Carcinogens induce reversion of the mouse pink-eyed unstable mutation

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Communicated by Gerald N. Wogan, Massachusetts Institute of Technology, Cambridge, MA, February 24, 1997 (received for review July 9, 1996)

ABSTRACT Deletions and other genome rearrangements are associated with carcinogenesis and inherited diseases. The pink-eyed unstable (p<sup>un</sup>) mutation in the mouse is caused by duplication of a 70-kb internal fragment of the p gene. Spontaneous reversion events in homozygous p<sup>un</sup>/p<sup>un</sup> mice occur through deletion of a duplicated sequence. Reversion events in premelanocytes in the mouse embryo detected as black spots on the gray fur of the offspring were inducible by the carcinogen x-rays, ethyl methanesulfonate, methyl methanesulfonate, ethyl nitrosourea, benzo[a]pyrene, trichloroethylene, benzene, and sodium arsenate. The latter three carcinogens are not detectable with several in vitro or in vivo mutagenesis assays. We studied the molecular mechanism of the carcinogen-induced reversion events by cDNA analysis using reverse transcriptase–PCR method and identified the induced reversion events as deletions. DNA deletion assays may be sensitive indicators for carcinogen exposure.

Tumor cells frequently contain genome rearrangements such as deletions (1–4). Furthermore, an elevated frequency of recombination and genome rearrangements is found in cells from patients suffering from cancer prone diseases such as Ataxia Telangiectasia (5), Li–Fraumeni syndrome (6), Blooms syndrome (7), and Werner's syndrome (8). About 25% of the human genome consists of repetitive DNA sequences, which may be either tandem repeats or interspersed repetitive elements (9). The large number of repetitive sequences scattered throughout the human genome create the substrates for intrachromosomal recombination events between direct repeats (e.g., ref. 10) and may lead to various genetic disorders if an essential locus is deleted or disrupted during the process.

Because of the association of genome rearrangements with cancer, Schiestl et al. (11) constructed a system in the yeast Saccharomyces cerevisiae that selects for deletions by intrachromosomal recombination, termed the DEL assay. DEL recombination is inducible by a wide variety of carcinogens, including carcinogens that are negative in most other short-term tests (12–15). In addition, deletion events of one copy of a partial duplication of the hprt gene are inducible by several carcinogens in CHO cells (16) and in human cells (17). Finally, reversion of an internal duplication at the pink-eyed dilution (p) gene in the mouse is inducible by x-rays (18).

We determined the effect of carcinogen exposure on the frequency of deletion events between two alleles of a gene duplication in vivo. As an assay system we chose reversion events of the pink-eyed unstable (p<sup>un</sup>) mutation in the mouse. The p gene encodes a melanosomal integral membrane protein that is required for the assembly of a high molecular weight melanogenic complex giving rise to the black coat color of wild-type (wt) mice (19). The p<sup>un</sup> mutation causes a dilution of the pigment in coat color and eye color. The p<sup>un</sup> mutation is a deletion disruption of the pink-eyed dilute locus creating a DNA sequence duplication of about 70 kb, which is a head-to-tail duplication (Fig. 1) as determined by a genome scanning method and by cloning of the duplicated sequence (23, 24). Spontaneous reversions of p<sup>un</sup> occur via deletion of one copy of the duplicated sequence (Fig. 1) resulting in accumulation of wt melanin in melanocytes. Although wt p transcript is 3.3 kb long (25), p<sup>un</sup> animals have a 4.8-kb transcript that contains a 1.3-kb tandem duplication of the sequences between nucleotides 765 and 2067 of the p transcript, including exons 6–18, with apparent breakpoints in introns 5 and 18 (21). Reversion of the p<sup>un</sup> mutation is easily scorable as black spots on the dilute coat. Its reversion frequency is at least three to five orders of magnitude greater than other recessive mutations at other coat-color loci (26). A range of 1.8% (25) to 3.8% (27) to 5.6% (18) of homozygous C57BL/6J p<sup>un</sup>/p<sup>un</sup> mice have patches of wt color in their coats and are thus mosaic revertants.

