Mutagenic Properties of the T-C Cyclobutane Dimer

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Determined the properties of cytosine-containing cyclobutane pyrimidine dimers is perhaps the most important element in efforts to understand the mechanisms responsible for the spectrum of mutations induced by 254-nm UV irradiation. In most organisms and genes studied, 60 to 90% of the mutations induced by UV can be unambiguously assigned to a bifurcated target site in T-C and C-C sequences (4, 5, 7, 11, 14, 17, 18, 20, 23, 27, 28), and since 70 to 80% of these mutations can typically be abolished by enzymatic photolyase activation (15), most of them are likely to have been caused by cyclobutane dimers.

Several reasons for the high degree of mutability at T-C and C-C bipyrimidine sites have been suggested, though the issue has not yet been resolved and remains controversial. One explanation invokes the high rate of deamination of cytosine to uracil as a possible cause, but other results appear to indicate that the rate of deamination is too low for this to be significant in *Escherichia coli*. If deamination is not the cause, the high degree of mutability must presumably reflect the inherent properties of T-C and C-C dimers. We investigated this question by transfecting excision-deficient and excision-proficient strains of *E. coli* with single-stranded vectors that carried a site-specific cis-syn T-C cyclobutane dimer and by analyzing the nucleotide sequences of replicated vector products. We found that replication past the T-C dimer, like replication past its T-T and U-U counterparts, is in fact >95% accurate and that the frequencies of bypass are also very similar for these photoproducts. Since the T-C dimer appears to be only weakly mutagenic, the high frequency of UV-induced mutations at T-C sites presumably depends on some other process, such as deamination, although the mechanism remains to be established.

G · C→A · T transitions within T-C or C-C bipyrimidine sequences are by far the most frequent class of mutation induced by 254-nm UV irradiation in most genes and species investigated, but the reason for the high degree of mutability and specificity at these sites is uncertain. Some data implicate the deamination of cytosine to uracil as a possible cause, but other results appear to indicate that the rate of deamination is too low for this to be significant in *Escherichia coli*. If deamination is not the cause, the high degree of mutability must presumably reflect the inherent properties of T-C and C-C dimers. We investigated this question by transfecting excision-deficient and excision-proficient strains of *E. coli* with single-stranded vectors that carried a site-specific cis-syn T-C cyclobutane dimer and by analyzing the nucleotide sequences of replicated vector products. We found that replication past the T-C dimer, like replication past its T-T and U-U counterparts, is in fact >95% accurate and that the frequencies of bypass are also very similar for these photoproducts. Since the T-C dimer appears to be only weakly mutagenic, the high frequency of UV-induced mutations at T-C sites presumably depends on some other process, such as deamination, although the mechanism remains to be established.

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MATERIALS AND METHODS

Bacterial strains and vector. The bacterial strains SMH10 and SMH99 are both derivatives of AB1157. SMH10 is F⁻ Δ(pro-lac) uvrA6 lex⁻ ung⁺, and SMH99 is F⁺ lacZ ΔM15 pro⁺ (pro-lac) ungA⁺ lex⁺ ung⁻. M13mp7L2 was derived from M13mp7 by inserting a G-T sequence immediately 5’ to the 5’ EcoRI site nucleotides and changing the A-C sequence 5’ to the G-T insertion to C-A (which lengthens the base-paired region of the polylinker) and by replacing the two original gene amber mutations found in M13mp7 with wild-type sequences.

Preparation of an 11-mer with a site-specific T-C cis-syn dimer. To prepare the dimer-containing 11-mer, a solution containing 25 μg of the kinase-treated 11-mer 5’ GCAAGTCGGAG 3’ and 86 μg of the non-kinase-treated 19-mer 5’ AAATTCCGACTTGCCACT 3’ in 100 μl of water and 50 mM NaCl was incubated at 45°C for 15 min and slowly cooled to 21°C, evaporated to 50 μl, and transferred to a glass vial. After addition of 45 μl of acetone and 5 μl of 200 mM phosphate buffer, pH 7.0, the solution was made anoxic by exposure to nitrogen and the sealed vial was exposed to radiation (310 to 315 nm) from 40-W UVB fluorescent tubes for 2 h at −5°C. Temperatures lower than this reduced the yield of dimer. After irradiation, 1 ml of cold ethanol was added, the solution was cooled to −80°C, and the sample was completely dried with intermittent recollection to −80°C. The dimer-containing species was purified by high-pressure liquid chromatography (HPLC) at 15°C with a precooled Novapak C18 radial-compression 8-mm by 10-cm column (Waters, Milford, Mass.) and linear gradients of
precooled triethylammonium acetate, pH 7.0, and precooled acetonitrile. The gradients increased from 7 to 8% acetonitrile over the first 20 min, from 8 to 12% acetonitrile over the next 25 min, from 12 to 20% acetonitrile over the next 5 min, and from 20 to 70% acetonitrile over the final 5 min, all at 1 ml/min. The dimer-containing species (~6% yield) was frozen at ~80°C immediately after collection and completely dried with intermittent refreezing. Samples were redissolved and immediately repurified by the same method. Dried samples or solutions of the modified 11-mer were stored at ~80°C.

