Proc. Natl. Acad. Sci. USA
Vol. 75, No. 7, pp. 3037–3041, July 1978
Biochemistry

Mechanism of ultraviolet-induced mutagenesis: Extent and fidelity of *in vitro* DNA synthesis on irradiated templates*

(pyrimidine dimers/DNA polymerases/proofreading exonuclease/misincorporation)

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Communicated by Walter Gilbert, February 21, 1978

ABSTRACT The effect of UV irradiation on the extent and fidelity of DNA synthesis *in vitro* was studied by using homopolymers and primed single-stranded ϕX174 phage DNA as substrates. Unfractionated and fractionated cell-free extracts from *Escherichia coli pol*$^+$ and *polA1* mutants as well as purified DNA polymerase I were used as sources of enzymatic activity. (DNA polymerases, as used here, refer to deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7.) The extent of inhibition of DNA synthesis on UV-irradiated ϕX174 DNA suggested that pyrimidine dimers act as an absolute block for chain elongation by DNA polymerases I and III. Experiments with an irradiated poly(dC) template failed to detect incorporation of noncomplementary bases due to pyrimidine dimers. A large increase in the turnover of nucleoside triphosphates to free monophosphates during synthesis by DNA polymerase I on irradiated ϕX174 DNA has been observed. We propose that this nucleotide turnover is due to idling by DNA polymerase (i.e., incorporation and subsequent excision of nucleotides opposite UV photolesions, by the 3′→5′ "proofreading" exonuclease) thus preventing replication past pyrimidine dimers and the potentially mutagenic event that should result. In support of this hypothesis, DNA synthesis by DNA polymerase from avian myeloblastosis virus and by mammalian DNA polymerase α, both of which are devoid of any exonuclease activity, was found to be only partially inhibited, but not blocked, by UV irradiation of the template and accompanied by an increased incorporation of noncomplementary nucleotides. It is suggested that UV mutagenesis in bacteria requires an induced modification of the cellular DNA replication machinery, possibly an inhibition of the 3′→5′ exonuclease activity associated with DNA polymerases.

UV irradiation of λ and ϕX174 bacteriophages results in killing, but not mutagenesis, of phage after infection of untreated host cells (1, 2), whereas UV irradiation of the host cells causes mutagenesis of both untreated and irradiated phage (1–4). Furthermore, UV-irradiated single-stranded ϕX174 DNA extracted from untreated host cells was found to be replicated only to the first pyrimidine dimer (5, 6). Caillet-Fauquet *et al.* (6) found that UV irradiation of the host cell leads to an enhancement of the DNA synthesis on UV-irradiated ϕX174 DNA *in vivo*, which led them to propose that pyrimidine dimers in the ϕX174 DNA can become mutagenic by causing misincorporation of deoxyribonucleotides opposite them.

This inducible mutagenic system is thought to be part of a complex cellular emergency response ("SOS induction") that is triggered by unrepaired DNA lesions (such as pyrimidine dimers). The response also includes the arrest of cellular division, the arrest of aerobic metabolism, and the induction of prophage in lysogenic bacteria (7, 8).

DNA polymerases (deoxynucleosidetriphosphate:DNA deoxynucleotidyltransferase, EC 2.7.7.7) are known to be able to control the fidelity of DNA synthesis by selection of the correct nucleotide (9) and by possession of a "proof-reading" 3′→5′ exonuclease activity that can detect and excise 3′-terminal mismatched bases (10, 11).

We have sought to understand the molecular mechanisms underlying UV-induced mutagenesis by an examination of the role of DNA polymerases in the *in vitro* replication of damaged DNA templates.

MATERIALS AND METHODS

Bacterial and Phage Strains. *Escherichia coli* HF 4714 carrying suppressor II (suII$^+$) and HF 4704, *thyA*$^-$ lacking a suppressor mutation (su$^-$) were used as permissive and nonpermissive host strains, respectively, for ϕX174 Eam 3 phage. *E. coli* GC 714 (*his-4 proA2 argE3 lac*$^+$ *galK2 str-3 sfiA11 tif-1 uvrA*), *E. coli* DM 1180 (*thr leu his ilv*$_{ts}$ *str*^{R} *tif-1 sifA11 lexA3*), and *E. coli* HMS50 (lacking DNA polymerase I and endonuclease I and requiring thymine) were provided by J. George, D. Mount, and C. Richardson, respectively.