In our study of the inducibility of p<sup>un</sup> reversions, we selected chemicals with “sufficient evidence for carcinogenicity” (28, 29). Epidemiological studies have shown that x-rays (e.g., ref. 30), benzene (BEN), and arsenate cause cancer in humans (29). Animal carcinogenesis tests designate ethylmethane sulfonate (EMS), methylmethane sulfonate (MMS), ethyl nitrosourea (ENU), benzo[a]pyrene (BeP), and trichloroethylene (TCE) as carcinogens (28, 29). Sodium arsenate (SOA), BEN, TCE, and BeP are among the 12 highest-ranking chemicals on the EPA Priority List of Hazardous Substances (31). In the short-term Salmonella assay, BEN, TCE (32), and SOA (33) test negative, whereas x-rays (34), EMS, MMS, and BeP (35) test positive. ENU gives a very weak response in the standard Salmonella assay (36, 37). Among these agents, γ-rays, EMS, MMS, and benzene have been tested with the yeast DEL assay and give positive responses (12–14). In addition, γ-rays, MMS, and benzene induce deletions in human cells (17).

METHODS

Determination of Frequency of p<sup>un</sup> Reversions. Mice homozygous for pink-eyed dilution unstable (C57BL/6J p<sup>un</sup>/p<sup>un</sup>) and p<sup>−</sup> mice C57BL/6J-p (control mice, containing a p mutation without sequence duplication) were obtained from The Jackson Laboratory and bred at our facility. An increase in the frequency of reversion events in the premelanocytes in the embryo gives rise to an increase in the number of offspring showing dark patches on their fur. The protocol used in this test was similar to the “mouse spot test” (for review, see, e.g., refs. 38 and 39). Matings were set up between p<sup>un</sup> mice, p<sup>−</sup> mice, or between p<sup>un</sup> and p<sup>−</sup> mice, and pregnancy was timed from the discovery of a vaginal plug. First and second litters

Abbreviations: wt, wild type; BEN, benzene; EMS, ethylmethane sulfonate; MMS, methylmethane sulfonate; ENU, ethyl nitrosourea; BeP, benzo[a]pyrene; TCE, trichloroethylene; SOA, sodium arsenate; RT-PCR, reverse transcriptase–PCR.

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FIG. 1. $p^{un}$ structure and possible mechanisms of intrachromosomal recombination resulting in deletions (according to refs. 11, 17, and 20). In the center the $p^{un}$ structure is shown with exons 6–18 duplicated (21). (A) Intrachromatid crossing-over occurs after pairing of the two copies of the $p^{un}$ duplication in a looped configuration (11). Crossing over results in deletion of one of the two copies giving rise to reversion to $p^{+}$. (B) Single-strand annealing is initiated by a double-strand break between the duplicated exons (20, 22); DNA ends are degraded by a 5′–3′ single-strand specific exonuclease to expose the flanking homologous sequences. Annealing of the complementary single strands occurs, and the nonhomologous ends are removed followed by DNA synthesis and ligation. (C) Unequal sister chromatid exchange occurs as crossing over between one copy of the exon duplication on one sister chromatid and the other copy of the exon duplication on the other sister chromatid. Reciprocal products are the deletion of one copy of the exon 6–18 duplication resulting in reversion to $p^{+}$ and the triplication of exons 6–18. (D) Sister chromatid conversion events can occur after unequal pairing of the homologous portions of both copies of the exon duplication on one sister chromatid with either one of the two copies on the sister chromatid having the duplicated sequence in a looped-out configuration (11). Double crossover or gene conversion may lead to a conversion event during which one of the two copies of exons 6–18 is lost. The other sister chromatid maintains its original configuration. This event may also be initiated by a double-strand break between the duplicated copies on one sister chromatid degradation with a single-strand exonuclease until to the region of homology after which invasion, D-loop formation, and repair synthesis might happen from the sister chromatid (11).

were used and gave similar results. Sperm entry into the egg was assumed to have occurred in the early morning hours of the day on which the plug was found, and noon of that day was defined as 0.5 days postconception. Offspring were examined for spots at 12–14 days of age, when spots are most easily visible. Two subsequent examinations were performed, the last one at 4–5 weeks. Control values were obtained from mice bred at the same time as the experimental mice. Animal care and experiments were carried out according to institutional guidelines.