Construction of M13mp7L2 vector molecules containing a site-specific T-C dimer. Construct molecules, produced by ligating the dimer-containing 11-mer into linearized single-stranded DNA of the hybrid phage vector, were prepared by a modification (Fig. 1) of the method of Banerjee et al. (2). Linearized M13mp7L2 viral DNA, produced by cutting at the EcoRI site within the polylinker hairpin region, was recircularized by annealing with a 31-mer, rather than a 51-mer, scaffold oligomer. Recircularization was carried out at a vector concentration of 100 ng/μl, with a twofold molar excess of scaffold, in 25 mM NaCl overnight at room temperature. Reducing the region of base pairing between the vector and the scaffold from 20 to 10 by each end facilitated later removal of the scaffold but did not appear to otherwise impair recircularization. The 31-mer scaffold contained a U-U, rather than a C-C, mismatch opposite the T-C dimer, which discouraged any of the scaffold molecules that escaped removal by denaturation, but it nevertheless appears to recircularize the 31-mer scaffold together with alkali rather than heat denaturation to minimize deamination included ligation for 30 min with 6 U of ligase/μl. Other measures to minimize deamination included ligation for 2 min, neutralization with sodium acetate (final concentration, 500 mM), and 50-fold dilution with cold TE (1 mM Tris HCl [pH 7.5], 0.1 mM EDTA). The solution was stored at 80°C immediately after ligation.

Photoreactivation, transfection, and analysis of replicated phage. Photoreactivation treatments were carried out before alkali denaturation. A 50-μl reaction mixture containing 1.6 μg of ligated construct (either the dimer-containing construct or the dimer-free control), 50 mM Tris HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, 11 μg of bovine serum albumin, and 12 μg of E. coli photolyase (kindly supplied by Aziz Sancar, University of North Carolina, Chapel Hill) was divided into two equal aliquots, each placed in a glass vial. One vial of each pair was completely wrapped in aluminum foil, and both were exposed to filtered black-light radiation (340 to 390 nm) for 30 min at room temperature. Samples were handled under light from gold fluorescent lamps at this and all subsequent steps. The fact that >95% of the T-C dimers could be photoreactivated with this amount of enzyme confirmed that this photoproduct was the cis-syn isomer; the trans-syn isomer cannot be detectably photoreactivated under these conditions (unpublished data). Bacterial cells were made competent and transfected with the hybrid phage constructs as described previously (2). Nucleotide sequences were determined by the dideoxy method in replicated products isolated from a random set of plaques over a region starting ~25 nucleotides 3’ to the T-C dimer site and ending ~100 nucleotides 5’ to this site.

RESULTS

Production of vectors with a site-specific T-C dimer. Vector molecules containing a specifically located T-C cis-syn dimer were constructed basically by the method described previously (2), which entails the production of an 11-mer carrying a uniquely located photoproduct and the ligation of this oligomer into a single-stranded vector. However, the low yield and extreme lability of the T-C dimer necessitated a variety of modifications to the standard procedure. The basic method involves linearizing single-stranded DNA of the M13mp7L2 hybrid phage with EcoRI, which cuts at a unique site within the small hairpin region, and then recircularizing the vector by annealing it with a scaffold oligomer. The ends of the scaffold are complementary to the ends of the cut vector, but the scaffold leaves an 11-nucleotide gap into which the modified 11-mer can be efficiently ligated. The scaffold is then removed by denaturation in the presence of a large excess of the anti-scaffold, an oligomer of complementary sequence, which minimizes reannealing of the scaffold to the vector.

