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Genetics

Spectra of spontaneous and mutagen-induced mutations in the *lacI* gene in transgenic mice

(germ cell/mutagenicity/*N*-ethyl-*N*-nitrosourea/benzo[*a*]pyrene/λ shuttle vector)

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ABSTRACT **Transgenic mice with a λ shuttle vector containing a *lacI* target gene were generated for use as a short-term, *in vivo* mutagenesis assay. The gene is recovered from the treated mice by exposing mouse genomic DNA to *in vitro* packaging extracts and plating the rescued phage on agar plates containing 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal). Phage with mutations in the *lacI* gene form blue plaques, whereas phage with a nonmutated *lacI* form colorless plaques. Spontaneous background mutant rates using this system range from 0.6×10^{-5} to 1.7×10^{-5}, depending upon tissue analyzed, with germ cells exhibiting less than one-third the background rate of somatic tissue. Treatment of the mice with *N*-ethyl-*N*-nitrosourea (EtNU), benzo[*a*]pyrene (B[*a*]P), or cyclophosphamide caused an induction of mutations over background. Recovery of the *lacI* target for sequence analysis was performed by genetic excision of a plasmid from the phage using partial filamentous phage origins. The predominant mutations identified from untreated and treated populations were base substitutions. Although it has been shown by others that 70% of all spontaneous mutations within the *lacI* gene, when replicated in *Escherichia coli*, occur at a hot spot located at bases 620–632, only 1 of 21 spontaneous mutations has been identified in this region in the transgenic mouse system. In addition, 5 of 9 spontaneous transitions analyzed occur at CpG dinucleotides, whereas no transition mutations were identified at the prokaryotic deamination hot spots occurring at *dcm* sites (CCA/TGG) within the *lacI* gene. For EtNU, approximately equal amounts of transitions and transversions were observed, contrasting with B[*a*]P-induced mutations, in which only transversions were obtained. In addition, B[*a*]P mutagenesis showed a predominance of mutations (81%) involving cytosines and/or guanines, consistent with its known mode of action. The discovery of a spontaneous mutation spectrum different from that of bacterial assays, coupled with the concordance of EtNU and B[*a*]P base mutations with the known mechanisms of activity for these mutagens, suggests that this transgenic system will be useful as a short-term, *in vivo* system for mutagen assessment and analysis of mechanisms leading to mutations.**

The *lacI* gene has been used extensively as a target for the identification and analysis of spontaneous and induced mutations, due in part to the ease of using a colorimetric assay to rapidly screen for mutations. Genetic and sequence analysis of spontaneous and induced mutations detected in several systems utilizing *lacI* (1–5) has resulted in an extensive accumulation of data regarding the sequence specificity of spontaneous and induced mutations in *lacI*, which can be of considerable comparative value through the use of the *lacI* target gene in whole animal assay systems.

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To combine the cost-saving aspects of short-term assays with the predictive power of whole animal assays, we previously described the development of a system that depends upon efficient recovery of a λ phage shuttle vector from transgenic mouse genomic DNA through the use of restriction-negative *Escherichia coli* strains (6, 7). This system has been used to observe dose-dependent induction of mutations over background levels using λ shuttle vectors containing a *lacZ* target gene (6, 8). We have extended our initial work on the *lacZ* λ mutagenesis system by incorporating a *lacI* target gene into a λ shuttle vector. The use of the *lacI* gene in this assay results in greatly simplified plaque detection (blue mutant plaques on a background of colorless nonmutant plaques) as well as allowing for higher phage plating densities (as much as 50,000 phage plaques per plate). In addition, the relatively small size of the target [1080 base pairs (bp) of coding region] facilitates sequence analysis and subsequent comparison to the large historical base of information available for *lacI* in several systems, allowing investigation of the underlying mechanisms leading to mutations.

Transgenic mice containing this λ vector were exposed to three well-studied and representative mutagens. *N*-Ethyl-*N*-nitrosourea (EtNU) has been shown to be a strong direct-acting alkylating agent of DNA in eukaryotic and prokaryotic systems (9, 10). Benzo[*a*]pyrene (B[*a*]P) is a member of an important class of environmental carcinogens, the polycyclic aromatic hydrocarbons, and has been shown to require metabolic activation resulting in the formation of a B[*a*]P diol-epoxide (BPDE) that covalently binds susceptible DNA targets (11). Cyclophosphamide (CP) is a bifunctional alkylating agent that is used as a chemotherapeutic. CP, which also requires metabolic activation, has been identified as a human carcinogen (12) and a potent teratogen (13). The two major cytotoxic metabolites of CP, acrolein and phosphoramide mustard, are alkylating agents of DNA and RNA (14).

