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DNA Sequence Context Affects UV-Induced Mutagenesis in *Escherichia coli*

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Abstract

We investigated the effect of altering the DNA sequence surrounding a mutable target site on the production of ultraviolet light (UV) induced mutations. Site-directed base substitutions were incorporated on both sides of a TAA sequence encoding a UAA nonsense defect in the *tyrA14* allele of *Escherichia coli*. This allele is readily revertible by UV and a total of eight different base substitution mutations can be recovered. Five different strains harboring DNA sequences allowing the formation of 5'-TT, 5'-CT and 5'-TA* photoproducts were constructed and exposed to UV. DNA sequence analysis was used to determine the spectrum of the revertants that were recovered. The results showed that changes at the 3' base of a TT site were predominately T to C transitions and T to A transversions. However, unlike the TT site, a 5'-CT site produced a relatively high frequency of T to G transversions. In addition, T to A transversions that could not have been targeted by a cyclobutane-type or [6–4]-type pyrimidine dimer were produced; this result suggested that these mutations may be targeted by a TA* photoproduct. Also, a distinct strand bias was noted for two mechanistically identical base substitutions in a strain having a palindromic target sequence; this result may reflect an unequal damage distribution or processing of photoproducts as a consequence of asymmetric DNA replication. Finally, our results show that DNA sequences expected to allow the greatest density of UV-induced DNA damage produce the highest mutation frequencies. Overall, these findings provide new insights regarding the role of DNA photoproducts in UV mutagenesis.

Keywords

ultraviolet; mutagenesis; cyclobutane; photoproduct; base substitution

1. Introduction

Ultraviolet light (UV) induced mutagenesis is thought to result from the production of DNA damage followed by trans-lesion DNA synthesis (TLS; for reviews see [1–4]). UV produces a number of DNA photoproducts [5,6]; the cyclobutane pyrimidine dimer (CPD) and the pyrimidine [6–4] photoproduct are responsible for much of the mutagenesis. CPDs may occur at any di-pyrimidine sequence; about 50% of the CPDs formed by germicidal UV (254 nm wavelength) involve TT sequences, while 40% involve TC or CT sequences and 10% involve CC sequences [6]. The [6–4] photoproduct, in contrast, only forms readily at TT and TC sequences, and, overall, represents about 10% of the total photoproducts formed, although there is considerable site to site variability [7]. Both products effectively block normal DNA

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replication, yet DNA synthesis mediated by the *umuDC* gene products (i.e. PolV) in *Escherichia coli* can bypass such lesions. This bypass, with the incorporation of incorrect nucleotide(s), is referred to as “targeted mutagenesis”, since the misincorporation occurs at the site of and as a consequence of the DNA damage. The bases that are incorporated opposite the DNA photoproduct, however, appear to be non-random.

Studies of UV-mutagenesis have revealed that the majority of base substitution events are G:C to A:T transitions at cytosine-containing photoproducts [8]. For CPDs, it has been suggested that rapid deamination of the cytosine to uracil could allow incorporation of adenine, giving rise to the transition [9–11]. However, mutations also occur at TT sequences, giving a very different mutational pattern. Studies using TT photoproducts incorporated at specific sites have revealed a number of non-random substitution patterns (Table 1). For example, Banerjee et al. [12] showed that of the base changes produced by a cis-syn TT cyclobutane dimer, 79% were 5'-TT to 5'-TA transversions while 17% were 5'-TT to 5'-TC transitions. Hence, 96% of all the mutations occurred at the 3'-base of the dimer. In contrast, the trans-syn form of the TT CPD produced 24% 5'-TT to 5'-AT transversions and 6% 5'-TT to 5'-CT transitions; 69% of the mutations were double base changes, frameshifts or deletions. Thus, of the single base change mutations produced by the trans-syn CPD, 95% occurred at the 5'-base of the dimer [13]. It is important to note that about 90% of the CPDs have the cis-syn conformation, while only about 10% are in the trans-syn form. In comparison, the [6–4] photoproduct was roughly 10 times more mutagenic than the cis-syn CPD and it produced a very different mutational spectrum; 94% of the mutations were 5'-TT to 5'-TC transitions (see Table 1) [14]. Therefore, a much greater diversity of changes are possible when considering photoproducts involving TT sequences, and the changes that occur at any particular site in the genome may reflect the contribution of all types of DNA damage.

