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Genetics

Comparison of somatic mutation in a transgenic versus host locus

(*lacI*/*Dlb-1*/small intestine/x-rays/ethyl nitrosourea)

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ABSTRACT **Somatic mutations can now be quantified in almost any cell type in mice carrying bacterial genes in a λ phage shuttle vector. Mutations induced *in vivo* are detectable *ex vivo*, after packaging host-cell DNA into phage that are grown on suitable bacteria. However, the transgenic DNA differs from many host loci in several ways: it (*i*) is prokaryotic DNA, (*ii*) is present in multiple tandem copies, and (*iii*) is heavily methylated and probably not expressed. Thus, mutation of a transgene may not be a suitable model of the host loci, which are eukaryotic, unique, and expressed. To test the relevance of the transgene mutation model, the frequencies of the bacterial *lacI*$^+$ to *lacI*$^-$ mutations induced in half of the small intestine were compared with the frequencies of the host *Dlb-1*^{b} to *Dlb-1*^{a} mutations induced in the other half. The loci responded similarly to ethyl nitrosourea (ENU) with respect to the animal's age and sex, sex of the parent transmitting the transgene, and expression time. ENU dose–response curves were similar. Furthermore, no difference was found at the *Dlb-1* locus between transgenic and nontransgenic siblings. In contrast, x-rays induced few *lacI* mutations but many *Dlb-1* mutations. Probably few large deletions are detectable at *lacI*, but many are detectable at *Dlb-1*. If so, an important class of mutation is not readily detected in these transgenic mice. With this exception, the transgene and host gene responded similarly in this somewhat limited trial, as is necessary if the transgenic mice are to be a useful model.**

Somatic mutation plays a critical role in formation of many cancers, but is not readily quantified in most somatic tissues. Recently, however, mouse strains containing bacterial transgenes have made it possible to detect mutation in any tissue or cell type (1–3). Although dose-dependent increases in mutation frequency have been detected in several tissues (1–4), there are several reasons to be concerned that a transgenic locus and a host locus may not respond alike. First, the location of the transgene can affect transgene expression (5) and mutational response. One strain of *lacZ* transgenic mice in which *lacZ* was located on the X chromosome produced higher mutation frequencies than other *lacZ* strains (6). Second, introduction of foreign genes into the mammalian genome may inactivate host genes (7–9) or result in DNA rearrangements at the insertional sites (10). The phenotypes of insertional mutations vary and may include recessive prenatal lethality, transmission distortion, and developmental abnormality (10). The construction of the transgenic mice may have altered host-cell DNA repair mechanisms, or other protective mechanisms, by inactivating one of the loci involved. This, in turn, could affect the mutational response. There is one report of a mutational "hot spot" in one strain of the *lacZ* transgenic mice in which 10% of mice contained deletions and/or duplications (6). Third, the unusual nature of the *lacI* transgene may affect DNA repair and thus the mutation frequency or even the mutation spectrum at this locus compared with host loci. Transgenes, for example, may be heavily methylated (11), leading to transcriptional inactivity (12, 13). Also it is known that repair of UV damage in mammalian cells can differ between the transcribed and untranscribed regions of the DNA (14), even between the sense and antisense strands, so foreign prokaryotic DNA might be repaired differently, especially if not expressed (15). Indeed, the chromatin architecture of the transgenic region may differ from normal host chromatin. Finally, the *lacI* transgene is in about 40 tandem copies, each about 45 kilobases, arranged as a head-to-tail concatamer at a single locus of mouse genome (2, 3, 16). Such a structure may provide unusual opportunities for recombination repair. It is conceivable, therefore, that the bacterial transgene is differentially repaired and that a different mutational response results. For these reasons it is essential to know whether or not mutation of the transgene parallels mutation of host loci. If not, the transgenic mice would be of dubious value as an assay for somatic mutation.

