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Biochemical analysis of UV mutagenesis in *Escherichia coli* by using a cell-free reaction coupled to a bioassay: Identification of a DNA repair-dependent, replication-independent pathway

(SOS mutagenesis/error-prone repair/UmuC/RecA/carcinogenesis)

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ABSTRACT **Incubation of UV-irradiated plasmid DNA with a protein extract prepared from *Escherichia coli* cells led to the production of mutations in the *cro* gene residing on the plasmid. The mutations were detected in a subsequent bioassay step, which involved transformation of an indicator strain with the plasmid DNA that was retrieved from the reaction mixture, followed by plating on lactose/MacConkey plates. UV mutations produced in this cell-free reaction required the *recA* and *umuC* gene products and were prevented by rifampicin, an inhibitor of RNA polymerase, which inhibited plasmid replication. Removal of pyrimidine photodimers from the plasmid by enzymatic photoreactivation after the *in vitro* stage, but prior to transformation, increased plasmid survival as expected. Surprisingly, it also caused a large increase in the frequency of UV mutations detected in the bioassay. This photoreactivation-stimulated *in vitro* UV mutagenesis was dependent on the excision repair genes *uvrA*, *uvrB*, and *uvrC* and occurred in the absence of DNA replication. This suggests that two distinct UV mutagenesis pathways occurred *in vitro*: a replication-dependent pathway (type I) and a repair-dependent pathway (type II). DNA sequence analysis of type II UV mutations revealed a spectrum similar to that of *in vivo* UV mutagenesis. When the photoreactivation step was included in the protocol, type II UV mutagenesis did not require the RecA and UmuC proteins. These results are in agreement with the *in vivo* delayed photoreactivation phenomenon, where the removal of photodimers after an incubation period eliminated the requirement for RecA and UmuC in UV mutagenesis. The above system will enable the biochemical analysis of UV mutagenesis and the isolation of proteins involved in the process.**

UV mutagenesis in *Escherichia coli* is an active process that requires the involvement of a specific set of proteins, occurs at a defined time span, and is manifested as a specific spectrum of DNA sequence alterations (1, 2). It is believed that UV mutagenesis occurs opposite a UV lesion located in a segment of single-stranded DNA (1, 3). Such a premutagenic site can be generated by interruption of DNA replication at the lesion or by excision repair of closely opposed UV lesions, where the removal of a UV lesion from one strand results in a single-stranded DNA excision gap containing the second UV lesion. Which of these mechanisms accounts for UV mutagenesis is an unsettled issue. On the basis of the kinetics of fixation of UV mutations, it was suggested that in wild-type (wt) cells UV mutagenesis occurs primarily at excision repair gaps, whereas in excision repair-deficient cells, mutations arise at replication forks that were stalled by UV damage (3–5).

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The key step in UV mutagenesis is thought to be a trans-lesion DNA synthesis reaction (also termed bypass synthesis), which converts the single-stranded DNA segment carrying the UV lesion into duplex form. According to this model, the reaction is carried out by a specialized form of DNA polymerase that has the capability of polymerizing through UV lesions (1, 3, 6, 7). The nature of this DNA polymerase is not clear, but it is likely to be derived from DNA polymerase III and may be assisted by SOS-inducible proteins. This suggestion is supported by the dependence of UV mutagenesis on the SOS-controlled genes *recA* (8, 9), *umuD*, and *umuC* (10, 11) and on the α subunit of DNA polymerase III holoenzyme (12–15), the major replicative polymerase in *E. coli* (16). The bypass synthesis step is likely to be similar in the two pathways of UV mutagenesis since in both wt and *uvr* mutants UV mutagenesis shows the same genetic dependence on *recA*, *umuC*, and *umuD*. It has been proposed that UV mutagenesis involves two distinct steps: misincorporation, in which a nucleotide is polymerized opposite the lesion in a *recA*- and *umuD/C*-independent reaction, and extension past the lesion, which requires the RecA and UmuD/C proteins (17, 18).

In an attempt to elucidate the molecular mechanism of UV mutagenesis, we have developed an assay system based on a cell-free UV mutagenesis reaction coupled to a bioassay. Using this system, we present evidence for the existence of both replication-dependent and repair-dependent pathways of UV mutagenesis.

