Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6182–6186, July 1992
Genetics

Genetic, cytogenetic, and molecular analyses of mutations induced by melphalan demonstrate high frequencies of heritable deletions and other rearrangements from exposure of postspermatogonial stages of the mouse

(nature of specific-locus mutations/qualitative analysis of mutations/effect of germ-cell stage)

L. B. RUSSELL, P. R. HUNSICKER, N. L. A. CACHEIRO, AND E. M. RINCHIK

Biology Division, Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831-8077

Contributed by L. B. Russell, March 17, 1992

ABSTRACT **Specific-locus experiments have previously shown melphalan to be mutagenic in all male germ-cell stages tested and particularly so in early spermatids. All but 2 of 24 specific-locus mutations recovered were tested genetically, cytogenetically, and/or molecularly. At least 12 of 15 tested mutations recovered from postspermatogonial stages but only 1 of 7 mutations recovered from stem-cell or differentiating spermatogonia gave evidence of being deletions or other rearrangements. Melphalan-induced mutations, thus, confirm the pattern of dependence of mutation structure on germ-cell stage that had been shown earlier for other chemicals. Results of the present investigation illustrate the capabilities of combined genetic, cytogenetic, and molecular analyses for characterizing the nature of specific-locus mutations. Fine-structure molecular mapping of long regions surrounding specific loci has been greatly facilitated by the availability of genetic reagents (particularly, deletion complexes) generated in specific-locus experiments over the course of decades. Reciprocally, this mapping permits increasingly detailed characterization of the nature of lesions induced by mutagenic exposures of germ cells, adding great powers for qualitative analysis of mutations to the specific-locus test. Cytogenetic and genetic investigations also provide evidence on lesion type, especially for loci at which mutations cannot yet be analyzed molecularly. Melphalan, like chlorambucil, can generate many mutations, a high proportion of which are deletions and other rearrangements, making this chemical valuable for generating mutations (at any locus) amenable to molecular access.**

Early germ-cell mutagenesis studies using the specific-locus test (SLT) were concerned primarily with determining whether a given treatment was mutagenic and with measuring mutation rates under various biological and/or physical treatment conditions (1, 2). However, the very design of the SLT—specifically, the inclusion of markers at two very closely linked loci—provided the means for obtaining at least crude *qualitative* information about the mutations recovered (3, 4). Over the decades, large numbers of independent mutations involving each of the loci were propagated in breeding stocks and analyzed genetically; complex sets of overlapping deletions were demonstrated, and units of functional significance were mapped in the regions surrounding several of the specific loci (5, 6). After molecular access to these regions was obtained, the overlapping deletions provided highly valuable reagents for fine-structure physical and functional mapping (7, 8).

The DNA probes that have been generated during this work are, in turn, furnishing the means for analyzing in more detail the structural nature of specific-locus mutations. In the simplest application these probes can be used to diagnose whether such mutations are deletions; but the molecular reagents are already available (and will become more numerous) for determining the extent of any deletions and for detecting other types of rearrangements associated with specific-locus mutations.

Before the present study, only selected radiation-induced mutations (for review, see ref. 8) and mutations induced by the chemical chlorambucil (CHL) (9) had been analyzed molecularly. By applying genetic criteria, specific-locus results obtained with several other chemicals, as well as with radiations, were retrospectively characterized as to the probable nature of the mutations (10, 11). These various assessments have suggested that mutations induced in postspermatogonial stages are primarily large lesions (LLs), whereas those induced in spermatogonia are primarily "other" lesions (OLs) (i.e., not identifiable as deletions or other rearrangements by crude criteria) (11).

Critical for such germ-cell-stage comparisons are agents that prove to be mutagenic *both* in spermatogonia and postspermatogonial stages. Melphalan (MLP) (Chemical Abstracting Service no. 148-82-3) has recently been added to a very short list of such chemicals (11, 12). MLP resembles the closely related CHL in producing a high frequency of mutations in postspermatogonial stages, the peak mutagenic response being elicited by exposure of early spermatids (12, 13). To date, these are the only two chemicals tested in the SLT that produce this "type-2" mutation-rate pattern (11). CHL was found to induce almost exclusively LL mutations in postspermatogonial stages (9). Such mutations can be valuable reagents for molecular analyses of genes or regions of interest throughout the genome.