An additional UV-induced photoproduct, thymidyl-(3'-5')-deoxyadenosine, termed the TA* photoproduct, results from the cycloaddition of the 5,6 double bonds of thymine and adenine [15,16]. Despite the fact that the quantum yield of this product was estimated to be 10–100 fold less than CPDs or [6–4] products [17], this largely unstudied minor photoproduct may have important mutagenic properties [18]. Specifically, Zhao and Taylor [18] illustrated that 82% of the plaques recovered following transfection of an SOS-induced *E. coli* host with a TA*-containing phage genome resulted in a targeted mutation and of these, 67% were a 5'-TA to 5'-TT change (Table 1).

The studies reported herein determined the frequencies and specificities of base substitution events occurring at 5'-TT, 5'-CT and 5'-TA sequences following UV-irradiation of *E. coli* cells. For these studies, the nonsense-defective (UAA) *tyrA14* gene was employed as a mutation target. Strains having site-directed changes in the DNA on either side of the TAA site were used to investigate the effect of altering the type of photoproducts that could form and potentially give rise to mutations. Thus, changes involving the 3'-base of both 5'-CT and 5'-TT sites, changes involving the 5'-base of TT sites and changes within 5'-TTT sequences were analyzed. In addition, we suggest a role for the TA* photoproduct in UV-induced mutagenesis. The results illustrate the types of base changes that can occur and the possible contributions of specific photoproducts to the overall spectrum of changes.

2. Materials and Methods

2.1 Bacterial Strains and Plasmids

The bacterial strains employed in this research included *E. coli* K-12 CJ236 (CGSC# 6793, *dut ung*), BMH71-18 (*mutS::Tn10*), XL-1 Blue (Stratagene) and NK6024 (CGSC# 6178 *pheA18::Tn10*), which were used for the propagation of plasmid DNAs and the construction of B/r strains FX-11-CA, TA, TC, and AT as described below. FX-11-CT is identical to parental

strain FX-11, and has been previously described [19]. FX-11 was excision repair defective (*uvrA115*), auxotrophic for tyrosine (a UAA nonsense defect) and contained a UAG-suppressing, glutamine-inserting tRNA mutation (*supE*) that suppressed a UAG-defective gene for leucine biosynthesis.

FX-11 derivatives containing base-substitution mutations in the DNA adjacent to the UAA-encoding sequence (Figure 1) were created by *in vitro* site-directed mutagenesis [20], *in vivo* gene replacement [21] and P1 transduction [22], as previously described [23]. The strain designations reflect the 5' and 3' bases neighboring the TAA target site, respectively. Thus, FX-11-CT has the DNA sequence 5'-CTAAT-3', as shown in Figure 1. DNA sequencing was used to confirm the transfer of the specifically altered *tyrA* genes into FX-11. These new strains had different base-substitutions adjacent to the UAA-encoding DNA sequence in the *tyrA* gene, (see Figure 1). In addition, Figure 1 illustrates the positions of potential CPD and [6-4] photoproducts within the target sequences and indicates the possible base substitution mutations in each of the FX-11 strains. For all the strains, a 5'-TT photoproduct (CPD or [6-4]) could be produced in the transcribed DNA strand. A misincorporation event opposite the 5'-base of this photoproduct (5'- $\bar{T}\bar{T}$) could give rise to the third position mutations, TAG, TAT, or TAC, while changes at the 3'-base (5'- $\bar{T}\bar{T}$) could produce a second position mutant (TTA or TCA). Formation of this lesion might be enhanced in strains FX-11-CA and FX-11-TA because of the additional thymine residue (5'- $\bar{T}\bar{T}\bar{T}$). Further, formation of a second 5'-TT photoproduct is possible in these strains so that the TAG, TAT and TAC mutations might also arise as a consequence of misincorporation opposite the 3'-base of this lesion (5'- $\bar{T}\bar{T}\bar{T}$). Strains FX-11-CA and FX-11-CT could allow the formation of a 5'-CT photoproduct (most probably a CPD, since [6-4]s do not readily form at such sequences) in the non-transcribed DNA strand that could generate the first position CAA, AAA, or GAA mutations via misincorporation opposite the 3'-base of the lesion (5'- $\bar{C}\bar{T}$). Similarly, the same mutations in FX-11-TA and FX-11-TC could result from a 5'-TT photoproduct (5'- $\bar{T}\bar{T}$). Although not highlighted in Figure 1, 5'-TA* photoproducts could also form in either DNA strand, and these could potentially target first or second position mutations.