Enzymes, Homopolymers, and Other Materials. *E. coli* DNA polymerase III was fraction V of the purification scheme of Livingston and Richardson (12); DNA polymerase I "large fragment," according to Setlow *et al.* (13), was purchased from Boehringer Mannheim. One unit of enzyme activity incorporates 10 nmol of dNMP into acid-insoluble material during 30 min at 37° using the reaction conditions of Richardson *et al.* (14). DNA polymerase α from calf spleen was purified through DEAE-cellulose, phosphocellulose, hydroxylapatite, and DNA-cellulose column chromatography (15). *E. coli* DNA polymerase I and AMV (avian myeloblastosis virus) DNA polymerase (reverse transcriptase) purified to apparent homogeneity (16), were kind gifts from L. Loeb. Photoreactivating enzyme purified from *Streptomyces griseus* was a gift of G. Veldhuisen, University of Leyden. *Blu* II restriction enzyme, which is an isoschizomer of *Hae* III restriction enzyme (17), was provided by M. Van Montagu. Poly(dC) and oligo(dG)$_{12-18}$ were from Collaborative Research. Poly[^{14}C](dT) and oligo[^{14}C](dA) were kindly prepared by F. Campagnari. Unlabeled deoxyribonucleotide triphosphates were purchased from Boehringer Mannheim. [^{3}H]- and [^{32}P]deoxyribonucleoside triphosphates and [^{14}C]- and [^{3}H]-thymidine were purchased from Amersham Radiochemical Center (U. K.). Polyethyleneimine-impregnated cellulose thin-layer plates (TLC plastic sheets, PEI-cellulose F) were from Merck.

Primed ϕX174 DNA. Template–primer complex was constructed by using phage ϕX174 [^{14}C]DNA (single-stranded) to which short complementary fragments of ϕX174 RF-I DNA

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Abbreviation: AMV, avian myeloblastosis virus.

* This is the second paper in the series "Molecular Mechanisms of Induced Mutagenesis." The first paper is ref. 6.

(covalently closed, replicative form) were annealed. Preparation of the two DNA species has been described (18). The specific radioactivity was 4×10^{-7} cpm per single-strand molecule. Purified RF-I supercoiled DNA was nicked by either pancreatic DNase (*Method A*) or *Blu* II restriction enzyme (*Method B*) (details will be published elsewhere).

Method A. Nicked RF-I DNA molecules were mixed in equal nucleotide amounts with [^{14}C]DNA (single-stranded; final concentration, 5 μg/ml) in 10 mM Tris·HCl, pH 8/10 mM EDTA/10 mM NaCl. DNA was denatured by addition of NaOH (to 0.1 M final concentration) at 42° for 10 min. The mixture was neutralized by 1:10 dilution with 1.8 M Tris·HCl/0.2 M Tris base, pH 6.1. Annealing of homologous DNA fragments was achieved by addition of 50% formamide (Merck) for 2 hr at room temperature and followed by dialysis against 50 mM Tris·HCl, pH 8/1 mM EDTA/10 mM NaCl. The resultant DNA was characterized and purified by sedimentation through a neutral sucrose gradient. A sedimentation coefficient ($s_{20,w}$) of 24.8 S corresponds to ϕX174 single-stranded molecules which are, on average, 15% double-stranded. Visualization of this DNA by electron microscopy, kindly performed by G. Michel-Maenhaut, has shown mostly circular phage ϕX174 DNA with one double-stranded fragment.

Method B. ϕX RF-I DNA at 50 μg/ml was cleaved to completion by *Blu* II restriction endonuclease in 10 mM Tris·HCl, pH 7.5/6.6 mM $MgCl_2$/6 mM 2-mercaptoethanol for 4 hr at 37°. The reaction was stopped by addition of EDTA to a final concentration of 150 mM. All DNA was cleaved into 11 fragments, as observed in 1.5% agarose gel electrophoresis, mixed in a 1:3 ratio with ϕX174 [^{14}C]DNA (single-stranded), and annealed according to *Method A*.

UV Irradiation of Primed Templates. UV irradiation of homopolymers in 50 mM Tris·HCl, pH 8/20 mM KCl and of primed ϕX174 [^{14}C]DNA in 50 mM Tris·HCl, pH 8/1 mM EDTA/10 mM NaCl was carried out as described (6). Fluxes were measured by a Latarjet dosimeter.

