THE ROLE OF PYRIMIDINE DIMERS AS PREMUTAGENIC LESIONS: A STUDY OF TARGETED *VS.* UNTARGETED MUTAGENESIS IN THE *lacI* GENE OF *ESCHERICHIA COLI*

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ABSTRACT

We have employed conjugal transfer of an F′ *lac* episome to examine targeted and untargeted mutagenesis in the *lacI* gene of *Escherichia coli* and to determine the relative importance of pyrimidine dimers as premutational UV lesions compared to (6-4) photoproducts that also may have a mutational role. This conjugal system allowed us to assess the premutagenic role of UV lesions independently from any role as inducers of SOS functions. F′ DNA was transferred to an SOS-induced recipient strain from: unirradiated donor cells, UV-treated donor cells or donor cells that were irradiated and then exposed to photoreactivating light. The results indicate that SOS-related, untargeted events may account for as much as one-third of the nonsense mutations (*i.e.*, base substitutions) recovered after undamaged F′ DNA is transferred to UV-irradiated recipients. When the donor strain also is irradiated, in excess of 90% of the mutations detected following conjugation appear to be targeted. Photoreactivation of the UV-treated donor cells, prior to F′ transfer to the SOS-induced recipient strain, demonstrated that in this experimental system virtually all recovered UV-induced mutations are targeted by photoreactivable lesions. We presume that these lesions are pyrimidine dimers because (6-4) photoproducts are not photoreactivable.

ULTRAVIOLET light (UV)-induced mutagenesis has been studied in *Escherichia coli* for more than a third of a century. Much has been learned about the kinetics and host requirements for mutagenesis and about the macromolecular basis of repair (for reviews, see WITKIN 1976; KIMBALL 1978; HALL and MOUNT 1981; LITTLE and MOUNT 1982; HASELTINE 1983). Yet, the precise premutagenic lesion responsible for UV mutagenesis continues to be the subject of speculation, and our knowledge of the molecular processes involved in mutation fixation remains fragmentary. On the basis of a number of observations (see HASELTINE 1983), it has long been assumed that pyrimidine-pyrimidine cyclobutane dimers (henceforth referred to as pyrimidine dimers) represent the major premutagenic lesion induced by UV. However, recent findings suggest that pyrimidine-pyrimidine (6-4) UV photoproducts [hereafter

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designated (6-4) photoproducts] may play an important role in UV mutagenesis (LIPPKE *et al.* 1981; BRASH and HASELTINE 1982; HASELTINE 1983).

Pyrimidine dimers, but not (6-4) photoproducts, are photoreactivable (D. E. BRASH and W. A. HASELTINE, cited in HASELTINE 1983). Thus, the question of whether pyrimidine dimers or (6-4) photoproducts are the major premutagenic lesions might be investigated by examining UV mutational spectra following photoreactivation. Unfortunately, photoreactivation of pyrimidine dimers prevents the induction of error-prone SOS repair functions (RADMAN 1974; WITKIN 1976) as evidenced by the concomitant loss of expression of genes or processes coregulated with the SOS response (D. E. BRASH and W. A. HASELTINE, cited in HASELTINE 1983; G. C. WALKER, personal communication; B. W. GLICKMAN, unpublished results). Since SOS induction is a prerequisite for mutation induction by UV (WITKIN 1976; KATO and NAKANO 1981), reduction in UV-induced mutagenesis by photoreactivation might not reflect loss of pyrimidine dimers as potential premutagenic lesions, but rather elimination of the SOS-inducing signal.

In an attempt to examine the role of pyrimidine dimers in UV-induced mutagenesis, we have employed a system in which the photoreactivation of pyrimidine dimers in a target gene can be accomplished without loss of the SOS-inducing signal. The system involves UV treatment of a donor cell carrying a target gene (in this case *lacI*) on an F′ episome and subsequent transfer of the F′, via conjugation, to a recipient cell UV induced for SOS functions. In this manner, irradiation of the donor cell followed by photoreactivation prior to conjugation permits the production of UV photoproducts and the subsequent specific reversal of the pyrimidine dimers. Thus, by transferring the UV-irradiated and then photoreactivated F′ DNA to recipient cells in which SOS repair has been elicited by UV treatment, we can assess the significance of pyrimidine dimers as premutagenic lesions independently of their role as inducers of SOS functions.

This experimental system also has allowed us to study what has been termed "direct" or "targeted" *vs.* "indirect" or "untargeted" mutagenesis (DRAKE 1974; WITKIN and WERMUNDSEN 1978). It has been suggested that hot spots for UV-induced forward mutations in the *lacI* gene of *E. coli* might reflect hot spots for UV-induced DNA base damage (BRASH and HASELTINE 1982). On the basis of this finding and the mutational specificities of several agents, it has been argued that most mutations induced in *E. coli* by UV and certain chemical carcinogens occur at the sites of DNA damage, *i.e.*, are targeted at specific DNA lesions (EISENSTADT *et al.* 1982; FOSTER, EISENSTADT and CAIRNS 1982; MILLER 1982; FOSTER and EISENSTADT 1983; FOSTER, EISENSTADT and MILLER 1983). On the other hand, UV irradiation of *E. coli*, prior to infection with bacteriophage λ, leads to increased mutation of the λ DNA by *recA*-dependent SOS processes (JACOB 1954; DEVORET 1965; DEFAIS *et al.* 1971; ICHIKAWA-RYO and KONDO 1975). Similarly, when *tif-44* (*recA441*) or $dnaB_{ts}$ strains of *E. coli*, thermoinducible for SOS functions, are incubated under restrictive conditions, mutagenesis is enhanced markedly (WITKIN 1974, 1976; WITKIN and WERMUNDSEN 1978). Such findings have been taken to indicate that there is a

mutator activity associated with the inducible SOS system (RADMAN 1974; WITKIN 1976). If so, a component of UV mutagenesis may be untargeted, *i.e.*, occur at undamaged sites in DNA. However, it has been difficult to determine quantitatively the degrees to which targeted and untargeted events may contribute to mutagenesis at specific sites within a single gene. To probe this question, targeted mutagenesis was studied by irradiating donor cells and selecting *lacI*$^-$ mutations in the recipient strain after F′ transfer. To test for untargeted mutagenesis, recipient cells were UV irradiated to induce SOS functions, and then undamaged F′ DNA was introduced via conjugation.