MATERIALS AND METHODS

Materials. Sources were as follows: unlabeled deoxynucleoside triphosphates and ribonucleoside triphosphates, Pharmacia; [α-^{32}P]dATP (400 Ci/mmol; 1 Ci = 37 GBq), Amersham; Hepes, polyethylene glycol 8000, creatine kinase, and creatine phosphate, Sigma; ammonium sulfate, ICN; proteinase K and RNase A, Boehringer Mannheim; *S*-adenosylmethionine, New England Biolabs; Bacto MacConkey agar, Difco.

Bacterial Strains and Plasmids. The *E. coli* K-12 bacterial strains and their relevant genetic characteristics are AB1157, wt; SR1165, same as AB1157 but *umuC122*::Tn*5* (19); SR559, wt; SR260, same as SR559 but Δ*uvrB* (20); N3137 and N3124, same as AB1157 but *uvrA*::Tn*10* and *uvrC*::Tn*10*, respectively; IT1870, wt; EST1779, same as IT1870 but Δ*recA* (21); TK701, wt; TK702, same as TK701 but *umuC36* (22). Strain WBM535 [Δ*recA*::Tn*10* Δ(*pro–lac*) (λ200 *imm21 ind*) F′*lacI*^{q}*Z*$^-$*Y*$^+$*pro*$^+$] was constructed as follows. A *recA* deletion was introduced into *E. coli* CSH26 Δ(*pro–lac*) by P1 transduction. F′*lacI*^{q}*Z*$^-$*Y*$^+$*Pro*$^+$ (23) was introduced into the resultant Δ*recA* strain by conjugation, after which the cells

Abbreviations: wt, wild type; EPR, enzymatic photoreactivation.
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were lysogenized with phage λ200 *imm21 ind* that carried an operator/promoter (O_RP_R)–*lacZ* fusion (24).

Plasmid pOC2 is a pBR322 derivative carrying the *cro* repressor gene of phage λ and the *bla* and *kan* genes, which encode ampicillin and kanamycin resistance, respectively. It was constructed by eliminating from plasmid pAP101 (25) the *Eco*RV–*Sty* I DNA fragment carrying most of the *tet* gene and replacing it with the 1.4-kilobase *Hae* II fragment carrying the *kan* gene from plasmid pACYC177.

UV Irradiation of Plasmid DNA. DNA (113 fmol of circles per μl) in 10 mM Tris·HCl, pH 7.5/1 mM EDTA was spread on Parafilm as 3-μl droplets and UV irradiated at 254 nm on ice by using a low-pressure mercury lamp. The dose rate was 0.6 $J \cdot m^{-2} \cdot sec^{-1}$ as determined by a UV-products radiometer using a UVX-25 sensor. Under these conditions, an average of 0.05 photodimers per pOC2 molecule per $J \cdot m^{-2}$ was produced, determined as described (26).

Preparation of the Protein Extract (Fraction II). The protein extract (70–100 mg/ml, determined according to Bradford; ref. 27) was prepared essentially as described by Fuller *et al.* (28), except that 0.47 g of ammonium sulfate was added to each ml of fraction I.

The *in Vitro* Reaction. The standard replication mixture (25 μl) contained 40 mM Hepes·KOH (pH 7.6), 10 mM $MgCl_2$, 40 mM KCl, 2 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 0.5 mM UTP, 100 μM dATP, 100 μM dCTP, 100 μM dGTP, 100 μM dTTP, 200 μM *S*-adenosylmethionine, 40 mM creatine phosphate, 4 mM dithiothreitol, creatine kinase at 0.2 mg/ml, 5% polyethylene glycol 8000, 0.3 μg of plasmid pOC2, and ≈300 μg of protein (fraction II). When required, 2–10 μCi of [α-^{32}P]dTTP were added. The reaction components were mixed on ice and then incubated at 30°C for 40–60 min. Incorporation of the radiolabeled dNTP into the acid-insoluble material was determined as described (26).

The *cro* Mutagenesis Bioassay. After completion of the *in vitro* reaction, the mixtures were heat inactivated for 10 min at 65°C, and the DNA was purified as follows: the proteins were digested by proteinase K treatment (final concentration of 0.4 mg/ml, 1 hr at 37°C), after which the samples were extracted with phenol and precipitated with ethanol. RNA originating from the protein extract was digested with RNase A, followed by another phenol extraction and ethanol precipitation. After this purification, whenever desired, photodimers were eliminated from the DNA by enzymatic photoreactivation (EPR) by using *E. coli* DNA photolyase (a generous gift from A. Sancar, University of North Carolina) as described (29). Samples containing ≈150 ng of the purified DNA were used to transform the WBM535 indicator strain, using the Ca/Mops method (30). The cells were plated on lactose/MacConkey indicator plates containing kanamycin at 50 μg/ml, and colonies were scored after an incubation period of 20 hr at 37°C.

