Mutational Mechanisms Deduced From 4-Aminobiphenyl-Induced Mutation Spectra in Salmonella

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ABSTRACT

We used colony probe hybridization and polymerase chain reaction/DNA sequence analysis to determine the mutations in ~2,400 4-aminobiphenyl (4AB) +S9-induced revertants of the +1 frameshift allele hisD3052 and of the base-substitution allele hisG46 of Salmonella typhimurium. Most of the mutations occurred at sites containing guanine, which is the primary base at which 4AB forms DNA adducts. A hotspot mutation involving the deletion of a CG or GC within the sequence CCGCGCGCG accounted for 100 and 99.9%, respectively, of the reversion events at the hisD3052 allele in the pKM101 plasmid-minus strains TA1978 (uvr) and TA1538 (ΔuvrB). In strain TA98 (ΔuvrB, pKM101), which contained the SOS DNA repair system provided by the pKM101 plasmid, ~85% of the revertants also contained the hotspot deletion; the remaining ~15% contained one of two types of mutations: (1) complex frameshifts that can be described as a –2 or +1 frameshift and an associated base substitution and (2) deletions of the CC or GG sequences that flank the hotspot site (CCCGCGCGCG). We propose a misincorporation/slippage model to account for these mutations in which (1) pKM101-mediated misincorporation and translesion synthesis occurs across a 4AB-adducted guanine; (2) the instability of such a mispairing and/or the presence of the adduct leads to strand slippage in a run of repeated bases adjacent to the adducted guanine; and (3) continued DNA synthesis from the slipped intermediate produces a frameshift associated with a base substitution. This model readily accounts for the deletion of the CC or GG sequences flanking the hotspot site, indicating that these mutations are, in fact, complex mutations in disguise (i.e., cryptic complex frameshifts). The inferred base-substitution specificity associated with the complex frameshifts at the hisD3052 allele (primarily G:C → T:A transversions) is consistent with the finding that 4AB induced primarily G:C → T:A transversions at the hisG46 base-substitution allele. The model also provides a framework for understanding the different relative mutagenic potencies of 4AB at the two alleles in the various DNA repair backgrounds of Salmonella.

THE determination of mutation spectra in reverse-mutation systems has provided considerable insights into mutational mechanisms, particularly those associated with frameshift mutation (Ripley 1990). The hisD3052 allele of Salmonella typhimurium, which is a –1 frameshift that was induced by the acridine nitrogen mustard ICR-354-OH (Oeschger and Hartman 1970; Hartman et al. 1986), has been used more than any other frameshift allele for the identification of mutagenic agents (Kier et al. 1986). In addition, the availability of this allele in strains containing different DNA repair backgrounds (Inman et al. 1983; Maron and Ames 1983) has permitted the study of the influence of DNA repair on the mutability of this allele.

Various methods have been applied during the past 20 years to evaluate revertants of the hisD3052 allele at the molecular level, including (1) deduction of the DNA sequence from the amino acid sequence of the histidinol dehydrogenase polypeptide coded by revertants of the hisD3052 allele (Isono and Yoruno 1974) and (2) cloning and DNA sequence analysis (Fusco et al. 1988; O'Hara and Marnett 1991). However, the ability to analyze hisD3052 revertants in numbers sufficient to construct informative mutation spectra has become practical only recently with the development of a colony probe hybridization procedure to identify a common hotspot mutation (Kupchella and Cebula 1991) and the application of polymerase chain reaction (PCR)/DNA sequence analysis to identify the remaining frameshifts (Cebula and Koch 1990b; Kupchella and Cebula 1991; Bell et al. 1991; DeMarini et al. 1992). Thus, we have used these methods to examine the influence of DNA repair on the mutation spectrum of the hisD3052 allele after reversion by a common environmental mutagen/carcinogen, 4-aminobiphenyl (4-AB), whose mutagenic potency at this allele is highly influenced by the DNA repair status of the cell.

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4-AB is a well documented bladder carcinogen in laboratory animals and humans, and it is genotoxic in a wide variety of organisms and endpoints (IARC 1987a,b). More recent studies show that it is mutagenic in various strains of Salmonella (IOANNIDES et al. 1989) as well as in Drosophila (TRIPATHY et al. 1990) and mammalian cells (OLLER et al. 1989; BOOKLAND et al. 1992a). 4-AB also induces micronuclei (CLJET et al. 1989; SHELBY et al. 1989) and unscheduled DNA synthesis (ASHBY and MOHAMMAD 1988; STEINMETZ et al. 1988).

The metabolism of 4-AB via N-oxidation results in reactive electrophilic species capable of forming adducts with hemoglobin and DNA (BELAND and KADLUBAR 1990; KADLUBAR et al. 1991). Metabolically activated 4-AB forms a profile of DNA adducts that is similar in various species/tissues; the adducts are predominantly (70%) C(8) adducts on guanine, with minor adducts at the C(8) position of adenine (15%) and at the N\textsubscript{6} exocyclic position of guanine (5%) (BELAND and KADLUBAR 1990). Treatment of Salmonella strain TA1585 (hisD3052, rfa, ΔuvrB) with N-hydroxy-4-AB gives a similar adduct profile, with C(8) guanine accounting for 71% of the adducts (KADLUBAR et al. 1982).

Despite the wealth of studies demonstrating the carcinogenicity, mutagenicity, and DNA-adduct-forming ability of 4-AB, only one study has determined at the DNA sequence level the types of mutations induced by a DNA-reactive form of 4-AB. LASKO et al. (1988) sequenced 20 mutants of the lacZ\textsubscript{a} gene fragment of Escherichia coli bacteriophage M13mp10 DNA treated with N-acetoxy-N-trifluoroacetyl4-aminobiphenyl. As expected, G\textendash C base pairs were the major targets for base-substitution mutations. The addition of an SOS DNA repair system provided by the pGW16 plasmid, which is active electrophilic species capable of forming adducts (KADLUBAR et al. 1991). The present study extends these observations by examining the role of DNA repair on the mutagenic specificity of this agent. As demonstrated by IOANNIDES et al. (1989), 4-AB is mutagenic at both a frameshift (hisD3052) and a base-substitution (hisG46) allele of Salmonella. Both of these alleles are available in strains that vary in their DNA repair background according to the presence or absence of nucleotide-excision repair (uvrB) and the presence or absence of the SOS DNA repair system provided by the pKM101 plasmid. Thus, we have constructed 4-AB-induced mutation spectra in five strains of Salmonella, involving two alleles and four different DNA repair backgrounds, permitting us to examine the influence of these factors on the mutagenic specificity of 4-AB.