The controls for these experiments involve the transfer of unirradiated DNA to unirradiated recipient cells. We (KUNZ and GLICKMAN 1983) found this transfer to be considerably less accurate than vegetative replication. This decrease in replication fidelity was independent of *recA* gene function in either or both the donor and recipient strain.

The results presented here indicate that untargeted mutagenesis may contribute significantly to the production of nonsense mutations in undamaged F′ DNA transferred into UV-irradiated recipients. This untargeted component of mutagenesis is quantitatively much less important when the donor strain also is irradiated. In the latter situation, targeted events gave rise to in excess of 90% of the total nonsense mutations detected. Finally, photoreactivation of UV-irradiated cells revealed that, in the conjugal system employed here, virtually all UV-induced mutations were targeted at photoreactivable lesions, presumably pyrimidine dimers.

EXPERIMENTAL RATIONALE

The *E. coli lacI* system allows the detection of nonsense mutations at a large number of sites within the *lacI* gene; specific mutations can be identified by analysis of suppression pattern and deletion mapping (COULONDRE and MILLER 1977a,b; MILLER *et al.* 1977; SCHMEISSNER, GANEM and MILLER 1977). As the DNA sequence of the nonsense mutations has been determined, each mutation can be correlated with a specific base change (FARABAUGH 1978; MILLER, COULONDRE and FARABAUGH 1978). An essential feature of this system is that the *lacI* gene is situated on the F′ episome and, hence, can be transferred. Moreover, since the *lac* operon is deleted from the chromosomal DNA of both the donor and recipient, transmission of UV-induced DNA lesions to the F′ copy of the *lacI* gene via recombination cannot occur. Therefore, any mutations arising within the *lacI* gene must be the result of DNA synthesis or repair processes acting directly on F′ DNA.

During conjugal transfer of the F′, a single DNA strand is passed from donor to recipient, and this strand is the one having its 5′ end at the origin of transfer (OHKI and TOMIZAWA 1968; RUPP and IHLER 1968; IHLER and RUPP 1969). The transferred strand is replaced in the donor cell by conjugal DNA synthesis. A complement to the donor strand is synthesized in the recipient (OHKI and TOMIZAWA 1968; VAPNEK and RUPP 1970, 1971).

For quantitative measurements of untargeted events giving rise to *lacI*$^-$ mutations, unirradiated, *i.e.*, undamaged F′ DNA is transferred to recipients in-

duced for SOS functions. Any increase in mutagenesis greater than that detected using uninduced recipients is ascribed to untargeted mutation. For quantitative measurements of *lacI*$^-$ mutations due to targeted events, UV-irradiated F′ DNA is transferred to recipient cells. Since the particular F′ DNA strand transmitted during conjugation and its sequence are known, it is possible to correlate specific target sites for UV damage with the induction of certain *lacI* nonsense mutations. To determine whether these mutations arise as a consequence of UV-induced pyrimidine dimers or (6-4) photoproducts, UV-irradiated donor cells are exposed to photoreactivating light prior to conjugation with recipients in which SOS functions have been induced by UV treatment. If the premutagenic lesions are mainly pyrimidine dimers, photoreactivation of the irradiated donor strain is expected to result in a mutational spectrum similar to that for F′ transfer from unirradiated donors to UV-treated recipients. However, if (6-4) photoproducts constitute the bulk of the premutagenic damage, the resultant mutational spectrum is expected to resemble that observed for conjugation involving UV-irradiated donor cells.

MATERIALS AND METHODS

The methods of bacterial conjugation, *lacI*$^-$ mutant selection, media and buffers have been described in detail (see KUNZ and GLICKMAN 1983). Briefly, exponentially growing donor and recipient cells were concentrated by centrifugation and mated at 37° for 60 min in nutrient broth (donor to recipients = 1:10). Selection for F′ transfer depended upon the transfer of *pro*$^+$ carried on the F′, whereas *lacI*$^-$ mutants were selected by their growth on phenyl-β-D-galactoside. Growth of the recipient was prevented by the absence of proline in the selection plates; growth of the donor was prevented by the addition of streptomycin to the selection plates. The results presented are from at least three independent experiments; in the cases in which the donor strain was unirradiated (and thus the mutation frequency low), the experiments were repeated at least nine times. Data concerning the frequency and specificity of *lacI* mutations arising in crosses without irradiation in the wild-type strain and in the presence of *recA* mutations have been presented previously (KUNZ and GLICKMAN 1983).

Strains and media: Unless otherwise stated, bacterial strains, media and techniques for the *lacI* system were the same as those described by COULONDRE and MILLER (1977a,b) and TODD and GLICKMAN (1982). The wild-type strains KMBL3835 [F′ *pro-lac/ara*, Δ (*pro-lac*), *thi*, *trpE9777*] and S90cN [F$^-$ Δ (*pro-lac*), *ara*, *thi*, *strA*, *nal*^{R}] have been described previously (GLICKMAN 1979; KUNZ and GLICKMAN 1983).