2.2 Mutagenesis Assay

The FX-11 strains were subjected to a standard mutagenesis assay, as previously described [19]. Briefly, overnight cultures of the FX-11 strains were diluted and grown in A-0 buffer (10 ml) containing glucose (0.4%), phenylalanine and tyrosine (20 μ g/ml each) to an OD₄₅₀ = 0.5; the cells were then centrifuged and resuspended in 5 ml A-0 buffer. The cell suspension was placed in a 10 cm watch glass with a small stir bar and set on a magnetic stir plate about 35 cm from a germicidal lamp. UV fluence was measured using an International Light radiometer; a dose rate of 0.1 W/m² was employed. Cells were sampled before and after irradiation (10 secs or 1 J/m² final dose). Samples of the bacteria were then withdrawn to assay viability and mutagenesis using A-0 semi-enriched (0.02% nutrient broth) minimal agar plates containing leucine (20 μ g/ml) and phenylalanine (20 μ g/ml), but lacking tyrosine. After incubation at 37°C for 48 hours, revertant colonies were counted and subsequently isolated onto minimal agar media lacking nutrient broth. Purified revertants were tested with T4 phage to distinguish tRNA suppressor mutations from base substitutions within the UAA-encoding sequence in *tyrA14* (hereafter referred to as "backmutants"). At least 60 such revertants per mutagen dose were tested. Revertants that were identified as backmutants were phage tested again. PCR-based dideoxy DNA sequencing [24] was employed for the identification of base changes producing backmutations, as previously described [23,25]. Viability was determined by plating appropriately diluted suspensions of the treated and untreated cells on semi-enriched agar media and colonies were counted after 24 hours incubation at 37°C. Three or four independent experiments per strain were performed and the results were averaged.

3. Results

The objective of this research was to examine the changes in base substitution patterns that might result from changes in the DNA sequence that could alter the production of different types of UV photoproducts. Five different FX-11 strains (CA, CT, TA, TC and AT) that contained different bases neighboring the TAA site in the *tyrA14* gene were exposed to 0 or 1 J/m² germicidal UV light and plated onto agar plates lacking tyrosine (see Materials and Methods). Tyrosine prototrophs were isolated and then subjected to phage testing to delineate backmutants from suppressor mutants. Two different suppressor mutations (GlnU^o and GlnV^o) and eight possible base changes (AAA, CAA, GAA, TCA, TTA, TAC, TAG, TAT) at the TAA site in *tyrA14* were analyzed.

Viability and the frequency of tRNA suppressor mutations were not expected to differ from strain to strain as a consequence of the site-directed changes at the TAA target site. Consistent with this notion, the surviving fraction for each FX-11 strain following a dose of 1 J/m² UV was 66% ($\pm 4\%$). No significant (t-test, $p < 0.01$) differences in the response between the different strains was observed (data not shown). Likewise, the mutation frequencies (MFs) for tRNA suppressors were very similar. The average frequency of the GlnV^o suppressors was $5.5 \pm 0.6 \times 10^{-6}$ and the average frequency for the GlnU^o suppressors was $6.1 \pm 1.2 \times 10^{-6}$ (data not shown). No significant (t-test, $p < 0.01$) differences between strains was observed.