Described here are the effects of mutagenic exposure of the transgenic animals monitored by phenotypic alterations of the target *lacI* gene as well as sequence analysis of selected clones resulting from spontaneous and induced mutation of the target gene. Mutations identified here are then correlated with spontaneous and induced mutation data derived from existing whole animal and *in vitro* assays.

MATERIALS AND METHODS

λ Shuttle Vector. The λ vector used in these studies, λ LIZα (*lacI*^{q}, *lacZ*α) (Fig. 1), contains the entire *lacI*^{q} gene (15), the amino-terminal 675 nucleotides of *lacZ* (*LacZ*α), an ampicillin-resistance gene, and a ColE1 origin of replication, all flanked by the initiator and terminator halves of the f1

Abbreviations: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; B[*a*]P, benzo[*a*]pyrene; BPDE, B[*a*]P diol-epoxide; EtNU, *N*-ethyl-*N*-nitrosourea; CP, cyclophosphamide.

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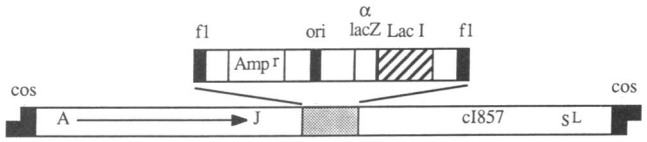

FIG. 1. The λ vector used to generate the transgenic mice, λ LIZα, is ≈42.7 kilobases (kb). The shaded region of the vector has been expanded to detail the plasmid that is generated by genetic excision using the partial filamentous phage origins flanking the region. ori, ColE1 origin of replication; Amp^r, ampicillin-resistance gene.

filamentous phage origin. The positioning of the halves of the f1 origin allows a plasmid containing the target gene to be excised from the surrounding λ arms by superinfection with helper phage (16), allowing rapid subcloning for sequence characterization of identified *lacI* mutants.

Purification of Genomic DNA from Tissues. Genomic DNA was prepared from somatic tissues as described (6, 17). Isolation of seminiferous tubules containing germ cells was done by decapsulating testes followed by collagenase digestion (type II, Sigma) to release the tubules. The tubules were washed two times in phosphate-buffered saline and then incubated in a lysis buffer (0.2 mg of proteinase K per ml/2% 2-mercaptoethanol/10 mM Tris·HCl, pH 8.0/100 mM NaCl/10 mM EDTA/0.5% SDS) for 3 hr at 50°C. Subsequent DNA isolation procedures were performed as described (6, 17). This purification results in a genomic DNA sample containing ≈95% germ-cell DNA (18).

Mutagenesis Assay. The entire assay procedure using the *lacZ* gene as the mutagenic target has been described in detail (6, 17). The shuttle vector was recovered from genomic DNA by mixing with restriction-negative, β-galactosidase-negative *in vitro* λ packaging extract (Transpack, SCS). The packaged phage were then preadsorbed to *E. coli* SCS-8 [*recA1, endA1, mcrA*, Δ(*mcrBC-hsdRMS-mrr*), Δ(*argF-lac*)*U169*, φ80*dlacZ*Δ*M15*, Tn*10* (tet^r)], mixed with top agar containing 2 mg of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal; SCS) per ml of top agar, and poured onto assay dishes containing nutrient agar. Point mutations, deletions (≤5 kb), and insertions (≤8 kb) are detectable in the *lacI* promoter, *lacI* target gene, and/or the *lacZ* operator, which serves as the binding site for the *lac* repressor molecule (Fig. 1; sizes for deletions and insertions are based upon λ phage packaging constraints). This is accomplished by α complementation with the *lacZ*Δ*M15* in SCS-8, resulting in a blue plaque (19). Plates were examined for the presence of blue mutant plaques on a background of nonmutant colorless plaques. The ratio of blue plaques to colorless plaques was taken as a measure of the mutagenicity of the compound. Density of plaques was limited to a maximum of 50,000 plaque-forming units per plate (25 cm × 25 cm) to ensure accuracy in detection of plaques with the mutant phenotype.