***In Vitro* Photoreversal of Pyrimidine Dimers.** Photoreactivation of UV-irradiated ϕX174 DNA was carried out in 0.15 M NaCl/0.015 M sodium citrate, pH 7.0, for 1 hr by flash photolysis at 37° in the presence of saturating amounts of photoreactivating enzyme. The flash light, 10 kW-sec (1 kW-hr = 3.6×10^6 J), was filtered through glass and water layers.

Cell-Free Extracts. Cells were grown to a concentration of 5×10^8 bacteria per ml in rich medium [1% (wt/vol) bactotryptone/0.5% (wt/vol) yeast extract, 1% (wt/vol) NaCl] supplemented with 15 μg of thymidine per ml when required, centrifuged at room temperature for 10 min at 8000 × *g*, resuspended in 0.02 vol of 10% (wt/vol) sucrose/50 mM Tris·HCl (pH 7.5) at room temperature, quickly frozen in liquid nitrogen, and stored at −20° until used. Cell-free extracts were prepared as described (19).

Assays for DNA Synthesis. The assay mixtures (0.1 ml) for *E. coli pol*+ cell-free extract or purified DNA polymerase I contained 50 mM Tris·HCl(pH 8), 6 mM $MgCl_2$, 1 mM dithiothreitol, 2 nmol of each of the four deoxyribonucleoside triphosphates, 100–200 pmol of primed ϕX174 [^{14}C]DNA, and either 50–100 μg of protein from cell-free extracts or 0.07 unit of DNA polymerase I. The assay mixture (0.1 ml) for *E. coli polA1* cell-free extract was the same as for purified DNA polymerase III and contained 50 mM Tris·HCl, (pH 7.4), 10 mM $MgCl_2$, 5 mM dithiothreitol, 5 mM ATP, 5 mM spermidine·HCl, 5 nmol of each of the four deoxyribonucleoside triphosphates, 400–500 pmol of primed ϕX174 [^{14}C]DNA, and 50–100 μg of protein from cell-free extracts. The ^{3}H-labeled deoxyribonucleoside triphosphate had a specific activity of 500–1000 cpm/pmol. Reactions were carried out at 30°, aliquots were withdrawn at indicated times and spotted onto GF/C glass fiber filters, which were washed by the method of Bollum (20), and radioactivity was measured in Omnifluor scintillation fluid.

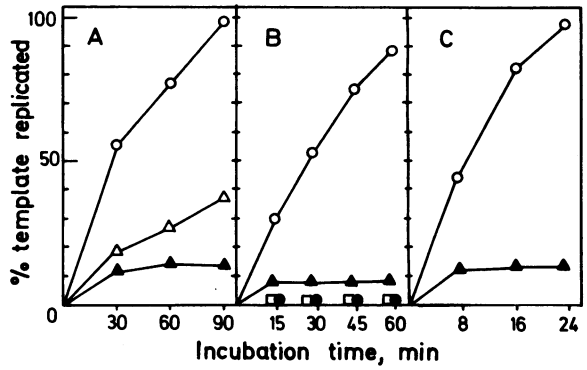

FIG. 1. Extent of DNA synthesis on intact and UV-irradiated primed ϕX174 phage DNA. Sources of DNA polymerase activity are: (*A*) cell-free extract of *E. coli pol*+, (*B*) cell-free extract of *E. coli polA1*−, (*C*) DNA polymerase I (large fragment). Polymerization in *A* was carried out essentially by DNA polymerase I (see *Materials and Methods*) and in *B*, by DNA polymerase III complex because the reaction conditions were optimal for this enzyme and the activity was inhibitied by 0.2 M KCl (22). Knowing both the specific radioactivity of the ϕX174 DNA template and the fraction covered by the primer fragments, it was possible to express DNA synthesis as percentage acid-insoluble template replicated. Because a substantial fraction of the template was covered by primer fragments, both 5′→3′ exonucleotic degradation and strand displacements of these primer fragments could increase the amount of template available for DNA synthesis. Thus, the estimates of the residual DNA synthesis on irradiated templates (see Table 1) may be overestimated. ○, Intact template; ▲, UV-irradiated template (100 J/m^2); △, UV-irradiated (100 J/m^2) photoreactivated template; □, reaction without template; ●, reaction in the presence of 0.2 M KCl.

Assays for Misincorporation. Assays for misincorporation are described in the legend to Table 2.