Twenty-four mutations recovered from exposure of various male germ-cell stages to MLP are analyzed in the present study by using genetic, cytogenetic, and molecular techniques. By means of this combined characterization, we can demonstrate a major qualitative difference between mutations induced in spermatogonial and postspermatogonial stages; most of the latter, but only one of the former, gave evidence of being deletions or other rearrangements.

METHODS

The derivation of mutant mice is described elsewhere (12). Each primary mutant was crossed with appropriate stocks to confirm allelism with one of the specific-locus markers. Confirmed mutations were propagated in breeding stocks. Crosses were made to generate homozygotes from each mutation; breeding protocols varied according to the locus at which the mutation had occurred (9).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MLP, melphalan; CHL, chlorambucil; SLT, specific-locus test; LL, large lesion; OL, other lesion.

Presumed mutants that were sterile or had reduced numbers of offspring were studied cytogenetically in Giemsa-banded mitotic metaphase preparations derived from cultured kidney fibroblasts (14). For several of the mutants that transmitted chromosome rearrangements, meiotic preparations (15) were analyzed for multivalent associations in diakinesis/metaphase I.

High-molecular-weight DNA was obtained from distal tail-tip biopsies from either the primary mutants and/or their direct descendents. Southern blot analyses were done with probes for the *b*, *c*, and *d* loci by methods described for CHL-induced specific-locus mutations (9).

RESULTS

Mutations Involving the Marker Loci. Table 1 lists the 24 specific-locus mutations recovered in the MLP experiments and summarizes the genetic, cytogenetic, and molecular information available for each. Six of the mutations were immediately classifiable as deletions through the use of molecular probes available for the *b*, *c*, and *d* loci. Primary mutants carry the newly arisen mutation, induced by MLP in either a C3H or 101 chromosome, opposite the tester chromosome inherited from the T stock. Therefore, we tested either for loss of C3H/101 ("wild-type") restriction-fragment-length polymorphisms in DNA from the primary mutants or, when possible, for deletion of the probe sequence in homozygous-mutant DNA. For example, Fig. 1*A* shows that DNA derived from the single (homozygous-viable) *c*-locus mutation, $c^{OR\text{-}2Mm}$, is deleted for all coding regions of the tyrosinase (*c*) gene. None of the wild-type *Eco*RI fragments recognized by the tyrosinase cDNA clone MTY811 (17) are present in homozygous-mutant DNA; only the 2.3-kilobase (kb) *Eco*RI fragment recognized by a chromosome-9 control probe, p94.1, is observed.

Fig. 1*B* shows that two primary *b*-locus mutants, $b/b^{OR\text{-}5Mm}$ and $b/b^{OR\text{-}3Mc}$, are deleted for the 4.0- and 1.2-kb wild-type *Taq* I fragments recognized by a 250-base pair (bp)

Table 1. Genetic, cytogenetic, and molecular analyses of heritable MLP-induced mutations involving specific loci

Mutation*	Week of origin†	Survival of homozygotes‡	Fertility of heterozygotes§	Karyotype	Deletion detectable with probe¶			Lesion size‖
					MTY811	MT4	0.3	
Postspermatogonial origin								
$a^{OR\text{-}1Mk}$	1	LD	SS**	In(2E1–H1)				LL
$a^{jOR\text{-}2Mj}$	3	Lethal	PF	NRO				LL
$b^{OR\text{-}1M5}$	3	Viable	SS	NRO	—	No	No††	OL?
$b^{OR\text{-}5Mm}$	4	Lethal	F‡‡	T(4E1;7C)	—	Yes	—	LL
$b^{OR\text{-}3Mc}$	5	Lethal	F		—	Yes	—	LL
$c^{OR\text{-}2Mm}$	3	Viable	F		Yes	—	—	LL
$d^{opOR\text{-}3Mf}$	3	Juvenile lethal	SS	In(9A4;9E2)	—	No††	No	LL
$d^{opOR\text{-}4Mg}$	4	Juvenile lethal	F		—	No††	No	OL
$Df(dse)^{OR\text{-}1Mc}$	2	LD	St	NRO	—	No††	Yes	LL
$Df(dse)^{OR\text{-}2Mg}$	3	Lethal	F		—	No††	Yes	LL
$se^{OR\text{-}1Mm}$	3	LD	LD§§					NC
$s^{OR\text{-}1Mf}$	1	Lethal	F					LL
$s^{OR\text{-}2M5}$	3	Lethal?¶¶	F‡‡					LL?
$s^{OR\text{-}4Mf}$	3	LD	St	Del(14D3–E4)				LL
$s^{OR\text{-}2Mh}$	3	LD	St	T(1;11,2;14;15,5;10,Y;16)				LL
$s^{OR\text{-}2Ml}$	3	Lethal	F‡‡	T(10D2;11B2);Del(3H1–H2)				LL
$s^{OR\text{-}5Mf}$	4	LD	LD§§					NC
Spermatogonial origin								
$b^{OR\text{-}2M10}$	>7	Viable	F		—	No	—	OL
$b^{xOR\text{-}2M7.5}$	>7	Viable	F	NRO	—	No	No††	OL
$d^{opOR\text{-}1M7.5}$	>7	Juvenile lethal	F		—	No††	No	OL
$d^{OR\text{-}3M5}$	>7	Lethal	F		—	No††	Yes	LL
$p^{xOR\text{-}2Me}$	6	TNC	F					OL
$p^{xOR\text{-}3Mb}$	>7	Viable	F					OL
$s^{OR\text{-}10Mb}$	>7	Viable	F					OL