Calculation of Error Rates. Mutant frequencies are expressed with SEMs at 95% confidence. The SEM is calculated as follows: $2(SD \div \sqrt{n})$, where n equals the number of mice in a sample. Approximately equal numbers of plaques were analyzed from each mouse in a sample; therefore, the SEM reflects mouse-to-mouse variation in measured mutant frequencies.

Sequence Analysis of Mutants. *In vivo* excision of the plasmid containing the entire target gene region (including the *lacI* promoter and *lacZ* operator) from the λ phage was accomplished as described (16). Recovered plasmid DNA was prepared by a boiling miniprep procedure (20) followed by extraction with HF-silica resin (Strataclean, SCS) (21). Double-stranded dideoxy sequencing (22) was then performed. DNA sequence is numbered with base 1 being the site of mRNA initiation. All mutations are listed as changes in the coding strand.

RESULTS

Characterization of Transgenic Mice. Three transgenic C57BL/6 offspring were identified by slot blot analysis. Germ-line transmission was confirmed for two lines, and one of these, A1, was selected for further characterization. The copy number of the shuttle vector in the A1 lineage was estimated by slot blot analysis to be ≈30 copies, and Southern blot analysis of *Bam*HI-digested A1 genomic DNA indicated that the shuttle vectors integrated as a head-to-tail concatamer at a single locus without detectable rearrangement (data not shown). The A1 founder mouse was bred to a nontransgenic C57BL/6 mouse to establish Mendelian inheritance of the λ shuttle vector, and 6- to 8-week-old inbred F_1 offspring were used in subsequent experiments.

Spontaneous Mutation Rates. Three untreated mice and three treated with phosphate buffer at 10 ml/kg of body weight were used as controls to establish spontaneous background mutation rates for the A1 lineage (Table 1). Approximately 3.5×10^6 plaques were analyzed from genomic DNA prepared from purified male germ cells, yielding a mean spontaneous mutation rate of $0.6 \times 10^{-5} \pm 0.1 \times 10^{-5}$ (SEM). To compare this rate to that obtained from somatic tissue, ≈2.6×10^6 plaques were rescued from spleen genomic DNA isolated from the same mice. The mean spontaneous mutation rate from the spleen was $1.7 \times 10^{-5} \pm 0.6 \times 10^{-5}$ (SEM). Analysis of somatic tissues other than spleen also show

Table 1. Mutant frequencies (MF) from analysis of germ cells and spleen after indicated treatments

Plaques × 10^6	Mutants	MF × 10^{-5}*
Induction of mutations in germ cells		
Control		
3.50	20	0.57 ± 0.2
EtNU		
1.30	13	1.00 ± 0.4
(125 × 1)		
0.64	17	2.66 ± 0.6
(250 × 1)		
Induction of mutations in spleen		
Control		
2.65	46	1.74 ± 0.6
EtNU		
0.62	25	4.03 ± 1.0
(125 × 1)		
0.71	68	9.58 ± 4.0
(250 × 1)		
0.76	113	14.86 ± 4.0
(50 × 5)		
CP		
1.12	22	1.96 ± 0.4
(20 × 1)		
0.71	33	4.65 ± 1.2
(100 × 1)		
B[*a*]P		
0.67	87	12.98 ± 2.8
(500 × 1)		
0.55	118	21.46 ± 6.4
(100 × 5)		

For germ cells, five male mice were used as controls, and three male mice each were treated with the listed doses of EtNU. For spleen, six mice (five male and one female) were used as controls, and three mice each were treated with the listed doses of EtNU, CP, and B[*a*]P. In all cases, mice were sacrificed 3 days following dosing.
*With SEM to 95% confidence.

significantly higher background rates than that observed for germ cells (data not shown).

Mutagenicity Testing. *EtNU.* The effects of EtNU were analyzed in spleen and germ-cell genomic DNA (Table 1). EtNU was administered to three mice each at single doses of 125 and 250 mg/kg of body weight. Analysis of male germ cells showed dose-dependent inductions of ≈2-fold and ≈5-fold, respectively, for each dose of EtNU. In addition to the above dosing, three mice were treated with five daily doses of 50 mg of EtNU per kg of body weight and analyzed for mutations in the spleen. An approximate 2-fold, 5-fold, and 8-fold induction, respectively, was seen for each dose level tested.