The primary observation from this study is the enhanced frequency of 4-AB-induced complex frameshift mutations in the hisD3052 strain carrying plasmid pKM101 (strain TA98). Each of these mutations can be described as a frameshift mutation with a flanking or nearby base substitution. We present a mutational model that accounts for these complex frameshifts as well as provides a framework for understanding the different mutagenic potencies of 4-AB in various DNA repair backgrounds.

MATERIALS AND METHODS

Mutagenicity assay: S. typhimurium strains TA1978 (hisD3052, rfa), TA1538 (hisD3052, rfa, ΔuvrB), TA98 (hisD3052, rfa, ΔuvrB, pKM101), TA1975 (hisG46, rfa), TA1535 (hisG46, rfa, ΔuvrB), and TA100 (hisG46, rfa, ΔuvrB, pKM101) were kindly provided by B. N. AMES, Biochemistry Department, University of California, Berkeley. Strains UTH8413 (hisD3052, rfa, pKM101) and UTH8414 (hisG46, rfa, pKM101) were provided by T. H. CONNER and T. S. MATNEY, Graduate School of Biomedical Sciences, University of Texas, Houston. The standard plate-incorporation assay (MARON and AMES 1989) was performed using three plates/dose and 10\textsuperscript{9} cells/plate. After 3 days of incubation, the revertant colonies (rev) were counted, and the results were expressed as rev/plate. 4-AB (Sigma, St. Louis, Missouri) was diluted in dimethyl sulfoxide (DMSO, Burdick & Jackson, Muskegon, Michigan) and evaluated in the presence of Sprague-Dawley aroclor 1254-induced male rat liver S9 (1.8 mg of S9 protein/plate) that was prepared as described (MARON and AMES 1983).

 Colony purification, DNA isolation, PCR and DNA sequencing: Approximately 2,200 hisD3052 and 200 hisG46 4-AB-induced, independent revertants were streaked onto minimal medium supplemented with biotin (MARON and AMES 1983) and incubated for 2 days at 37°C in order to purify each revertant clone and to assure that no nonrevertant cells from the background lawn were present. The target sequence of the hisG46 allele is a CCC codon, and particular base substitutions at the first or second position of this codon produce the revertants described here. The purified revertants of the hisG46 allele (strains UTH8414 and TA100) were analyzed by the colony probe hybridization procedure described by CEBULA and KOCH (1990a) with slight modifications. The probes were the 5'-nucleotide oligomers described by CEBULA and KOCH (1990a). The filters were hybridized with the probes for 2 h at 37°C; then the filters were washed in 1 x SSC (0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) as follows: CTC, CAC and CCC at 47°C for 25 min; TCC at 47°C for 50 min; ACC at 50°C for 20 min; and GCC at 55°C for 24 min. Those revertants that hybridize to the probe containing CCC contain the original hisG46 sequence; such revertants contain a suppressor mutation in the anticodon region of either of two tRNA\textsuperscript{Thr} genes and represent a TA → GC transversion (KUPCHELLA et al. 1994).

 Purified revertants of the hisD3052 allele (strains TA1978, TA1538 and TA98) were first screened for the presence of a hotspot GC or CG deletion by means of the colony hybridization procedure described by KUPCHELLA and CEBULA (1991). The hybridization procedure was similar to that for the hisG46 allele except that an additional unlabeled probe was used to enhance the hybridization reaction due to the presence of repeated sequences within the hotspot region (KUPCHELLA and CEBULA 1991).

 Those revertants that did not contain the hotspot deletion were subjected to PCR and DNA sequence analysis as described by BELL et al. (1991). Briefly, revertant colonies were boiled for 10 min in 200 μl of TE buffer, centrifuged for 10 min, and 5–10 μl of the supernatant were used as the Salmonella genomic DNA in an asymmetric PCR in which the primers were present
at a ratio of 1:100. After 40 cycles of heating and cooling, the reaction was subjected to ultrafiltration, and the amplified ssDNA was sequenced using dITP termination mixes by the method of SANGER et al. (1977).

Statistical analysis: Statistical comparisons of the hisD3052 mutation spectra were performed using the program of ADAlhs and SKOPEK (1987), which produces a Monte Carlo estimate of the $P$ value of the hypergeometric test (a generalization of Fisher's exact test). Comparisons of the hisG46 mutation spectra were performed by chi-square analysis using the Stat-Sak program (GERARD E. DALLAL, 53 Betran Street, Malden, Massachusetts 02148).

RESULTS AND DISCUSSION

Dose-response curves and mutagenic potency: Figure 1 shows representative 4-AB-induced mutagenicity dose-response curves from which revertants were picked for analysis. Mutagenic potencies, calculated from the slope of the linear portions of these curves, are listed in Table 1 for the set of experiments reported in this study. For the frameshift allele hisD3052, the average mutagenic potency of 4-AB in the excision repair-proficient strain TA1978 was 0.7 rev/μg. Addition of the pKM101 plasmid did not cause much change in the mutagenic potency (0.6 rev/μg). However, the excision repair-deficiency in strain TA1538 (ΔuvrB) increased the potency of 4-AB by ~17-fold (12 rev/μg). The combination of nucleotide excision-repair deficiency (ΔuvrB) and the presumed error-prone translesion synthesis provided by the pKM101 plasmid (strain TA98) doubled this value to 24 rev/μg.

4-AB was unable to revert the base-substitution allele hisG46 in the absence of the plasmid regardless of the nucleotide excision-repair background (strains TA1975 and TA1535 in Figure 1). In the presence of the plasmid (strain UTH8414), 4-AB induced 1.4 rev/μg; whereas, the combination of nucleotide excision-repair deficiency and plasmid pKM101 (strain TA100) increased the mutagenic potency to 43 rev/μg (Figure 1 and Table 1). Our results for strains TA1535, TA1538, TA98 and TA100 are similar to those reported in other studies (HAWORTH et al. 1983; STEELE and IOANNIDES 1986; IOANNIDES et al. 1989; IARC 1987a); results for 4-AB in strains TA1978, UTH8413 and UTH8414 have not been reported previously. These results show that 4-AB reverts both alleles, especially in the absence of the uvrABC
excision-repair system. Although 4-AB is mutagenic at the hisD3052 allele in the presence or absence of plasmid pKM101, the plasmid is required for all 4-AB-induced mutagenesis at the hisG46 allele.

