Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4123–4127, July 1982
Genetics

Mutational specificity of UV light in *Escherichia coli*: Indications for a role of DNA secondary structure

(UV mutagenesis/excision repair/mutational hotspots)

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Communicated by Evelyn M. Witkin, March 29, 1982

ABSTRACT **We used the *lacI* forward mutagenesis system to determine the mutational specificity of UV-induced mutation in a repair-proficient (Uvr^+) and a repair-deficient (ΔUvrB) strain of *Escherichia coli*. The spectra recovered at similar levels of mutagenesis were similar, the exception being a mutational hotspot at site A24 specific to the ΔUvrB strain. Mutations induced at this hotspot, as well as those induced at other mutational hotspots that were found to be common to both the Uvr^+ and Uvr^- strains, involve G·C → A·T transitions. All of the hotspots are at sites of potential dipyrimidine photoproducts, such as thymine-cytosine and cytosine-cytosine dimers, or of pyrimidine-cytosine photoproduct Py-C* lesions. Each of these hotspots occurs at a site in the potential hairpin loop of quasi-palindromic sequences. These observations suggest an important role for DNA structure in determining the fate of UV-induced premutational lesions.**

The *lacI* system of *E. coli* provides a method for determining UV-induced mutational specificity at a large number of sites (1–3). In contrast, earlier studies in other systems generally relied upon the analysis of reversion at a rather limited number of sites (4–6). Often the mutants analyzed in such reversion studies were originally induced by the mutagen (7); therefore, the possibility existed that preferentially mutable sites or hotspots had been selected and that these might behave atypically. Alternatively, the original mutation might have removed a critical DNA target sequence rendering the site practically immutable. Moreover, in studies of the reversion of nonsense mutations, the majority of "revertants" often occur not in the structural gene but at suppressor loci, which behave differently in their responses to UV light (4, 8, 9). The *lacI* system allows an examination of forward mutagenesis at 65¶ individual sites where nonsense mutations can arise by a single base substitution. Because both the DNA sequence and the location of the nonsense mutations have been established (10), each mutation can be attributed to a specific transition or transversion event.

Detailed knowledge of the sites at which mutagenesis occurs and of the specific base changes produced may yield important clues about the premutagenic lesions and how they are processed. In the case of UV irradiation, the mutational mechanism is poorly understood. Photoreactivation experiments suggest that mutagenesis in both excision-proficient and excision-deficient strains requires pyrimidine dimers (11, 12). Nevertheless, considerable evidence exists that not all UV-induced mutagenesis occurs at the sites of induced lesions (13). For example, undamaged bacteriophage λ plated on UV-irradiated hosts show high levels of $recA^+$-dependent mutagenesis ("indirect mutagenesis") (14). In a current model, UV-induced mutagenesis in *E. coli* proceeds through a $recA^+$-dependent, inducible error-prone repair pathway called "SOS" repair [reviewed by Witkin (13)]. It is postulated that, where alternative repair pathways are unable to function (e.g., at gaps resulting from blocked DNA replication at pyrimidine dimers), SOS repair permits bypass of the nontemplating damage but does so at the cost of replicational fidelity (15, 16). This model suggests that lesions such as pyrimidine dimers, capable of blocking DNA replication, would be the premutagenic lesions but makes no clear prediction as to whether most mutations would be directly "targeted" or "untargeted" (perhaps the result of reduced replicational fidelity or of otherwise cryptic lesions) and would occur at more distant sites. Thus, studies on mutational specificity may provide clues as to the nature of the responsible photolesion and the mechanism of mutagenesis.

However, the situation is more complex. Evidence has accumulated suggesting that pathways for UV-induced mutagenesis differ in excision-proficient and excision-deficient strains (11, 17, 18). In the case of repair-proficient strains, it is hypothesized that mutations arise at sites within structural genes during excision-repair gap filling. This process is ($lexA^+$ $recA^+$)-dependent, but protein synthesis is not required for mutation fixation, and the process may be considered constitutive (18). However, mutagenesis in excision-deficient cells and in excision-proficient strains at particular sites, such as suppressor tRNA loci, appear to involve error-prone postreplication repair, which also requires the $lexA^+$ $recA^+$ genotype, but through a process requiring protein synthesis. Moreover, because of the low level of UV-induced mutagenesis in Uvr^+ bacteria, it has been postulated that excision-proficient bacteria have an error-free post-replication excision-dependent repair process that is absent in excision-deficient cells (19).

