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Transcription-repair coupling determines the strandedness of ultraviolet mutagenesis in *Escherichia coli*

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ABSTRACT We have analyzed the spectra of UV-induced mutations in the *lacI* gene of a wild-type and an *mfd* strain of *Escherichia coli*. *mfd* strains have been recently proposed to be deficient in a factor coupling DNA repair and transcription. Analysis of UV-induced mutations occurring at adjacent pyrimidines showed that mutations in the wild-type strain arose largely from the nontranscribed strand but arose predominantly from the transcribed strand in the *mfd* strain. The overall strand switch was 14-fold. One mutation, G·C → A·T in the *lacI* initiation codon, showed a >300-fold shift. No effect was observed for mutations at non-pyrimidine-pyrimidine sequences. These results provide *in vivo* evidence for a key role of the *mfd* gene in controlling the strandedness of mutagenesis and support the proposed role of the *mfd* gene product in directing DNA excision repair to the transcribed strand of a damaged gene.

Heterogeneity in DNA repair, in prokaryotic and eukaryotic cells, has been observed at several levels: chromatin-domain-specific repair (1–3), preferential repair of active genes (4–10), and strand-specific repair (4, 11, 12). These different levels of repair specificity may be interrelated. Actively repaired chromatin domains often include actively transcribed genes, whereas most gene-specific repair may be explained by preferential repair of the transcribed strand of the gene.

A current model for preferential repair of the transcribed strand stems from the observation that UV light-induced pyrimidine dimers, as well as other lesions, effectively block transcription (13, 14). A stalled RNA polymerase–DNA complex may act as a signal to direct excision-repair enzymes to the transcribed strand of a gene, enhancing its repair (4). Precisely how this can be achieved is unclear, but one possibility is the existence of a transcription-repair coupling factor (TRCF). Recently, such a factor was purified from *Escherichia coli* using an *in vitro* strand-specific repair assay (15, 16). This factor was able to direct nucleotide excision-repair (UvrABC) to the transcribed strand of a gene, resulting in preferential repair of this strand. Furthermore, a defect in transcription-stimulated repair was discovered in extracts from an *E. coli mfd* strain that could be complemented by the TRCF purified from wild-type cells (16).

The *mfd* strain of *E. coli* was first characterized by Witkin (17) as a derivative unable to display the phenomenon of mutation frequency decline (MFD). MFD is the rapid loss of certain UV-induced nonsense suppressor mutations upon transient inhibition of protein synthesis following irradiation (17–19). This phenomenon was determined to be a manifestation of excision repair because (*i*) it was not observed in excision-repair-deficient (*uvr*) strains (17, 20) and (*ii*) the *mfd* strain displayed a reduced rate of excision repair (21, 22). Bockrath and coworkers (23, 24) noted that MFD only occurred when the lesion putatively responsible for the suppressor mutation resided on the transcribed strand of the tRNA gene, suggesting that MFD might be a strand-specific phenomenon.

Clearly, strand-specific repair will have profound consequences for the distribution of mutations. UV-induced mutations occur preferentially at sites of adjacent pyrimidines, consistent with the two main UV photoproducts, the cyclobutane pyrimidine dimer and the 6-4 pyrimidine-pyrimidone photoproduct. These mutations have been shown in a number of cases (23, 25, 26) to arise preferentially at dipyrimidines residing in the nontranscribed strand, consistent with the preferential repair of lesions in the transcribed strand. To examine the mutational consequences of strand-specific repair in detail, we have characterized the spectrum of UV-induced mutations in the *lacI* gene in a wild-type and an *mfd* strain. The *lacI* gene (encoding the repressor of the *lac* operon) has been used previously to determine the UV mutational spectrum in cells proficient and deficient in excision repair (27).

Two specific predictions for the spectrum of mutations in the *mfd* strain may be advanced. First, in the absence of TRCF, the bias in favor of mutations at dipyrimidines in the nontranscribed strand, as observed in the wild-type strain (25, 27), should disappear. Second, a bias in favor of mutations at dipyrimidines in the transcribed strand may occur. The latter prediction is based on the observation *in vitro* of an *inhibitory* effect of transcription on excision repair of the transcribed strand (but not the nontranscribed strand) in the absence of TRCF (14). Our results show that both predictions are fulfilled.