The fold over background at which revertants were picked for molecular analysis is indicated by arrows in Figure 1. Revertants of wild-type strain TA1978 were picked at 1000 pg/plate, which was considerably higher than the doses at which revertants were picked for the other strains, but there was no indication of toxicity at this high dose. Other than for strain TA1978, for which ~84% of the revertants were induced by 4-AB (based on a 6-fold increase over the number of background revertants), greater than 93% of the revertants in the other strains were induced by 4-AB.

**Mutation spectrum at the hisG46 allele:** The base-substitution mutational specificity of 4-AB at the hisG46 allele was determined by analyzing background and 4-AB-induced revertants of UTH8414 (pKM101) and TA100 (ΔuvrB, pKM101) selected at 8- and 11-fold, respectively, over the background (Figure 1). The number of revertants analyzed and the number (and percentage) in each category are shown in Table 2. The yield of revertants in each category is shown in Figure 2. The results showed that 78% (TA100) to 86% (UTH8414) of the base substitutions induced by 4-AB at the hisG46 allele were G-C → T-A transversions. Chi square analysis indicated that the background and 4-AB-induced mutation spectra (Figure 2) were significantly different in UTH8414 (χ² = 18.03, P = 0.002) as well as in TA100 (χ² = 52.90, P < 0.001). Not surprisingly, the 4-AB-induced mutation spectra were significantly different between the two strains (χ² = 16.16, P = 0.006) because the excision-repair deficiency in TA100 increased the mutant yield by 10-fold. In contrast, the background mutation spectra were not significantly different between the two strains (χ² = 5.04, P = 0.41).

The finding that the majority of the 4-AB-induced base substitutions in these pKM101-containing strains were G-C → T-A transversions is consistent with previous studies using other mutagens or background mutants in *E. coli* (FOWLER et al. 1979; Mattern et al. 1985) and *Salmonella* (EISENSTADT et al. 1989; CEBULA and KÖCH 1990a; PRIVAL and CEBULA 1992) that show that the

**FIGURE 2.—Distribution of base-pair substitutions at the hisG46 allele recovered among background (open bars) and 4-AB-induced (filled bars) revertants of strains UTH8414 (pKM101) and TA100 (ΔuvrB, pKM101) of Salmonella in the presence of 89. Revertants were picked at the doses and the fold increases over the background indicated by arrows on the dose-response curves in Figure 1. The CCC class represents reversion events at an extragenic suppressor tRNA, and CCC is the target sequence of the hisG46 allele. The rev/plate of each class of mutation were calculated by distributing the total number of rev/plate obtained under each condition (Figure 1) among the classes of mutations based on the frequency (%) at each class of mutation occurred (Table 2). The total rev/plate for UTH8414 were 18 (control) and 137 (4-AB); for TA100 they were 144 (control) and 1560 (4-AB). pKM101 plasmid primarily enhanced the frequency of transversions. Likewise, Lasko et al. (1988) found that most of the N-hydroxy-4-AB-induced mutants in bacteriophage M13mp10 were also transversions and required the presence of the pKM101-derived plasmid pGW16.

**Mutation spectra and classification of mutants at the hisD3052 allele:** The 4-AB-induced mutation spectra at

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**TABLE 2**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total mutants</th>
<th>TCC</th>
<th>ACC</th>
<th>GCC</th>
<th>CTC</th>
<th>CAC</th>
<th>CCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTH8414</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>109</td>
<td>17 (15.6)</td>
<td>19 (17.4)</td>
<td>2 (1.8)</td>
<td>26 (25.9)</td>
<td>42 (38.5)</td>
<td>3 (2.8)</td>
</tr>
<tr>
<td>4-AB</td>
<td>99</td>
<td>4 (4.0)</td>
<td>26 (26.2)</td>
<td>0 (0.0)</td>
<td>9 (9.1)</td>
<td>59 (59.6)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>TA100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>192</td>
<td>22 (11.5)</td>
<td>30 (15.6)</td>
<td>7 (3.6)</td>
<td>39 (20.0)</td>
<td>90 (46.9)</td>
<td>4 (2.0)</td>
</tr>
<tr>
<td>4-AB</td>
<td>94</td>
<td>6 (6.4)</td>
<td>23 (24.5)</td>
<td>1 (1.0)</td>
<td>13 (15.8)</td>
<td>51 (54.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>
the hisD3052 allele in three DNA repair backgrounds are shown in Figure 3. The frequencies at which various classes of mutations were recovered in each strain are shown in Table 3. A complete mutation spectrum was not constructed for revertants of strain UTH8414 (pKM101) because 4-AB induced a mutant yield in this strain that was only 3-fold above the background, preventing the construction of a meaningful mutation spectrum. However, we determined the frequency of the hotspot mutation in this strain, which was 25% for the background and 65% for 4-AB.

In the wild-type strain (TA1978), the hotspot mutation (-CG or -GC at CGCGCGCG) accounted for 87.4% of the total. Among the remaining mutations, nearly twice as many duplications as deletions were recovered; whereas, no insertions or complex mutations (frameshifts involving a base substitution, see below) were recovered. Among the deletions and duplications, a wide range of sizes was observed, including the largest deletion (35 bases) and the largest duplication (46 bases) detected in any strain in this study.

Only one of the 396 revertants in the TA1978 mutation spectrum was a true revertant, i.e., one that reverted by the addition of a C at the site of the original -C mutation in this allele (the missing C is noted by a dash after nucleotide 893 in Figure 3). The frequencies and types of mutations induced by 4-AB were estimated by subtracting the background mutation spectrum using the method described in the legend of Table 4. The 4-AB-induced revertants of strain TA1978 were picked at 6-fold over the background. Thus, it was probable that one mutation out of six (i.e., 66 of the 396 mutations) was actually a background mutation. After subtracting the background (Table 4), we determined that 4-AB induced only the hotspot deletion in TA1978; the other mutations present in the spectrum were presumptive background mutations.