In this study we made use of the *lacI* system to investigate UV mutational specificity in both excision-repair-proficient and excision-repair-deficient strains of *E. coli*.

MATERIALS AND METHODS

Strains, Media, and the *lacI* System. Unless otherwise stated, the materials and techniques for the *lacI* forward-mutation system were the same as those used by Miller *et al.* (1) and Coulondre and Miller (2, 3). The wild-type strain NR3835 (F′ *pro-lac/ara*, Δ(*pro-lac*), *thi*, *trpE9777*) has been described (20). Strain NR3951 [same as NR3835, except (*bioFCD*, *uvrB chlA*)] was constructed by P1*vir*-mediated transduction with

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Abbreviation: MFD, mutation frequency decline.

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¶ There are 72 characterized base pair substitutions that can give rise to an amber or ochre mutation by a single base change, but only 65 of these are independent sites. Seven sites correspond to the tyrosine codons TAT and TAC, which can be converted to an amber or ochre codon by an alteration at the third position.

C261 [*str*^{r}, *his*, Δ(*bioFCD*, *uvrB chlA*)] as donor (21); chlorate-resistant transductants of NR3835 were selected and screened for UV sensitivity. Biotin was provided at 0.5 μg/ml.

UV Irradiation and Mutant Selection. UV irradiation was carried out as described (22), and mutational spectra were prepared from 10–15 independent cultures grown to 4–6 × 10^8 cells per ml. *lacI* mutants were selected by plating 10 μl of the irradiated cell suspension in 3 ml of soft agar on plates containing phenyl-β-D-galactoside. Sufficient growth occurred on the selective plates to ensure full expression of all mutants because the addition of limiting amounts of glucose or glycerol as alternative carbon sources did not increase either the spontaneous or induced mutation frequency. The spontaneous mutational spectra obtained by this method did not differ from that found previously (ref. 2; unpublished results). At the UV fluences used to produce the spectra (100 J·m^{-2} for the Uvr$^+$ and 5 J·m^{-2} for the ΔUvrB strains), the contribution of spontaneously arising mutants was less than 1%. Since the mutants were selected directly following irradiation by plating in top agar, each induced mutant was of independent origin. Furthermore, each independent culture used to produce the UV spectra was screened for spontaneous jackpots which would have interfered with the induced spectra.

RESULTS

The mutational spectra for the Uvr$^+$ strain (Figs. 1 and 2) were obtained after a UV fluence of 100 J·m^{-2}. This dose resulted in a cell survival of about 3%, which is similar to the survival levels used by Coulondre and Miller (3). The induced *lacI*$^-$ mutation frequency was 55 × 10^{-6} per survivor, and the frequency of amber and ochre mutants was 8.1 × 10^{-6} per survivor, which is slightly lower than reported by Coulondre and Miller (3) and may reflect our use of exponentially growing rather than stationary-phase cells. Nonetheless, the UV spectra obtained by direct plating of the Uvr$^+$ strain were very similar to those of Coulondre and Miller (2, 3). Mutational hotspots were observed at sites A23, O24, and O27 and in similar ratios as reported by