Carcinogen Exposure. The animals were exposed to x-rays or to an acute dose of the chemical carcinogens by i.p. injection at 10.5 days postconception. The carcinogens were dissolved either in saline or corn oil, and up to 0.2 ml of solution was injected. The highest purity grades of the carcinogens were used: EMS, MMS, ENU, SOA, and BaP were from Sigma; C57BL/6

Reverse Transcriptase–PCR (RT-PCR) Detection of Deletion Events. For molecular characterization, 3- to 4-day-old C57BL6/10 strain $p^{un}$ mice were sacrificed, and black patches and control pieces of gray skin were excised. As a positive control, pieces of black skin of wt C57BL/6J mice were used. Total RNA was isolated using guanidinium thiocyanate-phenol extraction (40). The first-strand cDNA synthesis was performed using the SuperScript II reverse transcriptase preamplification system and oligo(dT)$_{12-18}$ (GIBCO/BRL). The first-strand cDNA synthesis reaction contained 5 μg total RNA/1 × PCR buffer (GIBCO/BRL)/2.5 mM MgCl$_2$/0.5 mM dNTP mix/10 mM DTT. The PCR amplification of the $p$ and $p^{un}$ cDNA was performed using Taq DNA polymerase (GIBCO/BRL) and specific primers. The primers were homologous to sequences outside of duplicated regions: 3′ primer, CAA CCA GAT GCC ACC CAG AAT AGC; 5′ primer, CTG TGT CAC CGC TGG AAA ACT ACT. The PCR contained one-tenth of cDNA reaction mixture/1 × PCR buffer (GIBCO/BRL)/1.5 mM MgCl$_2$/200 μM dNTP mix/100 nM of each primer/2 units of Taq DNA polymerase. After initial denaturation for 3 min, 35 PCR cycles were performed using the ELLONGASE Amplification System (GIBCO/BRL). The ELLONGASE enzyme mix, which consists of a mixture of Taq and Pyrococcus species GB-D thermostable DNA polymerases, allows to effectively amplify DNA fragments up to 30 kb long. These PCRs contained 10 mM of dNTP mix, 10 μM of both primers, 2–4 μg of cDNA sample, and 1.6 mM of Mg$^{2+}$ in total volume of 50 μl. The cycling program consisted of a total of 35 cycles. Each cycle contained denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec, and synthesis at 68°C for 30 sec.
for 2.5 min. The PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining.

RESULTS

Mice homozygous for p\textsuperscript{mun}/p\textsuperscript{mun} were assayed for the frequency of spontaneous and carcinogen-induced spots. Because the spontaneous frequency varied between 4 and 11% (Table 1), we obtained spontaneous values from mice bred at the same time as the experimental mice. The data for x-rays show a highly significant increase of p\textsuperscript{mun} reversion after irradiation with 1 Gy. Animals were exposed to carcinogens by single i.p. doses at levels based on published fetotoxicity data. These doses, in many cases, approach maximum acute exposure levels tolerated by the fetus. We did not observe toxic effects to the female adult mice by these treatments, with the exception of TCE, which acted as sedative for several hours due to its anaesthetic effect. Carcinogen exposure decreased the litter size in all cases (Table 1).

In the first experiment we used the alkylating agents EMS, MMS, and ENU. Sixty-two of 585 control offspring (11%) developed spots. A 100 mg/kg EMS exposure caused spotting in 29% of the offspring; 100 mg/kg MMS, 25%; and 25 mg/kg ENU, 53%. In a separate experiment, SOA increased the spotting frequency from 5.3% to 29%. In the third experiment we used hydrophobic compounds, which we dissolved in corn oil before injection. Control animals injected with corn oil alone had a spotting frequency of 3.9%. BaP (150 mg/kg) caused 63% spotting; 200 mg/kg of TCE caused 32% spotting; and 200 mg/kg of BEN caused 27% spotting. All carcinogen results tested as highly significant by \( \chi^2 \) distribution values. Even when the highest control value (11%) was used in the analysis of all three experiments, we still found highly significant differences (\( P \ll 0.001 \)).

Gondo et al. (24) showed that spontaneous reversion of the p\textsuperscript{mun} mutation to wt is due to intrachromosomal recombination. However, after irradiation or chemical exposure, other different genetic events could happen. Possibilities include induction of other mutations or activation of genes that lead to bypass suppression as seen with the \( dsu \) gene (dilute suppressor), which suppresses the dilute coat-color phenotype of mice homozygous for the dilute leaden and ashen mutations (41). In addition, at least in humans, melanogenesis is inducible by UV light. Hence, we used control mice and molecular characterization to ensure that deletion events resulting from intrachromosomal recombination are responsible for induced spotting.