Assay for Incorporation and Hydrolysis of Newly Incorporated Nucleotides. Both incorporation and subsequent hydrolysis of newly incorporated deoxyribonucleotides were assayed by using one-dimensional thin-layer chromatography to separate the radioactive DNA template, labeled dNTP, and dNMP derived from labeled dNTP after the reaction was completed. Polyethyleneimine thin-layer chromatography was performed as described by Hershfield (21), except that the areas corresponding to dNMP, dNTP, and DNA template (the origin) were scraped off and their radioactivity was measured directly in Omnifluor scintillation liquid. The substrate was primed ϕX174 [^{14}C]DNA and the labeled nucleotides were [^{3}H]dTTP and [^{3}H]dATP.

RESULTS

Inhibition of *In Vitro* DNA Synthesis by *E. coli* DNA Polymerase I and III on UV-Irradiated ϕX174 DNA. Single-stranded ϕX174 phage DNA was primed by annealed fragments of ϕX174 RF-I DNA. Up to 100% of the input nonirradiated ϕX174 DNA template can be replicated in polymerization reactions by cell-free extracts from *E. coli pol*+ (Fig. 1*A*; ref. 22) or from *E. coli* lacking DNA polymerase I (Fig. 1*B*) or by purified *E. coli* DNA polymerase I "large fragment" (Fig. 1*C*). In agreement with previous studies (23–26), all three polymerization reactions were similarly inhibited by UV irradiation of the ϕX174 DNA template. The dose response of the inhibition of DNA synthesis suggests that each pyrimidine

Table 1. Relationship between the numbers of pyrimidine dimers per genome, lethal hits, and the extent of DNA synthesis by *E. coli* DNA polymerases on primed φX174 DNA

UV dose, J/m²	No. of Pyr-Pyr*	No. of lethal hits†	Residual synthesis‡ Cell extracts *Pol*+	*Pol*A1	DNA pol I large fragment
0	0	0	1	1	1
50	4	5	ND	0.24	0.23
100	8	10	0.15	0.12	0.15
200	16	§	ND	0.08	0.09
500	32	§	ND	0.041	0.035

ND means not done.

* No. of pyrimidine dimers (Pyr-Pyr) per φX174 DNA from ref. 1.

† Number of lethal hits equals −ln of surviving fraction at the indicated doses. The survival curve of the φX174 phage (exponential up to 100 J/m²) was kindly provided by P. Caillet-Fauquet and M. Defais.

‡ The residual DNA synthesis on irradiated DNA may be slightly overestimated (see legend to Fig. 1).

§ Survival curve is not exponential at these doses.

dimer is both an absolute block to polynucleotide chain elongation *in vitro* and a lethal hit *in vivo* (Table 1), as was shown by *in vivo* studies (6). This conclusion is supported by the finding that *in vitro* monomerization of pyrimidine dimers by photoreactivation enzyme could restore approximately 25% of the template activity (Fig. 1*A*). The probable reason that the restoration of template activity was not more efficient is that only one of the four possible isomers of pyrimidine dimers induced by UV irradiation of the single stranded DNA can be photoreactivated (27).

Turnover of Nucleoside Triphosphates during DNA Synthesis by DNA Polymerase I on UV-Irradiated Template. *E. coli* DNA polymerases I and III possess the "proofreading" 3′→5′ exonuclease activity which could clearly be involved in the failure of these enzymes to replicate a damaged template. To examine this possibility, both incorporation of nucleoside monophosphates from labeled triphosphates into primed φX174 DNA and subsequent production of free nucleoside monophosphates from incorporated labeled nucleotides were followed in a single assay during synthesis by DNA polymerase I (large fragment) using unirradiated and irradiated DNA as templates (see *Materials and Methods* and legend to Fig. 2).

This analysis was done with a purified DNA polymerase fraction that was free of DNA-independent triphosphatases. In addition, the large fragment of DNA polymerase I (13), which lacks the 5′→3′ exonuclease (nick translation) but possesses the 3′→5′ exonuclease activity, was used to avoid possible production of free monophosphates by the 5′→3′ exonuclease degradation of newly incorporated nucleoside monophosphates. Results obtained with [³H]dTTP (Fig. 2*A*) and [³H]dATP (Fig. 2*B*) as precursors clearly showed an increased, continued production of free monophosphates even after a complete arrest of DNA synthesis on UV-irradiated φX174 DNA. In contrast, DNA synthesis on nonirradiated DNA produced only small amounts of free monophosphates. This result indicates that the arrest of DNA synthesis by pyrimidine dimers is accompanied by an increased turnover of nucleoside triphosphates. When total nucleotide turnover was calculated from Fig. 2, no preference for dATP turnover over dTTP turnover was observed with UV-irradiated template. The differences observed using unirradiated template are not very accurate in view of the small amounts of free monophosphates produced.