In contrast to the tRNA suppressor mutations, the backmutants showed strain to strain differences. A total of 340 backmutants from three or four separate experiments using each FX-11 strain were subjected to DNA sequence analysis to determine the specific base changes that occurred. These results are listed in Table 2. It should be noted that UV-induced mutation frequencies were at least 40-fold greater than the spontaneous frequencies. Therefore, very few of the sequenced mutations could have been of spontaneous origin.

Base substitutions occurring at the first position of the TAA sequence (CAA, AAA and GAA) were recovered from most of the FX-11 strains, but there were distinct differences. For example, the frequency of CAA mutations was greatest in FX-11 strains TA and TC, where the targeting lesion was expected to be a TT photoproduct rather than a CT photoproduct. The mutation occurs at the 3'-base of the dinucleotide sequence (5'-TT). Similarly, the frequency of AAA mutations was also greater in FX-11 strains TA and TC than in strains FX-11-CA and FX-11-CT. These results were consistent with the specificities of the TT CPD and the TT [6-4] photoproducts (see Table 1). However, the frequency of GAA mutations was greater in FX-11 strains CA and CT, suggesting that the 3'-base of a 5'-CT photoproduct was more able to target T to G transversions. Moreover, the frequency of AAA mutations in FX-11-AT was similar to the frequency in FX-11-TA, and was actually greater than the frequency in either FX-11-CA or FX-11-CT. Since neither a 5'-TT or 5'-CT photoproduct could target this mutation, this finding may suggest a role for the TA* photoproduct. The possibilities that i) a potential 5'-CT photoproduct produced the GAA mutations and ii) a TA* adduct contributed to the formation of AAA mutations are discussed below.

An analysis of base substitutions at the second position of the TAA site (TTA and TCA) revealed that the frequency of TTA mutations was greater than the frequency of TCA mutations in all the strains tested. Both mutations occur at the 3'-base of the central TT sequence (5'-TT) of the TAA site, again suggesting that TT photoproducts produced primarily T to A transversions rather than T to G transversions. Interestingly, in strains FX-11-CA and FX-11-TA, where the mutation occurs at the terminal T of a 3-base run (5'-TTT), no TCA mutations were recovered. It should also be noted that a T:A to C:G transition at the second position would produce a TGA mutation, which cannot be recovered in this assay.

At the third position (TAG, TAT and TAC mutations), the frequency of TAG and TAT mutations was greatest in FX-11-CA and FX-11-TA, where the probability of TT photoproduct formation would be expected to be enhanced by a TTT sequence. Here, the mutation could occur at either the 3'-base or the 5'-base of a TT photoproduct. Again, however, TAC mutation frequencies (T to G transversion) were low.

To view the contribution of each type of dinucleotide sequence to the formation of specific base substitutions, the data was reorganized as shown in Table 3. In addition, the strand containing the potential photoproduct is indicated. Since the FX-11 strains employed are excision-repair defective (*uvrA*), we do not expect transcription-coupled DNA repair to operate. Therefore, strand-specific differences may reflect the directionality of the replication fork. An analysis of these data revealed several interesting results. First, for potential lesions in the non-transcribed strand, 5'-C $\bar{T}$ sequences produced much greater frequencies of T to G transversions than 5'-T $\bar{T}$ sequences, but about 3.5-fold lower frequencies for T to C transitions and T to A transversions (Table 3, compare rows 1 and 2). Further, T to C transitions occurred about twice as often as T to A transversions regardless of the dipyrimidine site. Second, the 5'-T $\bar{T}$ sequence in the transcribed strand gave frequencies of T to A transversions that were reduced about 2-fold when compared with the same lesion in the non-transcribed strand (Table 3, compare rows 2 and 3). However, these frequencies increased 2-fold if a 5'-TT $\bar{T}$ site was available (Table 3, compare rows 3 and 4). Third, mutations at the 5'-base of a TT site were relatively sparse and included mostly T to C transitions and T to A transversions (Table 3, row 5). Finally, mutations within the 5'-TTT sequence (T $\bar{T}$ T) that could represent changes at either the 3'-base or the 5'-base of a TT photoproduct were increased compared with the 5'-T $\bar{T}$ sites (Table 3, compare rows 5 and 6). Also, the frequency of T to C transitions equaled the frequency of T to A transversions, and T to G transversions were not observed (Table 3, row 6). These results show that the spectrum of base substitutions produced by UV can be altered by the actual DNA sequence that affects the potential for formation of different types of UV-induced damage.