Somatic mutation of a host gene can be readily quantified in the epithelial cells of the small intestine by an assay that detects the loss of the binding site for the lectin *Dolichos biflorus* on the surface of cells in the villi (17). The presence of the antigen in the small intestine is determined by an autosomal dominant allele, *Dlb-1*^{b}. Mice homozygous for the *Dlb-1*^{a} allele do not express the antigen on the villi, although they do express it elsewhere (18). In *Dlb-1*^{a}/*Dlb-1*^{b} mice, mutation of the *Dlb-1*^{b} allele in a stem cell results in a ribbon of nonstaining cells on the villus. Because the epithelium is completely renewed every 4 or 5 days, the mutant ribbons are evident within 1 week. These ribbons are easily enumerated in whole mounts stained with a *Dolichos biflorus* agglutinin (DBA)–peroxidase conjugate. The locus has been well characterized as a mutational target (17, 19–23). Although the *Dlb-1* locus is known to be on chromosome 11 (24), the gene has not been cloned so that the mutation spectrum at this locus cannot be determined at the sequence level.

In these experiments mutations of the *lacI* transgene and the *Dlb-1* host locus were quantified in the small intestine of the same animals, both untreated and mutagenized. The mice used were the F_1 generation obtained from crossing the transgenic mice and SWR mice. The F_1 mice were heterozygous at the *Dlb-1* locus and hemizygous for the transgene. The main experimental variables were mutagen, time after exposure to the mutagen, sex of the animal, sex of the transgenic parents, age of the animal, and region of the small intestine.

MATERIALS AND METHODS

Animals. The hemizygous *lacI* C57BL/6 transgenic mice were obtained from Stratagene. These mice are *Dlb-1*^{b} homozygotes. SWR (*Dlb-1*^{a}/*Dlb-1*^{a}) and C57BL/6J (*Dlb-1*^{b}/*Dlb-1*^{b}) mice were obtained from The Jackson Laboratory.

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Abbreviation: ENU, ethyl nitrosourea.

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All mice were housed in standard plastic cages with wire lids and wood-chip bedding. A 12/12-hr light/dark cycle was used. The room was maintained at 22 ± 2°C and 70% humidity. Water and mouse chow [PMI Feeds (St. Louis), Laboratory rodent diet no. 5001] were supplied ad libitum. All housing and treatment protocols were reviewed and approved by an independent Animal Care Committee. After a week-long acclimatization period, the two strains were mated. The F_1 mice were tested for the presence of the *lacI* gene with slot blots by Stratagene. Because we could not obtain sufficient animals of the same age at one time, we stratified animals by age into five groups, 6–7, 9–10, 12–14, 20–22, and 25–26 weeks old. The test groups were structured to contain similar contributions from each stratum, and the treatments were assigned at random to the groups thus constructed. Only female mice were used for time-course experiments, and only male mice were used for dose–response experiments. There were three animals per group in the time-course and ethyl nitrosourea (ENU) dose–response experiments and five animals per group in x-ray dose–response experiments. The ENU dose–response curve for the parental *lacI* C57BL/6 mice had three males and three females per group. Mice were sacrificed by cervical dislocation 1–8 weeks after the completion of treatment.

Mutagens. ENU was obtained from Sigma. Test solutions were freshly made and injected intraperitoneally (i.p.). The x-rays used were generated from a Phillips x-ray machine (Phillips Electronic Instruments, Toronto) at 160 kV and 18.6 mA with 0.5-mm aluminum filtration at 1.85 Gy/min, calibrated with a Victoreen R-meter.