The absence of nucleotide excision repair (strain TA1538) strongly increased the mutagenic potency of 4-AB and permitted analysis of revertants selected at 18-fold above background. This resulted in a 4-AB-induced spectrum that was composed nearly entirely of the hotspot deletion (99.3%). Of 912 revertants analyzed, only 6 non-hotspot mutations were recovered, 5 of which were deletions, and the remaining one was a complex mutation (discussed below). No large duplications were observed in this (or the TA98) spectrum due to the large increase above the background produced by 4-AB in the uvrB strains and the relatively low background frequency of duplications in the uvrB strains (Table 4). After subtracting the background mutations (Table 4), we determined that 4-AB induced primarily the hotspot deletion (along with the single complex mutation) in this strain.

The spectrum of 4-AB-induced revertants in strain TA98, which contained the error-prone translesion synthesis capability provided by the pKM101 plasmid, contained many more complex frameshift mutations, most of which can be defined as (+1) or (-2) frameshifts associated with a flanking base substitution, e.g., CGC → A at position 880–882 (Figure 3). As argued below, this class also includes the apparent deletion of the CC or GG flanking the hotspot site, which we consider to be cryptic complex frameshifts. After subtracting the background revertants from this spectrum (Table 4), we determined that 4-AB induced only two classes of mutations: the hotspot deletion (~85%) and the complex frameshifts (~15%). The complex mutations occurred primarily at two sites and consisted of either (1) a 2-base deletion and a contiguous base substitution at the hotspot, (2) the apparent deletion of the CC or GG that flank the hotspot site, or (3) a 1-base duplication and a base substitution separated by one base at the TGA stop codon 3' from the hotspot site (Figure 3).

**Hotspot mutations at the hisD3052 allele:** Since the initial work of ISONO and YOUNO (1974), evidence has accumulated showing that a large percentage of the spontaneous and induced revertants of the hisD3052 allele revert as a result of a hotspot mutation, which consists of a 2-base deletion (−CG or −GC) within the sequence CGCGCGCG (FUSCOE et al. 1988; CEBULA and KOCH 1990a; BELL et al. 1991; KUPCHELLA and CEBULA 1991; O'HARA and MARNETT 1991; DEMARINI et al. 1992). Based on the analysis above, the 4-AB-induced revertants of this allele are no exception (Table 4).

The high frequency of hotspot mutations induced by 4-AB is typical of other polycyclic planar mutagens with reactive side groups that permit the formation of DNA adducts. A previous study by ISONO and YOUNO (1974) that was confirmed by KUPCHELLA and CEBULA (1991) showed that 5 out of 5 4-nitroquinoline-N-oxide-, 6 out of 6 2-nitrosophluorene-, and 5 out of 5 hyacanthone-induced revertants contained this hotspot mutation. FUSCOE et al. (1988) found this mutation among 5 out of 5 PHIP-, 13 out of 13 IQ-, 3 out of 3 MeIQ-, and 3 out of 3 aflatoxin B1-induced revertants.

With the development of colony probe hybridization procedures to simplify the identification of the hotspot mutation (KUPCHELLA and CEBULA 1991), larger sample sizes (200 or more induced revertants) have been analyzed. CEBULA and KOCH (1990b) found that 97% of Adriamycin-92% of daunomycin-, 86% of aflatoxin B1-, 92% of N-2-acetylaminofluorene- (2-AAF), and 74% of benzo(a)pyrene-induced revertants contained the hotspot mutation. A recent review of work from our laboratory (DEMARINI et al. 1993) shows that 86% of ellipticine-, 94% of 1-nitropyrene, 98% of Glu-P-1, 89% of main- and side-stream cigarette smoke condensate-90% of urban air particulate organic-, and 88–98% of municipal waste incinerator particulate organic-induced revertants contained the hotspot mutation.

Three exceptions to this general trend have been
FIGURE 3.—Mutation spectra of 4-AB-induced revertants of strains TA1978, TA1538 and TA98 of Salmonella in the presence of S9. The dash after position 893 represents the −1 deletion of a C that constitutes the hisD3052 allele. Open bars, deletions; filled bars, duplications; open bars with attached triangles, complex mutations involving deletion, addition, and/or base substitution; mutations connected by a line, complex mutations involving deletion or duplication plus a base substitution at a nearby site. Each symbol represents the mutation present in a single revertant. The TA1538 and TA98 spectra were each composed of three separate collections of revertants. Application of the statistical program of Adams and Skopek (1987) showed that the three spectra generated for a particular strain were not significantly different ($P > 0.5$); thus, they were combined to form the spectra shown here.
### TABLE 3

Percentage (frequency) of classes of mutations in 4-AB mutation spectra at the hisD3052 allele

<table>
<thead>
<tr>
<th>Mutation class</th>
<th>TA1978 (wild-type)</th>
<th>TA1538 (ΔωurB)</th>
<th>TA98 (ΔωurB, pKM101)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deletions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hotspot*</td>
<td>87.4 (346/396)</td>
<td>99.3 (906/912)</td>
<td>83.1 (679/817)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td>0.5 (2/396)</td>
<td>0.2 (2/912)</td>
<td>0.5 (4/817)</td>
</tr>
<tr>
<td>-5</td>
<td>0.5 (2/396)</td>
<td>0.1 (1/912)</td>
<td>0.2 (2/817)</td>
</tr>
<tr>
<td>-8</td>
<td>1.8 (7/396)</td>
<td>0.1 (1/912)</td>
<td></td>
</tr>
<tr>
<td>-11</td>
<td>0.8 (3/396)</td>
<td>0.1 (1/912)</td>
<td></td>
</tr>
<tr>
<td>-14</td>
<td>0.3 (1/396)</td>
<td>0.1 (1/912)</td>
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<tr>
<td>-17</td>
<td>0.3 (1/396)</td>
<td>0.1 (1/912)</td>
<td></td>
</tr>
<tr>
<td>-35</td>
<td>0.3 (1/396)</td>
<td>0.1 (1/912)</td>
<td></td>
</tr>
<tr>
<td><strong>Additions</strong></td>
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</tr>
<tr>
<td>Duplications</td>
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</tr>
<tr>
<td>+1</td>
<td>4.8 (19/396)</td>
<td></td>
<td>1.3 (11/817)</td>
</tr>
<tr>
<td>+4</td>
<td>2.3 (9/396)</td>
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<tr>
<td>+19</td>
<td>0.3 (1/396)</td>
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<td>+28</td>
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<tr>
<td>+48</td>
<td>0.3 (1/396)</td>
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<td><strong>Insertions</strong></td>
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</tr>
<tr>
<td>+1</td>
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<td></td>
<td>0.2 (2/817)</td>
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<td>Complex 5</td>
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</tr>
<tr>
<td>-2</td>
<td>3.7 (30/817)</td>
<td>7.5 (61/817)</td>
<td></td>
</tr>
<tr>
<td>(Cryptic) 5</td>
<td>0.1 (1/912)</td>
<td>2.0 (16/817)</td>
<td></td>
</tr>
<tr>
<td>-2, +bps</td>
<td>1.2 (10/817)</td>
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<td><strong>Totals</strong></td>
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<tr>
<td>Deletions</td>
<td>87.4 (346/396)</td>
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<td>83.1 (679/817)</td>
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<tr>
<td>Other</td>
<td>4.0 (16/396)</td>
<td>0.5 (5/912)</td>
<td>1.0 (8/817)</td>
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<tr>
<td>Additions</td>
<td>7.8 (31/396)</td>
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<td>0.2 (2/817)</td>
</tr>
<tr>
<td>Complex</td>
<td>0.8 (3/396) 4</td>
<td>14.4 (117/817)</td>
<td></td>
</tr>
</tbody>
</table>