RESULTS

The *cro* Bioassay System. In the system described below, UV mutations were generated during the incubation of a pBR322-derived plasmid carrying the *cro* gene of bacteriophage λ with a protein extract from *E. coli* cells. Mutations in *cro* were detected in a subsequent bioassay step, which involved the introduction of the plasmid into the appropriate indicator strain, followed by plating and screening, which allowed the phenotypic detection of mutant colonies. The *cro* mutagenesis assay system is described in detail elsewhere (31). Briefly, the indicator strain WBM535 carries a Δ(*lac–pro*) chromosomal mutation, a λ *imm21* prophage that contains a single copy of the *lacZ* gene under the control of the O_RP_R operator/promoter of bacteriophage λ, and a Δ*recA* mutation that renders the cell nonmutable by UV light. In cells harboring the *cro* gene on a plasmid, expression of the chromosomal *lacZ* gene is repressed by the Cro repressor, leading to the inability of the cells to ferment lactose. Thus, transformation of the indicator strain with a *cro* plasmid leads to the appearance of white colonies on lactose/MacConkey indicator plates. When an indicator cell is transformed by a plasmid that carries a mutation in *cro* that sufficiently reduces the amount of repressor or its binding to the operator, O_R, *lacZ* is expressed, thereby allowing lactose fermentation and leading to the appearance of red colonies on the indicator plates. The plasmid used was pOC2, a pBR322 derivative that carries the *cro* gene along with the *bla* and *kan* genes encoding resistance to ampicillin and kanamycin, respectively.

The bioassay consisted of the transformation of competent *E. coli* WBM535 cells with plasmid pOC2, followed by plating on lactose/MacConkey plates containing kanamycin. Transformation efficiency for nonirradiated CsCl-purified plasmid was typically 2–5 × 10^6 transformants per μg of DNA. It decreased 2-, 4-, and 8-fold for plasmids irradiated at 100, 200, and 400 $J \cdot m^{-2}$, respectively, due to inactivation of the plasmid by UV radiation. Thus, the transformation efficiency of a UV-irradiated plasmid represents its UV survival. The mutation frequency was calculated by dividing the number of red colonies (cells harboring plasmid with a mutated *cro* gene) by the overall number of transformants. Usually several hundred thousand transformants were obtained for each plasmid sample. Each *in vitro* UV mutagenesis experiment was performed three or four times, and the results were averaged. The deviation between experiments was ±40% when performed without EPR and ±20–30% in experiments that included EPR, where larger numbers of mutants were obtained (see below). The basal mutation frequency for a nonirradiated CsCl-purified plasmid pOC2 was 2.0 × 10^{-5} per transformant. This mutation frequency remained essentially unchanged for plasmids that had been UV irradiated with a dose of up to 400 $J \cdot m^{-2}$, as expected for a Δ*recA* strain (Fig. 1). Thus the indicator strain itself cannot produce mutations in the UV-irradiated plasmid.

The *in Vitro* UV Mutagenesis Reaction. The reaction conditions that were employed are based on the *in vitro* repli-

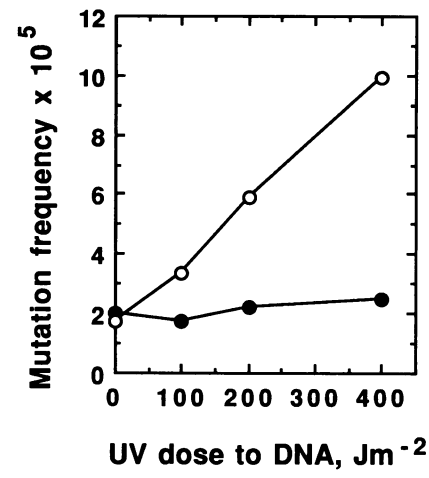

FIG. 1. Production of mutations in a UV-irradiated plasmid upon incubation with a protein extract. UV-irradiated plasmid pOC2 was incubated for 60 min at 30°C with 300 μg of fraction II protein extract prepared from *E. coli* TK701 wt cells. After incubation, the DNA was retrieved and bioassayed for the presence of mutations by transformation of the indicator strain *E. coli* WBM535, followed by plating and scoring mutant and wt colonies on lactose/MacConkey plates containing kanamycin (○). The details of the reaction mixture and the bioassay are given in *Materials and Methods*. UV-irradiated plasmid pOC2 that was not incubated with the protein extract served as a control (●).