UV irradiation and photoreactivation: A 30-watt GE germicidal lamp emitting mainly 254 nm light was used for UV irradiation. The incident dose rate was adjusted to 1 $Jm^{-2}sec^{-1}$ as measured with an International Light IL570 germicidal radiometer. Experiments were carried out under yellow light to avoid unintentional photoreactivation. Exponential phase cells were washed twice with and resuspended in cold Vogel-Bonner buffer (KUNZ and GLICKMAN 1983) at 3–4 × 10^8 cells/ml. Cell suspensions were agitated during UV exposure and were kept on ice following UV treatment. The UV doses resulted in surviving fractions of approximately 70 and 60% for the donor and recipient strains, respectively. A lamp containing two Sylvania Blacklite Blue F15T8-BLB bulbs emitting mainly 380 nm light was used for photoreactivation. Photoreactivation was carried out immediately following UV irradiation in covered 10-cm plastic Petri dishes (Falcon) containing 10-ml suspensions of irradiated cells. Exposure was at 37° for 10 min, 20 cm from the light source.

RESULTS

Untargeted mutagenesis—the transfer of undamaged F′ DNA into SOS-induced recipient cells: In these experiments we compared mutation frequencies follow-

ing F′ transfer to unirradiated (SOS "off") or UV-irradiated (SOS "on") recipient cells. The *lacI* mutational responses are summarized in Table 1. As can be seen here and as we reported previously (KUNZ and GLICKMAN 1983), transfer of the F′ is itself mutagenic (compare row 2 with row 1). The reduced fidelity following conjugal transfer is not due to the induction of "SOS" phenomena by F′ transfer as it also was observed in a RecA$^-$ recipient (KUNZ and GLICKMAN 1983). Unfortunately, the high level of mutation largely obscures the effect of transfer to an irradiated host in which untargeted mutagenesis might be expected. Comparing row 3 with row 2, we can see that UV irradiation of the recipient did not affect the *lacI*$^-$ frequency, at least within the statistical variation allowed by these measurements. Yet, the fraction of *lacI*$^-$ mutations that were nonsense mutations increased significantly ($P < 0.005$; based upon a χ^2 comparing the number of nonsense mutations recovered among the total *lacI*$^-$ mutants obtained following transfer to unirradiated *vs.* UV-irradiated recipients). This modest increase of a factor 1.4 ($\pm$ 0.1 at the $P < 0.05$ level) in the frequency of nonsense mutations suggests that about 40% of the nonsense mutations may have arisen as a consequence of untargeted mutagenesis. In addition, the untargeted events show an altered specificity from that seen when the recipient is unirradiated.

The increase in nonsense mutations observed when transfer is to an irradiated host is not large. However, at low doses untargeted mutagenesis would be significant. For example, after a UV dose of 10 Jm^{-2} the total *lacI* mutation frequency is not increased significantly, whereas the frequency of ambers and ochres has doubled (compare row 4 with row 1 in Table 1). As the mutational spectra obtained after this treatment lack the hot spots usually observed after UV irradiation (B. W. GLICKMAN and R. L. DUNN, unpublished observations), a significant proportion of the increased mutations may reflect untargeted events.

TABLE 1

Frequencies of UV-induced nonsense mutations

	UV fluence[a] (Jm^{-2})			Amber mutants		Ochre mutants		Total nonsense mutants		No. of *lacI*$^-$ mutants screened
Row	F′	F$^-$	*lacI*$^-$ frequency[b]	%	Frequency[b]	%	Frequency[b]	%	Frequency[b]	
1	0	(no transfer)	3.2	0.96	0.03	0.61	0.02	1.6	0.05	67,568
2	0	0	5.6	10	0.56	3.7	0.21	13.7	0.77	2,931
3	0	20	6.2	10	0.64	7.2	0.45	17.2	1.1	3,532
4	10	(no transfer)	3.4	1.7	0.06	1.2	0.04	2.9	0.10	10,312
5	10	0	40	31	12	15	5.9	46	18	972
6	10	20	139	29	41	14	20	43	60	979

[a] F′ (donor) and/or F$^-$ (recipient) strains were irradiated prior to conjugation.

[b] Frequencies are per 10^6 survivors in rows 1 and 4 (no transfer of the F′) or per 10^6 F′ transferred in rows 2, 3, 5 and 6. Each frequency is the average of at least three independent experiments. In cases in which there was no transfer, the results are an average of 30 (no UV) and ten (UV) experiments.

The specificity of untargeted mutagenesis: To investigate further the nature of the additional nonsense mutations recovered in the SOS-induced recipient, the amber mutations were analyzed and the responsible base substitutions identified. Amber rather than ochre mutations were selected for characterization because of their prevalence and their facile analysis. The spectrum for the amber mutations is shown in Figure 1, where it is compared with that obtained previously (KUNZ and GLICKMAN 1983) for F′ transfer into unirradiated recipient cells. Most sites behaved similarly, and it would appear that the two spectra are rather alike in broad outline. However, the mutation frequencies at a number of amber sites increased up to sixfold over the corresponding values for F′ transfer to unirradiated recipients. The largest increases occurred at the amber 24 and 31 transition sites. Previously, these sites have been found to be relatively refractory to both spontaneous and UV-induced mutation in wild-