CP. CP was administered to three mice at 20 mg/kg of body weight and to three mice at 100 mg/kg of body weight. Spleen genomic DNA was isolated from the treated animals to examine mutagenic effects in this tissue. No induction over background was seen for the low dose of CP, whereas a 2.4-fold induction was observed for the 100 mg/kg dose (Table 1).

B[a]P. Mutagenesis in B[*a*]P-treated mice was studied in spleen tissue, with three mice being administered a single dose of 500 mg B[*a*]P/kg body weight, and three mice receiving five single daily doses of 100 mg/kg body weight (Table 1). Inductions of approximately 7-fold and 11-fold over background, respectively, were seen for this compound.

Sequence Analysis of *lacI* Mutants. *Spontaneous mutants.* Twenty-one spontaneous mutants were selected from clones rescued from several tissues (ovary, testis, spleen, lung, brain, and liver) for sequence analysis. The types of mutations identified from analysis of these mutants are listed in Table 2 (summarized in Table 5). Base substitutions throughout the gene comprise a majority (16/21 or 76%) of the spontaneous mutations. Of these base substitutions, transitions make up 9/16 or 56%, whereas transversions comprise 44%. In addition, we have identified a single 4-base insertion (1/21 or ≈5%) at base 622, the site of a repeated tetramer of the sequence 5′-TGGC-3′. This finding differs markedly from studies in *E. coli* systems that have shown that this site accounts for up to 70% of all spontaneous mutations (2, 23).

Deletions account for the remainder of mutations identified, 4/21 or 19% of the spontaneous mutations analyzed, compared to *lacI* genes in *E. coli*, where deletions comprised the largest non-hot spot mutation class (37%) (2). In addition, five of nine spontaneous transitions identified (56%) are G·C–A·T transitions that may have resulted from spontaneous deamination of methylated cytosines at CpG dinucleotides. However, none of these transitions occurred at the previously identified hot spots for spontaneous deamination in *lacI* (24).

Induced mutants. Mutant phage resulting from EtNU or B[*a*]P treatment were selected from male germ cells and spleen tissue for sequence analysis. To minimize the possibility of these mutants arising from spontaneous, and not induced, mutations, mutant phage were selected only from the doses of mutagen that resulted in the largest inductions over background levels.

(*i*) EtNU. Twenty-four mutants arising from analysis of germ and spleen tissue (15 from germ cells, 9 from spleen) were selected for sequence analysis [Table 3 (summarized in Table 5)]. The predominant class of mutations identified were base substitutions (21/24 or 88%), whereas the remaining 3 mutations identified were deletions (12%). Seven of the 21 base substitutions (33%) were G·C–A·T transitions, the pre-

Table 2. Sequence analysis of spontaneous mutants derived from several tissues of the A1 mice, separated by mutation class

Clone	Tissue	Base no.	Mutation	Amino acid change
Transversion				
F11	Testis	49	G·C–T·A	Tyr-stop
F18	Testis	202	G·C–T·A	Ala-Glu
AA6	Ovary	702	G·C–T·A	Gly-Val
BB12	Ovary	857	G·C–T·A	Glu-stop
D119	Spleen	582	T·A–G·C	Leu-stop
D109	Spleen	194	G·C–C·G	Leu-Val
V3	Spleen	794	G·C–C·G	Ala-Pro
Transition				
133	Testis	92	G·C–A·T	Arg-Cys
AA2*	Ovary	93 to 94	G·C–A·T	Arg-His
CC4	Lung	93	G·C–A·T	Arg-His
CC15	Lung	185	G·C–A·T	Ala-Thr
D112	Spleen	329	G·C–A·T	Arg-stop
CC6	Brain	329	G·C–A·T	Arg-stop
I35	Testis	882	G·C–A·T	Pro-Leu
BB19	Liver	83	A·T–G·C	Thr-Arg
CC19	Brain	168	A·T–G·C	Tyr-Cys
Insertion				
C62	Spleen	621	(+4) TGGC	Frame shift
Deletion				
F12	Testis	362 to 363	(−2) CA	Frame shift
D117	Spleen	541–562	(−22)	Frame shift
F15	Testis	578 to 579	(−2) CT	Frame shift
CC13	Liver	811–818	(−8)	Frame shift

Mutants were selected at random from each of the control mice. Deletions are listed as number of bases deleted and position of deletion.
*Clone AA2 contains a mutation of G → A and C → T.