LD, lacking data; TNC, test not complete; NRO, no rearrangement observed in mitotic karyotypes or in meiosis (diakinesis/metaphase I).

*The following superscripts are used for gene symbols: *x*, allele with expression intermediate between that of marker and wild type; a^j, nonagouti jet (phenotypically resembles a^e); d^{op}, dilute opisthotonic. The portion of the superscript that follows OR (Oak Ridge) is the mutant designation.

†Postinjection interval in which mutant was conceived is indicated. The following germ-cell stages are exposed (16): week 1 (days 1–7), mature spermatozoa; week 2 (days 8–14), late spermatids; week 3 (days 15–21), early spermatids; week 4 (days 22–28), late spermatocytes; week 5 (days 29–35), early spermatocytes; weeks 6 and 7 (days 36–49), differentiating spermatogonia; week >7 (days >49), spermatogonial stem cells.

‡Lethal, prenatal lethality; Juv. lethal, lethality between weeks 3 and 6, postnatally; LD indicates that homozygotes were unobtainable because the primary mutant died before test, was sterile, or produced no surviving carrier offspring.

§Fertility of primary mutant and/or heterozygous descendants (unless shown otherwise) is indicated as follows: F, fertile; St, sterile; SS, semisterile (low littersize); PF, poorly fertile (infrequent matings).

¶Only *c*, *b*, and *d* mutations were analyzed with molecular probes. MTY811 is a partial tyrosinase cDNA clone (*c* locus) (17); MT4-Pv.25 is a 250-bp subclone of a tyrosinase-related protein cDNA (*b* locus) (18, 19); 0.3 is a *d-se*-region probe (20). —, Not done.

‖Conclusion drawn (from the various lines of evidence in table) concerning size of lesion involving specific locus. Symbols are as defined by Russell *et al.* (11): NC, no conclusion possible.

**Primary mutant is semisterile; presumed heterozygous offspring are runty and die before sexual maturity. In combination with the closely linked (0.3 centimorgan) gene *bp* (brachypody), mutation produces extreme runting and abnormal feet.

††DNA from mice carrying this mutation was included as a control for this probe.

‡‡Although the primary mutant was semisterile, semisterility subsequently segregated independently of the specific-locus mutation.

§§Mutant died before breeding test.

¶¶Mutant allele was poorly transmitted to heterozygotes; carriers were not mated.

Pvu II subclone of the pMT4 clone; just the 5.2-kb fragment associated with the tester *b* chromosome is evident. The pMT4 clone (21) encodes tyrosinase-related protein (TRP-1), the product of the *b* locus (18, 19). Both $b^{OR\text{-}5Mm}$ and $b^{OR\text{-}3Mc}$ are homozygous lethal (Table 1).

Analysis of the primary *d–se*-region mutants involved hybridizing Southern blots of *Eco*RI-digested mutant DNAs with probe p0.3, a 2.6-kb *Eco*RI–*Pst* I fragment derived from the site of the *Emv-3* provirus integration within the *d* locus (20, 22). Fig. 1*C* demonstrates that one *d* and two *d se* mutations were deleted for p0.3 (note the absence of the wild-type 9-kb *Eco*RI fragment in these mutants). Two of these mutations were homozygous lethal prenatally; the third (a *d se*) was untestable genetically because the primary mutant was sterile.