4. Discussion

The results described in this paper illustrate that changes in DNA sequence that alter the potential for UV photoproduct formation impact the frequency and types of base substitution mutations that occur. Several specific points are noteworthy.

The first observation concerns strain FX-11-AT, where the formation of a TT or CT photoproduct could not have targeted the first position changes (AAA, CAA, GAA). In this strain, the frequency of AAA mutations was, on average, similar to the frequency in the other strains tested. Here, the formation of a 5'-TA* photoproduct in the transcribed DNA strand could potentially target the AAA mutation. This result is consistent with the mutation spectrum produced by this adduct [18]. Further, Bose and Davies [17] noted the results of LeClerc and Istock [26] showing that irradiation of M13*lac* DNA resulted in two tandem base substitution mutations at TA sites and suggested that the TA* photoproduct may have been responsible. They also suggested that this photoproduct may have important implications in eukaryotic cells where the TATA box is involved in gene regulation. Little information is available regarding the potential role of this photoproduct in eukaryotic systems. However, Otoshi et al. [27] showed that UV-irradiated plasmid DNA that was treated with both CPD and [6-4] DNA photolyases prior to transfection into human cells was still able to produce a high percentage of T to A transversions at TA sites. As a result, they suggested a role for the TA* photoproduct in the production of these mutations. Obviously, more research is required to fully address the contribution of minor photoproducts to UV mutagenesis both in prokaryotic and eukaryotic systems.

On the other hand, we cannot rule out the possibility that the AAA mutations in strain FX-11-AT resulted from “non-targeted” mutagenesis. Error-prone replication opposite a TT photoproduct in the transcribed strand may have continued past the photoproduct to introduce the requisite base change. In addition, we cannot determine the contribution, if any, of a TA* adduct in the transcribed strand to the frequency of AAA mutations in the other FX-11 strains, or the contribution of a TA* adduct in the non-transcribed strand to the occurrence of TTA mutations. Additional experiments are underway in order to help clarify these alternatives.

The second observation reflects the data from strains FX-11-CA and FX-11-CT, where a 5'-CT photoproduct could form in the non-transcribed DNA strand. Here, the frequency of GAA mutations was significantly greater than in strains FX-11-TA and FX-11-TC, where a TT photoproduct could form instead (Figure 1 and Table 2). This suggested that the potential 5'-CT product had a much greater capacity for producing T to G transversions opposite the 3'-base of the lesion. The data of Banerjee et al. [12,13] indicate that TT-CPDs do not produce these changes. While the formation of a TA* photoproduct in either the non-transcribed strand (TA to GA change) or the transcribed DNA strand (TA to TC change) could possibly target the GAA mutation, the data in Table 1 show that i) TA to GA changes were not seen and ii) TA to TC changes were only 6% of the total mutations recovered. In addition, the frequency of GAA mutations in strain FX-11-AT is only 25% of that in strains FX-11-CA and FX-11-CT. Therefore, 5'-CT dimers may present a different obstacle for DNA polymerase, altering the base selection process. Since [6-4] products probably do not readily form at 5'-CT sequences [7,28], the premutagenic lesion is likely a CPD.