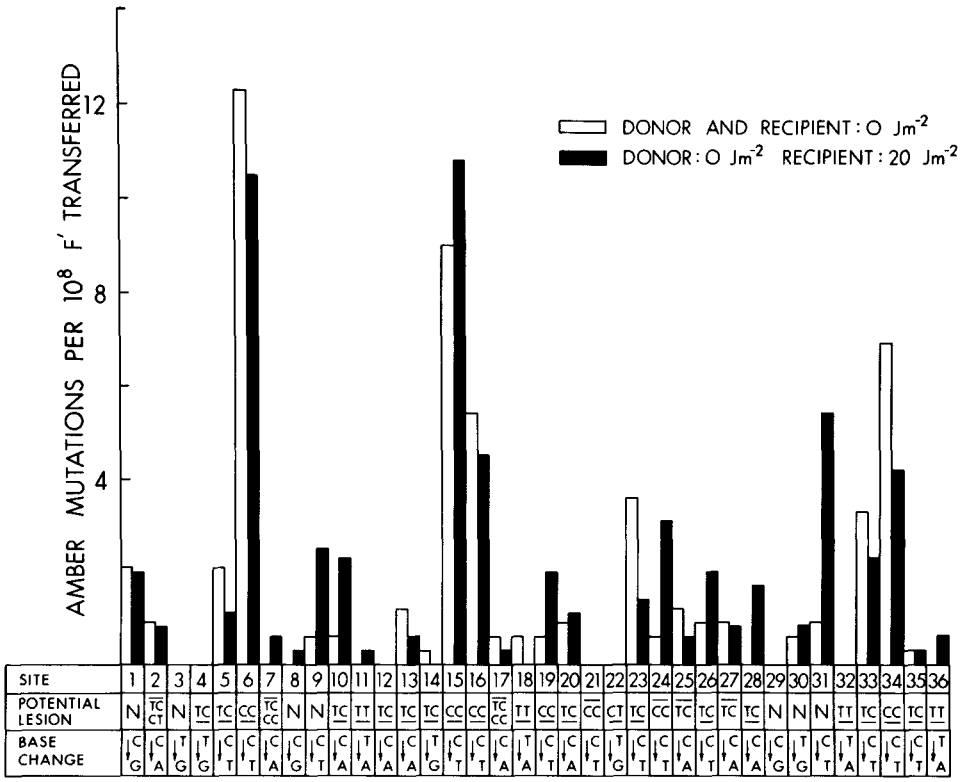

FIGURE 1.—Spectra of *lacI* amber mutations selected after F′ transfer from unirradiated donors. The open bars represent mutations obtained following transfer of the F′ from donor strain KMBL3835 to recipient strain S90cN (186 mutations analyzed). The solid bars represent mutations obtained following F′ transfer between the same strains except that the recipient was UV irradiated (20 Jm^{-2}) prior to conjugation (226 mutations analyzed). There are 36 characterized amber sites within the *lacI* gene. The potential lesions at these sites are either nondimer targets (N) or dimer targets (TT, TC, CT, CC). A dimer target in the transferred F′ DNA strand is underlined and a dimer target in the nontransferred strand is overlined. At sites where potential dimers of more than one class may form, both potential dimers are given. The base changes indicated for each site are those that convert the wild-type codon to the nonsense codon.

type strains (COULONDRE and MILLER 1977b; TODD and GLICKMAN 1982; KUNZ and GLICKMAN 1983). The most significant difference between the spectra may be that, after F′ transfer to SOS-induced recipients, mutations were recovered at several transversion sites (ambers 7, 8, 11, 28 and 36) not found following conjugation with unirradiated recipients. This is of interest in that, among these new sites, all but amber 8 represent transversion events in which an A:T base pair replaces C:G or T:A base pairs. Thus, the specificity of untargeted mutagenesis may have a bias toward transversion events involving the insertion of A:T base pairs. This bias toward transversion events also is reflected by the finding that the amber transversion frequency increased by 40% after F′ transfer to SOS-induced recipients, whereas the amber transition frequency increased by only 9%. The increase in nonsense mutations (preceding section) and the altered mutational specificity, taken together, provide quantitative evidence for untargeted mutagenesis.

Targeted mutagenesis—F′ transfer from UV-irradiated donor cells: When the donor but not the recipient strain was irradiated before F′ transfer, there was a 180-fold increase in the frequency of nonsense mutations (Table 1, row 5) over that observed for UV without conjugation (Table 1, row 4). Moreover, almost half of the *lacI*$^-$ mutants were nonsense mutants! This is the highest percentage of *lacI* nonsense mutations obtained in any *lacI* experiment to date (B. A. KUNZ and B. W. GLICKMAN, unpublished observations). Although conjugal transfer of UV-irradiated F′ DNA has been found to elicit a number of *recA*-dependent SOS functions in recipient cells (indirect induction), it has not been demonstrated previously that a mutator activity is among these indirectly induced processes (BOREK and RYAN 1958; GEORGE and DEVORET 1971; GEORGE, DEVORET and RADMAN 1974; MOREAU, PELICO and DEVORET 1982).

UV treatment of the recipient cells to induce SOS functions prior to conjugation with irradiated donors led to a further 3.5-fold enhancement of the nonsense mutation frequency (compare row 6 with row 5 in Table 1). This increase is not due to untargeted mutation. The predominance of potential dimer sites in the mutational spectra (Table 4) and effective reversal of mutation by photoreactivation (Table 6 and Figure 3) argue against this; rather, it appears that under conditions in which SOS is "more turned on," DNA repair becomes even more error-prone.

The specificity of targeted mutagenesis: As described before, a specific single strand of the F′ episome is transferred from donor to recipient during conjugation. The particular F′ strand that is transferred in the system used here does not have potential pyrimidine dimer targets at 14 of the 36 characterized amber sites. Ambers 1, 3, 8, 9, 29, 30 and 31 are nondimer sites, *i.e.*, present no potential target for pyrimidine dimer formation in either DNA strand. Amber sites 2, 7, 17, 21, 24, 25 and 27 present potential pyrimidine dimer target sites only in the F′ DNA strand that is not transmitted during conjugation. Thus, if UV-induced nonsense mutations in the *lacI* gene are targeted by pyrimidine dimers, then mutations at these 14 amber sites should be conspicuously absent from the spectrum obtained following F′ transfer from UV-irradiated donors.