cation system initially developed for *oriC* plasmids (28) and later adapted for pBR322 (32). Under these conditions, between 500 and 800 pmol of nucleotides was incorporated into the DNA when 900 pmol (as nucleotides) of substrate nonirradiated plasmid DNA was used. This DNA synthesis originated almost exclusively (95%) from DNA replication, which was semi-conservative, and had the characteristics of *in vivo* pBR322 replication: it was unidirectional; it started from the pBR322 origin of replication; and it depended on RNA polymerase, DNA polymerase I, and DNA polymerase III holoenzyme (unpublished results). The extent of DNA synthesis decreased to 40%, 20%, and 10% when using pOC2 DNA irradiated at 100, 200, and 400 $J \cdot m^{-2}$, respectively, which was most likely due to termination at UV lesions (33, 34).

After incubation, the plasmid was isolated from the reaction mixture and assayed for the presence of mutations in *cro* as described above. As can be seen in Fig. 1 and in Fig. 2*A*, incubation of the plasmid with protein extracts from two different wt cells (TK701 and IT1870, respectively) led to a 5- to 10-fold increase in the frequency of *cro* mutations in a UV dose-dependent manner. Remarkably, the *in vitro* replication of nonirradiated plasmid had no effect on the frequency of *cro* mutations, indicating that the fidelity of DNA replication in the extract was as high as the *in vivo* replication.

The addition of rifampicin, an inhibitor of RNA polymerase, to the reaction mixture prevented the production of UV mutations (Fig. 2*A*). Since replication of pOC2 is rifampicin sensitive (data not shown), these results suggest that UV mutagenesis requires DNA replication, or else it involves another rifampicin-sensitive step.

UV Mutagenesis Requires the *recA* and *umuC* Gene Products. To examine the involvement of the *recA* and *umuD/C* gene products in the cell-free UV mutagenesis reaction, we have used fraction II from mutant strains deficient or mutated in these proteins. As shown in Fig. 2*A*, UV mutations increased up to 10-fold for a plasmid irradiated at 400 $J \cdot m^{-2}$ when incubated with an extract from a wt strain (IT1870). Essentially no UV mutations were produced when the plasmids were incubated with a protein extract prepared from an isogenic Δ*recA* derivative (EST1779) (Fig. 2*A*). The level of DNA synthesis in the two extracts was similar (data not shown), implying that the difference in mutagenesis did not arise from a difference in the extent of DNA replication. Thus we conclude that the *in vitro* process responsible for the generation of UV mutations required the RecA protein.

Similar experiments were carried out with protein extracts obtained from strains TK701 (wt) and TK702 (*umuC36*). As shown in Fig. 2*B*, UV mutagenesis was reduced, but not completely eliminated, when assayed with extracts prepared from the *umuC36* strain. The residual UV mutagenesis observed could have resulted from residual activity of the mutated UmuC protein in the extract, or else it may represent a *umuC*-independent branch of UV mutagenesis. Such pathways were shown to exist for UV mutagenesis of phage S13 (21) and the F episome (35). Thus, *in vitro* UV mutagenesis requires the RecA and UmuC proteins, similar to the *in vivo* situation.

Photoreactivation of the DNA Prior to Transformation-Stimulated *in Vitro* UV Mutagenesis. UV-irradiated plasmid DNA retrieved from the *in vitro* reaction mixture still contained UV lesions. Although the indicator strain could not generate UV mutations due to its *recA* deficiency, we were concerned that there might have been some processing of UV lesions into mutations in the indicator strain. In an attempt to clarify this point, we have eliminated the photodimers from the plasmid by EPR with purified *E. coli* DNA photolyase (29). This was done after incubation with the protein extract and prior to transformation. As expected, due to the elimination of inactivating photodimers, the EPR increased plasmid survival up to that of nonirradiated DNA (data not shown). Surprisingly, the EPR also caused a 20- 30-fold increase in the frequency of UV mutations as a function of the UV dose, from 2×10^{-5} with nonirradiated DNA up to $40–60 \times 10^{-5}$ with DNA originally irradiated at 400 $J \cdot m^{-2}$ (Fig. 3). UV-irradiated DNA that did not undergo the *in vitro* reaction but was photoreactivated showed the background mutation frequency of 2×10^{-5}. This result indicates that UV mutations were indeed produced during the *in vitro* reaction but were not efficiently detected in the absence of EPR, possibly due to the low survival of plasmids carrying a relatively high number of photodimers. As will be described below, these mutations may represent a pathway of UV mutagenesis (type II UV mutagenesis) different from the rifampicin-sensitive pathway observed in the absence of EPR (type I UV mutagenesis).