MATERIALS AND METHODS

Strains and Media. B/r strain WU3610-45 (*tyr, leu, mfd*) (22) obtained from C. Selby (University of North Carolina) was the source of *mfd*. All other strains used are K-12. NR10121 (*ara, thi, zcf-117*::Tn*10*, *mfd*, Δ*prolac*) and NR10125 (*ara, thi, zcf-117*::Tn*10*, Δ*prolac*) are derived from KA796 (*ara, thi,* Δ*prolac*) (28) by P1 transductional transfer of *mfd* linked with transposon *zcf-117*::Tn*10* (29). NR10130 and NR10134 are identical to NR10121 and NR10125 except they contain F′*prolac* (F′128-27). This F′ carries the i^Q (*lacI*) and L8 (*lacZ*) promoter mutations that facilitate the scoring of *lacI* mutations (30) as well as two silent *lacI* base substitutions (*lacI204*) that abolish a frameshift hotspot (31). NR10149 (*uvrB mfd*) and NR10148 (*uvrB*) are identical to NR10130 and NR10134, respectively, except for the Δ(*uvrB-bio*) marker derived from NR3951 (27). LB broth was as described (32). Minimal (MM) plates contained 1× VB salts (33), 1 μg of thiamine per ml, 0.1 μg of biotin per ml, and 0.2% glucose. P-gal plates, used for selection of *lacI* mutants, are MM plates containing 750 μg of phenylgalactoside (P-gal) per ml instead of glucose. All strains were grown at 37°C.

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Abbreviations: TRCF, transcription-repair coupling factor; MFD, mutation frequency decline; P-gal, phenylgalactoside; YY, adjacent pyrimidines.

UV-Light Irradiation and Selection of *lacI* Mutants. Overnight cultures were diluted in fresh LB broth, grown to 2–3 $\times 10^8$ cells per ml, centrifuged, resuspended in half the volume of ice-cold 0.9% (wt/vol) NaCl. An aliquot (10 ml) was irradiated in a Petri dish with UV light from a 782L10 bulb (American Ultraviolet, Murray Hill, NJ) (1.2 $J \cdot m^{-2} \cdot sec^{-1}$). Duplicate samples were plated before and after irradiation on LB plates to determine survival. All procedures were performed in subdued light. For *lacI* frequency determination, 0.5 ml ($\approx 2 \times 10^8$ cells) of irradiated cells was added to 5 ml of LB broth, grown overnight, and plated on MM and P-gal plates; on P-gal only *lacI* (and *lacO*) mutants are able to grow (30). To collect mutants for mutational spectra, eight independent cultures from each strain (wild type and *mfd*) were irradiated (72 $J \cdot m^{-2}$), diluted 5-fold in LB broth, and immediately distributed in aliquots (0.2 ml) over the wells of a 96-well microtiter dish (one dish per culture, $\approx 3 \times 10^7$ cells per well). Survival was 17% and 1.2% for wild type and *mfd*, respectively. After overnight growth, 5 μl from each well was spread on a quarter section of a P-gal plate. One or two *lacI* colonies per quarter section were picked randomly (1168 for wild type and 1068 for *mfd*) and gridded on a series of P-gal plates (100 colonies per plate), which were then replica-mated into strains CSH52 for determination of dominant *lacI* mutants (i^{-d}) (31, 34) and S90C for recombinational transfer of the mutant (i^{-d}) genes from F′*prolac* to single-stranded phage vector mRS81 for DNA sequencing (28, 32). Only one i^{-d} mutant was selected from each well.

DNA Sequencing. DNA sequencing of mutant *lacI* genes was performed on phage vector mRS81 as described (28, 32) using Sequenase 2.0 (United States Biochemicals). A total of 273 (wild type) and 235 (*mfd*) independent i^{-d} mutants was sequenced, among which 246 (wild type) and 204 (*mfd*) yielded a mutation within the *lacI* dominant target (in 10–15% of the mutants no mutation was detected in the i^{-d} region).