In the first control, p\textsuperscript{mun} mice that lack the p gene fragment duplication were used. These p\textsuperscript{mun} mice showed black streaks in their hair shafts similar to the ones reported previously for the p\textsuperscript{mun} mice (18), suggesting that these events are not specific for the p\textsuperscript{mun} allele. Thus, the previously reported increase in the frequency of black streaks induced by x-rays is probably not due to recombination events. However, no black spots were found among 152 offspring mice. In addition, after exposure to x-rays and BaP no spots were found among the offspring (Table 1). When p\textsuperscript{mun} and p\textsuperscript{mun} mice were crossed, the spontaneous spotting frequency was 6.6% (6/91 offspring) versus 78% (7/9) after BaP exposure of the dams, a highly significant difference (Table 1). Hence, carcinogen-induced spots on the fur of the offspring are specific for reversion events of the p\textsuperscript{mun} allele, and a single p\textsuperscript{mun} allele on one homolog is sufficient to give induced reversion events.

Because spontaneous reversion of the p\textsuperscript{mun} mutation to wt is due to intrachromosomal recombination (Fig. 1; ref. 24), we wanted to determine whether the carcinogen-induced spots were also due to recombination events resulting in wt p sequences in these spots. By genome scanning and molecular cloning techniques, the p\textsuperscript{mun} DNA has been shown to carry a head-to-tail tandem duplication of \( \sim 70 \) kb, and the spontaneous reversion events are due to the loss of one copy of this duplicated DNA (24, 25). Southern blotting could not be used because of the limited amount of DNA that can be harvested from the spots, and PCR of genomic DNA was not feasible because of the large size of the duplication. However, in p\textsuperscript{mun} animals the p gene is disrupted and contains a 4.8-kb transcript rather than the 3.3-kb transcript present in the wt or the revertant (25). Because these duplication breakpoints have been cloned and sequenced (21, 42) we designed primers so that we could analyze spots by RT-PCR for reversion events. These primers amplify a 1.3-kb fragment from p wt cDNA (Fig. 2, lane 1), whereas p\textsuperscript{mun} transcript results in a 2.6-kb fragment (Fig. 2, lanes 2–4). However, in p\textsuperscript{mun} skin sample cDNA, we also found the 1.3-kb fragment at a ratio of roughly 1:1. This may be due to the fact that about 1 in 10\(^5\) cells are of the revertant phenotype (27) and that the shorter 1.3-kb fragment may be

### Table 1. Effect of carcinogens on intrachromosomal recombination

<table>
<thead>
<tr>
<th>Strain of mice</th>
<th>Chemical</th>
<th>Dose</th>
<th>No. of mice treated</th>
<th>No. of live offsprings</th>
<th>Average litter size</th>
<th>No. of spotted offsprings</th>
<th>Frequency, %</th>
<th>Significance, ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>p\textsuperscript{mun}/p\textsuperscript{mun}</td>
<td>Control</td>
<td>0</td>
<td>89</td>
<td>498</td>
<td>5.2</td>
<td>28</td>
<td>5.6</td>
<td>(&lt;10^{-6})</td>
</tr>
<tr>
<td></td>
<td>X-rays</td>
<td>100 cGy</td>
<td>64</td>
<td>172</td>
<td>2.7</td>
<td>40</td>
<td>23</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>101</td>
<td>585</td>
<td>5.8</td>
<td>62</td>
<td>11</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>EMS</td>
<td>100 mg/kg</td>
<td>21</td>
<td>94</td>
<td>4.5</td>
<td>27</td>
<td>29</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>MMS</td>
<td>100 mg/kg</td>
<td>22</td>
<td>83</td>
<td>3.8</td>
<td>21</td>
<td>25</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>ENU</td>
<td>25 mg/kg</td>
<td>18</td>
<td>57</td>
<td>3.2</td>
<td>30</td>
<td>53</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0</td>
<td>59</td>
<td>337</td>
<td>5.7</td>
<td>18</td>
<td>5.3</td>
<td>(&lt;0.0005)</td>
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<tr>
<td></td>
<td>SOA</td>
<td>20 mg/kg</td>
<td>17</td>
<td>56</td>
<td>3.3</td>
<td>16</td>
<td>29</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>Corn oil control</td>
<td>0.2 ml</td>
<td>10</td>
<td>51</td>
<td>5.1</td>
<td>2</td>
<td>3.9</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td>150 mg/kg</td>
<td>10</td>
<td>32</td>
<td>3.2</td>
<td>20</td>
<td>63</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>TCE</td>
<td>200 mg/kg</td>
<td>18</td>
<td>41</td>
<td>2.3</td>
<td>13</td>
<td>32</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>BEN</td>
<td>200 mg/kg</td>
<td>15</td>
<td>48</td>
<td>3.2</td>
<td>13</td>
<td>27</td>
<td>(&lt;0.01)</td>
</tr>
<tr>
<td>p\textsuperscript{mun}/p\textsuperscript{mun}</td>
<td>Control</td>
<td>0</td>
<td>30</td>
<td>152</td>
<td>5.1</td>
<td>0</td>
<td>0</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>X-rays</td>
<td>100 cGy</td>
<td>5</td>
<td>31</td>
<td>6.2</td>
<td>0</td>
<td>0</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td>150 mg/kg</td>
<td>2</td>
<td>6</td>
<td>3.0</td>
<td>0</td>
<td>0</td>
<td>(&lt;0.0005)</td>
</tr>
<tr>
<td>p\textsuperscript{mun}/p\textsuperscript{mun}</td>
<td>Control</td>
<td>0</td>
<td>15</td>
<td>91</td>
<td>6.1</td>
<td>6</td>
<td>6.6</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td></td>
<td>BaP</td>
<td>150 mg/kg</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>7</td>
<td>78</td>
<td>(&lt;0.0001)</td>
</tr>
</tbody>
</table>