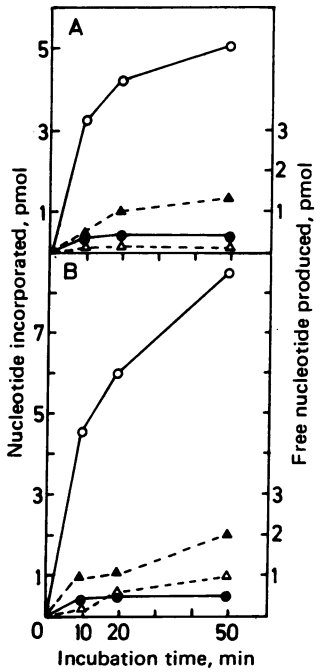

FIG. 2. Incorporation and subsequent hydrolysis of the newly incorporated deoxyribonucleotides by purified DNA polymerase I (large fragment) on intact and UV-irradiated primed φX174 DNA. The assay mixture (30 μl) contained 50 mM Tris·HCl (pH 8), 10 mM $MgCl_2$, 1 mM dithiothreitol, bovine serum albumin at 100 μg/ml, 500 pmol of each of the four deoxyribonucleoside triphosphates, 60 pmol of nucleotide of primed φX174 [¹⁴C]DNA, and 0.03 unit of DNA polymerase I (large fragment). The ³H-labeled deoxyribonucleoside triphosphate, either dTTP (*A*) or dATP (*B*) had a specific activity of 1000 cpm/pmol. Reactions were carried out at 30° and, at indicated times, 6-μl aliquots were spotted on polyethyleneimine-cellulose plates and the chromatography was performed. No significant conversion of dNTP to dNMP was found in the absence of either enzyme or substrate. ○, Incorporation on intact template; ●, incorporation on irradiated template (500 J/m²); △, free dNMP production on intact template; ▲, free dNMP production on irradiated template.

The observed production of nucleoside monophosphates by DNA polymerase I on a damaged template, even in the absence of detectable DNA synthesis, could be explained by repeated attempts of the polymerase to incorporate nucleotides opposite pyrimidine dimers, followed by exonucleolytic 3′→5′ excision of the resulting nucleoside monophosphates. We will refer to this phenomenon as polymerase "idling."

Extent of DNA Synthesis on UV-Irradiated φX174 DNA by AMV DNA Polymerase. If the 3′→5′ exonuclease activity of *E. coli* DNA polymerases I and III were to remove nucleotides inserted opposite (or near) pyrimidine dimers, thereby effectively blocking replication past dimers, the DNA polymerases lacking the 3′→5′ exonuclease may replicate a damaged template more effectively than polymerases having this activity. Fig. 3 shows that this is true for AMV reverse transcriptase, an error-prone DNA polymerase devoid of any exonuclease activity (16), which copies UV-irradiated φX174 DNA far above the level copied by *E. coli* DNA polymerase I.

Fidelity and Extent of DNA Synthesis on UV-Irradiated Poly(dC) Template. Pyrimidine dimers are bulky structural deformations in DNA and are the principal UV-induced mutagenic lesions (8) that drastically affect hydrogen bonding properties of the involved bases. If they were frequently copied, the overall effect would be a higher rate of incorporation of incorrect bases than on an unirradiated template. To study the fidelity of *in vitro* DNA synthesis, three different DNA