Colony Hybridization. To detect the G·C → A·T mutation at base pair 31, *lacI* colonies were gridded on a nylon membrane (NEN) placed on an LB plate and probed as described in Halliday *et al.* (35) using an oligonucleotide complementary to base pairs 21–39 of the (−) strand of *lacI* but with A instead of G at base pair 31. Several positive colonies were sequenced to confirm the identification.

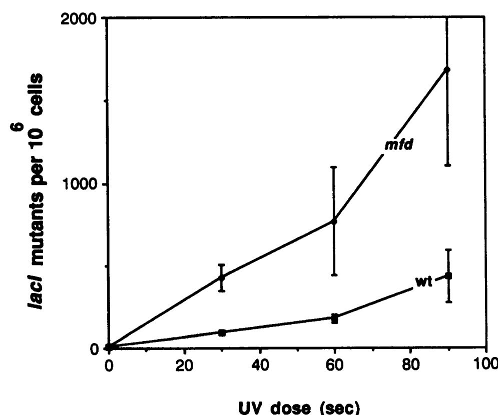
RESULTS

The *E. coli mfd* strain showed a slightly higher UV mutability for forward *lacI* mutations than the parental strain (Fig. 1), consistent with previous observations using other markers (17, 22, 36). In addition, it showed a somewhat higher sensitivity to the killing effects of UV irradiation (Fig. 1). The latter observation differs from earlier reports in which no difference in killing was observed between wild-type and *mfd* strains (17, 22). We observed increased UV sensitivity on LB and MM media and in K-12 and B/r backgrounds (not shown). However, no increased UV sensitivity was observed for a *uvrBmfd* strain compared to a *uvrB* strain (not shown), suggesting that the origin of the increased UV sensitivity resides in the pathway of excision repair.

To isolate *lacI* mutants for DNA sequence analysis, we selected a dose of 72 $J \cdot m^{-2}$, at which *lacI* mutant frequencies were obtained of 100 (± 17) $\times 10^{-6}$ and 356 (± 66) $\times 10^{-6}$ for wild type and *mfd*, respectively, a 100- to 300-fold enhancement over the background. To facilitate the DNA sequencing (the *lacI* gene being 1100 base pairs long), we concentrated on those mutants carrying a mutation in the first 200 base pairs of the gene, encoding the operator-binding domain of the protein (30). This subset of mutants (termed i^{-d}) can be easily identified by their dominance over the wild type in a genetic complementation test (34). This approach has the advantage of a reduced target size combined with a high density of detectable mutable sites (31, 34).

Both strains had a similar proportion of dominant mutants, 29% (wild type) and 27% (*mfd*). The sequence analysis of 246 (wild type) and 204 (*mfd*) independent dominant mutants is summarized in Table 1. For both strains, the majority were base-pair substitutions, followed by smaller contributions of frameshifts, double mutations, and deletions. The complete spectra of the base-pair substitutions and frameshifts are presented in Fig. 2. Differences between the strains can already be gleaned from Table 1. In the wild-type strain, transitions (44%) were more frequent than transversions (35%), whereas in the *mfd* strain transversions (60%) were more frequent than transitions (26%).

Striking differences between the wild-type and *mfd* strains become apparent when inspecting the DNA sequence dependence of the mutations (Fig. 2). Over 85% of the UV-induced base-pair substitutions occurred at sites of adjacent pyrimidines (YY, where Y indicates pyrimidine), in the wild-type (171/194) and the *mfd* (153/177) strains. This is consistent with the two major UV photoproducts occurring at YY sequences—i.e., the cyclobutane pyrimidine dimer and the (6-4) pyrimidine-pyrimidone product, both of which are mutagenic (37). This correlation allows an assignment of the strand from which the mutations arise. Mutations at YY sequences in the nontranscribed strand (NTS) will appear at C or T bases in Fig. 2 (since the nontranscribed strand is the strand displayed). Conversely, mutations arising from YY sequences in the transcribed strand (TS) will appear at G or A bases. Table 2 shows all base-pair substitutions and frameshifts as they occur at C, T, G, or A sites. Among the