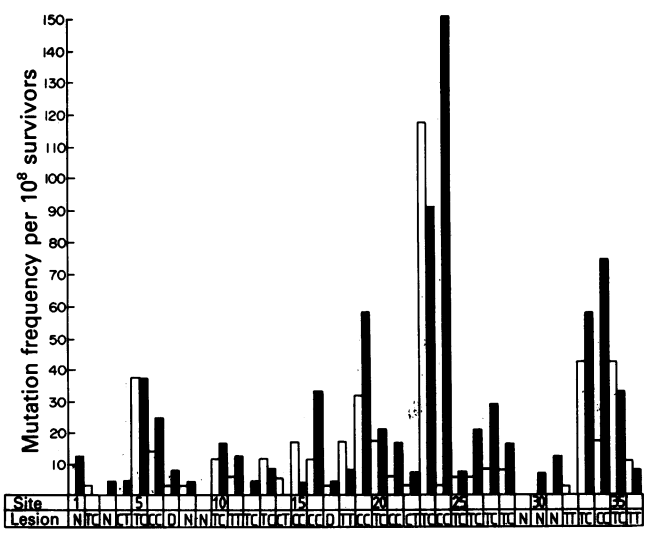

FIG. 1. Amber *lacI*$^-$ mutant spectra for the wild-type and ΔUvrB strains. □, Frequency distribution of 160 *lacI*$^-$ wild-type mutants among the 36 available amber sites after a UV dose of 100 J·m^{-2}, resulting in a total frequency of *lacI*$^-$ mutants of 55 × 10^{-6}; ■, frequency distribution of 193 amber mutants obtained in the ΔUvrB strain after a UV dose of 5 J·m^{-2}, resulting in a *lacI*$^-$ mutation frequency of 75 × 10^{-6} mutants per survivor. The potential pyrimidine dimer available at each site is indicated. N, sites where a pyrimidine dimer cannot form; D, sites where both C-C and T-C dimers could potentially form.

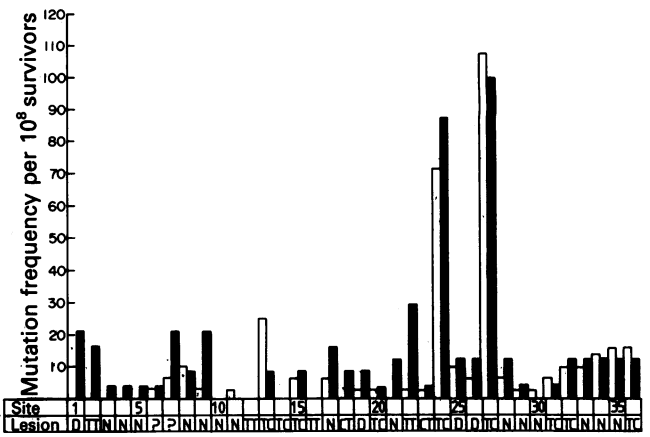

FIG. 2. Ochre *lacI*$^-$ mutant spectra for the wild-type and ΔUvrB strains. □, Frequency distribution of 108 *lacI*$^-$ ochre mutants in the wild-type strain after a UV dose of 100 J·m^{-2}; ■, frequency distribution of 120 ochre mutants in the ΔUvrB strain after a UV dose of 5 J·m^{-2}. For details see Fig. 1. Sites O6 and O7 have not yet been assigned specific positions in the *lacI* gene (10, 23), and the base change is uncertain.

Coulondre and Miller (2, 3). These hotspots arise as a consequence of G·C → A·T transitions at thymine-cytosine sites. The 5 J·m^{-2} dose used to produce the UV spectra for the ΔUvrB strain was chosen to generate a frequency of induced amber and ochre mutants similar to those produced by 100 J·m^{-2} in the Uvr$^+$ strain. The spectra for the ΔUvrB strain are given alongside those of the Uvr$^+$ strain in Figs. 1 and 2.

A comparison of the spectra obtained with the Uvr$^+$ and ΔUvrB strains reveals a new hotspot at site A24 in the ΔUvrB strain. This site accounted for almost 19% of all mutants arising among the 36 potential amber sites. The frequency with which A24 mutants were induced in the ΔUvrB strain was 149 × 10^{-8} compared with 2.9 × 10^{-8} in the wild type. A24 mutants arise as a consequence of G·C → A·T transitions at a cytosine-cytosine site.

Because the mutational responses at individual sites might show different dose–response characteristics, UV spectra were determined at lower UV fluences (10 J·m^{-2} and 0.5 J·m^{-2} in the case of the Uvr$^+$ and ΔUvrB strains, respectively). At these doses, cell survival is almost unaffected, and the mutation frequency is less than double the spontaneous level. Therefore, the spectra obtained largely reflect spontaneous occurrences; however, the induction of mutants at specific sites (particularly hotspots) might be discernible. The spectra for the amber and ochre mutants produced in the Uvr$^+$ strain did not show any indication of specific mutagenesis at the A23, O24, and O27 hotspots (data not shown). However, in the case of the ΔUvrB strain (Fig. 3), the hotspot A24 accounted for almost 20% of the total mutants. This is at least 10-fold greater than expected from the spontaneous spectrum and is even higher than the spontaneous "deamination" hotspots at A6, A15, and A34 (23).