Mice homozygous for pink-eyed dilution unstable (C57BL/6J p\textsuperscript{mun}/p\textsuperscript{mun}) were used to determine the frequency of revertant spots on their coat in response to carcinogen treatment as described (18). Data for x-rays for p\textsuperscript{mun}/p\textsuperscript{mun} were taken from Schiestl et al. (18) for comparison. Control animals C57BL/6J p\textsuperscript{mun}/p\textsuperscript{mun} and C57BL/6J p\textsuperscript{mun}/p\textsuperscript{mun} were used, and control counts were taken at the time the exposures were performed.
Therefore, test negative in the assay as well as in many other short-term tests. Initially, there was some hope that the aforementioned transgenic mutation assays would detect such carcinogens. Unfortunately, too little data have been accumulated to assess the sensitivity of the transgenic mouse mutation assays to such Salmonella-negative carcinogens. Preliminary data with Salmonella-negative carcinogens, such as carbon tetrachloride and peroxisome proliferators, yielded negative results with the transgenic mouse mutation assays (47, 48), which has led to the general belief that these chemicals may be undetectable with the transgenic mouse mutation assays (43). Because Salmonella-negative as well as Salmonella-positive carcinogens have been shown to induce DEL recombination in yeast (12–14) and in human cells (17), it was important to determine the effect of selected examples of both classes of carcinogens on DEL recombination in our in vivo mouse model. The present paper shows that DEL recombination in vivo in the mouse is, in fact, inducible by examples of both classes of carcinogens.

**Comparison with Other Mouse in Vivo Mutagenesis Assays.** X-ray exposure of up to 6 Gy does not induce mutations in the lacI mouse, probably because λ packaging requires DNA of a certain size and may not tolerate deletions (49). However, 5 × 0.5 Gy x-rays induce deletions in a plasmid-based LacZ transgenic mouse that tolerates large deletions (50). In comparison, in our results, 1 Gy x-rays caused a 4-fold increase in p ammunition. MMS fails to induce lacI mutations in mice even when administered at 20 mg/kg per day for up to 21 days (47). In comparison, a single dose of 100 mg/kg MMS induced a high frequency of p ammunition reversions (Table 1). ENU, EMS, and BaP are positive for induction of mutations in transgenic mice (43, 44).

We also compared our data with data for the “mouse-specific locus” test (germ line mutation events) and the “mouse spot test” (somatic mutation events in the embryo). ENU is the most potent mutagen in the mouse-specific locus test (51). However, the doses necessary for induction of specific locus mutations are about 50 mg/kg; at doses of 25 mg/kg (52) no effect was found. In fact, these and other data are in agreement with a threshold of ENU mutagenic effect between 34 and 39 mg/kg (53). In comparison, at 25 mg/kg, ENU is one of the most potent inducing agents for p ammunition reversions (Table 1). EMS, MMS (54), as well as BaP (38), which otherwise are potent mutagens, are negative in the mouse-specific locus test. In the mouse spot test, x-rays, EMS, MMS, ENU, BaP, and TCE give positive result (39). Thus, the majority of chemicals that were positive for p ammunition reversions were also positive in the mouse spot test. It seems interesting that the mouse spot test detects not only forward mutations but also recombinational events in embryos (55), which might be the reason for the high concordance between the two assays.