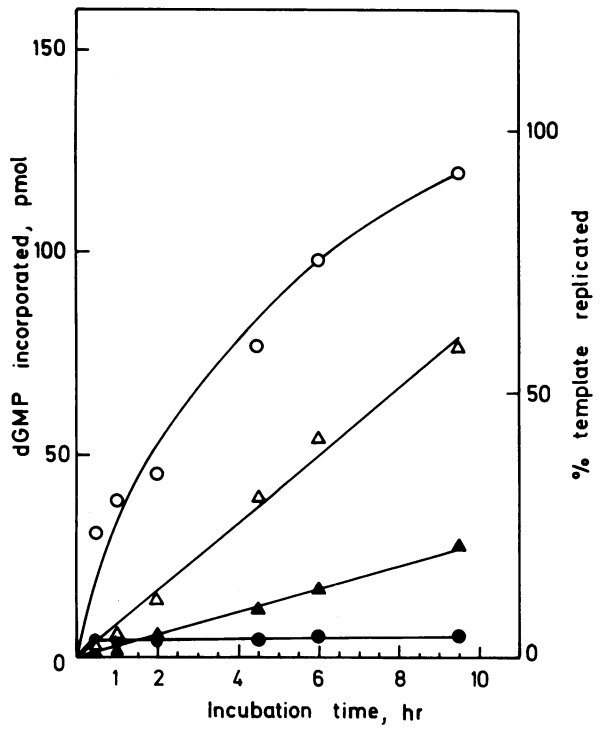

FIG. 3. Extent of DNA synthesis by AMV DNA polymerase ("reverse transcriptase") on intact and UV-irradiated primed ϕX174 phage DNA. The assay mixture (0.2 ml) contained 50 mM Tris·HCl (pH 8), 5 mM $MgCl_2$, 5 mM dithiothreitol, 20 mM KCl, 5 nmol each of the four deoxyribonucleoside triphosphates, 5 nmol of nucleotide of primed ϕX174 [^{14}C]DNA, and 2 μg of AMV DNA polymerase or 2 units of *E. coli* DNA polymerase I. The specific activity of [^{3}H]dGTP was 1000 cpm/pmol. Reactions were carried out at 30°, aliquots (20 μl) were withdrawn at indicated times, and acid-insoluble counts were determined. Curves show incorporation by *E. coli* DNA polymerase I on intact template (○) and on UV-irradiated (200 J/m^2) template (●). They also show incorporation by AMV DNA polymerase on intact template (△) and on irradiated template (▲).

Table 2. Fidelity and inhibition of DNA synthesis on UV-irradiated poly(dC) template

Enzymes	UV dose, J/m^2	Incorporation, pmol dGMP	Incorporation, pmol dAMP	Error frequency × 10^4
E. coli DNA pol III	0	796	~0.009	~0.1
	10,000	149	ND	ND
	50,000	17	ND	ND
AMV reverse transcriptase	0	3,599	1.03	2.8
	10,000	1,471	0.59	4.0
	50,000	711	0.65	9.1
Calf spleen DNA pol α	0	613	0.10	1.6
	10,000	688	0.25	3.6
	50,000	159	0.20	12
E. coli DNA pol III*	0	495	~0.021	~0.42
	5,000	320	~0.011	~0.34
	15,000	73	~0.008	~1.1

The reaction mixtures for *E. coli* DNA polymerase III and AMV reverse transcriptase are described in *Materials and Methods* and the legend to Fig. 3, respectively. DNA polymerase α was assayed in 30 mM phosphate buffer, pH 7.2/1 mM dithiothreitol/0.1 mM EDTA/10 mM $MgCl_2$/250 μg bovine serum albumin per ml/6% glycerol. All reaction mixtures contained 250 μM poly(dC)·oligo(dG) hybridized in a 2:1 ratio, 50 μM [^{3}H]dGTP (50 cpm/pmol) as correct nucleotide, and 30 μM [^{32}P]dATP (10,000 cpm/pmol) as incorrect nucleotide and were incubated for 4 hr at 30°, during which all reaction kinetics were found to be linear. Reactions were stopped by addition of 100 μl of carrier DNA at 1 mg/ml and 2 ml 10% perchloric acid and pelleted by centrifugation. To eliminate excess soluble radioactivity, pellets were resuspended in 0.2 M NaOH, reprecipitated twice with perchloric acid, and finally spotted onto GF/C glass fiber filters (20). The subtracted blank values (no template added) for dATP were 400–700 cpm.

* Additional results of synthesis by *E. coli* DNA polymerase III on templates at low UV fluxes are given in order to facilitate comparison of error frequency at levels of inhibition of DNA synthesis similar to those found for the other polymerases.

polymerases were tested with UV-irradiated and intact poly(dC) (Table 2) and poly(dT) (results not shown) homopolymers as templates. Synthesis by *E. coli* DNA polymerase III showed the highest fidelity and the greatest sensitivity to inhibition by UV-irradiation, whereas synthesis by purified, exonuclease-free, mammalian DNA polymerase α and by AMV reverse transcriptase was less inhibited by UV-irradiation and showed a high increase in misincorporation.

