replication forks.

In light of the above results, it was important to verify that the pyrimidine dimers were still present in phage DNA extracted from bacteria.

We attempted to determine the amount of pyrimidine dimers by using the T4 endonuclease V assay (35). We found that when replicating phage DNA (HL peak) was extracted from cell lysates, the enzyme was able to recognize only one-fourth of the sites that it recognized when the DNA was extracted from purified phage UV irradiated at the same fluence (data not shown). This was also true when we tried to measure the amount of pyrimidine dimers in the unreplicated DNA (HH peak). This insensitivity of UV lesions to dimer-specific enzyme has already been reported for dimer-containing DNA in *E. coli* B/r DNA after replication (36). When these bacteria receive a sublethal UV dose before UV irradiation they do not efficiently excise the lesion. The processing of the lesions evidently modifies them in such a way that they become insensitive to *Micrococcus luteus* enzyme but are still detectable by radiochromatography. We thus decided to measure the amount of thymine dimers by HPLC. We first prepared and purified *cis-syn* thymine dimers to be used as chromatographic markers (see Materials and Methods). Irradiation of phage-labeled DNA in vitro produced the expected amount of thymine dimers (Fig. 6).

FIG. 6. Reverse-phase chromatography of a hydrolysate of UV-irradiated lambda DNA. [^3H]thymidine-labeled lambda phage were irradiated in Tris-10 mM MgSO_4 with 100 J/m^2 . Lambda DNA was phenol extracted, lyophilized, hydrolyzed as described in Materials and Methods, and analyzed on HPLC. Thymine dimers represent 0.175% of the label. The theoretical value is 0.236%.

However, to detect the thymine dimer peak, after an irradiation of 40 J/m^2 , phage DNA representing about 4×10^6 cpm was charged on the column (Fig. 6). In *in vivo* experiments, 50 times less phage had to be used to keep a low multiplicity of infection. In such a case, the thymine dimers peak was hardly detectable. We thus labeled bacteria and measured the persistence of thymine dimers in bacterial DNA immediately and 15 min after irradiation, a time well beyond the phage incubation times. For this purpose, bacteria were labeled overnight and during exponential growth and shifted to 42°C for 90 min, as during the lambda replication experiments. The cultures were irradiated and divided in two portions, one of which was immediately lysed and phenol extracted, while the other was further incubated for 15 min before lysis. Samples were then treated as described in Materials and Methods. Thymine dimers were present in equal amounts in bacterial DNA in both cases (Table 1). This shows that the *uvrA* mutation of the host cell was not leaky. We assumed then that dimers were still present in equal amounts in lambda phage DNA under our experimental conditions (1) and were only partially sensitive to specific enzymes possibly because of their processing.

DISCUSSION

It is thought that UV mutagenesis occurs via an error-prone bypass of UV lesions (32, 42). This translesion DNA

TABLE 1. HPLC thymine dimer quantification in *E. coli* DNA

Incubation time (min)	Radioactivity (cpm)		% Thymine dimers ^a
	Total thymine	Thymine dimers	
0	1.183×10^6	5.77×10^3	0.487
15	1.003×10^6	4.5×10^3	0.450

^a Theoretical value, 0.5%.

synthesis could be facilitated by transient inhibition of the 3'→5' proofreading exonuclease activity of DNA polymerases (38, 40). It has been shown that SOS induction results in a better efficiency of replication of damaged single-stranded phage DNA (Weigle reactivation) (6). To gain more information on the mechanism of Weigle reactivation on double-stranded DNA, we have examined the early replication of UV irradiated bacteriophage lambda DNA. We report here that in *uvrA dnaA* strains, SOS induction leads to a measurably increased capacity to replicate past pyrimidine dimers in UV-irradiated lambda phage DNA. However, we observed that the presence of 20 pyrimidine dimers per phage DNA molecule did not seem to represent a strong block for replication, even in unirradiated cells (Fig. 3 and 5). In fact, there was no significant difference in the amount of replicated irradiated phage DNA in the uninduced or induced host at early times after infection, although more DNA was replicated at later times under SOS-induced conditions (Fig. 3).