*a Deletion of either CG or GC from the sequence CCGCGCGCG located at nucleotides 878-885.

*b A frameshift mutation with an associated base substitution.

*c A subset of complex frameshift mutations that appear as the deletion of either the CC or GG that flank the hotspot site (CCCGCGCGCGG).

*d No sequence available.

Observed. Only 4% of mitomycin C- (KUPCHELLA and CEBULA 1990) and β-methoxy-acrolein- (O'HARA and MARNETT 1991) induced revertants of the hisD3052 allele might be explained most simply by the model of STREISINGER et al. (1966) and STREISINGER and OWEN (1985) in which misaligned replication intermediates, derived from slippage of one strand relative to the other, might be stabilized within iterated sequences. Indeed, the hotspot sequence provides more opportunity than does any other sequence within the target for the occurrence of slipped mispairing within an iterated sequence during replication. The enhancement of this 2-base deletion by mutagens such as 4-AB that form DNA adducts at the C(8) position of guanine may then be explained by a correct incorporation/slippage model proposed by SCHAAPER et al. (1990) and tested experimentally by LAMBERT et al. (1991). This model is illustrated in Figure 4 in which cytosine is incorporated correctly opposite a 4-AB-adducted guanine; however, base pairing involving an adducted guanine may be unstable and/or progression of the DNA polymerase may be hindered by the adduct, increasing the probability of strand slippage on the repeated GpC motif. A new stabilized primer terminus is then formed, and extension of this terminus yields the 2-base hotspot deletion.

Evidence supporting this model includes theoretical and spectral studies showing that 4-AB adducts at the C(8) position of guanine can cause the adducted guanine to assume either the anti or syn conformation, and that while in the anti conformation, the adducted guanine can pair correctly with cytosine (BROYDE et al. 1985; SHAPIRO et al. 1986). The model’s requirement for correct insertion opposite the adducted guanine to produce the hotspot mu-
tation is consistent with studies of base insertion opposite other dG-C(8) adducts in which cytosine is the base inserted most frequently opposite the adducted guanine (RABKIN and STRAUSS 1984; MICHAELS et al. 1991).

Application of the model to the specific sequence of the hotspot leads to the prediction that adducts on G_3, G_5, G_6 or G_7 (but not on G_1) can produce the hotspot deletion (Figure 4). An adduct on G_1 may permit correct incorporation of a cytosine, but there is no suitable sequence 5' of the adducted G_1 on which appropriate slippage can occur to produce a 2-base deletion. This model is similar to that suggested by LAMBERT (1992a,b) for -1 frame shifts within a contiguous run of guanines.

The pathway delineated in Figure 4 is but one of a large number that can be drawn, all ultimately leading to the same observed 2-base deletion. These pathways differ by the number of nucleotides incorporated following the insertion of a C opposite the adducted G and by the precise conformation assumed following the 2-base slippage. For example, in the case of the depicted G_3 adduct, either 0, 1, 2, or 3 subsequent nucleotides could be incorporated prior to slippage. All cases would result in the same observed 2-base deletion. Furthermore, following slippage, the misaligned intermediate could assume one of several conformations in which the adducted G_3 is either intra- or extrahelical. In this regard, GARCIA et al. (1993) have shown that a monotonic run of guanines containing a 2-AAF adduct was stabilized when the adducted guanine was extrahelical.

At this time, we have no strong arguments to favor one specific pathway over the other. Instead, we wish to stress that all pathways proceed by a common mechanism comprised of (1) the incorporation of a C opposite the adducted G and (2) a subsequent 2-base slippage promoted and directed by the relative instability of the primer terminus and the specific sequence context. It is likely that the high degeneracy of the intermediate state is a contributing factor to the high frequency of the -2 event, in conjunction with a total of eight Gs (four in each strand) as targets for adduction that could be potential initiators of the event.

The repeated GpC motif within the hotspot may permit additional mechanisms to operate. Such sequences can form Z-DNA, which is unstable and may result in a high frequency of spontaneous 2-base deletions (FREUND et al. 1989). The formation of Z-DNA by the presence of this motif three times within the hotspot sequence may account for the high background frequency of 2-base deletions at the hisD3052 allele. Theoretical and spectroscopic studies by BROVDE et al. (1985) and SHAPIRO et al. (1986) indicate that 4-AB guanine adducts at the C(8) position can readily adopt the syn conformation, which may promote the B- to Z-DNA transition in a manner similar to that proposed for 2-acetylaminofluorene (BURNOUF et al. 1989). Support for this suggestion comes from ABUAF et al. (1987) who showed by circular dichroism that modification of poly(dG-dC) by a reactive form of 4-AB induced the B- to Z-DNA transition. Thus, the 2-base hotspot deletion may result from the structural features of Z-DNA and/or the action of certain Z-DNA-binding proteins (e.g., FISHEL et al. 1988) on such structures, especially on C(8) adducts on guanine in Z-DNA. The importance of the DNA sequence context is illustrated by the finding that 4-AB adducts in a 15-mer duplex not containing a repeating GpC motif yield a DNA polymer that has primarily a B-conformation (MARQUES and BELAND 1990; CHO et al. 1992).