The EPR treatment leaves minor UV lesions in the DNA, such as 6-4 pyrimidine adducts (36), which may have been

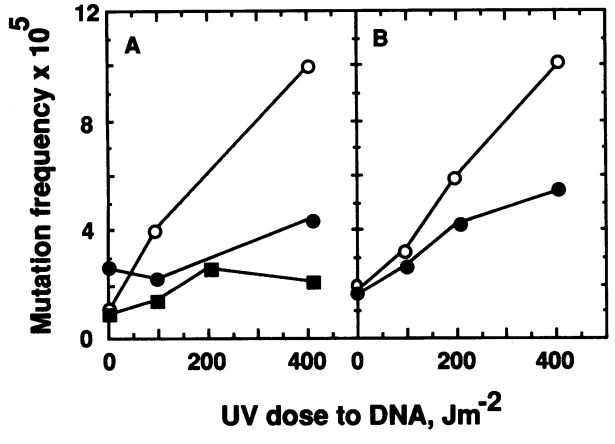

FIG. 2. *In vitro* UV mutagenesis depends on RecA and UmuC and is rifampicin sensitive. UV-irradiated plasmid pOC2 was incubated with the protein extracts indicated below and bioassayed as described in the legend to Fig. 1 and in *Materials and Methods*. (*A*) The *in vitro* reactions were carried out in the absence (○) or presence (■) of rifampicin (final concentration of 5 μg/ml) with fraction II prepared from *E. coli* IT1870 wt cells. In parallel, the *in vitro* reaction was performed with a cell extract prepared from *E. coli* EST1779 Δ*recA* cells (●). (*B*) The *in vitro* reactions were carried out with fraction II from *E. coli* TK701 wt cells (○) or TK702 *umuC36* cells (●).

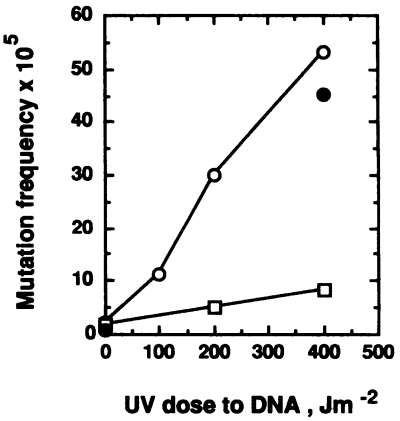

FIG. 3. The effect of EPR on *in vitro* UV mutagenesis. UV-irradiated plasmid pOC2 was incubated with 240 μg of fraction II proteins prepared from *E. coli* AB1157 wt cells and bioassayed as described in *Materials and Methods*. The assays were performed without EPR (□) or with EPR after the *in vitro* reaction but prior to transformation (○). The effect of rifampicin was examined by including it in the *in vitro* reaction and assaying the plasmid DNA for mutations after an EPR treatment (●).

mutagenic in the indicator Δ*recA* strain if not repaired. When a *uvrA6* Δ*recA* indicator strain that we have constructed was transformed with UV-irradiated plasmid pOC2, both with or without an EPR treatment prior to transformation, no UV mutations were observed in *cro* (data not shown). This rules out the possibility that the indicator strain can process nonphotoreactivatable UV lesions into mutations.

Photoreactivation-Stimulated *in Vitro* UV Mutagenesis Does Not Depend on DNA Replication and Requires the *uvr* Gene Products. The addition of rifampicin to the *in vitro* reaction mixture had no effect on EPR-stimulated *in vitro* UV mutagenesis (Fig. 3), indicating that unlike type I UV mutagenesis, type II UV mutagenesis did not depend on DNA replication. When the assay was performed with extracts prepared from a *uvrA* strain, EPR-stimulated UV mutagenesis was greatly reduced (Fig. 4*A*). The same was found with extracts prepared from a *uvrB* or a *uvrC* strain (Fig. 4*A*). Thus, the pathway stimulated by EPR was dependent on the excision repair genes and did not depend on DNA replication. These biochemical characteristics define it as a separate pathway, termed type II UV mutagenesis. The residual UV mutagenesis observed with the *uvr* extracts (Fig. 4*A*) is likely to be of the type I.