Third, the frequency of individual mutations in strain FX-11-TC, where 5'-GTTAAC sites are available in both DNA strands, showed a distinct asymmetry. The first position mutations (CAA, AAA, GAA) occurred with a much greater frequency (87% of the total) than second and third position mutations (TTA, TCA, TAG, TAT and TAC). More specifically, Figure 1 illustrates that, in strain FX-11-TC, the AAA and TTA mutations would be expected to be produced by identical mechanisms; a T to A transversion at the 3'-side of a TT photoproduct or either base of a TA* photoproduct. However, Table 2 shows that the frequency of the AAA mutation was 4.70×10^{-6} , while the frequency of the TTA mutation was 1.25×10^{-6} ; a 3.8-fold difference. Since FX-11 strains are excision repair defective, this difference cannot reflect transcription coupled repair. Moreover, since the target sites are identical, differential photoproduct formation seems unlikely, especially considering the fact that transcription of the *tyrA* gene is expected to be repressed under the growth conditions used prior to UV exposure. However, the damage distribution might be affected by ongoing DNA replication. Specifically, the generally more single-stranded nature of the discontinuously replicated template strand may alter the formation of DNA photoproducts. Patrick and Rahn [6] and Bose and Davies [17] noted differences in the yields of different photoproducts under native (dsDNA) or denatured (ssDNA) conditions. Alternatively, our results may reflect differential processing by the DNA replication machinery. A number of other studies have noted differences between mutagenesis occurring in opposite strands [29-31]. In contrast, Gawel et al. [32] did not observe any strand bias for UV-induced mutations in a variety of *lacZ* targets that were incorporated into the *E. coli* genome in opposite orientations. However, their targets did not include a TT site and their assays did not look at transversion mutagenesis. We are currently furthering our investigation of this asymmetry.

The fourth set of observations suggest that a greater probability for DNA damage at a particular site produces a greater frequency of mutations at that site. For example, the frequencies of CAA and AAA mutations are much greater at the 5'-TT site than at the 5'-CT site (Table 3, compare rows 1 and 2). This might indicate a greater capacity for DNA damage, since TT-CPDs occur more often than CT-CPDs, combined with the knowledge that [6-4] products can form at TT sites, but are unlikely at 5'-CT sites [28]. The increase in the proportion of CAA

mutations is consistent with the specificity of the TT [6–4] product (Table 1). In addition, the frequencies of 5'-base substitutions at TT sites were about 3-fold lower than the frequencies of 3'-base substitutions at TT sites (Table 3, compare rows 3 and 5, and rows 4 and 6). The data of Banerjee et al. [12,13] and LeClerc et al. [14] illustrate that only trans-syn CPDs produce a significant number of 5'-base substitutions. Since these would be expected to be relatively rare products of UV-irradiation, a lower frequency might be anticipated. Similarly, while the frequencies of TTA, TCA, TAG, TAT and TAC mutations vary from strain to strain (Table 2), it is apparent that when a TTT site was available to target the TAG, TAT and TAC mutations at either the 3'- or 5'-side of a TT photoproduct, the frequencies were generally greater. In particular, the frequency of TAG mutations, which could result from a TT [6–4] product, were highest in FX-11-CA and FX-11-TA. Again, a greater probability for damage may produce a greater frequency of mutation.

Finally, the frequency of mutation seems to vary with the overall probability of photoproduct formation within the target region *as a whole*. For example, strain FX-11-TA would provide the greatest density of reactive sites (three TT sites and two TA sites). Since TT cis-syn CPDs would be expected to predominate, fewer other types of damage may result. Thus, the majority of events produced would be primarily 3'-T to A (AAA, TTA and TAT mutations) and 3'-T to C (CAA and TAG mutations) and this is what was recovered. In contrast, strain FX-11-AT could only allow the formation of a single TT product plus two TA* adducts. Therefore, the greater probability of formation of minor products (trans-syn TT or TA*) might produce a greater diversity of mutations, and this is also what was observed.