**Mechanism of complex frame shifts:** The model described above (Figure 4) for the hotspot deletion (*i.e.*, correct incorporation of a cytosine opposite an adducted guanine followed by slippage) can be modified to involve misincorporation opposite an adducted guanine followed by slippage to explain the complex frame shifts (Figure 5). This model is based on (1) the for-
Complex Frameshift Mutations

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![Diagram of GC/CG Frameshift at Hotspot](image)

**Figure 4.**—Proposed mechanism of hotspot deletion involving correct incorporation of a cytosine opposite a 4-AB-adducted guanine. Such base pairs may be unstable and/or progression of the DNA polymerase may be hindered by the adduct, increasing the probability of strand slippage on the repeated GpC motif. A new stabilized primer terminus is then formed, and extension of this terminus yields the 2-base hotspot deletion.

mutation of DNA adducts by 4-AB in Salmonella predominantly at the C(8) position of guanine (KADLUBAR et al. 1982), (2) the finding that such adducts cause the guanines in the majority of 4-AB-adducted poly (GpG) molecules (a sequence motif found at the complex frameshift hotspot) to reside in the syn conformation (BROYDE et al. 1985; SHAPIRO et al. 1986), (3) the possibility for the O6 and N-7 atoms of modified guanines in the syn conformation to mispair with the N-1 and N2 of guanine or with the N6 and N-1 of adenine, resulting in transversions (DRAKE and BALITZ 1976; TOPAL and FRESCO 1976), (4) the observation that in the presence of pKM101, ~80% of the 4-AB-induced base substitutions are transversions (Figure 2), and (5) the well documented ability of DNA polymerases (when copying undamaged templates in vitro) to produce frameshift errors that are mediated by a nucleotide misinsertion and a subsequent slippage event (KUNKEL and SONI 1988; BEBENEK and KUNKEL 1990; KUNKEL 1990; BEBENEK et al. 1992).

Nearly all of the complex frameshifts in the 4-AB mutation spectrum in TA98 are accounted for by our proposed misincorporation/slippage model. The four general categories of complex frameshifts that are explained by the model are illustrated in Figure 5 and discussed below.

For complex frameshifts at the hotspot, which generally consist of 2-base deletions and a contiguous base substitution, we postulate that (1) misincorporation occurs opposite an adducted guanine, (2) two or more additional (correct) bases are incorporated, (3) a 2-base slippage occurs on the adducted strand, (4) extension continues, and (5) replication of the nonadducted strand results in a complex frameshift consisting of a 2-base deletion and a contiguous base substitution (Figure 5A). The model predicts that adducts on only G3, G4, or G5 can participate in the generation of these complex frameshifts. As stated previously for the 2-base deletion at the hotspot site, the possible intermediates that can be drawn are numerous, depending on the extent of synthesis following misincorporation (1, 2, or 3 nucleotides in the case of an adducted G3) and the precise nature of the bases in the bulge.

An additional feature of the 4-AB-induced mutation spectrum in strain TA98 is the presence of two clusters of mutations flanking the hotspot (Figure 3): the apparent deletion of CC at the 5' end of the hotspot region, and the apparent deletion of GG at the 3' end of the hotspot region (CCGCCGCCGG). Although these mutations appear at first to be simple 2-base deletions, they are not explicable by simple slippage models because there is no additional CC or GG sequence adjacent to the two sites on which an appropriate 2-base slippage can occur. The absence of these two mutations from the 4-AB-induced mutation spectra in the two pKM101-minus strains (TA1978 and TA1538), as well as their rare occurrence among background revertants of TA1978 and TA1538 (in preparation), suggest that the frequency of these mutations is enhanced by the presence of the pKM101 plasmid and that these mutations may be complex mutations in disguise (i.e., cryptic complex frameshifts).

Indeed, the cryptic complex frameshifts may be explained in the same way as the other complex frameshifts; however, they represent a restricted set of complex frameshifts at the hotspot site. The model predicts that these mutations occur when a guanine is misincorporated opposite an adducted G3, followed by a 2-base slippage. Misincorporation of a guanine opposite an adducted G3 residing on the nontranscribed (-) strand, followed by a 2-base slippage, produces the -CC mutation (Figure 5B); misincorporation of a guanine opposite an adducted G3 on the transcribed (+) strand, followed by a 2-base slippage, produces the -GG mutation (Figure 5C).

In contrast to the complex frameshifts at the hotspot, which generally consist of a 2-base deletion and a contiguous base substitution, the complex frameshifts at or
FIGURE 5.—Illustration of the misincorporation/slippage model for (A) a common complex frameshift at the hotspot, (B) the −CC cryptic complex frameshift at the hotspot, (C) the −GG cryptic complex frameshift at the hotspot, and (D) a common complex frameshift at the stop codon.
near the TGA stop codon 3' of the hotspot involve a 1-base duplication and a base substitution separated by one base. The misincorporation/slippage model explains these mutations by postulating that after misincorporation, a 1-base slippage occurs on the nonadducted strand (as opposed to a 2-base slippage on the adducted strand for the complex frameshifts at the hotspot). Extension followed by replication produces a 1-base duplication and a base substitution separated by one nucleotide. Figure 5D illustrates the model for the most common mutation of this category.

Application of the misincorporation/slippage model to all of the complex frameshifts at the hotspot site, including the cryptic complex frameshifts at the hotspot site, permits an analysis of the relative frequency at which misincorporation/slippage events occur at each of the nucleotides in the two strands, as well as the preference of misincorporation at each of these sites. The results (Table 5 and Figure 6A) show that there is a clear strand bias, such that 84% of the complex frameshifts at the hotspot are due to misincorporations opposite adducted guanines located on the nontranscribed (-) strand. This may be due to (1) the presence of more 4-AB adducts on the nontranscribed (-) strand than on the transcribed (+) strand, (2) a replication asymmetry that causes adducts on the lagging strand to be more mutagenic than those on the leading strand (Veauve and Fuchs 1993), or (3) DNA sequence context effects that extend beyond the immediately adjacent nucleotides.