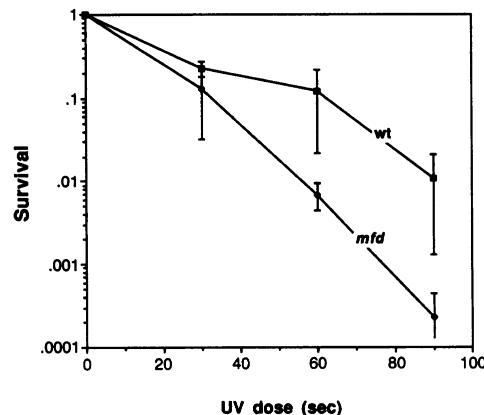


FIG. 1. Mutation frequency and survival in UV-treated *E. coli* K-12. Cells were exposed to a UV flux of 1.2 $J \cdot m^{-2} \cdot sec^{-1}$. wt, Wild type. Values (±SD) are the average of three (*mfd*) or five (wt) determinations.

Table 1. UV-induced i^{-d} mutations in wild-type (wt) and *mfd* strains

Mutation	wt (%)	*mfd* (%)
Base-pair substitution	194 (79)	177 (87)
G·C → A·T	92	34
A·T → G·C	17	20
Transitions	109 (44)	54 (26)
A·T → C·G	16	31
G·C → T·A	16	27
A·T → T·A	21	15
G·C → C·G	32	50
Transversions	85 (35)	123 (60)
Frameshift*	32 (13)	8 (4)
Double mutation	15 (6)	18 (9)
Tandem	12	7
Non-tandem	3	11
Deletion	5 (2)	1 (0.5)
Total	246	204

*All frameshifts were the loss of a single base except for one A·T base-pair addition between base pairs 124 and 125 that occurred in the wild-type strain (see Fig. 2).

mutations at YY sites in the wild-type strain, there is a 3.2-fold bias in favor of the nontranscribed strand (NTS/TS = 152/48). However, the opposite is observed in the *mfd* strain, a 4.5-fold bias in favor of mutations in the transcribed strand (NTS/TS = 29/130). Thus, a 14-fold switch in the strand specificity of the mutations at YY sites has taken place. In contrast, no significant changes are observed in the distribution of mutations over the four bases at non-YY sites. The latter events (for which no strand assignment can be made) may represent examples of "untargeted" mutagenesis resulting from the SOS mutator effect (38, 39).

The second analysis that we performed was of mutations at base pair 31, the third base of the *lacI* translation initiation codon (Fig. 2). A G·C → A·T transition at this site was found in a previous study of UV mutagenesis as the most frequent mutation (5%) in a Δ*uvrB* strain, while being absent in the wild-type strain (27). The G·C base pair at this position is part of a dipyrimidine sequence in the transcribed strand, and one possible explanation for this difference is that this site is an efficient substrate for transcription-directed repair. Since this mutation does not confer dominance over the wild-type repressor, we used a colony-hybridization method for its detection. The results were striking (Table 3). No mutations were found among 1168 *lacI* colonies in the wild-type strain, but 290 were detected among 1068 in the *mfd* strain. Thus, there is at least a 300-fold difference in the relative proportion of this mutation (<0.09% vs. 27%).

Since the G·C → A·T transition at base pair 31 proved to be extremely sensitive to the state of the *mfd* gene, we used it as a marker to determine the dependence of the *mfd* effect on the UvrABC excision-repair system. We isolated a new set of UV-induced *lacI* mutants in a Δ*uvrB* strain and an Δ*uvrB mfd* strain and again analyzed them by colony hybridization (Table 3). In the Δ*uvrB* strain 3% (21/800) of the *lacI* mutants had a G·C → A·T at base pair 31. This percentage is similar to the 5% observed previously (27) and confirms this site as a hot spot for mutation in an excision-repair deficient strain (>30-fold increase over the wild-type strain). Interestingly, in the Δ*uvrB mfd* strain a similar 4% (33/800) had this mutation. Thus, the dramatic effect of the *mfd* gene product on mutations at this site (>300-fold) depends on the excision-repair system. This is analogous to the excision-repair dependence of MFD (17).