***lacI* Transgenic Mouse Assay.** Genomic DNA was extracted from small intestinal epithelial cell suspensions. After sacrifice of the mice, the whole length of the small intestine was carefully removed from the mesentery and divided into two parts, proximal and distal. The proximal section was flushed with Douncing buffer (pH 8.0) and then inverted. To separate the cells from the muscle layer, the inverted intestine was placed in 3 ml of Douncing buffer with DNase-free RNase A (100 μg/ml; Sigma) and forced in and out of a 5-ml needle-less tuberculin syringe about 10 times. Genomic DNA was purified by treating the cell suspension with proteinase K solution (2 mg/ml; Sigma), followed by phenol chloroform extraction and ethanol precipitation as described by Kohler *et al.* (2, 3). The λ phage shuttle vector, which contains the entire *lacI* mutational target gene and the amino-terminal 675 nucleotides of the *lacZ* reporter gene (*lacZα*; refs. 2 and 3), was recovered from the isolated mouse DNA by *in vitro* packaging with packaging extracts (Transpack; Stratagene). The *lacI* mutations were tested on *Escherichia coli* (SCS-8; Stratagene) which contain the remaining carboxyl-terminal portion of the *lacZ* gene. Functional β-galactosidase is produced by intraallelic complementation of the phage-packaged transgene (*lacZα*) with the remaining portion of the *lacZ*. The infected bacteria were grown on NYZ agar containing 70 mg of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal, Stratagene) per assay plate. The X-Gal is cleaved by β-galactosidase to produce a blue substance. Thus, plaques in which a mutational event rendered the *lacI* repressor gene inoperative are easily detected as blue plaques on a background of clear nonmutant plaques. The number of plaques analyzed from each animal varied substantially. In most cases, more than 30,000 plaques were analyzed from each animal (average about 51,000 plaques per animal). Sixty-two mutant plaques were replated to confirm the mutant phenotype; 97% of these mutant plaques yielded blue mutant plaques on replating.

***Dlb-1* Locus Assay.** Whole mounts of the small intestine were prepared as described (17, 21) with a few modifications. Briefly, after careful removal of the small intestine from the mesentery, the distal section was carefully flushed with cold phosphate-buffered saline (PBS, pH 7.4) to get rid of the food particles. Subsequently, the small intestine was flushed with 10% formal saline by using a syringe with a blunt needle. Then one end was sealed between two microscope slides clipped by binder clips, and the intestine was inflated by injecting 10% formal saline and fixed for at least 3 min. The small intestine was cut along the mesenteric side, placed on a microscope slide, stretched, and held at both ends by plastic coated paper clips under which a small piece of coverslip was placed. The slides were then put in 10% formal saline to fix for 1 hr, rinsed with PBS, and incubated for 45 min in 20 mM DL-dithiothreitol (Sigma) dissolved in 20% ethanol/80% 150 mM Tris (pH 8.2). Mucus was removed by pipetting the solution over the intestinal tissue.

Before being stained, the slides were rinsed three times with PBS and incubated in 0.1% phenylhydrazine hydrochloride (Sigma) in PBS for 30 min to block endogenous peroxidases. After three washes with PBS and a 10-min incubation in PBS containing 0.5% albumin (fraction V, Boehringer), the slides were stained with the *D. biflorus* agglutinin–peroxidase conjugate (Sigma) at 5 μg/ml in the PBS/albumin (fraction V). The peroxidase was developed by using 3,3′-diaminobenzidine (Sigma) solution for 20 min. The slides were rinsed twice with PBS and then put in a 100-mm^2 plastic dish containing PBS (to prevent drying of the tissue) for cell counting.

The slides were coded and scored with a dissecting microscope at a magnification of ×50. Scoring criteria have been established (17, 19, 20). The $Dlb\text{-}1^a/Dlb\text{-}1^b$ epithelial cells stained dark brown; mutant cells (which had no lectin-binding sites) appeared as unstained vertical stripes on the villi. Fifty fields defined by the square field in an eyepiece graticule were scored, each containing about 200–250 villi, to yield about 10^4 villi per animal. The total number of villi was estimated from the mean of duplicate counts of the number of villi in the first and last fields.