Throughout this paper, we have attempted to make correlations between presumed damage potential and specific mutation frequencies. In some cases, however, there are differences that we cannot easily explain. For example, the frequency of TTA mutations occurring in strain FX-11-CA was greater than the frequency in strain FX-11-TA (Table 2). This difference cannot be accounted for by differences in the neighboring DNA sequence of the transcribed DNA strand and, hence, the presumed targeting photoproduct (TT-CPD). The same is true when comparing TTA mutations between strains FX-11-CT and FX-11-AT. It is possible that some of the TTA mutations could be the result of differential formation of a TA* photoproduct in the non-transcribed strand of the different strains as a result of the base pair located upstream of the TAA site. It is interesting to note that when this was a G:C base pair (as in FX-11-CA and FX-11-CT), the frequency of TTA mutations was greater. However, in the absence of direct measurements of CPDs, (6–4)s and TA* photoproducts within the target sequences of the individual strains, it is difficult to be certain. Such measurements would be exceptionally useful to this analysis, both in terms of confirming our suggestions and providing insight into those differences that we cannot currently explain. Despite this caveat, the results provide several interesting ideas that warrant further consideration.

Overall, our results point out that 1) the TA* photoproduct may contribute to UV mutagenesis, 2) the potential 5'-CT photoproduct (likely a CPD) may promote T to G transversions unlike its TT counterpart, 3) differential production or processing of photoproducts located in opposite strands may affect mutational outcome and 4) the local sequence environment may affect the types of photoproducts that form and, therefore, the spectrum of mutations that result. In total, these results extend our understanding of UV mutagenesis and provide testable hypotheses for further investigation of this complex process.

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					C						CAA
					A						AAA
					G						GAA
FX-11-CA	nt	5'–	g	c	T	A	A	a	t	–3'	
	tr	3'–	c	g	A	T	T	t	a	–5'	
FX-11-CT	nt	5'–	g	c	T	A	A	t	t	–3'	
	tr	3'–	c	g	A	T	T	a	a	–5'	
FX-11-TA	nt	5'–	g	t	T	A	A	a	t	–3'	
	tr	3'–	c	a	A	T	T	t	a	–5'	
FX-11-TC	nt	5'–	g	t	T	A	A	c	t	–3'	
	tr	3'–	c	a	A	T	T	g	a	–5'	
FX-11-AT	nt	5'–	g	a	T	A	A	t	t	–3'	
	tr	3'–	c	t	A	T	T	a	a	–5'	
					A						TTA
					G						TCA
					C						TAG
					A						TAT
					G						TAC

Figure 1.

DNA sequences, sites of CPD and [6–4] photoproducts and possible mutations in FX-11 strains. For each FX-11 strain, the sequence of the non-transcribed (nt) and transcribed (tr) strands of the *tyrA* gene is shown. Sites of CPD and [6–4] photoproducts potentially involved in targeted mutagenesis are shaded. Possible base changes and the resultant mutants are shown above and below their respective sites.

Table 1

Base changes produced at UV photoproducts in SOS-induced *E. coli*^a

Base Change	Thymine-thymine products ^b						
	cis-syn CPD		trans-syn CPD		[6-4] product		TA * adduct ^c
TT	2443	94%	536	89%	16	9%	55%
AT	0	0%	16	3%	0	0%	4%
CT	6	< 1%	4	1%	2	1%	5%
GT	0	0%	0	0%	2	1%	5%
CA	0	0%	0	0%	0	0%	0%
TA	130	5%	0	0%	0	0%	2%
TC	28	1%	1	< 1%	0	0%	18%
TG	0	0%	0	0%	158	85%	5%
Other ^d	1	< 1%	47	8%	1	1%	2%
Total	2608	100%	604	100%	185	100%	9%
							100%

^aData from [14,18,33].

^bValues reflect the number of progeny phage recovered and identified, followed by the percentage of the total and the percentage of the total that were mutant.

^cValues are a percentage of the total progeny analyzed, followed by the percentage of the total that were mutant.

^dOther includes multiple changes, deletions and insertions.