On the basis of cytogenetic evidence, an additional four mutations were also found to be gross rearrangements. These included a sterile *s*-locus mutant that had a deletion of bands 14D3–14E4 (Fig. 2*A*), which encompass the probable site of the *s* locus (23). Another sterile *s* mutant carried multiple reciprocal translocations, involving nine chromosomes (including chromosome 14, with the breakpoint in the general region thought to contain the *s* locus), as follows: T(1;11), T(2;14;15), T(5;10), and T(Y;16). A female *a*-locus mutant was found to have a chromosome-2 inversion with breakpoints in bands 2E1 and H1; the latter includes the probable

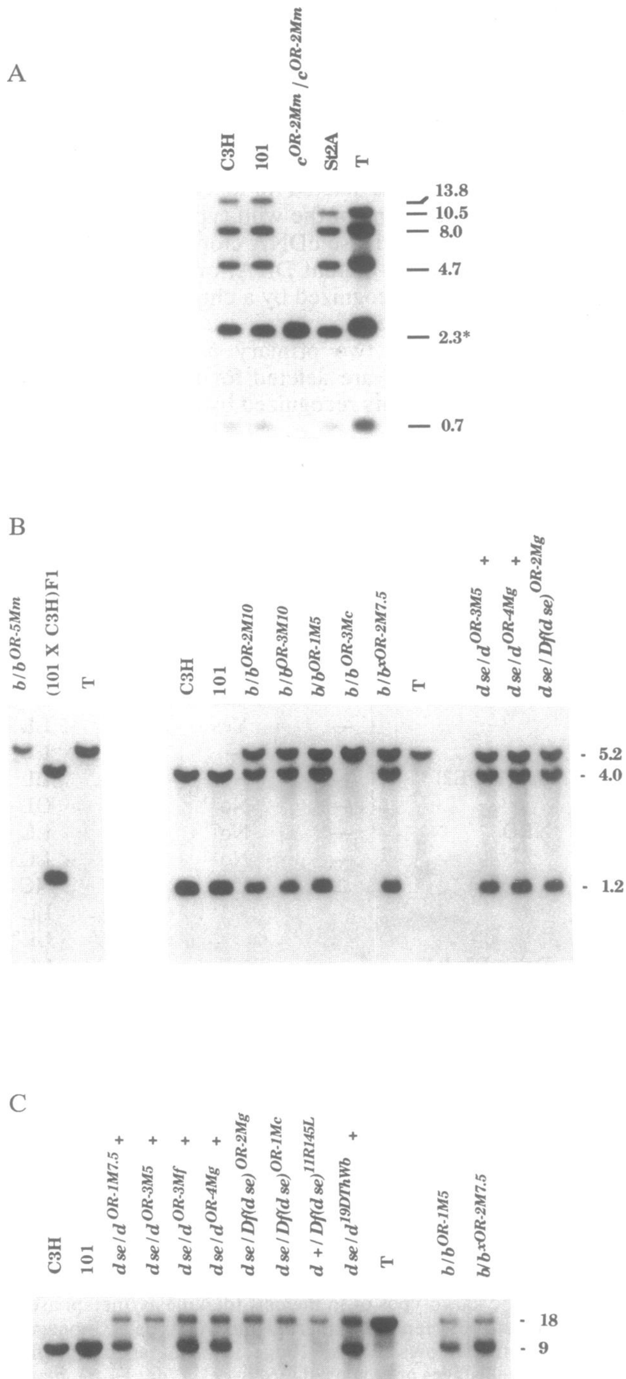

FIG. 1. Molecular analysis of MLP-induced specific-locus mutations. DNA from the indicated genotypes was digested with restriction enzymes, electrophoresed in agarose, blotted to nylon membranes, and hybridized with ^{32}P-labeled DNA probes. Sizes of restriction fragments, given in kilobase pairs, are indicated at right of each panel. (*A*) *Eco*RI digests hybridized with a mixture of MTY811 (tyrosinase cDNA; *c* locus) and p94.1 (a chromosome-9 control probe). The 2.3-kb fragment marked by the asterisk identifies the chromosome-9 locus recognized by p94.1. (*B*) *Taq* I digests hybridized with a 250-bp *Pvu* II fragment of pMT4 (*b* locus). (*C*) *Eco*RI digests hybridized with p0.3, a 2.6-kb *Eco*RI–*Pst* I fragment derived from the *Emv*-3 integration site (*d* locus). The *d–se*-region mutations in *B* (last three lanes) and the *b*-region mutations in *C* (last two lanes) are included as nondeletion controls for the *b*- and *d*-region probes, respectively. T, T stock.