We found it necessary to modify this method extensively when investigating the properties of the T-C dimer. Initial attempts to induce this photoproduct at the unique T-C target site in the 11-mer with an acetone-UVB photosensitized reaction yielded <1% of the dimer-containing species; this was apparently the consequence of the competitive formation of another photoproduct. This photoproduct may be cytosine hydrate, because an oligomer carrying it could be converted by drying to a material with properties identical to those of the unmodified 11-mer. Since the yield of hydrate is lower in duplex DNA, we carried out the photosensitized reaction with the 11-mer annealed to a complementary strand. A 19-mer that was complementary to the 11-mer but with extensions of four nucleotides at each end was used for this purpose to facilitate separation of the two strands during HPLC purification. Use of the duplex increased the yield of the dimer-containing species to 5 to 7%. Deamination was minimized by treatment with kinase and purification of the oligomer before treatment to induce the dimer, by buffering the DNA solution at pH 7.0 (6, 19), and by maintaining it at ~5°C while carrying out the photosensitized reaction. Temperatures lower than this reduced the yield of dimer. Deamination was also minimized by using 2-h exposures to unfiltered radiation (310 to 315 nm) from UVB fluorescent tubes for the photosensitized reaction, rather than 24-h exposure to sunlamps, and by carrying out HPLC purification at 15°C, the lowest temperature at which good separation could be achieved. Samples were kept frozen while being dried and were stored at ~80°C. Other measures to minimize deamination included ligation for 30 min with 6 U of ligase/μl, rather than overnight, and the use of a 31-mer scaffold together with alkali rather than heat denaturation to remove the scaffold (Fig. 1). The 31-mer scaffold is complementary to only 10 nucleotides at each end of the linearized single-stranded vector, rather than to 20 nucleotides as with the 51-mer used previously, making the scaffold easier to remove by denaturation, but it nevertheless appears to recircu-
larize the vector with equal efficiency. Finally, we used samples of the vector carrying a site-specific T-C dimer, and also of the dimer-free control vector, to transfet SOS-induced and uninduced cells of E. coli, with the aim of estimating two of the properties that contribute to mutability, namely, the frequency of bypass replication and the error frequency of bypass. The first property is important in its own right as a major factor in determining mutagenic potential, but it is also important because of the suggestion that T-C dimers constitute unusually potent blocks for bypass replication and can be readily bypassed only after deamination (25). The second property was examined to investigate the possibility that the T-C dimer is unusually prone to inducing mutations as a consequence of a high error rate during bypass replication.

**Frequency of dimer bypass.** The proportion of construct molecules in which dimer bypass replication had occurred was estimated from the number of plaques resulting from transfection with the construct carrying the photoproduct normalized to the number of plaques obtained with an exactly equivalent dimer-free control (2). Dimer bypass frequency was determined in three transfection experiments, each using independently made samples of the modified 11-mer and vector; two of the samples employed the excision-deficient (uvrA6) strain SMH10, and the other employed the excision-proficient strain SMH99, which carried the ung mutation but was otherwise isogenic. As shown in Table 1, bypass frequencies were 22 to 24% in the excision-deficient strain SMH10 and 42% in the excision-proficient strain SMH99, values that are very similar to those observed previously for T-T and U-U dimers in these strains (1, 10). SOS-induced bypass frequencies are about twice as high in excision-proficient strains as in excision-deficient cells, probably because SOS induction is more efficient. Such results suggest that the T-C dimer blocks replication to no greater, and also no less, extent than any of the other dimers studied. This is likely to be true even though ~20% (19 of 103) of the dimers in the SMH99 experiment and ~40% (13 of 32 and 15 of 40) of the dimers in the SMH10 experiments were converted to the T-U dimer replication product (Table 1), and thus the bypass frequencies are an average for a mixed population of T-C and T-U dimers. In particular, such data appear to be incompatible with the proposal that the T-C dimer blocks replication almost completely (25). Since the frequencies of replication past uracil-containing and thymine-containing dimers are virtually identical (1, 10), bypass frequencies would have been about 8% [(60% T-C × 0% bypassed) + (40% T-U × 20% bypassed)] rather than the observed 22 to 24% in SMH10 and <8% [(82% T-C × 0% bypassed) + (18% T-U × 40% bypassed)] rather than the observed 42% in SMH99 if the T-C dimer could not be bypassed at all. The T-C dimer bypass frequency was in fact likely to be at least equal to the lower value observed for other dimers, that is, 15% in SMH10 and 35% in SMH99, to be compatible with the observed results, particularly those from SMH99. Estimates in Table 1 of the proportion of T-C dimers that were deaminated to T-U were obtained as fractions of replicated vectors with T-T at the photoproduct target site, since uracil within dimers pairs with adenine ~95% of the time (10). Finally, the higher-than-usual bypass frequencies in uninduced cells reflected a higher-than-normal contamination of the dimer-carrying 11-mer by non-dimer-containing oligomer, rather than true dimer bypass. Higher-than-normal contamination was likely because the inability of the T-C dimer precluded the rigorous, repetitive purification used with the stable photoproducts. Almost all of the replication products from uninduced cells carried the T-C sequence at the target site, the only exceptions being a single T-T product (among 55 sequenced) in one of the SMH10 experiments and two T-T products (among 54 sequenced) in the SMH99 experiment. Deliberate deamination by heating the construct at 85°C for 6 min before transfection neither reduced the amount of bypass in uninduced cells nor converted the material bypassed to a species that generates the T-T sequence, though such a conversion occurred in virtually all of the material bypassed in SOS-induced cells (Table 1). It is therefore likely that most of the plaques derived from uninduced cells resulted from non-dimer-containing constructs and that in the absence of SOS induction the T-C dimer blocks replication as completely as the other dimers studied.