The spectra for amber mutations selected subsequent to conjugation between irradiated donors and unirradiated or UV-treated recipients are presented in Figure 2. Irradiation of the donor strain prior to conjugation produced a mutational spectrum considerably different from that seen without irradiation. For example, hot spots were observed at the amber 23 and 33 sites. These sites also are hot spots for UV mutagenesis in the absence of conjugation (COULONDRE and MILLER 1977b; TODD and GLICKMAN 1982). Irradiation of both the donor and recipient cells prior to F′ transfer resulted in an additional increase in the mutation frequencies (compare row 6 with row 5 in Table 1) but caused no other obvious alteration in the mutational spectrum (Figure 2). For both spectra, at least 97% of the amber mutations recovered occurred at sites for which potential pyrimidine dimer targets are situated in the F′ DNA strand that is transferred (Table 3). This is in marked contrast to the results for conjugation involving unirradiated donors in which 14% (unirradiated recipient) and 27% (irradiated recipient) of the amber mutations detected were

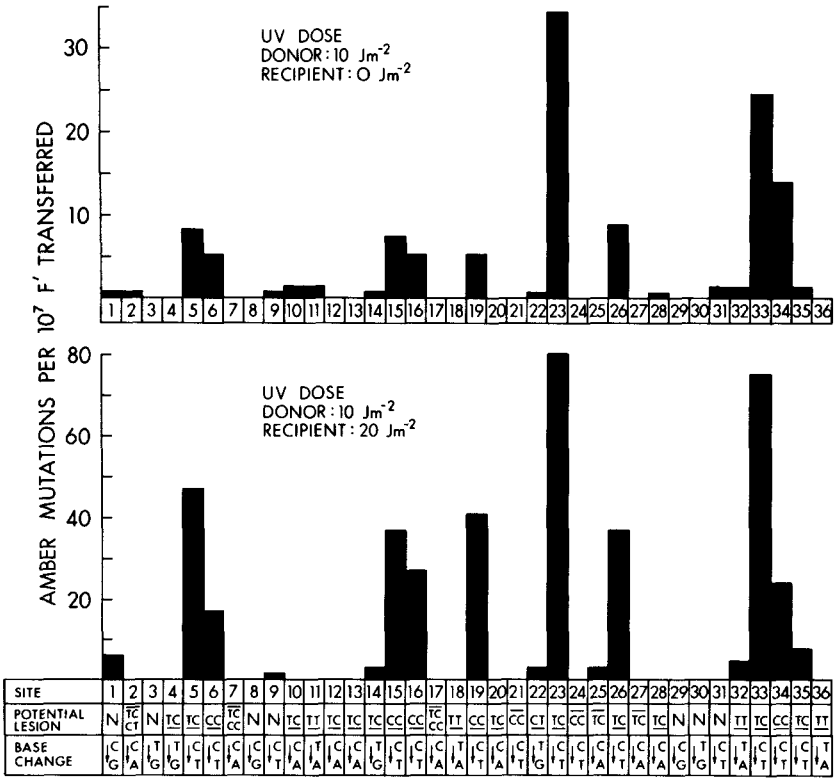

FIGURE 2.—Spectra of *lacI* amber mutations selected after F′ transfer from UV-irradiated donors. The upper spectrum represents mutations obtained following transfer of the F′ from the irradiated (10 Jm^{-2}) donor strian KMBL3835 to the unirradiated recipient strain S90cN (167 mutations analyzed). The lower spectrum represents mutations obtained following F′ transfer between irradiated donors (10 Jm^{-2}) and recipients (20 Jm^{-2}) (270 mutations analyzed). For other details, see the legend to Figure 1.

at sites where the transferred strand does not bear potential pyrimidine dimer targets (data drawn from Table 3).

To elucidate more fully the nature of the events that gave rise to the amber mutations, the spectra were analyzed in greater detail. When the donor strain was irradiated prior to conjugation, approximately 95% of the mutations resulted from G:C → A:T transitions (Table 2). Thus, the fraction of base substitution events which are G:C → A:T transitions is much higher than that for F′ transfer from unirradiated donors (Table 2) or than the corresponding percentage observed for UV treatment without subsequent conjugation (GLICKMAN 1983). The preponderance of transitions is best seen in Table 3, from which it also can be concluded that the transitions occurred far more frequently at potential dimer sites than at nondimer sites. (Note, however, that

TABLE 2

Distribution of base substitutions leading to amber mutations

Base substitution	No. of sites available	No. of sites occupied							
		UV[a] to F′:	0		0		10		10
		UV to F⁻:	0		20		0		20
G:C → A:T	14		13	(82.8)[b]	13	(78.3)	12	(94.0)	11 (95.9)
G:C → T:A	10		7	(11.3)	9	(13.7)	3	(2.4)	2 (1.1)
A:T → T:A	4		1	(1.1)	3	(1.8)	2	(2.4)	1 (1.1)
A:T → C:G	5		2	(1.6)	1	(1.3)	2	(1.2)	1 (0.4)
G:C → C:G	3		1	(3.2)	2	(3.5)	1	(0.6)	1 (1.5)
Totals	36		24		28		20		16
Total no. of ambers examined			186		226		167		270

[a] UV dose in Jm^{-2} given to F′ (donor) or F⁻ (recipient) prior to conjugation.
[b] Numbers in parentheses are percentages of nonsense mutants.

TABLE 3

Percent transitions and transversions among dimer and nondimer sites in the transferred F′ DNA strand

Potential sites	UV fluence[a] (Jm^{-2})		Transition sites available	Transitions[b] (%)	Transversion sites available	Transversions %	Total ambers examined
	F′	F⁻					
Pyrimidine dimer	0	0	10	79.0	12	6.5	186
	0	20	10	61.1	12	10.6	226
	10	0	10	92.2	12	4.8	167
	10	20	10	95.6	12	2.2	270
Nondimer[c]	0	0	4	3.8	10	10.8	186
	0	20	4	17.3	10	9.7	226
	10	0	4	1.8	10	1.2	167
	10	20	4	0.4	10	1.9	270

[a] UV dose given to F′ (donor) or F⁻ (recipient) prior to conjugation.
[b] Percent of total occurrences observed for each set of treatment conditions.
[c] Nondimer sites include those for which a potential dimer target occurs only in the nontransferred F′ DNA strand.

only four nondimer sites are available in the strand transferred!) Although more than 92% of the mutations arose via transition events, no mutations were detected at two of the 14 transition sites: ambers 21 and 24 (Figure 2). These are the only amber transition sites for which the potential pyrimidine dimer targets are in the nontransferred strand. Thus, when the potential dimer sites are not transferred, and, therefore, not available for targeted mutagenesis, mutation is rarely induced.