DISCUSSION

The Nature of the A24 Mutational Hotspot. The single striking difference between the mutational spectra obtained for the Uvr$^+$ and ΔUvrB strains is the A24 hotspot found only in the repair-defective strain (Fig. 1). This site is further unique in that the induction of A24 mutants was detected in the ΔUvrB strain at UV fluences at which mutagenesis at other hotspots was not observed (Fig. 3). The A24 hotspot is produced by a G·C → A·T transition at a cytosine-cytosine site. Five other transition sites also involve a cytosine-cytosine target, yet A24 clearly behaves

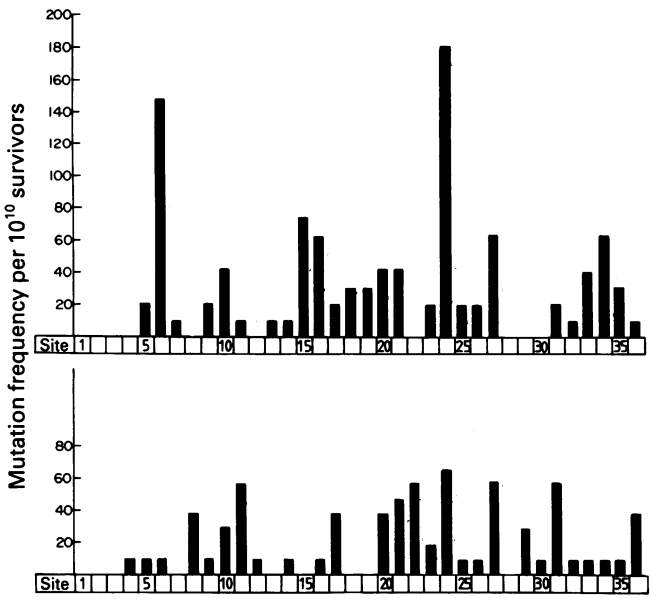

FIG. 3. Amber (*Upper*) and ochre (*Lower*) mutational spectra obtained in the ΔUvrB strain after a UV dose of 0.5 J·m^{-2}; 102 amber and 71 ochre mutants were analyzed. At this dose, survival is not significantly affected, and the frequency of nonsense mutations is approximately double the spontaneous level.

differently. Upon examining the nucleotide sequence surrounding the A24 site, we found that A24 occurs in an unpaired terminal loop of a potential hairpin structure of a quasi-palindromic sequence (see position 7 in loop B of Fig. 4). The location of the A24 hotspot within a configuration of potential secondary structure is particularly intriguing in view of the high UV mutability of suppressor loci and their location within the loops of potential secondary structure (25).

We do not yet know the molecular events leading to mutations at the A24 hotspot (or at any other site). The high mutability of this site in the Uvr$^-$ background might be related to a predisposition for the formation of a premutagenic lesion that is excised by the UV-endonuclease in the Uvr$^+$ strain. Alternatively, complex differences in the structures and processing of premutagenic lesions might be responsible for the differences in mutational outcome in the two genetic backgrounds. Indeed, evidence has accumulated that the mechanism(s) of mutagenesis in the Uvr$^-$ strain may differ from those of the Uvr$^+$ strain, even though both error-free and error-prone modes of repair are available in both genetic backgrounds (11, 13, 17–19).

One model deserving of consideration is that the transient formation of DNA secondary structure during DNA replication might reduce the efficiency of error-free postreplicational recombination repair (13, 26), if secondary structures inhibited strand exchanges. Clearly, a low level of error-free repair strongly predicts the high level of mutation at the A24 site seen after low UV fluences (Fig. 3), where the cell's error-free repair capacity is unlikely to be overtaxed.

Possibly the absence of the A24 hotspot in the Uvr$^+$ strain was related to the action of mutation frequency decline (MFD), which occurs only in excision-proficient strains at specific sites within tRNA genes (25, 27–30). MFD is very rapid: up to 50% of the mutants can be lost within 2 min (25, 27). Although the mechanism of MFD remains unclear and its occurrence within a structural gene is questionable (31), the location of the A24 site in the loop of a hairpin might be analogous to the situation in tRNA and suggests the possibility that MFD might be responsible for the loss of the A24 mutants in the repair-proficient strains. MFD is characteristically blocked by the addition of nutrient broth or Casamino acids to the post-irradiation growth medium. Neither the addition of nutrient broth or Casamino acids to the selection plates nor up to 18 hr of incubation in media containing these components increased the frequency of A24 mutants in the Uvr$^+$ strain (data not shown). Therefore, it seems unlikely that MFD is responsible for the absence of this hotspot in the Uvr$^+$ strain.