As UV-irradiated phage DNA started to replicate, DNA synthesis intermediates could indirectly induce the SOS response. This indirect induction might allow *E. coli* replicative machinery to synthesize DNA on the damaged phage genome. It was previously shown that UV-irradiated lambda is able to partially induce a *sfiA::lacZ* fusion (11). Another argument in favor of indirect SOS induction by irradiated lambda is the slight increase in mutagenesis observed in phage progeny when irradiated lambda infects intact host (7, 13). Thus, the presence of the SOS proteins partially induced by the replicating phage would allow bacterial replication machinery to bypass a certain number of lesions (Table 1) and to synthesize phage DNA. When bacteria were induced before infection, lesions would be bypassed more efficiently. This would explain the longer newly synthesized strand in replicating DNA and the higher overall replication (Fig. 3 and 4).

On the other hand, it has been proposed that *E. coli* DNA polymerase III holoenzyme has the capability of synthesizing DNA through pyrimidine dimers (25, 26). Indeed, it was demonstrated that in vitro elongation on a damaged template is possible to a certain extent when single-strand-binding protein is present together with DNA polymerase III holoenzyme. If the same is true in vivo, it could explain why the size of the newly synthesized strand of irradiated phage infecting uninduced host cells during early replication is longer than the interdimer distance (Fig. 5). In addition, the appearance of a novel form of *E. coli* DNA polymerase I, PolI*, after induction of the SOS system has been reported (23, 24). This relatively low-fidelity polymerase could also account for the capacity of the cell to replicate damaged template. However this polymerase is not induced in *polA1* mutants, even though both Weigle reactivation and mutagenesis of lambda as well as bacterial UV mutagenesis occur in these mutants to the same extent as in excision-deficient strains (5, 44). It is then unlikely that PolI* is responsible for the SOS processing of the lesions.

Recombination repair could also contribute to Weigle reactivation of lambda. However, this did not seem to be a significant effect, since we could not detect any well-defined hybrid peak corresponding to parental molecules containing part of daughter strands in alkaline CsCl gradients (data not shown). An additional piece of information indicating that recombination does not play an important role in phage SOS repair comes from the observation that no difference was found in phage survival after infection of *recA430*, deficient

only in the coprotease activity, or *recA* deletion mutants in a *lexA(Def)* background (7).

The observation that the sedimentation profile of the replicating DNA of unirradiated lambda phage is different from that of the irradiated phage (compare Fig. 1A and B) was unexpected. A possible explanation could be that irradiated lambda undergoes a modification in its mode of replication, i.e., an early switch to rolling circle. An analogous change has been described previously for lambda molecules replicating in a thymine-requiring host in a low concentration of thymidine in which a dramatic increase in rightward unidirectionally replicating molecules was observed (39). This unidirectional mode of replication has also been demonstrated for *E. coli* itself during thymine deprivation (15). It is possible that this unidirectional mode of replication is not blocked by the presence of DNA damage, since it also occurs in UV-irradiated Hfr strains or F factors during indirect SOS induction (19).

In addition, it has been recently shown that bulky lesions present in only one strand of a duplex DNA trigger the selective loss of this strand in the progeny (22). When lesions are present in both strands, either one strand or the other but not both is conserved in the progeny. The authors proposed that when lesions are uniformly distributed, the conserved strand would be the one on which translation synthesis had proceeded first. This process could be favored by a unidirectional mode of replication.

In conclusion, by studying lambda replication intermediates during Weigle reactivation in the absence of pyrimidine dimer excision, we infer that replication occurs by a translation synthesis mechanism. This synthesis is likely *umuDC* dependent, since *umuDC* functions are required to obtain full Weigle reactivation in a *uvrA* background (20, 33). We could not verify this requirement for UmuDC proteins to replicate lambda under our conditions, since we were unable to isolate a *dnaA uvrA umuC* triple mutant, which appears not to be viable. Moreover, the higher efficiency of Weigle reactivation of double-stranded DNA phage compared with that of the single-stranded phage is probably due to inducible excision repair, which does not occur on single-stranded DNA (9, 10).

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