Photoreactivation-Stimulated *in Vitro* UV Mutagenesis Does Not Depend on the *recA* and *umuC* Gene Products. EPR-stimulated UV mutagenesis remained essentially unaffected when the reaction was performed with extracts prepared from cells carrying a Tn*5* insertion in the *umuC* gene or a Δ*recA* mutation (Fig. 4*B*). Thus, with EPR included in the protocol, type II UV mutagenesis was not dependent on the *recA* and *umuC* gene products. Since this pathway could be assayed only when the photoreactivation step was included, we do not know at this point whether it depends on RecA and UmuC in the absence of photoreactivation.

The Spectrum of EPR-Stimulated *in Vitro* UV Mutagenesis Is Similar to *in Vivo* UV Mutations. To determine the spectrum of *in vitro*-produced type II UV mutations, mutant plasmids (from red colonies) obtained from *in vitro* mutagenesis experiments carried out with fraction II from wt cells were isolated, and the identity of the mutations produced in *cro* was determined by DNA sequence analysis. Table 1 presents a total of 42 mutants: 28 UV mutations that were distributed among 14 sites and 14 background mutations that were distributed among 6 sites. Nearly 80% of the UV mutations were transitions, mostly G·C → A·T transitions. In contrast, most of the background mutations (80%) were transversions (G·C → C·G and G·C → T·A). All UV mutations were located at potential photodimer sites (two or more consecutive pyrimidines). Most of the mutations (21 out of 28) were formed at polypyrimidine runs of five to eight pyrimidines, consistent with the finding that such DNA sequences are hot spots for the formation of UV lesions, including closely opposed UV lesions (37). Interestingly, three tandem double-base substitutions were found among the UV mutations, but not among the background mutations (Table 1). The predominance of transitions, primarily G·C → A·T transitions and the appearance, at low frequency, of tandem double-base substitutions, is typical of *in vivo* UV-induced mutations (2).

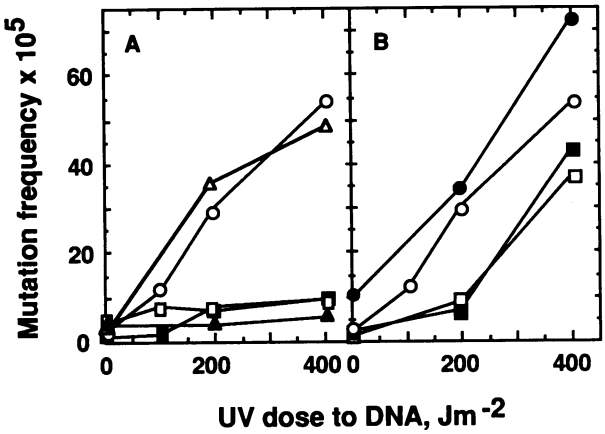

FIG. 4. Dependence of photoreactivation-stimulated *in vitro* UV mutagenesis on the *uvr* genes but not on the *recA* and *umuC* genes. EPR-stimulated UV mutagenesis was assayed with plasmid pOC2 as described in *Materials and Methods* with fraction II protein extracts prepared from the *E. coli* strains indicated. (*A*) ○, AB1157 (wt); ■, N3137 (*uvrA*::Tn*10*); □, N3124 (*uvrC*::Tn*10*); △, SR559 (wt); ▲, SR260 (Δ*uvrB*). (*B*) ○, AB1157 (wt); ●, SR1165 (*umuC122*::Tn*5*); □, IT1870 (wt); ■, EST1779 (Δ*recA*).

Table 1. DNA sequence specificity of *in vitro* UV mutagenesis

Mutation type	Background mutation		UV light mutation	
	No.	%	No.	%
Transition	2	14	22	79
G·C → A·T	2		21	
A·T → G·C	0		1	
Transversion	11	79	2	7
G·C → C·G	4		0	
G·C → T·A	7		2	
Double mutation	0	0	3	11
GG/CC → AA/TT	0		2	
GG/CC → TT/AA	0		1	
Frameshift	0	0	1	3
Inversion	1	7	0	0
Total	14	100	28	100

UV-irradiated (400 $J \cdot m^{-2}$) or nonirradiated plasmid pOC2 was incubated with fraction II prepared from *E. coli* AB1157 cells. After incubation the plasmids were purified, subjected to EPR, and bioassayed for the presence of mutations in the *cro* gene as described in *Materials and Methods*. The plasmid was extracted from individual mutant colonies, each obtained from a different transformation reaction, and the mutation was determined by DNA sequence analysis of the *cro* gene.