Even though most mutations arose at potential dimer sites, from Table 4 it is apparent that thymine-thymine (TT) dimers did not constitute significant target sites. This can be explained by the facts that most UV-induced mutagenesis favors transitions (DRAKE 1963; HOWARD and TESSMAN 1964; PERSON *et al.* 1974; COULONDRE and MILLER 1977b; LAWRENCE and CHRISTENSEN 1979; TODD and GLICKMAN 1982) and that in the *lacI* nonsense system, A:T → G:C transitions cannot be measured (MILLER, COULONDRE and FARABAUGH 1978). As a consequence, transitions at potential TT dimer sites cannot be monitored in this system. Regardless of whether the recipient strain was exposed to UV, when the donor strain was irradiated, the vast majority of mutations were recovered at potential cytosine-cytosine (CC) dimer sites and at potential thymine-cytosine (TC) dimer sites, including the UV hot spots at ambers 23 and 33 (Figure 2, Table 4). The CC and TC dimer sites also are potential (6-4) photoproduct sites (LIPPKE *et al.* 1981; BRASH and HASELTINE 1982). Thus, although the specificity data argue strongly that almost all of the amber mutations detected after F′ transfer from irradiated donors arose via targeted transition events, the results do not reveal the identity of the targeting lesion.

It should be pointed out that, whereas the *lacI* system allows the detection of the G:C → A:T transition and all four transversions, as the A:T → G:C transition cannot give rise to *de novo* nonsense mutations, this latter transition

TABLE 4

Distribution of amber mutations among potential dimer target sites in the transferred F′ DNA strand

Potential target	Sites available[a]	No. of sites occupied							
		UV to F′[b]	0		0		10		10	
		UV to F⁻	0		20		0		20	
TT	4		1	(1.1)[c]	3	(1.8)	2	(3.0)	1	(1.1)
TC	12		9	(23.1)	9	(20.0)	8	(65.0)	6	(60.7)
CC	5		5	(61.3)	5	(50.2)	5	(30.0)	5	(35.6)
CT	1		0		0		1	(0.6)	1	(0.4)
Total dimer sites	22		15		17		16		13	
Nondimer sites	14		9	(14.5)	11	(28.0)	4	(0.4)	3	(2.2)
Totals	36		24		28		20		16	
Total no. of ambers mapped			186		226		167		270	

[a] Nondimer sites include those for which a potential dimer target occurs only in the nontransferred F′ DNA strand.

[b] UV dose in Jm^{-2} given to F′ (donor) or F⁻ (recipient) prior to conjugation.

[c] Numbers in parentheses are percentages of total for each set of treatments.

goes undetected. As a consequence, the *lacI* system is unable to detect UV-induced transition events *at potential target sites not containing a C residue.* Thus, among potential dimers only TC and CC sites can be scored. As a consequence of this limitation, the potential role of (6-4) photoproducts (which are detected only at TC and CC sites in double-stranded DNA) and dimers cannot be directly distinguished.

Photoreactivation of UV-induced mutations: Twenty-nine of the *lacI* amber mutations occur at potential pyrimidine dimer sites. Of these, 24 also are potential sites for (6-4) UV photoproducts. Furthermore, after UV irradiation of the donor strain, the majority (2:1) of amber mutations occurred at potential TC dimer sites (Table 4); this is consistent with the finding that (6-4) photoproducts occur most frequently at TC sequences (LIPPKE *et al.* 1981). However, unlike pyrimidine dimers, (6-4) photoproducts are not photoreactivable (D. E. BRASH and W. A. HASELTINE, cited in HASELTINE 1983). Thus, in order to determine whether the *lacI* amber mutations selected after F′ transfer from irradiated donors arise as a consequence of pyrimidine dimers or (6-4) photoproducts, UV-treated donor cells were exposed to photoreactivating light prior to conjugation with SOS-induced recipients.

Table 5 shows that photoreactivation of the UV-treated donor strain, prior to conjugation with the irradiated recipient cells, led to greater than a 90% reduction in donor strain lethality and to a comparable decrease in the frequency of nonsense mutations when compared to F′ transfer without photoreactivation. The corresponding mutational spectra (Figure 3) show clearly that photoreactivation resulted in a substantial decrease in mutation frequencies at all sites recovered when the donor was UV irradiated but not photoreactivated. For example, the frequencies of mutation at the UV hot spots, ambers 23 and 33, were reduced by 97%. On the average, the amber mutation frequencies were diminished by 84%.

Following photoreactivation, the fraction of the total amber mutation frequency ascribable to events at the spontaneous hot spot sites, ambers 6, 15 and 34, is substantial. In addition, mutations were recovered at a number of sites not detected after F′ transfer from irradiated donors but found after conjugation when only the recipient strain was treated with UV, *e.g.*, at amber sites 2, 9, 24, 27 and 28. In other words, the photoreactivation spectrum generally resembles that for F′ transfer from unirradiated donors to irradiated recipients, thus confirming the extensive nature of the photoreversion. Further analysis of the mutational spectra substantiates this interpretation. Both the distribution of the classes of base substitutions leading to *lacI* amber mutations and the percentage of transitions and transversions among potential dimer sites are quite similar for the spectra obtained after photoreactivation or subsequent to transfer of undamaged F′ DNA to irradiated recipients (data not shown). This similarity is demonstrated in Table 6, which shows that photoreactivation reduces the relative contribution of amber mutations at potential CC and TC dimer sites. In other words, following photoreactivation, the spectrum appears closer to that seen without UV irradiation of the donor.