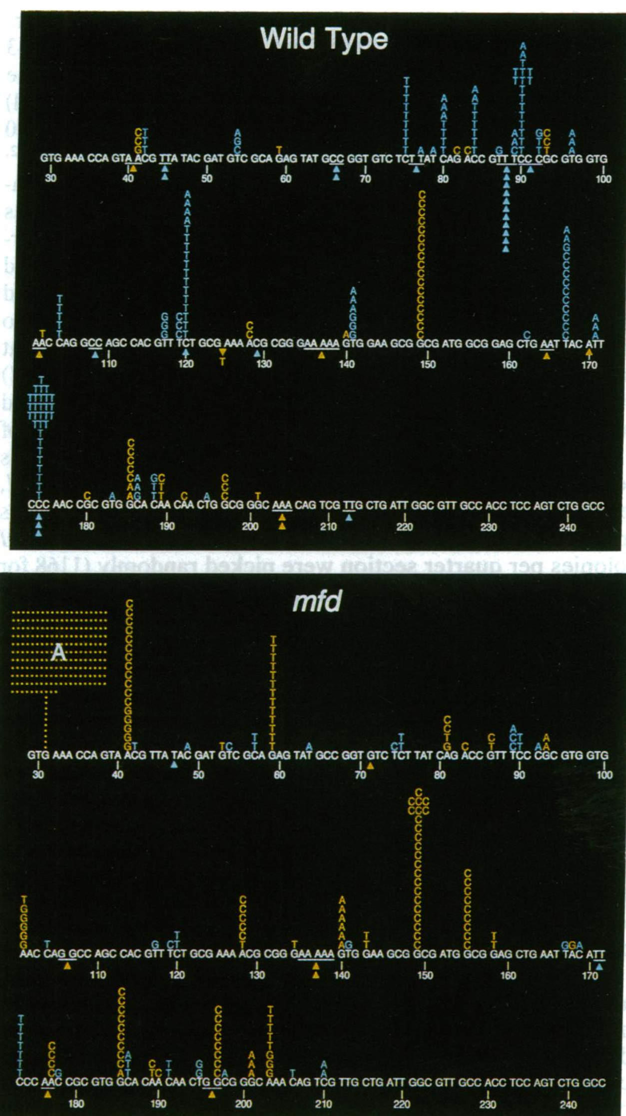


FIG. 2. Distribution of dominant *lacI* base-pair substitutions and frameshifts in the wild-type (*Upper*) and *mfd* (*Lower*) strains. Blue, mutations at C or T bases; yellow, mutations at G or A bases. Mutations above the DNA sequence are base-pair substitutions; those below are one-base frameshifts. Underlined bases indicate ambiguity in the position of the missing base. The G → A transition at position 31 is included for completeness, although this is not a dominant mutation.

DISCUSSION

The results of this paper provide *in vivo* evidence for a key role of the *E. coli mfd* gene in determining the strandedness of mutagenesis by UV light, and strongly support the proposed role of the *mfd* gene product as a TRCF (16) directing nucleotide excision repair to the transcribed strand of a damaged gene. Spectra of UV-induced mutations in wild-type and *mfd* strains revealed a pronounced mutational shift (14-fold) from dipyrimidines in the nontranscribed strand (wild type) to dipyrimidines in the transcribed strand (*mfd*). At one additional site, the *lacI* initiation codon, where the assigned lesion is in the transcribed strand, the loss of Mfd function increased the proportion of this mutation >300-fold. Together these data support the idea that in a wild-type strain, mutations arise largely from lesions in the nontranscribed strand because lesions in the transcribed strand are repaired preferentially. In the absence of the *mfd* gene product, the bias in favor of the nontranscribed strand is lost and actually reversed (see below).