The Other Sites. The mutational hotspots O24, O27, and A23, seen in both Uvr$^+$ and Uvr$^-$ strains, lie in the same region of the *lacI* gene as A24 does. In fact, almost 50% of all the non-

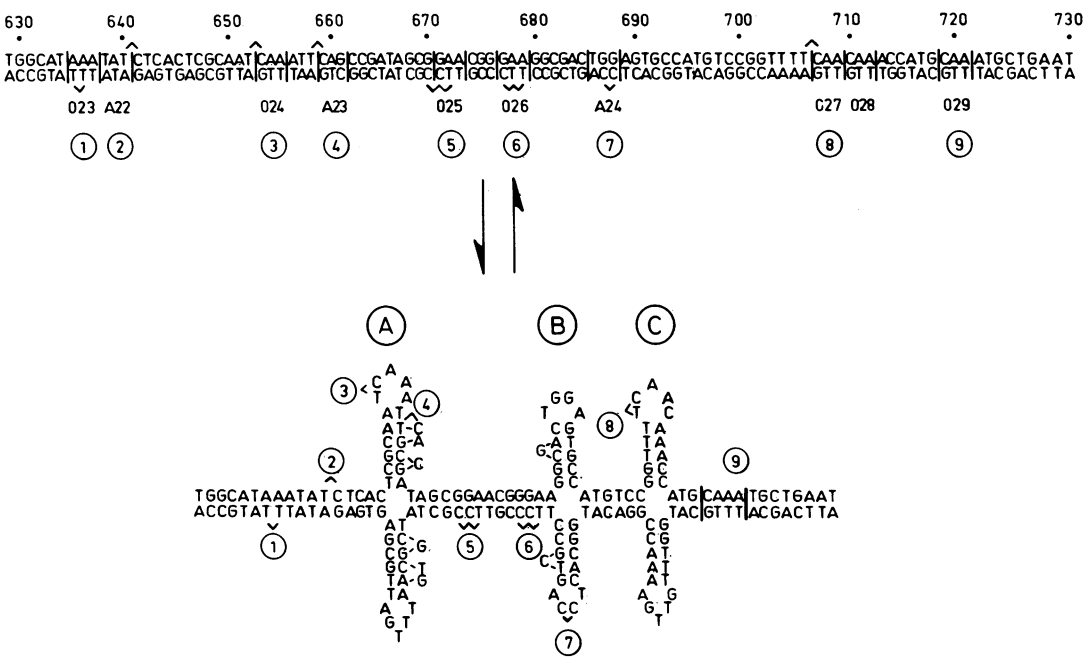

FIG. 4. A DNA secondary structure for the 101-base sequence, nucleotides 630–730, of the *lacI* gene. Sites 1–9 can yield amber and ochre codons by single base substitutions. A, B, and C potential hairpins are discussed in the text; ^ or ˇ, Sites of potential pyrimidine dimer formation. The DNA sequence and its numbering are from ref. 24.

sense mutants arose in a segment spanning only 6% of the DNA. These three hotspots involve G·C → A·T transitions at DNA sequences where thymine-cytosine dimers could form. Intriguingly, each of these hotspots is also located at unpaired sites within potential secondary structures. Therefore, these hotspot sites could reflect also a decreased probability of error-free repair in regions of DNA secondary structure. The fact that these three sites differ from site A24 in that they are readily mutated in both the Uvr^+ and Uvr^- background must reflect either differences in the premutagenic lesion between A24 and the other hotspot sites or unique interactions between site A24 and the *E. coli* repair systems.

The spectra produced by UV treatment in the Uvr^+ and Uvr^- strains are (with the exception of the A24 site) very similar. However, we do not know the basis of this similarity. These similarities in genetic consequences may simply reflect the fate of a common lesion processed in a similar fashion, but in no way excludes the possibility that the processing of the same or even different DNA lesions by different mechanisms might be responsible.