Table 3. Mutations identified as a result of EtNU treatment and analysis of male germ cells and spleen

Clone	Base no.	Mutation	Amino acid change
		Germ cells	
Transversion			
A8	928	G·C–T·A	Ser-Arg
A11	633	T·A–G·C	His-Pro
B14	64	T·A–A·T	Tyr-stop
A9	79	T·A–A·T	Tyr-stop
A3	406	T·A–A·T	Tyr-stop
A1	894	T·A–A·T	Ile-Asn
B16	−19	G·C–C·G	Promoter
A4	928	G·C–C·G	Ser-Thr
Transition			
B2	42	G·C–A·T	Thr-Met
A10	329	G·C–A·T	Arg-stop
B6	329	G·C–A·T	Arg-stop
B1	377	G·C–A·T	Gln-stop
B17	83	A·T–G·C	Thr-Ala
Deletion			
A12	188–190	(−3) CAA	Deletion of Gln
A2	206 to 207	(−2) CA	Frame shift
		Spleen	
Transversion			
D28	92	G·C–T·A	Arg-Ser
C13	221	G·C–T·A	Gly-Cys
C40	786	G·C–T·A	Ala-Glu
C8	219	T·A–A·T	Ile-Asn
C38	463	T·A–G·C	Leu-stop
Transition			
D35	57	G·C–A·T	Ala-Val
D12	92	G·C–A·T	Arg-Ser
D47	329	G·C–A·T	Arg-stop
Deletion			
C7	570	(−1) A	Frame shift

Mutants were selected from highest doses of mutagen in male mice for germ cells and in male and female mice for spleen. Deletions are listed as number of bases deleted and position of deletion.

dominant (75%) type of base substitution reported in the *lacI* gene in *E. coli* after EtNU treatment (25, 26). Our analysis of germ-cell mutations also identified 7 of 13 base substitutions (54%) as occurring at G or C residues. In comparison to the small amount of existing germ-cell sequence data for EtNU, six specific locus mutations have been characterized, and each of them has involved A or T residues (27–29).

(*ii*) B[*a*]P. Eleven mutants were selected from analysis of spleen tissue [Table 4 (summarized in Table 5)]. The most prevalent type of mutations (7/11 or 64%) in this case were also base substitutions, whereas 4 deletion mutants were identified (36%). Of the base substitutions, all were transversions (7 of 7), consistent with previous data for the sequence specificity of B[*a*]P mutations in *lacI*, where transversions were predominantly found (30). In addition, 9 of 11 mutations (82%) involved guanines or cytosines. This is in agreement with the mechanism of B[*a*]P mutagenesis, where the active metabolite of B[*a*]P, BPDE, is known to bind principally to the exocyclic amino group of guanine (31).

DISCUSSION

We have used a λ/*lacI* mutagenesis system in transgenic mice to identify and characterize spontaneous and induced mutations resulting from mouse exposure to EtNU, B[*a*]P, and CP. The system allows for easy characterization of mutation events colorimetrically and for generation of rapid sequence data to correlate with existing data on the well-studied *lacI* gene.

Spontaneous background mutant rates in the germ line showed an approximate 3-fold lower rate than obtained with somatic tissue (spleen). Such a difference in background rates was identified by us previously (6), although in that study, whole testis had been analyzed, not purified germ cells. Although the observation of a lower mutation rate in germ cells is teleologically satisfying for the preservation of heritable genetic integrity, the reason for this difference is not known. Variation in the number of cell divisions required in the germ line and spleen lineage to produce a mature cell, as well as the relative amounts of each precursor cell type present at a given time, may contribute to this difference. Differences in the types of and capacities of repair pathways could also account for the variation between germ-cell and spleen background rates. The presence of a mutagen barrier in the testis, possibly the Sertoli cell layer of the seminiferous tubules (32), may provide a protection against the effects of weak mutagens in food or drinking water. Whatever the mechanism or combination of mechanisms, it remains to be determined whether these differences will result in altered mutational spectra between germ cells and somatic cells for a given compound. More extensive sequence analysis should provide insight into this issue.

Table 4. Mutations identified from B[*a*]P treatment and analysis of spleen

Clone	Base no.	Mutation	Amino acid change
Transversion			
C105	86	G·C–T·A	Val-Phe
C85	528	G·C–T·A	Thr-Lys
C152	782	G·C–T·A	Gly-Val
C86	167	T·A–A·T	Tyr-Asn
C103	66	C·G–G·C	Ala-Gly
C88	92	C·G–G·C	Arg-Gly
C150	928	C·G–G·C	Ser-Arg
Deletion			
C104	93 to 94	(−2) GC	Frame shift
C81	270	(−1) C	Frame shift
C151	883	(−1) G	Frame shift
C87	968	(−1) A	Frame shift

Mutants were selected from highest doses of mutagen in male and female mice. Deletions are listed as number of bases deleted and position of deletion. No transitions were observed in this analysis.