Application of the model also predicts that adducts located on guanines at the 3' end of the sequence (i.e., G₃) account for more of the mutations than those located at the 5' end of the sequence (G₅) (Table 5, Figure 6B). This observation is reminiscent of the greater mutagenic effectiveness of adducts at the 3' end of a contiguous run of guanines in E. coli as demonstrated by Lambert et al. (1992b). Perhaps adducts located at the 3' end of an iterated sequence provide a greater number of slipped intermediates than would adducts located at the 5' end of the sequence. Slipped intermediates resulting from 3' adducts may also be more stable due to the availability for base pairing of more sequence 5' to the slipped site.

Application of the model to the complex frameshifts at the hotspot also shows that the polymerase preferentially misincorporates adenine at G₃ + G₅: A (74%) > T (17%) > G (9%) (Table 5, Figure 6C). These relative frequencies of misincorporation in the presence of pKM101 at the hotspot of the frameshift allele hisD3052 in strain TA98 are similar to those produced by 4-AB in the presence of pKM101 at the base substitution allele hisG₄6 allele in strain TA100 (Figure 2): A (79%) > T (20%) > G (1%). This is consistent with the role of the pKM101 plasmid in facilitating error-prone translesion synthesis, resulting in these base substitutions at both alleles.

Application of the model to the complex frameshifts resulting from 4-AB adducts on G₃ reveals the following relative frequencies of misincorporation: G (68%) > A (50%) > T (2%) (Table 5, Figure 6C). These values suggest a high frequency of misincorporation of guanine opposite an adducted G₃, which leads to the production of the cryptic complex frameshifts. The sequence context of G₃ is different from that of G₄ and G₅, which could affect misincorporation ratios as well as slippage tendencies. We note that in a different DNA sequence context (hisG₄6), there was no evidence for the misincorporation of guanine opposite a 4-AB-adducted guanine because 4-AB did not induce GC → CG transversions at the hisG₄6 allele (Figure 2). Figure 7 illustrates the polarity of adduction/misincorporation and the frequency with which different nucleotides may be misincorporated opposite specific adducted guanines in a manner consistent with the model and the 4-AB-induced TA98 mutation spectrum.

Our recovery of one complex frameshift mutation in a plasmid-minus strain (TA1538), along with the recovery of several cryptic complex frameshifts among spontaneous revertants of TA1978 and TA1538 (D. M.
J. G. Levine, R. M. Schaaper and D. M. DeMarini

DeMarini, manuscript in preparation) indicates that the pKM101 plasmid is not absolutely required for the production of complex frameshifts. In the absence of the plasmid, these mutations may be mediated by SOS functions that are present at a low level in pKM101-minus strains of Salmonella (Eisenstadt 1987; Smith and Eisenstadt 1989; Smith et al. 1990; Noimi et al. 1991; Woodgate et al. 1991). However, the present study demonstrates clearly that the pKM101 plasmid greatly enhances the production of complex frameshift mutations. The pKM101 plasmid contains the mucAB genes, which appear to be at least partial functional analogues of the E. coli umuDC genes, which participate in the SOS response in E. coli (Walker 1984; Blanco et al. 1986).

Previous observations are consistent with the conclusion that pKM101 enhances the production of complex frameshift mutations. Felton et al. (1989) recovered two benzo(a)pyrene-induced complex frameshift mutations in strain TA98 (out of an unspecified total) but none out of 24 benzo(a)pyrene-induced revertants of the plasmid-minus strain TA1538. Cebula and Koch (1991) reported the recovery of complex frameshift mutations that may have been promoted by pKM101 or other SOS functions in frameshift strains of Salmonella. O'Hara and Marnett (1991) found no complex frameshifts among 37 background or 27 mutagen-induced revertants of the hisD3052 allele in a pKM101-minus strain of Salmonella. In E. coli bacteriophage M13, the ability of SOS, induced by UV irradiation or the mucAB-containing plasmid pGW270, to enhance aflatoxin B1 (AFB1)-induced complex mutations has also been noted (Refolo et al. 1987; Sambamurti et al. 1988; Sahasrabudhe et al. 1989; Bennett et al. 1988, 1991). Some of the mechanisms proposed to explain the AFB1-induced complex frameshifts in M13 (Refolo et al. 1987) share features with the mechanisms proposed here for 4-AB-induced complex frameshifts in Salmonella.

Mutagenic potency as a function of mutational mechanisms: 4-AB displays a 60-fold (or larger) range of mutagenic potencies in the present study, depending on the DNA repair background of the strain of Salmonella and the allele used to measure the specificity (Table 1). Because the strains are otherwise isogenic, the differences in mutagenic potency at any one allele are likely due to the number of unrepaired adducts and the probability of the steps that are necessary to convert an adduct to a selectable mutation in each repair background. As discussed below, the mutagenic mechanisms derived from the mutation spectra can provide a framework for understanding the relative mutagenic potency of 4-AB among the different strains.

Reversion of the hisD3052 frameshift allele may be initiated by 4-AB-adducted guanines that promote slipped/mispairing. In an excision repair-proficient background, there are relatively few unrepaired 4-AB-adducted guanines to promote the required incorporation and slippage events, and the added ability to perform error-prone synthesis past such adducts by the
addition of the pKM101 plasmid does not enhance the mutagenic potency. This is consistent with the view that most of the 4AB-induced mutations at the hisD3052 allele, i.e., the hotspot CG or GC deletion, occur by correct incorporation of a C opposite 4AB-adducted guanines. The addition of the ΔuvrB allele increases the number of 4AB-adducted guanines available to promote slippage, leading to a strong enhancement of mutagenic potency. The potency is increased further by the addition of the pKM101 plasmid, which facilitates error-prone translesion synthesis. Although misincorporation by itself is insufficient to revert the frameshift allele, it promotes slippage, leading to a strong enhancement of mutagenic potency.