DISCUSSION

E. coli DNA polymerases I and III possess the same enzymatic activities (12). Once initiated, DNA synthesis by these two enzymes appears to be similar by all measureable parameters (12, 22) and their behaviors toward pyrimidine dimers also appear to be similar in two different experiments (Fig. 1 and Table 1). Therefore, all we have learned about DNA synthesis by DNA polymerase I on irradiated template may also apply to DNA polymerase III,† which is more likely to be involved in UV-induced mutagenesis (28).

Because there is an absolute blockage of DNA synthesis (Fig. 1) and an excessive turnover of nucleoside triphosphates (Fig. 2) with *E. coli* DNA polymerase I at pyrimidine dimers and because such blockage is not observed with purified AMV DNA polymerase (Fig. 3) and human DNA polymerases α, β, and γ (15, 24, 26) devoid of detectable 3′→5′ exonuclease activity, it is likely that the 3′→5′ exonuclease (proofreading) activity is responsible for the lack of DNA synthesis past pyrimidine dimers *in vitro* and *in vivo*. We propose that the 3′→5′ exonuclease activity provokes idling of DNA polymerase at each nonpairing DNA lesion, due to repeated excision of all nucleotide residues incorporated opposite such DNA lesions. In this way, the exonuclease activity prevents chain elongation. The same proofreading activity could clearly account for lack of mutagenesis of UV-irradiated phage in unirradiated host cells (1, 2). The observed increased efficiency of repair and mutagenesis of irradiated phage in irradiated cells (1–3, 6) could be due to inhibition of the 3′→5′ exonuclease activity, which would be consistent with results indicating a decreased fidelity of DNA synthesis by crude extracts of "SOS-induced" bacteria (24). Such an inhibition of proofreading exonuclease activity could then allow DNA synthesis across pyrimidine dimers (survival) accompanied by an incorporation of noncomplementary nucleotides (mutagenesis) opposite pyrimidine dimers and also elsewhere on undamaged DNA. This proposal is in agreement with recent findings that there is a certain lack of base specificity in UV-induced mutagenesis in *E. coli* (30).

There is a high proportion of double-neighbor base changes originating at the sites of pyrimidine doublets (J. Miller, personal communication) that could originate from a double

† Our attempts to measure nucleoside triphosphate turnover during synthesis that was catalyzed by DNA polymerase III failed because only a purified, complete system for *in vitro* synthesis of ϕX174 DNA can be used for this purpose (29).

misincorporation event opposite a pyrimidine dimer. A smaller proportion of UV-induced mutations in *E. coli* does not occur in sequences containing pyrimidine doublets (J. Miller, personal communication). These mutations could originate from the proposed diminished fidelity caused by inhibition of the proofreading exonuclease (6, 26), as well as from lesions other than pyrimidine dimers.

Genetic studies have suggested that there is a class of "nonpairing" (as distinguished from mispairing) DNA lesions, such as pyrimidine dimers, that induce a mutagenic capacity (SOS repair) in bacteria (7, 8, 15). The production of free monophosphates by DNA polymerases idling at such nonpairing DNA lesions may well be the initial inducing signal leading to the inactivation of λ repressor (31) and of cellular SOS repressors (26, 32), probably by proteolytic cleavage. If SOS induction produced an inhibition of the 3′→5′ exonuclease activity, this would lead to diminished production of free nucleoside monophosphates. It would also permit DNA synthesis across DNA lesions, and hence turn off the inducing signal. This simple mechanism would account for the observation that mutagenic DNA repair is turned on after damage to DNA and then off again once DNA synthesis is restored.

A discussion relating results of this paper to DNA repair and mutagenesis of double-stranded DNA genomes of both bacterial and mammalian cells by UV light and other mutagens can be found elsewhere (15, 26).

Drs. K. Backman, M. Errera, A. Falaschi, M. S. Fox, I. Molineux, and, in particular, Drs. L. Loeb and D. Mount and Mrs. C. de Buyl have contributed to the quality of this manuscript. G.V. and S.B. were Euratom fellows. This research was supported by Fonds Cancérologique of the Caisse Genenerale d'Epargne et de Retraite of Belgium and by Research Contract Euratom–Université Libre de Bruxelles 224-76-I Biology Belgium.

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