Statistics. Before analysis, the *Dlb-1* data were transformed logarithmically to equalize the variances. It was not necessary to transform spontaneous data and the *lacI* data. The mutant frequencies in transgenic and nontransgenic mice as a function of time were compared with regression analysis based on two-way ANOVA with unequal cell numbers (25) because one mouse died after ENU treatment and one after x-ray treatment. The same method was used for the analysis of imprinting. The dose–response and spontaneous data were tested for linearity. The lines did not differ from linearity, so they were compared with a single regression equation (25). The same method was used to compare the response of the two sexes with one another, to compare different sections of the intestine, and to compare the parental and F_1 *lacI*. The degrees of freedom reflect the occasional loss of a sample from a treatment group during the experiment. When other statistical methods were used, this information is given with the results.

RESULTS

***lacI* Transgene Assay.** In preliminary experiments (data not shown), the frequency of ENU-induced *lacI* mutation was virtually identical in the two sexes ($F = 0.48$; df = 2, 18; $P = 0.63$). The mutant frequencies in the proximal and the distal halves of the small intestine of parental transgenic mice were similar ($F = 0.32$; df = 2, 38; $P = 0.73$). Furthermore, the response to ENU of the F_1 mice was not significantly different from that of the parents ($F = 1.08$; df = 2, 30; $P = 0.35$; Fig. 1). No difference was found in the spontaneous or induced frequencies (ENU or x-rays) of mutations in mice inheriting the transgene from the male or the female parent ($F = 0.006$; df = 1, 44; $P = 0.99$; data not shown). Although the mutant frequencies were very significantly different among

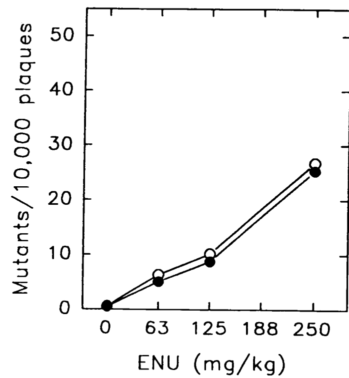

FIG. 1. Comparison of mutant frequency in transgenic parental mice (○) and transgenic F_1 offspring (●). The SEM are too small to be seen.

the treatments (control, x-rays, and ENU: $F = 174$; df = 2, 45; $P \ll 0.0001$), there was no statistical interaction between the treatment and inheritance of the *lacI* transgene ($F = 0.11$; df = 2, 42; $P = 0.9$).

***Dlb-1* Locus Assay.** As for the *lacI* locus, neither sex of the animals nor region in the small intestine significantly affected the spontaneous or the induced mutational response in the nontransgenic F_1 mice (ENU or x-rays; Fig. 2) as shown by multiway ANOVA. With respect to sex, the statistics are $F = 0.18$; df = 1, 72; $P = 0.67$. With respect to sections (proximal, middle, and distal), the statistics are $F = 1.41$; df = 2, 72; $P = 0.25$. Of course, the groups did differ significantly with respect to treatment ($F = 343$; df = 2, 72; $P \ll 0.0001$). No interactions were found between sex and treatment ($F = 1.43$; df = 2, 72; $P = 0.25$); section and treatment ($F = 0.4$; df = 4, 72; $P = 0.81$); and among sex, section, and treatment ($F = 0.51$; df = 4, 72; $P = 0.73$). Histologically, the middle and distal sections of the small intestine are better suited for the *Dlb-1* locus assay. Since the various parts respond similarly, the distal section of the small intestine was therefore chosen for the *Dlb-1* locus assay, while the proximal section was used for the *lacI* transgene assay.

Comparison of the Transgene and the Host Gene With Respect to Spontaneous Mutation. Spontaneous mutation frequencies (Fig. 3) in the *lacI* transgene did not increase significantly with age ($F = 1.18$; df = 1, 23; $P = 0.29$) although the regression has an upward slope. At the *Dlb-1* locus, spontaneous mutation frequencies increased slowly but significantly with age ($F = 16.8$; df = 1, 43; $P << 0.0001$). The difference in the relative frequency between the loci is about 6-fold. It is probable that the large variation at the *lacI* locus obscured a real increase with age. In the nontransgenic F_1 mice, the mutant frequencies at the *Dlb-1* locus were lower