**Error frequency of T-C dimer bypass and the timing of deamination events.** We next investigated whether the high frequency of mutations at T-C sites might reflect an inherently high error rate, independent of deamination, during the bypass of T-C dimers. This was examined by analyzing the nucleotide sequence in a region encompassing the T-C target site in replicated vectors derived from random sets of plaques. We also determined whether deamination occurred predominantly during the construction of the vector, or during transfection and in the cell after uptake, by enzymatically photoreactivating a sample of the construct in vitro before transfection. The sequence data (Table 2) show that replication past the T-C dimer is highly accurate and that the photoproduct is therefore not intrinsically very mutagenic. They also show that the T-U dimers in the sample occurred almost entirely by deamination during the in vitro preparation of the construct molecules

<table>
<thead>
<tr>
<th>Strain and relevant genotype</th>
<th>UV to cells (J/m²)</th>
<th>Bypass frequency (%)</th>
<th>% T-C→T-U deamination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>T-C dimer</td>
<td>T-T dimer</td>
</tr>
<tr>
<td>SMH10 (uvrA6 ung⁻)</td>
<td>0</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>100</td>
<td>22</td>
</tr>
<tr>
<td>SMH10 (uvrA6 ung⁻)</td>
<td>0</td>
<td>100</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>100</td>
<td>24</td>
</tr>
<tr>
<td>SMH99 (uvr⁺ ung)</td>
<td>0</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>100</td>
<td>42</td>
</tr>
</tbody>
</table>

*Typical values taken from previous results (references 1 and 10 and unpublished data). |
Maximum value for deamination in experimental constructs, estimated from the percent T-T progeny phage. |
Percent T-T progeny phage resulting from deliberate deamination of the constructs by heating at 85°C for 6 min before transfection. |

<table>
<thead>
<tr>
<th>Strain and relevant genotype</th>
<th>UV to cells (J/m²)</th>
<th>No. (%) of replicated vector molecules with indicated sequence at T-C photoproduct site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-C dimer</td>
<td>T-T</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>52 (96)</td>
</tr>
<tr>
<td>PR*</td>
<td>0</td>
<td>91 (82)</td>
</tr>
</tbody>
</table>