Excess of Mutations in the Transcribed Strand of the *mfd* Strain. In the absence of the *mfd* gene product, a >4-fold bias is observed in favor of mutations at YY sequences in the

Table 2. UV-induced mutations at C, T, G, or A sites at YY and non-YY sequences

Site	Mutation	Wild type				*mfd*			
YY	Base substitutions	C → T	84	G → A	3	C → T	14	G → A	10
		C → A	11	G → T	2	C → A	3	G → T	18
		C → G	1	G → C	26	C → G	1	G → C	48
		T → C	16	A → G	1	T → C	5	A → G	14
		T → A	11	A → T	3	T → A	1	A → T	9
		T → G	6	A → C	7	T → G	4	A → C	26
			129		42		28		125
	Frameshifts	ΔT	15	ΔG	0	ΔT	1	ΔG	2
		ΔC	8	ΔA	6	ΔC	0	ΔA	3
			23		6		1		5
Non-YY	Base substitutions	C → T	5	G → A	0	C → T	8	G → A	2
		C → A	2	G → T	1	C → A	4	G → T	2
		C → G	2	G → C	3	C → G	0	G → C	1
		T → C	0	A → G	0	T → C	0	A → G	1
		T → A	7	A → T	0	T → A	5	A → T	0
		T → G	3	A → C	0	T → G	1	A → C	0
			19		4		18		6
	Frameshifts	ΔT	0	ΔG	0	ΔT	1	ΔG	1
		ΔC	1	ΔA	1	ΔC	0	ΔA	0
			1		1		1		1

transcribed strand. Neither the DNA damage distribution (25) nor the distribution of selectable sites (from ≈4000 i^{-d} mutants isolated in our laboratory; refs. 31, 34, and 40 and unpublished data) can properly explain the excess of mutations in the transcribed strand in the *mfd* strain. One possible explanation for these results, supported by experimental data, is that in the absence of transcription-repair coupling, the repair of the transcribed strand is actually inhibited by the RNA polymerase stalling at the lesion. Using a defined *in vitro* DNA repair system containing ABC excinuclease, RNA polymerase, and UV-damaged DNA, Selby and Sancar (14) showed that RNA polymerase was effectively blocked at the sites of lesions in the transcribed strand and significantly inhibited their repair. No inhibition of transcription or of DNA repair occurred when the lesion resided in the nontranscribed strand. In fact, these results, in conjunction with the discovery that the *mfd* strain is deficient in TRCF, prompted a prediction for a reversed strand bias in the absence of Mfd (16).

A more detailed model within which to explain the strand biases relates to the precise mechanisms by which UV-induced mutations are thought to occur in *E. coli*. Two separate mechanisms have been proposed based on the ability of photoreactivation to reverse UV mutagenesis at various times following irradiation (41–43). In excision-repair-proficient strains, the majority of mutations occurs early—i.e., during excision repair and before resumption of DNA replication. Presumably, mutations are produced during the filling of excision-repair gaps, most probably when repair of a lesion in one strand encounters a lesion in the opposite strand (misrepair). In excision-repair-deficient strains, production of mutations occurs later, starting when DNA replication resumes. Here, mutations could occur either at the replication fork (misreplication) or during the filling of the daughter-strand gaps that are created when DNA replication is blocked at lesions and reinitiates downstream from the lesion.

If the misrepair model for repair-proficient strains also applies to *mfd* strains, then the question of which strand will produce the mutations must be viewed in terms of the *rate* of repair of one strand relative to the other. Removal of lesions from one strand will have two linked consequences: (*i*) removal of mutagenic lesions from that strand and (*ii*) simultaneous "fixing" of mutations due to a photoproduct in the opposing strand. In wild-type cells, the action of TRCF would lead to more rapid repair of lesions in the transcribed strand, causing an excess of mutations in the nontranscribed strand. In *mfd* cells, the inhibitory effect of transcription on repair of the transcribed strand would result in more rapid repair of lesions in the nontranscribed strand, causing an excess of mutations in the transcribed strand.

Table 3. G·C → A·T transitions at base pair 31 as detected by colony hybridization

Strain	Colonies tested	Colonies positive	Frequency
Wild-type	1168	0	<0.00068
mfd	1068	290	0.27
uvrB	800	21	0.03
uvrB mfd	800	33	0.04

UV dose for wild-type and *mfd* strains was 72 J·m^{-2}. For *uvrB* and *uvrB mfd*, two doses (0.7 and 7 J·m^{-2}) were used (400 colonies at each dose). Because of similar results, the data were combined.