Role for Pyrimidine Dimers in UV Mutagenesis? About 90% of the *lacI*$^-$ nonsense mutants recovered in either the Uvr^+ or Uvr^- strain occurred, primarily as transitions, at sites where pyrimidine dimers could form. This is similar to the observations of Miller *et al.* (10). Although this is consistent with a role for pyrimidine dimers as important determinants of the site of UV mutagenesis, this observation in no way proves their role. In fact, in the simplest case, one might expect the frequency distribution of mutations within a spectrum to reflect the frequency distribution of the different pyrimidine dimers (cytosine-cytosine, cytosine-thymine, thymine-cytosine, and thymine-thymine) in irradiated DNA (32). Clearly this is not the case. This disparity may reflect the modulating effect of DNA repair or the specificity of SOS in the determination of mutational spectra or might indicate that some other lesion contributes to mutagenesis at some or all sites. Recently a pyrimidine-cytosine photoproduct dubbed Py-C* has been shown to occur at physiologically relevant levels (33). As noted earlier, all the hotspot sites involve potential thymine-cytosine or cytosine-cytosine dimer targets and, therefore, are also potential Py-C* targets. Indeed, most mutagenesis in either the Uvr^+ and Uvr^- strain, including that at nonhotspot sites, involves G·C → A·T transitions at what could be either Py-C* or pyrimidine dimer sites. Unfortunately, because A·T → G·C transitions cannot generate amber or ochre mutants, the potential contribution of thymine-thymine and cytosine-thymine dimers to transition mutagenesis cannot be evaluated. This inability to measure A·T → G·C transitions means that the *lacI* system cannot yet be used to distinguish the potential contributions of Py-C* and thymine-thymine or cytosine-thymine dimers to mutagenesis.

Further Discussion. The localization of UV mutational hotspots in the *lacI* gene of *E. coli* at regions of potential secondary structure is not only interesting in terms of mutagenesis but also might prove to be a sensible indicator of its actual occurrence *in vivo*. Secondary structure has been predicted to play a role in a number of processes, including the initiation of DNA replication and the regulation of transcription. Cruciform structures have been invoked to explain certain nuclease-sensitive sites in plasmid DNA (34, 35). Although the energetic costs of forming such structures in double-stranded DNA is usually prohibitive, the formation of certain cruciform structures in underwound, superhelical DNA is favored (36). Furthermore, in single-stranded DNA as may occur during DNA replication, recombination, or repair, the secondary structure containing the A24 site should be stable because energy considerations [inferred from values calculated for tRNA (37) and, therefore, only approximate] yield a ΔG of -31 J·mole^{-1}. The other hairpin loops (labeled A and C in Fig. 4) also generate negative ΔG values and are, therefore, not unlikely to form in single-stranded DNA.

The apparent relationship between mutational hotspots and secondary structure prompted a computer-assisted search for such structures throughout the *lacI* gene. However, in only one such sequence was there a nonsense site (A35) present. Despite the fact that the local DNA sequence of the loop containing the A35 site is very similar to that of hotspot O24, and both contain potential thymine-cytosine targets (10, 24), site A35 does not constitute a hotspot (Fig. 1). Although we are currently unable to explain why A35 is less mutable than A23, O24, and O27 if secondary structure is a determinant of mutational hotspots, this may be only because we are largely ignorant of the factors determining the *in vivo* occurrence of secondary structure. The degree of local supercoiling or the binding of proteins that might facilitate or hinder the formation of secondary structure might be strongly influenced by nearby sequences. Alternatively, secondary structure might be favored in regions of gapped DNA such as might be associated with initiation or termination sites of Okazaki fragments.

The localization of mutational hotspots at sites within potential secondary structures in DNA is exciting. DNA structural isomers may transiently form during DNA replication, just when mutations may be fixed most readily by error-prone repair processes (13, 26, 38), or such isomers may provide unusual DNA templates for specific endonucleases that interfere with error-free repair. These structures also may exist as recombinational intermediates during a process that normally provides an opportunity for error-free repair (13). We propose, therefore, that DNA secondary structures can modulate the extent of both error-free and error-prone repair and, as a result, can contribute to the determination of mutational hotspots.

The authors appreciate the extensive discussions with many colleagues, including Drs. J. Brouwer, M. Schaaper, W. Haseltine, M. Radman, and J. Drake, on the mechanism and specificity of mutagenesis. Particular appreciation goes to Dr. L. Ripley for her extensive comments during the preparation of this manuscript. This work was supported by Koningin Wilhelmina Fonds Grant MG.79.81 to B.W.G.

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