DISCUSSION

A critical question that arises whenever a living cell is used to assay the products of an *in vitro* reaction is whether the entire reaction has indeed occurred in the test tube. The following arguments strongly suggest that at least some, if not all, of the key steps of UV mutagenesis occurred during the *in vitro* reaction. (*i*) The Δ*recA* indicator strain cannot process UV lesions into mutations. (*ii*) Incubation with the protein extract was required for the production of UV mutations. (*iii*) The production of UV mutations was dependent on the genotype of the cells from which the protein extracts were prepared. (*iv*) In the absence of photoreactivation, the mutagenic reaction was inhibited by the presence of rifampicin in the reaction mixture. (*v*) The elimination of most UV lesions by EPR prior to the bioassay did not reduce mutagenesis, as expected if UV lesions were somehow processed into mutations in the indicator cells; on the contrary, it increased the frequency of UV mutations.

The data presented in this study can be explained by the operation of two pathways of UV mutagenesis in the *in vitro* system: (*i*) a pathway that depends on DNA replication (type I UV mutagenesis) and (*ii*) a pathway that depends on the *uvr* excision repair genes and not on DNA replication (type II UV mutagenesis). Such pathways were suggested to exist in *E. coli* based on the kinetics of fixation of UV mutations in wt and *uvr* strains (3–5).

Type II UV mutagenesis could be assayed effectively only when photodimers were eliminated from DNA prior to trans-

formation, a treatment that greatly increased plasmid survival. This suggests that type II UV mutations have occurred in a subpopulation of the UV-irradiated plasmid molecules that contained a relatively high number of photodimers and thus survived in the indicator strain rather poorly. The dependence of type II UV mutagenesis on the excision repair genes *uvrA*, *uvrB*, and *uvrC* suggests that it occurred at excision repair gaps. A possible model that would explain these findings is that type II UV mutations occur at sites of two closely opposed UV lesions (3, 38–40). Removal of one of the UV lesions by the *uvr* system is expected to leave a short excision gap in the DNA containing the second UV lesion, a structure that cannot be repaired by excision repair. The filling-in of such an excision gap is likely to result in a mutation due to misincorporation opposite the UV lesion. There is evidence to support a similar excision repair-dependent mechanism for UV mutagenesis in yeast (41–43).

The fact that photoreactivation did not eliminate the mutations argues that the mutagenic step, most likely misincorporation of a dNMP residue opposite a UV lesion, was completed in the test tube prior to transformation. The lack of dependence of this step on the *umuC* and *recA* gene products suggests that they act in a separate later step, which is needed to complete the mutagenic process but is not associated with the actual production of the mutation. This later step, which was circumvented by EPR, occurs inefficiently in the test tube, most likely due to insufficient quantities of proteins such as UmuD/C in the extract. This finding is similar to the delayed photoreactivation phenomenon described by Bridges and Woodgate (17). They have found that photoreactivation, after an incubation period, of UV-irradiated *E. coli* cells released UV mutations from their dependence on the *recA* and *umuC* gene products.

In the experiments described here, the protein extracts were prepared from noninduced cells. This implies that the constitutive level of SOS-controlled proteins in these extracts was sufficiently high to promote the mutagenic reaction. Under these conditions, type II photoreactivation-stimulated UV mutations were produced much more efficiently than type I mutations (Fig. 3). This does not necessarily reflect their relative efficiency *in vivo*. Rather, it is likely to be primarily the result of circumventing the requirement for UmuD/C proteins by the EPR treatment. We anticipate that protein extracts prepared from SOS-induced cells will be more effective in carrying out type I UV mutagenesis.

Despite a considerable research effort, the molecular mechanism of UV mutagenesis is unknown. The availability of the assay system described above, in which UV mutations are produced efficiently in the test tube, will hopefully facilitate the biochemical dissection of UV mutagenesis and enable the elucidation of its molecular mechanism.