Photoreactivation clearly reverses much (≈90%) but not all of the mutage-

TABLE 5

Effect of photoreactivation of the irradiated donor strain on the frequencies of UV-induced nonsense mutations

Treatment[a]		Survival of donor strain (%)	*lacI*⁻ Frequency[b]	Amber mutants		Ochre mutants		Total nonsense mutants		No. of *lacI*⁻ mutants screened
F′	F⁻			%	Frequency[b]	%	Frequency[b]	%	Frequency[b]	
10	20	72	64.6	26.5	17.1	14.1	9.1	40.6	26.2	1474
10 + photoreactivation	20	99	11.1	10.3	1.1	9.7	1.1	20	2.2	1580

[a] Strains were irradiated, or irradiated and photoreactivated, prior to conjugation. UV dose in Jm^{-2}.
[b] Frequencies are per 10^6 F′ transferred.

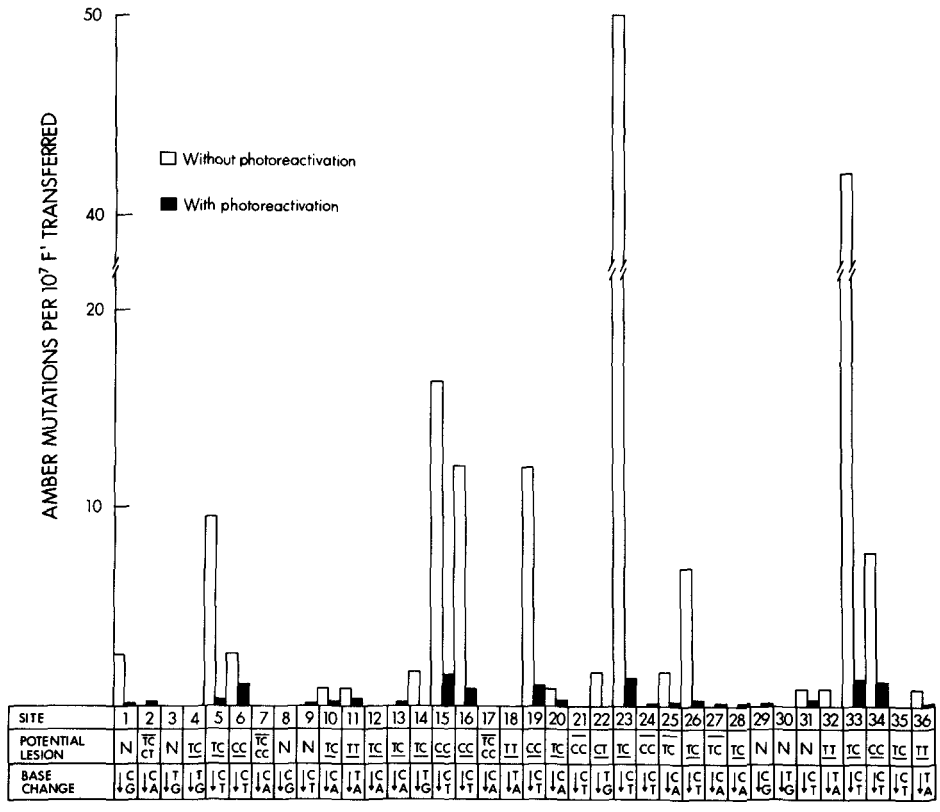

FIGURE 3.—Spectra of *lacI* amber mutations selected after F′ transfer from UV-irradiated or UV-irradiated and photoreactivated donors to irradiated recipients. UV doses were as in the legend to Figure 2. The open bars represent mutations obtained after F′ transfer from nonphotoreactivated donors (199 mutations analyzed). The closed bars represent mutations obtained after F′ transfer from photoreactivated donors (145 mutations analyzed). For other details, see the legend to Figure 1.

nicity of the UV treatment. The frequency of *lacI*$^-$ mutations after UV and photoreactivation treatment of the donor was 11.1×10^{-6} *vs.* 6.2×10^{-6} observed when the donor was not UV irradiated (in both cases the recipient received 20 Jm^{-2}). This residual mutagenesis may be due to the efficiency of photoreactivation being less than 100% or to the induction by UV or even the photoreactivating light of a small fraction of nonphotoreactivable lesions. In any event, the photoreactivation data demonstrate convincingly that, in the conjugal system studied here, most UV mutagenesis is targeted and that the target is photoreactivated efficiently. Thus, we conclude that pyrimidine dimers give rise to the majority of UV-induced *lacI* amber mutations.

DISCUSSION

Our data obtained from conjugal transfer suggest that as much as one-third of the amber mutations recovered following F′ transfer from unirradiated donors to SOS-induced recipients could have arisen as a consequence of un-

TABLE 6

Effect of photoreactivation of the irradiated donor strain on the distribution of amber mutations among potential target sites in the transferred F′ DNA strand

Potential target	Sites available	No. of occupied sites					
		UV to F′:[a]	0		10	10 + photoreactivation[b]	
		UV to F⁻:	20		20	20	
TT	4		3	(1.8)[c]	3 (1.6)	2	(4.1)
TC	12		9	(20.0)	8 (64.8)	8	(35.9)
CC	5		5	(50.2)	5 (29.6)	5	(49.7)
CT	1		0		1 (1.0)	0	
Total dimer sites	22		17		17	15	
Nondimer sites	14		11	(28.0)	3 (3.0)	8	(10.3)
Totals	36		28		20	23	
Total no. of ambers examined			226		199	145	

[a] UV dose in Jm^{-2} given to F′ (donor) and F⁻ (recipient) prior to conjugation.
[b] Photoreactivating light treatment given after UV irradiation.
[c] Numbers in parentheses are percentages of total occurrences observed for each set of treatments.

targeted mutagenesis, at least in the sense that these mutations occurred in nondamaged DNA. Interestingly, the specificity of untargeted mutagenesis appears to be biased in favor of transversions resulting from the incorporation of an A:T base pair. Such a preference has been observed previously in SOS-induced cells copying past apurinic sites during DNA synthesis (SCHAPPER, KUNKEL and LOEB 1983). It should be noted, however, that in the absence of UV treatment, conjugal DNA transfer is itself highly inaccurate (KUNZ and GLICKMAN 1983).