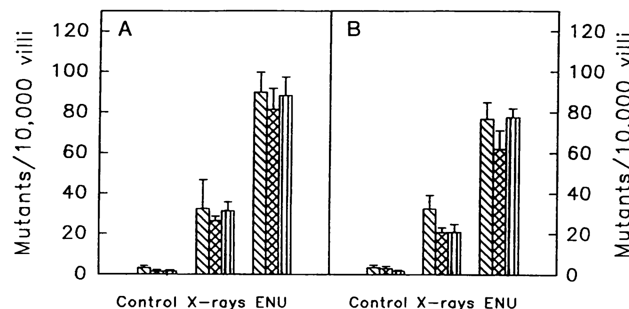

FIG. 2. Mean mutant frequencies (± SEM) at the *Dlb-1* locus in different sections of the small intestine of five nontransgenic animals 2 weeks after treatment with 6 Gy of x-rays or 100 mg of ENU per kg of body weight. (*A*) Female animals. (*B*) Male animals. ▧, Proximal; ▩, middle; ▥, distal.

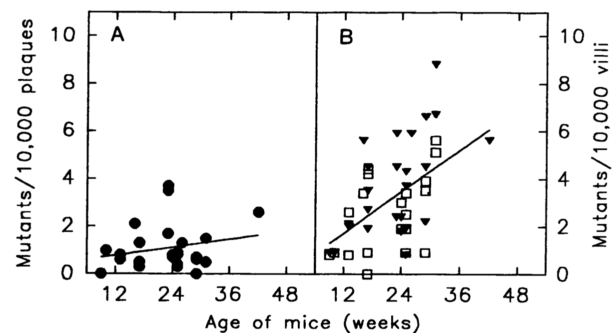

FIG. 3. Spontaneous mutations in the *lacI* transgene (*A*) and the *Dlb-1* host gene (*B*) in the mice of different ages. ▼, Transgenic mice; □, nontransgenic siblings.

but did not differ significantly from those in the transgenic mice ($F = 2.11$; df = 2, 41; $P = 0.13$). In the remaining figures, the scales for the two assays differ by a factor of 10. In most cases, about 10 times as many mutants were observed at the *Dlb-1* locus than at *lacI*, which probably reflects the number of mutable loci (see *Discussion*). The small number of phage analyzed for the *lacI* gene is probably a major factor in the variability that was observed.

Comparison of the Transgene and the Host Gene With Respect to ENU-Induced Mutation. The mutant frequencies as a function of time after treatment with a single dose of ENU are shown in Fig. 4. The mutant frequencies did not differ significantly with time after treatment at either the *lacI* locus ($F = 1.15$; df = 5, 11; $P = 0.39$) or the *Dlb-1* locus ($F = 2.05$; df = 5, 29; $P = 0.1$). The response of the transgenic and nontransgenic F_1 mice at the *Dlb-1* locus did not differ significantly ($F = 2.21$; df = 1, 28; $P = 0.15$). The dose–response curves obtained 2 weeks after treatment are shown in Fig. 5. The mutant frequencies were dose dependent at the two loci (*lacI*: $F = 232.8$; df = 1, 10; $P << 0.0001$ and *Dlb-1*: $F = 90.3$; df = 1, 22; $P \ll 0.0001$). The mutational response of the transgenic and nontransgenic animals at the *Dlb-1* locus again did not differ significantly ($F = 0.22$; df = 2, 20; $P = 0.8$).

Comparison of the Transgene and the Host Gene With Respect to X-Ray-Induced Mutation. The same comparisons have been conducted with x-rays. In this case, mutational response of the *lacI* transgene was different from that of the *Dlb-1* host gene in the time-course experiment. As shown in Fig. 6, the mutational response did not change with time at either locus (*lacI*: $F = 0.98$; df = 5, 11; $P = 0.47$ and *Dlb-1*: $F = 1.09$; df = 5, 29; $P = 0.39$) but the *lacI* mutant frequency was in the control range. Indeed, the results were not significantly different from the concurrent untreated controls ($F = 2.85$; df = 1, 16; $P = 0.11$). At the *Dlb-1* locus there was substantial variation, and the week 8 group had a low mutant