For 4AB to revert the hisG46 base-substitution allele, error-prone synthesis past a misincorporated nucleotide opposite a 4AB-adducted guanine is necessary to produce a revertant containing a base substitution and requires the pKM101 plasmid. The presence of more unrepaired 4AB adducts due to uvrB enhances the probability of this event, leading to a 30-fold increase in mutagenic potency. This is roughly the enhancement in the mutagenic potency of 4-AB (40-fold) produced by uvrB at the frameshift allele. This suggests, perhaps, that there are 30–40 times more unrepaired 4-AB adducts present in uvrB strains compared to DNA excision-repair-proficient strains. This conclusion for Salmonella is consistent with findings in E. coli for the repair of 4-AB adducts by the nucleotide excision-repair system (Tamura and King 1990; Suzuki et al. 1993). The mutagenic efficiency of N-hydroxy-4-AB in a uvrB strain (TA1538) is ~6% (Kadlubar et al. 1982; Beland et al. 1983). The mutation spectra presented here would indicate that this value reflects the ability of 4-AB-adducted guanines (Gp, Gs, Gv, and Gq) within the hotspot to promote a 2-base slippage, leading to the hotspot deletion.

CONCLUSIONS

In the absence of pKM101, 4-AB induces almost exclusively (100% for TA1978 and 99.9% for TA1538) the 2-base hotspot deletion at the hisD3052 allele. We propose that the formation by 4-AB of adducts at the C(8) position of guanines Gp, Gs, Gv and Gq within the repetitive CG/GC motif in the hotspot region promotes the hotspot mutation via the correct incorporation of a cytosine opposite the adducted guanine. This may result in a slightly unstable pairing and/or may hinder the progression of the DNA polymerase such that a 2-base slippage occurs (facilitated by the CG/GC repeat), leading to the 2-base hotspot deletion.

In the presence of pKM101, ~85% of the 4-AB-induced revertants of the hisD3052 allele also contain the hotspot mutation; the remaining revertants contain complex frameshift mutations, which consists of a frameshift and a base substitution. The pKM101 plasmid greatly enhances the production of these events, from 0% in TA1978 and 0.1% in TA1538 to 15% in TA98. The sequence changes and their location suggest a mutational model that involves misincorporation/slippage. At the hotspot, the complex frameshifts consist primarily of a 2-base deletion and a base substitution. 4-AB-adducted guanines in the CC/GC motif within the hotspot region may reside in the syn or the anti conformation and, thus, may mispair. Application of the misincorporation/slippage model to the 4-AB-induced mutation spectrum in strain TA98 predicts the following relative frequencies of misincorporation opposite C3 + G3; A (74%) > T (17%) > G (9%). These values are similar to those produced by 4-AB in the presence of pKM101 at the base-substitution allele hisG46, and they are consistent with the A rule. The model also predicts a polarity of mutagenic effectiveness, with adducts at the 3' end (e.g., Gs) accounting for more of the complex frameshifts at the hotspot than adducts at Gs or Gv. There is also a prediction of a strand bias, such that 84% of the complex frameshifts at the hotspot are due to adducts on the nontranscribed (-) strand.

The 4-AB mutation spectrum in the presence of the plasmid contains a class of mutations that we call cryptic complex frameshifts that appear as deletions of the CC and GC dinucleotides that flank the hotspot sequence. Because these mutations are induced by 4-AB only in the plasmid-containing strain, we explain their formation by the misincorporation of a guanine opposite an adducted Gs followed by a 2-base slippage on the adduct-containing strand. Thus, these apparent 2-base deletions are actually due to a G→C transversion and a CG (or GC) deletion.

The same model accounts for most of the complex frameshifts outside of the hotspot region, which are explained by misincorporation of adenine or thymine opposite an adducted guanine followed by a 1-base slippage on the nonadducted strand. This results in a 1-base duplication and a base substitution.

Finally, the mutational mechanisms postulated here provide a framework for understanding the relative mutagenic potency of 4-AB in the different DNA repair backgrounds. The results suggest that, regardless of the allele, there might be 30–40 times more unrepaired 4-AB adducts in uvrB strains than in excision repair-proficient strains. The presence of the plasmid presumably facilitates translesion synthesis across mispaired adducted guanines. The instability of such mispairs may promote more slippage than occurs in the absence of such mispairs, resulting in the formation of complex frameshifts and an enhancement in mutagenic potency. The high GC content of the hisD3052 target region (72%) and the presence of iterated sequences within this target, all acting in concert with DNA repair and replication enzymes, provide a rich substrate for 4-AB-adducted guanines to initiate the diversity of mutations observed in the present study.
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LITERATURE CITED


ASHBYJ., and R. MOHAMMAD, 1988 UDS activity in the rat liver of the

BEBENEK, B. BENNETT, JAMES C. FUSCOE, THOMAS A. KUNKEL,

744

BELL, D. rubian of poly(GG-CG) modified by the carcinogens N-methyl-

BENNETT, C. B., 1987 Analysis of mutagenesis, pp. 1016-1033 in


Bell, D. A., J. G. LEVINE and D. M. DEMARINI, 1991 DNA sequence

CHROMOSOMES MODIFIED BY THE CARCINOGENS N-METHYI-

CLiET, A., F. A. BELAND and M. MAQUES, 1992 NMR structural studies of a 15-mer DNA sequence from a ras protooncogene, modified at the first base of codon 61 with the carcinogen 4-aminobiphenyl. Biochemistry 31: 9587-9602.

CLET, I., E. FOURNIER, C. MELCION and A. CORIDER, 1989 In vivo micro-}


CHROMOSOMES MODIFIED BY THE CARCINOGENS N-METHYL-

Ciero and the tumor suppressor ras protoonco gene, modified at the first base of codon 61 with the carcinogen 4-aminobiphenyl, Biochemistry 31: 9587-9602.

CLET, I., E. FOURNIER, C. MELCION and A. CORIDER, 1989 In vivo micro-}


CHROMOSOMES MODIFIED BY THE CARCINOGENS N-METHYL-

Ciero and the tumor suppressor ras protoonco gene, modified at the first base of codon 61 with the carcinogen 4-aminobiphenyl, Biochemistry 31: 9587-9602.

CLET, I., E. FOURNIER, C. MELCION and A. CORIDER, 1989 In vivo micro-}


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CLET, I., E. FOURNIER, C. MELCION and A. CORIDER, 1989 In vivo micro-}


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Ciero and the tumor suppressor ras protoonco gene, modified at the first base of codon 61 with the carcinogen 4-aminobiphenyl, Biochemistry 31: 9587-9602.

CLET, I., E. FOURNIER, C. MELCION and A. CORIDER, 1989 In vivo micro-}


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