*PR, photoreactivation.
rather than during transfection or before replication of the vector in the cell. Sequence results from SOS-induced cells transfected with the nonphotoreactivated construct (Table 2) showed that 84 of 103 of the replicated vectors analyzed contained the T-C sequence at the photoprotein site, indicating that replication past the T-C dimer was at least 82% accurate, though as discussed below the true accuracy is probably >95%. The remaining sequences had T-T at the target site, and results with the photoreactivated construct suggest that virtually all of these sequences represent accurate replication past a T-U dimer formed in vitro during production of the 11-mer and its insertion into the vector. Transfection of uninduced cells with the construct photoreactivated in vitro before transfection gave exactly the same proportions of progeny molecules with T-C sequences (91 of 111; 82%) and T-T sequences (20 of 111; 18%) at the photoprotein site as were found with the nonphotoreactivated sample transfected into SOS-induced cells. Transfection of SOS-induced cells with the photoreactivated sample gave progeny that were 87% T-C and 13% T-T, results that are very similar and not statistically different, indicating that the proportion of T-T progeny unaccounted for by deamination in vitro is unlikely to exceed 5% and could be 0%. Photoreactivation was nearly complete because bypass frequencies were increased to 95% in uninduced cells and 94% in SOS-induced cells. If there is in fact a small fraction of T-T progeny unaccounted for by in vitro deamination, a result neither supported nor excluded by the data, it might derive from deamination products formed in the cell before replication or it might represent 3′ C→T mutations generated during replication past the T-C dimer. Although we have no means of differentiating between these mechanisms, we can nevertheless conclude that they are infrequent phenomena: neither is likely to occur in more than 5% of transfections. Apart from the T-C→T-T sequence change, no others that might be attributed to mutations induced by the T-C dimer were found, again suggesting that the inherent mutability of this photoprotein is low. In fact, no other sequence changes of any kind, targeted or untargeted, were found either in the experiment with SMH10 or those with SMH10. Although the T-U dimer is expected to induce mutations ~5% of the time (10, 13), mostly U→A and some U→C, such mutations were probably not detected because the number of products analyzed resulting from replication past this photoprotein was too small. Finally, analysis of samples from transfection with the dimer-free control (Table 2) provided no evidence of synthesis errors during production of the oligonucleotides or of mutagenic effects of the photoreactivation treatment.

**DISCUSSION**

Our results indicate that the mutagenic properties of the T-C cis-syn dimer are very similar to those of its T-U, U-U, and T-U counterparts (1, 10, 13). In particular, the T-C dimer does not, in the absence of deamination, exhibit an inherently high mutagenicity. The error rate for replication past this photoprotein is unlikely to exceed 5 to 7%, the value found with the other dimers, and since we were unable to unambiguously detect any mutations in the sample analyzed, it may in fact be much lower than this. Similarly, replication past the T-C dimer occurs at much the same frequency as with the other cis-syn dimers; the T-C dimer neither blocks replication to a greater extent than the other dimers, as suggested by Tressman and colleagues (25), nor is more readily bypassed. Since all cis-syn dimers studied have similar properties, the C-C and C-T photoproducts presumably also have such qualities. As a consequence, the high frequency of UV-induced mutations that are commonly observed within C-C and T-C bipyrimidine sequences is unlikely to be explained by the inherent properties of the cytosine-containing dimers.

A high proportion of UV-induced mutations at T-C and C-C sites has been observed in *E. coli*, though not in M13 or λ phages (Table 3), and is also found in results from a variety of other species, a representative sample of which are also given in Table 3. In *E. coli*, 84 to 86% of the single base pair substitutions that can be unambiguously assigned to one of the four bipyrimidine target sequences occur at T-C and C-C sites. Data from the ambiguous sites are also consistent with this observation, since the majority of mutations occur within T-C-C, T-C-T, and T-T-C sites (where the underlined nucleotide is the site of substitution). Since our data indicate that the inherent properties of the T-C dimer cannot explain this distribution, and by extension that the same is probably true of the C-C dimer, other possible explanations need to be examined: that T-C and C-C sites are favored because of the occurrence of nondimer UV photoproducts, because lesions at these sites are poorly repaired relative to those at T-T and C-T sites, or because of the deamination of cytosine.

It does not appear likely that the high incidence of mutations at these sites in *E. coli* is the consequence of events induced by nondimer photoproducts, or because T-C and C-C dimers are poorly repaired. The most likely nondimer lesion is the pyrimidine (6-4) pyrimidinone adduct, because the abundance of the T-C adduct is about equal to that of the corresponding dimer, the yield of the C-C adduct is only a few times lower than that of the C-C dimer (4), and the yield of adduct at C-T sites is low, in keeping with the low mutation frequency in this sequence. Nevertheless, even though the T-C pyrimidine (6-4) pyrimidinone adduct is much more mutagenic than the T-C or T-T dimer (12) and the same is probably true of its C-C counterpart, this lesion is unlikely to cause the majority of mutations at T-C and C-C sites. Photoreactivation of a UV-irradiated donor before conjugational transfer of an F′ lacZ to a fully SOS-induced recipient established that 70 to 80% of the lacZ