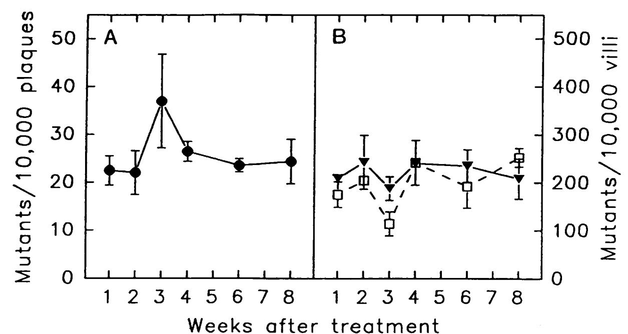

FIG. 4. Mean mutations (± SEM) induced by ENU (250 mg/kg) in the *lacI* transgene (*A*) and the *Dlb-1* host gene (*B*) at different times after treatment. ▼, Transgenic mice; □, nontransgenic siblings.

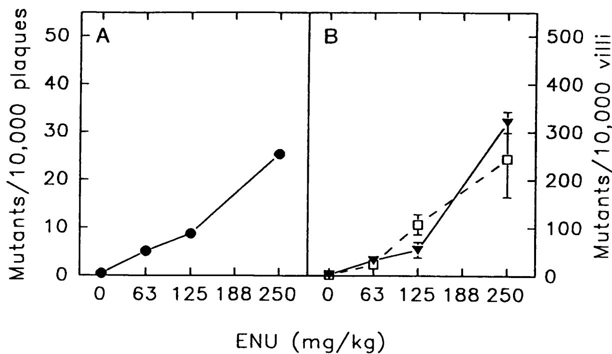

FIG. 5. Mean mutations (± SEM) induced by ENU in the *lacI* transgene (*A*) and the *Dlb-1* host gene (*B*). ▼, Transgenic mice; □, nontransgenic siblings.

frequency; this was the result of one animal with a low mutant frequency. A similar odd result was found at week 7 in the nontransgenic mice, which were otherwise similar to the transgenic mice in response to x-rays ($F = 0.02$; df = 1, 28; $P = 0.89$) but did not show this decline at week 8 (Fig. 6).

The dose–response curves, conducted in the other sex (males) gave a somewhat different result. Although the mutant frequencies increased only slightly with dose at the *lacI* locus, the results were very significant ($F = 13.7$; df = 1, 17; $P = 0.002$). Again, however, the *Dlb-1* locus was far more responsive ($F = 54.6$; df = 1, 18; $P \ll 0.0001$) (Fig. 7). It is noteworthy that in the time–response experiment, the dose used was 6 Gy, the highest dose used in the dose–response experiment, and more animals were used. It is likely that x-rays do mutate the locus at a low rate and that larger samples would establish this unequivocally. The dose–response at the *Dlb-1* locus of nontransgenic mice was lower than that of transgenic mice (Fig. 7). The two regression lines were parallel ($F = 1.9$; df = 1, 36; $P = 0.18$), but intercepts were unequal ($F = 5.03$; df = 1, 36; $P = 0.03$). Thus, the two regression lines were not coincident ($F = 3.46$; df = 2, 36; $P = 0.04$). This difference was not observed in the time-course experiment, which involved many more mice, so we do not attribute any biological meaning to it.