Table 5. Summary of mutational spectra

	Spontaneous		EtNU		B[*a*]P	
Mutation class	No.	%	No.	%	No.	%
Base substitution						
Transversion	7	44	13	62	7	100
Transition	9	56	8	38	0	0
Frame shift						
Insertion	1	20	0	0	0	0
Deletion	4	80	3	100	4	100

The data presented in Tables 2–4 are summarized, indicating relative frequencies of each type of mutation as a function of treatment.

Spontaneous background mutant rates obtained with the *lacI* system (spleen 1.7×10^{-5}; germ cells, 5.7×10^{-6}) are similar to what we obtained previously when using the *lacZ* gene as a target (spleen, 1.3×10^{-5}; testis, 8.1×10^{-6}) (6), despite the facts that the *lacI* target is approximately one-third the size of *lacZ* and a different transgenic line was used. This may be due in part to the increased sensitivity of *lacI* to mutation detection as well as the known tolerance of *lacZ* for base substitutions in the amino terminus. In addition, administration of EtNU to mice using the *lacI* system resulted in substantially similar induction rates (6).

Induction of mutations due to EtNU treatment in these experiments is significantly lower than specific locus data for EtNU reported by Hitotsumachi *et al.* (33). However, in experiments described here, animals were sacrificed only 3 days following completion of dosing, whereas in the specific locus experiments the animals were maintained for a period of 12–14 weeks following completion of treatment before mating to identify specific locus mutations. Recent experiments using similar alkylating agents in the *lacI* transgenic mouse system indicate that increased waiting time following dosing results in significantly higher induction rates than presented here for EtNU (G.S.P., S.W.K., and J.M.S., unpublished data). This time difference may allow for increased cell proliferation and DNA replication to fix adducts as mutations. Experiments are necessary to analyze EtNU mutagenesis after a prolonged waiting period following dose completion for a more relevant comparison.

B[*a*]P administration resulted in the most significant induction levels obtained in this study (11-fold). This increased response is in accordance with the strong effects observed in *in vitro* assays using BPDE, the mutagenic B[*a*]P metabolite (34). Although mutant induction in the spleen of CP-treated animals was lower relative to the other substances tested, it may be advantageous to examine tissues other than spleen that may be more greatly affected by CP.

Sequence analysis of spontaneous mutations revealed substantially different classes of mutations from that observed in other systems using *lacI*. Although we detected only a single spontaneous insertion at the repeated tetramer site in *lacI* (bases 620–632), this site accounts for up to 70% of all spontaneous mutations within the *lacI* gene studied in *E. coli* (2, 23). This result suggests that the mutations identified by the λ shuttle system in combination with SOS-deficient *E. coli* strains occurred in the animal.

Our analysis of EtNU mutations induced in germ cells and spleen identified G·C–A·T transitions that have been shown to be the most prevalent EtNU-induced mutations in other assays using *lacI* in *E. coli*; however, they comprised only 33% of total mutations observed for EtNU treatment in this eukaryotic system. Nevertheless, our results correlate well with EtNU-induced base substitutions detected in plasmid

shuttle vectors in Epstein–Barr virus-transformed human lymphoblastoid cells (35), suggesting a fundamental difference in mutagenesis for EtNU between prokaryotes and eukaryotes. In contrast, sequence analysis of B[*a*]P-induced mutations showed significant agreement with what has been shown previously for the metabolite BPDE, in studies on *lacI*, indicating the mechanism for BPDE action in whole animals may be similar to that in prokaryotes.

Additional sequence data are required to identify possible differences in mutation spectra as a function of compound as well as tissue type. This type of analysis may lead to a mechanistic understanding of the mutagenic action of a compound in whole animals. Future use of the *lacI* target in a transgenic mouse system will build on the valuable existing *in vitro* data base, with the added advantage of allowing correlation of the *in vivo* effects on mutation spectra. This system should also predict detailed analysis of tissue-specific mutational analysis, including rapid assessment of germ-line risk.

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