Table 2

Numbers and frequencies ($\times 10^{-6}$) of UV-induced mutations in FX-11 strains

Strain	Number of Mutants Sequenced								Total	
	CAA	AAA	GAA	TTA	TCA	TAG	TAT	TAC		
FX-11-CA	9	5	5	21	0	14	12	0	66	
FX-11-CT	30	13	14	32	6	14	12	4	126	
FX-11-TA	27	7	0	6	0	6	5	0	51	
FX-11-TC	24	15	0	4	1	0	1	0	45	
FX-11-AT	5	20	2	7	3	10	4	1	52	
Strain	CAA	AAA	GAA	TTA	Mutation Frequency ($\times 10^{-6}$) $\pm$ SEM			TAT	TAC	Total
					TCA	TAG	TAT			
FX-11-CA	2.01 $\pm$ 0.17	1.12 $\pm$ 0.10	1.12 $\pm$ 0.10	4.70 $\pm$ 0.41	0.00 $\pm$ 0.00	3.13 $\pm$ 0.27	2.69 $\pm$ 0.23	0.00 $\pm$ 0.00	14.7 $\pm$ 1.28	
FX-11-CT	2.20 $\pm$ 0.06	0.94 $\pm$ 0.03	1.05 $\pm$ 0.03	2.31 $\pm$ 0.07	0.42 $\pm$ 0.01	1.05 $\pm$ 0.03	0.84 $\pm$ 0.02	0.31 $\pm$ 0.01	9.12 $\pm$ 0.27	
FX-11-TA	7.72 $\pm$ 1.47	2.00 $\pm$ 0.38	0.00 $\pm$ 0.00	1.71 $\pm$ 0.33	0.00 $\pm$ 0.00	1.71 $\pm$ 0.33	1.43 $\pm$ 0.27	0.00 $\pm$ 0.00	14.5 $\pm$ 2.78	
FX-11-TC	7.52 $\pm$ 2.00	4.70 $\pm$ 1.25	0.00 $\pm$ 0.00	1.25 $\pm$ 0.33	0.31 $\pm$ 0.08	0.00 $\pm$ 0.00	0.31 $\pm$ 0.08	0.00 $\pm$ 0.00	14.1 $\pm$ 3.76	
FX-11-AT	0.71 $\pm$ 0.10	2.83 $\pm$ 0.40	0.28 $\pm$ 0.04	0.99 $\pm$ 0.14	0.42 $\pm$ 0.06	1.41 $\pm$ 0.20	0.57 $\pm$ 0.08	0.14 $\pm$ 0.02	7.35 $\pm$ 1.05	

Table 3
Average mutation frequencies ($\times 10^{-6}$) for different base substitutions at potential photoproduct sites

Row	Strand ^a	Site ^b	Strains	T to C		T to A		T to G	
				Mutation	Frequency ^c	Mutation	Frequency ^c	Mutation	Frequency ^c
1	nt	CT	CA,CT	CAA	2.1 ± 0.1	AAA	1.0 ± 0.1	GAA	1.1 ± 0.1
2	nt	TT	TA,TC	CAA	7.6 ± 1.1	AAA	3.4 ± 0.8	GAA	0.0 ± 0.0
3	tr	TT	CT,TC,AT	TGA	nr	TTA	1.4 ± 0.2	TCA	0.4 ± 0.0
4	tr	TTT	CA,TA	TGA	nr	TTA	3.2 ± 0.7	TCA	0.0 ± 0.0
5	tr	TT	CT,TC,AT	TAG	0.9 ± 0.2	TAT	0.5 ± 0.1	TAC	0.1 ± 0.0
6	tr	TTT	CA,TA	TAG	2.4 ± 0.4	TAT	2.1 ± 0.3	TAC	0.0 ± 0.0

^aTranscribed (tr) or non-transcribed (nt) strand of the *E. coli tyrA* gene.

^bSite of potential photoproduct formation and position of targeted base substitution. CT, 3'-base of a CT photoproduct; TT, 3'-base of a TT photoproduct; TTT, 3'-base of a TT photoproduct in a TTT sequence; TT, 5'-base of a TT photoproduct; TTT, 5'-base of a TT photoproduct in a TTT sequence.

^cAverage mutation frequency ± standard error of the mean. nr, non-recoverable.