Since there were no differences in mutant frequencies in expression-time experiments, the data could be pooled, and the mutagenic sensitivities to ENU and x-rays at different ages of animals could be tested at both loci. The results showed no significantly different response to ENU at either locus with respect to age (*lacI*: $F = 3.31$; df = 2, 17; $P = 0.06$ and *Dlb-1*: $F = 1.53$; df = 2, 38; $P = 0.23$). Similar results were obtained in animals treated with x-rays (*lacI*: $F = 0.44$; df = 2, 18; $P = 0.65$ and *Dlb-1*: $F = 0.93$; df = 2, 42; $P = 0.4$). The influence of plaque densities on mutant frequency could also be tested by pooling the data from all animals treated

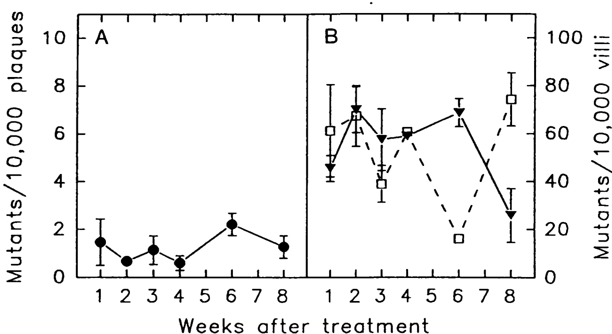

FIG. 6. Mean mutations (± SEM) induced by x-rays (6 Gy) in the *lacI* transgene (*A*) and the *Dlb-1* host gene (*B*) at different times after treatment. ▼, Transgenic mice; □, nontransgenic siblings.

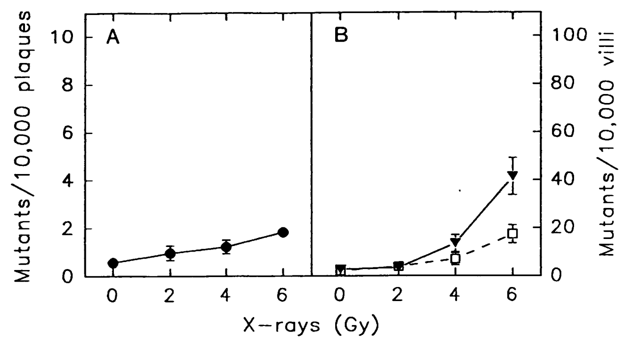

FIG. 7. Mean mutations (± SEM) induced by x-rays in the *lacI* transgene (*A*) and the *Dlb-1* host gene (*B*). ▼, Transgenic mice; □, nontransgenic siblings.

with 250 mg of ENU/kg of body weight. The result showed a very good correlation between the number of mutants and the number of plaques ($r = 0.85$, df = 79, $P < 0.01$). Plaque density *per se* is, thus, unimportant within the range tested, and low or high plaque densities can be used without influencing the mutant frequency (Fig. 8).

DISCUSSION

Before these experiments were initiated, there were many reasons for wondering if a transgene would respond like a host gene (see Introduction). We have been surprised by the similarity in the mutational response of the *lacI* transgene and the *Dlb-1* locus. Although the number of mutants observed at the two loci differs by a factor of 10, the actual mutation frequencies are very similar in most cases. Each plaque represents one mutable locus, but each villus is supplied by ≈10 stem cells and so represents about 10 mutable loci. This number can be derived from knowledge of the epithelial stem cells (26–29) but also is evident from the width of the mutant ribbons, which is about 1/10th of a villus on average (although there is considerable variability). The data presented for the two loci can be compared directly in the figures, since the *Dlb-1* data are plotted on a 10-fold larger scale (excepting only Fig. 3). The spontaneous mutant frequencies, although they vary with age, are different by about a factor of 6 (2.8×10^{-6} mutants per plaque per animal per wk in the *lacI* gene; 16×10^{-6} mutants per villus per animal per wk in the *Dlb-1* host gene). These averages are valid, in spite of the age variation, as each animal contributed to both measurements.