Strand Switch for G·C → C·G Transversions. The G·C → C·G transversions are an interesting case because they not only show the smallest switch between wild-type and *mfd* strains (1.8-fold) but they are also among the most frequent mutations (Table 2). It is tempting to speculate that they present a special case. Each G·C → C·G occurs at an identical sequence, G$\underline{G}$C (mutated base underlined). No cyclobutane dimer or (6-4) photoproduct has been observed at any of these sites (25). It cannot be excluded that other lesions, such as purine photoproducts (44), are involved. Mutations at these sites may not be subject to the Mfd effect, because the lesions at this site do not block transcription or are not subject to excision repair, or, alternatively, because damage is absent at these sites (untargeted mutation).

Effect of *mfd* at DNA Damage Sites. Two arguments indicate that the calculated overall 14-fold strand switch for UV-induced mutations underestimates the intrinsic effect of *mfd* at DNA damage sites. First, when considering the selectable YY sites for i^{-d} G·C → A·T transitions (the most frequent UV-induced mutation; refs. 27 and 45–47), the transcribed strand is at a serious disadvantage. It contains only three such sites (base pairs 140, 185, 201) compared to eight for the nontranscribed strand (base pairs 75, 80, 84, 90, 92, 104, 120, 174). Furthermore, the three sites are examples of $\underline{C}$C or $\underline{C}$T sites (mutated base underlined), which are generally less mutagenic than T$\underline{C}$ sites (27); the nontranscribed strand contains four T$\underline{C}$ sites. The importance of T$\underline{C}$ sites may be evidenced by the large effect observed for position 31.

Indeed, the observed switch from transition to transversion mutagenesis in the *mfd* strain (Table 1) is consistent with the paucity for G·C → A·T selectable sites in the transcribed strand.

Second, ≈12% of all UV-induced base substitutions in either wild-type or *mfd* strains occur at non-YY sites, where no cyclobutane dimer or (6-4) dipyrimidine products can be formed (Table 2). These untargeted events, presumably resulting from the SOS mutator effect (38, 39), are expected to occur also at YY sites and to be equally refractory to the Mfd effect at these sites. The average frequency of untargeted mutations per non-YY site can be calculated to be 0.74 at C, T sites and 0.42 at G, A sites (from Table 2 and analysis of selectable sites, data not shown). Subtracting the equivalent proportion expected at YY sites, a significant effect on the overall strand switch is observed. In the wild-type strain, the bias in favor of the nontranscribed strand increases slightly from 3.1 (NTS/TS = 129/42) to 3.7 (NTS/TS = 92/25), whereas in the *mfd* strain, the bias in favor of the transcribed strand increases strongly from 4.5 (125/28) to >100 (108/0) (loss of all nontranscribed-strand mutations). This would indicate an overall strand switch at DNA damage sites of >300-fold.

Mutation at Base Pair 31. The G·C → A·T at base pair 31 comprised <0.1% of the *lacI* mutants in the wild-type strain (no mutants observed) but comprised 27% in the *mfd* strain, a >300-fold effect (see Table 3). The local sequence is 5′-GTG̲AAA-3′ (mutated base underlined), GTG being the *lacI* translation initiation codon. The transcribed strand reads 5′-TTTC̲AC-3′. The study by Koehler *et al.* (25) detected a relatively frequent pyrimidine-pyrimidone (6-4) photoproduct at TC in this strand. It is an interesting question why this mutation can (*i*) be entirely absent among *lacI* mutants in the wild-type strain but (*ii*) comprise more than a fourth of all mutations in the *lacI* gene (1100-bp target) in the *mfd* strain. Experiments in an excision-repair-deficient background (*uvrB*) showed this site to comprise about 3% of all *lacI* mutations, regardless of whether the strain was mfd^+ or mfd^- (Table 3). Assuming that this percentage reflects the intrinsic mutability of this site relative to other sites, position 31 appears (in Uvr^+ backgrounds) to be very sensitive to transcription-stimulated repair (3% reducing to <0.1% in the mfd^+ strain) and very sensitive to transcription-induced inhibition of repair (3% increasing to 27% in the mfd^- strain). These two effects may well be related. A high affinity of RNA polymerase for this site (when damaged) may produce such effects by disproportionally stimulating repair in the presence of coupling factor and disproportionally inhibiting repair in its absence.

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