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1. Walker, G. C. (1984) *Microbiol. Rev.* **48**, 60–93.
2. Eisenstadt, E. (1987) in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington), pp. 1016–1033.
3. Witkin, E. M. (1976) *Bacteriol. Rev.* **40**, 869–907.
4. Nishioka, H. & Doudney, C. O. (1969) *Mutat. Res.* **8**, 215–228.
5. Nishioka, H. & Doudney, C. O. (1970) *Mutat. Res.* **9**, 349–358.
6. Bridges, B. (1978) *Nature (London)* **275**, 591–592.
7. Shwartz, H., Shavitt, O. & Livneh, Z. (1988) *J. Biol. Chem.* **263**, 18277–18285.
8. Miura, A. & Tomizawa, J. I. (1968) *Mol. Gen. Genet.* **103**, 1–10.
9. Witkin, E. M. (1969) *Mutat. Res.* **8**, 9–14.
10. Kato, T. & Shinoura, Y. (1977) *Mol. Gen. Genet.* **156**, 121–131.
11. Elledge, S. J. & Walker, G. C. (1983) *J. Mol. Biol.* **164**, 175–192.
12. Bridges, B. A., Motershead, R. P. & Sedgwick, S. G. (1976) *Mol. Gen. Genet.* **144**, 53–58.
13. Brotcorne-Lannoye, A., Maenhaut-Michel, G. & Radman, M. (1985) *Mol. Gen. Genet.* **199**, 64–69.
14. Hagensee, M. E., Timme, T. L., Bryan, S. K. & Moses, R. E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4195–4199.
15. Bryan, S. K., Hagensee, M. & Moses, R. E. (1990) *Mutat. Res.* **243**, 313–318.
16. Kornberg, A. (1988) *J. Biol. Chem.* **263**, 1–4.
17. Bridges, B. A. & Woodgate, R. (1985) *Mutat. Res.* **150**, 133–139.
18. Echols, H. & Goodman, M. F. (1990) *Mutat. Res.* **236**, 301–311.
19. Sargentini, N. J. & Smith, K. C. (1987) *Mutat. Res.* **179**, 55–63.
20. Sargentini, N. J. & Smith, K. C. (1981) *Carcinogenesis* **2**, 863–872.
21. Tessman, I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6614–6618.
22. Kato, T. & Nakano, E. (1981) *Mutat. Res.* **83**, 307–319.
23. Mieschendahl, M., Buchel, D., Bocklage, H. & Muller-Hill, B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7652–7656.
24. Meyer, B. J., Maurer, R. & Ptashne, M. (1980) *J. Mol. Biol.* **139**, 163–194.
25. Pakula, A. A., Young, V. B. & Sauer, R. T. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8829–8833.
26. Livneh, Z. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4599–4603.
27. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
28. Fuller, R. S., Kaguni, J. M. & Kornberg, A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7370–7374.
29. Sancar, A., Smith, F. W. & Sancar, G. B. (1984) *J. Biol. Chem.* **259**, 6028–6032.
30. Strike, P., Humphreys, G. O. & Roberts, R. J. (1979) *J. Bacteriol.* **138**, 1033–1035.
31. Skaliter, R., Eichenbaum, Z., Shwartz, H., Ascarelli-Goell, R. & Livneh, Z. (1992) *Mutat. Res.*, in press.
32. Masai, H., Bond, M. W. & Arai, K. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1256–1260.
33. Livneh, Z. (1986) *J. Biol. Chem.* **261**, 9526–9533.
34. Livneh, Z., Shwartz, H., Hevroni, D., Shavitt, O., Tadmor, Y. & Cohen, O. (1988) in *DNA Replication and Mutagenesis*, eds. Moses, R. E. & Summers, W. C. (Am. Soc. Microbiol., Washington), pp. 296–304.
35. Christensen, J. R., LeClerc, J. E., Valone Tata, P., Christensen, R. B. & Lawrence, C. W. (1988) *J. Mol. Biol.* **203**, 635–641.
36. Rahn, R. O. (1979) in *Photochemical and Photobiological Reviews*, ed. Smith, K. C. (Plenum, New York), pp. 267–330.
37. Lam, L. H. & Reynolds, R. J. (1987) *Mutat. Res.* **178**, 167–176.
38. Bresler, S. E. (1975) *Mutat. Res.* **29**, 467–472.
39. Sedgwick, S. G. (1976) *Mutat. Res.* **41**, 185–200.
40. Bridges, B. A. (1977) *Mutat. Res.* **44**, 149–163.
41. James, A. P. & Kilbey, B. J. (1977) *Genetics* **87**, 237–248.
42. James, A. P., Kilbey, B. J. & Prefontaine, G. J. (1978) *Mol. Gen. Genet.* **165**, 207–212.
43. Kilbey, B. J., Brychcy, T. & Nasim, A. (1978) *Nature (London)* **274**, 889–891.