The mutant frequency after ENU treatment did not vary with time in the 1- to 8-week interval at either locus, although expression time is known to be an important variable in

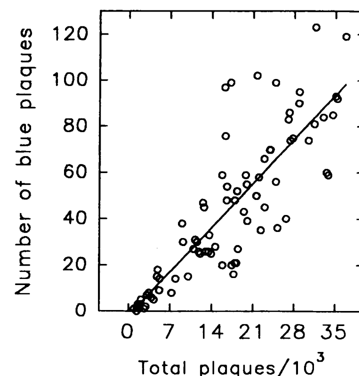

FIG. 8. Correlation between the number of mutant blue plaques and the number of total plaques from animals treated with 250 mg of ENU per kg. Each plate is a separate point; not all animals are represented by the same number of plates.

mammalian cell mutagenesis (2, 30). Jones *et al.* (30) concluded that the maximum frequency of mutations at the *hpt* locus in mouse lymphocytes occurred 10 weeks after ENU treatment and was "essentially stable at 20% of the maximum from 15 to 40 weeks." This difference from our results in the expression time is expected because the epithelial cells of the small intestine are proliferating much faster than the lymphocytes. The uniformity of the response we have observed at all of the sampling times permits the data to be aggregated and tested with respect to other variables. When this was done with respect to the influence of the sex of the parent donating the transgene, no difference was found, indicating that imprinting was not important. Similarly, the sex of the animal, the presence or absence of the transgene, and the age of the animal did not influence ENU-induced mutation at either locus. *Dlb-1* mutations per villus per animal were 8–9 times as frequent as *lacI* mutations per plaque per animal—a ratio that is similar to the ratio of spontaneous mutants and the estimated ratio of mutable loci. The slopes of the dose–response curves, which are roughly linear, differ by about a factor of 12.

In contrast, the two loci did not respond similarly to x-rays. Although *Dlb-1* mutants were frequent after x-ray treatment, *lacI* mutants were rare. A significant increase in the frequency of *lacI* mutants was detected in the dose–response experiment, but the expression-time experiment, which involved the highest dose from the dose–response experiment and many more animals, did not confirm this result. Significant increases were detected at the *Dlb-1* locus in both the dose–response and expression-time experiments, and the mutant frequencies observed were very similar. This difference between the loci is not merely the result of sample size, as many more plaques were analyzed than villi, so that the number of mutable loci at risk was similar. Again, no significant difference was found at different times after treatment at the *Dlb-1* locus. The response of animals with and without the transgene was similar. If mutations had been induced at *lacI* by x-rays at the same frequency per mutable locus as at the *Dlb-1* locus and eight mutable loci per villus are assumed, then the mutant frequency at 6 Gy would have been 6.8×10^{-4} mutants per plaque, whereas 1.3×10^{-4} mutants per plaque were observed (87 mutants per 677,179 plaques). This difference may represent the difficulty of detecting large deletions in the transgene, where a viable phage genome and the reporter gene must remain intact for a *lacI*$^-$ deletion to be recovered. Perhaps deletions extending into adjacent regions of the chromosome are detectable at the *Dlb-1* locus or the presence of introns increases its target size for deletion. The particular characteristics of each locus influence the response to mutagens. Other host loci and other transgenes, even the same transgene in the different locations, may respond differently. Clearly, a large body of comparative data is needed to establish the validity of transgenic assays and the reasons for differential response of loci.

Conclusion. The results obtained for the transgenic *lacI* locus and the *Dlb-1* host locus were similar in many respects, although not identical: (*i*) the sexes were similar both for spontaneous mutation at either locus and for ENU-induced mutation; (*ii*) the mutant frequency was constant with time after ENU treatment at both loci; (*iii*) there was no detectable difference in mutant frequency between the proximal and distal ends of the small intestine after ENU treatment at either locus; and (*iv*) the age of the animal did not influence the frequency of ENU-induced mutations at either locus. The two loci did differ in response to x-rays, however, since mutations were common at *Dlb-1* but rare at *lacI*. This difference, although explicable if x-rays produce a large proportion of deletions that are too large to be recovered as viable phage, indicates the need for further studies of this kind. In addition, we found that the presence of the shuttle vector did not influence mutation at the *Dlb-1* locus significantly, and there was no difference in mutation at the *lacI* locus with respect to the sex of the parent from which it was inherited, indicating no effect of imprinting. These data support the use of the transgenic mouse as a model for somatic mutations *in vivo*, although it is likely that some classes of mutations and some mutagens will not be detected readily.

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