**The Biological Activity of the Salmonella-Negative Carcinogens SOA, BEN, and TCE.** Arsenate is a human carcinogen (29), but SOA is actually not carcinogenic in mice (28). However, it is teratogenic in mice (56) and thus might be more toxic to embryos, which might explain our positive results for p ammunition reversions that happen in the embryo. SOA increases the frequency of eye spots in Drosophila that may be due to recombinational effects (57).

In an international collaborative study on the effect of different compounds in more than 100 different short-term assays carried out in 60 different laboratories, BEN tests negative with all bacterial assays and in more than 70% of all short-term tests (58). BEN is mutagenic for forward mutations in vivo (59) as well as in vitro in the mouse lymphoma assay in the presence of S9, but no DNA adducts are seen even at mutagenic levels. However, BEN causes oxidative damage (60), and in the presence of S9, it induces DNA strand breaks (61) and it is clastogenic (62). In fact, BEN induces gene-duplicating but not gene-inactivating mutations at the glycoprotein A locus in exposed humans, which has been explained by recombination or genome rearrangement (63).
TCE also induces clastogenic effects (64) such as an increased frequency of chromosomal aberrations and hyperploid cells (65) among occupationally exposed workers. Furthermore, single-strand breaks were detected in TCE-exposed mice (66). TCE also tests positive in the mouse spot test (67), which detects forward mutations as well as recombination events (55).

As suggested by the data described above, SOA, BEN, and TCE may cause DNA strand breaks that induce DEL recombination. DEL recombination assays detect Salmonella-negative carcinogens, many of which have been termed “non-mutagenic” or “nongenotoxic.” These and other data question the appropriateness of these terms, because deletions of many kilobases of DNA are prime examples of genotoxic mechanisms. In this respect, it seems interesting that benzene inhibits mutagenic “or” nongenotoxic.” These and other data question negative carcinogens, many of which have been termed “non-

Mechanism of Carcinogen-Induced p<sup>+</sup> Reversion. Using RT-PCR, we show that the carcinogen-induced spots result from genomic DNA deletions. In principle, the induced reversion events could occur between the two homologs as interchromosomal recombination either by crossing over by conversion, or within one chromosome (intrachromatid), as shown in Fig. 1. The mechanism responsible for induced interchromosomal reversion events of a similar duplication disruption in yeast has been studied in detail (11, 20). DEL recombination, in theory, could happen by intrachromatid crossing over (Fig. 1A) or single-strand annealing (Fig. 1B) in any phase of the cell cycle, including G<sub>1</sub>. On the other hand, unequal sister chromatid exchange (Fig. 1C) and sister chromatid conversion (Fig. 1D) are limited to S phase or G<sub>2</sub>. Data described in Galli and Schiestl (20) suggest that DNA double-strand breaks are involved in DEL recombination and that single-strand annealing, proceeding via a double-strand break intermediate, is a possible mechanism for induced DEL recombination events. It is not clear from the present data which mechanism occurs in the mouse. Because the same chemicals induce the deletion events in both yeast and mice and because at least BEN does not induce interchromosomal recombination events in yeast (14), the same mechanism might be involved.

With the model that DEL recombination depends on an initial DNA double-strand break, one can review the activity of the carcinogens. In fact, MMS, EMS, BeP, and BEN are positive in the micronucleus assay (62). X-rays induce double-strand breaks directly to lead to recombination by single-strand annealing (Fig. 1B). EMS, MMS, and ENU, alkylating agents that cause DNA adducts, might cause DNA strand breaks upon DNA repair or DNA replication. BeP metabolic products also cause DNA adducts or create radical cations (70) that may directly initiate DNA strand breaks. Finally, BEN and TCE may directly induce DNA strand breaks as described above. DNA double-strand breaks may lead to an increase in forward mutations, deletions, or recombination but may not lead to an increase in reversions as determined in the Salmonella assay. To detect the biological activity of the majority of carcinogens it is important to include assays that reproducibly detect Salmonella-negative carcinogens such as deletion assays.

We thank Murray Brilliant for suggestions, primers, and the communication of p<sup>+</sup> sequence data prior to publication. We thank Stephanie Kong and Richard Brennan for comments on the manuscript. This work was supported by grants from the American Cancer Society (CN-142) and the National Institutes of Health (ES06593) to R.H.S. and a fellowship from the Islamic Development Bank to F.K.