<table>
<thead>
<tr>
<th>Organism, target gene</th>
<th>% Single bp substitutions (G · C→A · T transitions) at Py-Py site</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C</td>
<td>10 (7) 14 (15) 2 (1) 14</td>
<td>35 (4) 25 (4) 17</td>
</tr>
<tr>
<td>T-C</td>
<td>11 (11) 15 (13) 0 39</td>
<td>30 (17) 4 (2) 27</td>
</tr>
<tr>
<td>C-T</td>
<td>15 (9) 9 (4) 4 (4) 40</td>
<td>21 (17) 11 (2) 28</td>
</tr>
<tr>
<td>T-T</td>
<td>34 (26) 23 (19) 2 (1) 8</td>
<td>24 (16) 9 (2) 23</td>
</tr>
<tr>
<td>E. coli, lacZ</td>
<td>39 (23) 18 (11) 5 (2) 7</td>
<td>23 (11) 9 (2) 18</td>
</tr>
<tr>
<td>Conjugating cells</td>
<td>24 (18) 30 (23) 4 (0) 6</td>
<td>24 (18) 13 (4)</td>
</tr>
<tr>
<td>S. cerevisiae, SUP40</td>
<td>14 (12) 38 (37) 2 (2) 25</td>
<td>21 (17) 1 (0) 14</td>
</tr>
<tr>
<td>Human, supF</td>
<td>13 (13) 48 (39) 0 7</td>
<td>32 (20) 0 5</td>
</tr>
<tr>
<td>Human XPA, supF</td>
<td>15 (15) 44 (44) 0 2</td>
<td>40 (40) 0</td>
</tr>
</tbody>
</table>

* Ambiguous sites flanked by different pyrimidines.
* Mutations at nonbipyrimidine sites.
* XPA, xeroderma pigmentosum cells.
mutations were induced by dimers (15), and 85% of these mutations occurred at T-C and C-C sites (18). Dimers are clearly implicated in the induction of mutations, rather than just in induction of the SOS response, because photoreactivation was confined to the donor cells. In addition, the preferential incidence of mutations at T-C and C-C sites cannot be explained by differential repair, because most mutations also occur within these sequences in excision repair-deficient strains.

The differing views on the role cytosome deamination might play in the production of mutations in UV-irradiated E. coli hinge on different estimates for the t½2 of this process. Tessman and colleagues suggested that ~70% of S13 phase mutations induced by UV are the result of cytosome deamination and that deamination occurs both in vivo and in vitro by marked step-function kinetics, with a t½2 of ~29 min for single-stranded S13 DNA and a t½2 of ~55 min for double-stranded S13 or λ phage DNA (25, 26). In the absence of sequence data, it is difficult to know if the mutations ascribed to deamination are in fact G · C→A · T transitions within appropriate bipyrimidine sequences. Mutations induced by UV in single-stranded M13 phage DNA or in double-stranded λ phage DNA are not predominantly G · C→A · T transitions of this kind (17, 27, 28) (Table 3). In fact, the observation that UV irradiation probably requires revisiting the issues discussed above. Most of the rate derived from the in vivo data of Ruiz-Rubio and colleagues suggests and may be too long to allow this factor to contribute significantly to UV mutagenesis in E. coli (3, 6, 8, 9, 19, 22, 24). In all of these experiments, deamination kinetics were first order and the estimates of the deamination step-function kinetics, with a 1/2 for deamination is much longer than the estimates of Tessman and more generally to the polarity of the environment (19), although the rate derived from the in vivo data of Ruiz-Rubio and Bockrath (22) is not substantially lower than those found in vitro studies (3), they were studying only one, possibly atypical, site in the glutamine tRNA gene. The fact that UV-induced mutations in the E. coli F' lacI plasmid are predominantly found at T-C and C-C sites in the F' plasmid of E. coli be explained? Attempts to answer this question probably require revisiting the issues discussed above. Most of the estimates for the t½2 of cytosome deamination have been obtained in vitro, raising the possibility that conditions in vivo, such as the presence of DNA-binding proteins, might accelerate this process. The deamination rate is sensitive to local pKs, and more generally to the polarity of the environment (19), which such proteins or other conditions might affect. Moreover, although the step derived from the in vivo data of Ruiz-Rubio and Bockrath (22) is not substantially lower than those found

Acknowledgment

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References