Conjugal transfer of the F′ subsequent to irradiation of the donor strain led to large increases in both *lacI*⁻ frequencies and the fraction thereof represented by nonsense mutations. This high level of mutation reflects not only the induction of DNA damage by the UV treatment but also the induction of SOS functions by the UV-damaged F′ DNA (indirect induction) (BOREK and RYAN 1958; GEORGE and DEVORET 1971; GEORGE, DEVORET and RADMAN 1974; MOREAU, PELICO and DEVORET 1982). Mutagenesis by the transfer of an irradiated F′ is, as expected, *recA* dependent (B. W. GLICKMAN and N. GUIJT, unpublished observation). Moreover, because of the nature of the system employed in this study (a single strand of F′ DNA being transferred to recipients lacking homologous chromosomal DNA), neither excision repair nor recombinational repair can act on the UV-treated and transferred F′ DNA (HOWARD-FLANDERS *et al.* 1968; GEORGE and DEVORET 1971). It seems reasonable to believe that the majority of nonsense mutations result from SOS-dependent incorporation of incorrect nucleotides opposite photoproducts in the DNA template, *i.e.*, the mutations are targeted (WITKIN 1976; CAILLET-FAUQUET, DEFAIS and RADMAN 1977; VILLANI, BOITEUX and RADMAN 1978; LACKEY, KRAUSS and LINN 1982; SCHAPPER, KUNKEL and LOEB 1983). The mutational

specificity data support this interpretation. Since only a specific single DNA strand of the F′ is transferred during conjugation, knowledge of the direction of transfer of the particular F′ used here and of the *lacI* DNA sequence enabled us to determine which *lacI* amber sites in the transferred strand also were potential pyrimidine dimer target sites. Analysis of the mutational spectra demonstrated that at least 97% of the *lacI* amber mutations recovered and characterized following F′ transfer from irradiated donors occurred at potential pyrimidine dimer target sites in the transferred strand. No mutations were detected at the two transition sites (ambers 21 and 24) for which the potential pyrimidine dimer targets are in the nontransferred strand, even though 92% of all of the recovered amber mutants were the result of transitions. Thus, we conclude that the UV-induced nonsense mutants were the result of mutagenesis targeted by specific UV photolesions.

The majority (95%) of the amber mutations detected after F′ transfer from irradiated donors occurred at potential TC and CC dimer sites, including the UV hot spots, ambers 23 and 33. On the basis of UV specificity alone, it seemed that either pyrimidine dimers or (6-4) photoproducts might be the targeting lesions. However, unlike pyrimidine dimers, (6-4) photoproducts are not photoreactivable (HASELTINE 1983), and it has been observed that UV mutagenesis in *E. coli* can be all but eliminated by photoreactivation. This would suggest that pyrimidine dimers are responsible for UV-induced mutation. Recently, it has been argued that the results of such photoreactivation experiments may be misleading (HASELTINE 1983). UV mutagenesis in *E. coli* is SOS dependent (WITKIN 1976; KATO and NAKANO 1981), and photoreactivation effectively removes the major portion of the DNA lesions responsible for the induction of SOS functions (D. E. BRASH and W. A. HASELTINE, cited in HASELTINE 1983; G. R. WALKER, personal communication; B. W. GLICKMAN, unpublished results). Therefore, photoreactivation might eliminate the SOS-inducing signal (pyrimidine dimers) but not the premutagenic lesions [(6-4) photoproducts]. This possibility was circumvented in our study by irradiation and subsequent photoreactivation of the F′ prior to transfer to recipients induced for SOS functions. Following this procedure, UV-induced DNA lesions not reversed by photoreactivation would remain as targets for SOS-dependent mutagenesis.

Photoreactivation of the irradiated donor strain prior to conjugation with UV-treated recipients resulted in a dramatic reduction (92%) in the frequency of *lacI* nonsense mutations recovered after F′ transfer. The mutation frequencies at the UV hot spots ambers 23 and 33 were decreased 97% and the total amber mutation frequency was decreased by 94%. From these results, we conclude that almost all of the amber mutations detected after conjugation involving UV-irradiated donors were targeted by pyrimidine dimers.

Photoreactivation did not completely reverse the mutagenic effect of irradiating the donor strain before conjugation. This residual mutagenesis might reflect the efficiency of photoreactivation or the induction of a small fraction of nonphotoreactivable lesions, possibly (6-4) photoproducts. However, it is clear that lesions other than pyrimidine dimers are responsible for at most only

a minor component of UV mutagenesis under the conditions employed here. Why then do other findings (BRASH and HASELTINE 1982; TODD and GLICKMAN 1982; B. W. GLICKMAN and R. L. DUNN, unpublished results) suggest that (6-4) photoproducts may play an important role in UV mutagenesis in the absence of F′ transfer? In this study, a much lower UV dose than used by other investigators was given, and as the UV dose decreases, relatively fewer (6-4) photoproducts are induced. In fact, doses less than 100 Jm^{-2} may produce only 10% as many (6-4) photoproducts as pyrimidine dimers (HASELTINE 1983). In addition, the relative mutagenic potential of dimers and (6-4) photoproducts may differ greatly in double- and single-stranded DNA. The (6-4) photoproducts are not reversed by the photoreactivation treatment. Nor are they destroyed since this occurs at wavelengths much lower than those used for photoreactivation (HARM 1980). Our results do not exclude the possibility that the (6-4) photoproduct or any other UV-induced DNA lesion may be responsible for a minor fraction of the nonsense mutations detected here or may play a more significant role in UV mutagenesis in other systems. However, our data indicate that, in the system studied here, pyrimidine dimers are the premutagenic UV lesions of major importance.

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