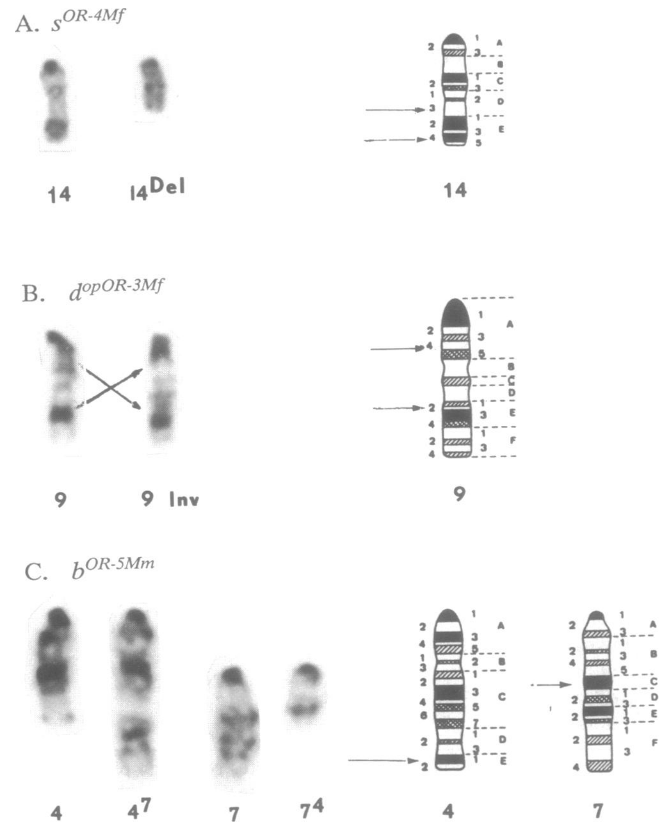

FIG. 2. Cytogenetic analyses of offspring of MLP-exposed males. Partial Giemsa-banded karyotypes of selected animals carrying specific-locus mutations. (*A*) Chromosome 14 from sterile *s*-locus mutant $s^{OR\text{-}4Mf}$, showing deletion (Del) of bands 14D3–E4 (arrows), which include the probable site of the *s* locus (23). (*B*) Inversion (Inv) in chromosome 9 in a male offspring of *d*-locus mutant $d^{opOR\text{-}3Mf}$. Breakpoints are in bands A4 and E2 (arrows). Band 9E is thought to contain the *d* locus (23). The original mutant also carried a translocation that has segregated independently of the *d*-locus mutation; the chromosomes involved in the translocation have not yet been definitively identified. (*C*) Reciprocal translocation between chromosomes 4 and 7 in the primary *b*-locus mutant $b^{OR\text{-}5Mm}$. Although the *b* locus is located in chromosome 4, the breakpoint for this rearrangement is distal to it. The translocation subsequently segregated independently of the *b*-locus mutation. Arrows indicate translocation breakpoints.

location of the *a* locus (23). Offspring presumed to carry this rearrangement opposite *a bp* (*bp*, brachypody, is 0.3 centimorgan distal to *a*) had abnormal appendages (suggesting that the *bp* as well as the *a* locus were affected by the rearrangement), were extremely runty, and died before weaning. Significantly, a mutation at *d* that was not deleted for the 0.3 probe, $d^{opOR\text{-}3Mf}$, was found to carry an inversion in chromosome 9, with one break in band 9E (Fig. 2*B*), which probably contains the *d* locus (23). The original mutant also carried a translocation that has segregated independently of the *d*-locus mutation (the chromosomes involved in the translocation have not yet been definitively identified).

A number of chromosome rearrangements unrelated to the marker loci were also found. For example, one *s*-locus mutant, $s^{OR\text{-}2Ml}$, carried a translocation as well as a deletion, neither of which involved chromosome 14. In another stock derived from an *s*-locus mutant, $s^{OR\text{-}2M5}$, semisterility segregated independently from the specific-locus mutation. Finally a *b*-locus mutant, $b^{OR\text{-}5Mm}$, carried a translocation involving chromosome 4, but with a breakpoint clearly distal to the *b* locus (Fig. 2*C*); this translocation subsequently segregated independently of the *b*-locus mutation.

In addition to the 10 mutants that, on the basis of molecular or cytogenetic evidence, can be classified as carrying deletions or other rearrangements involving a specific-locus marker, 3 others may probably be assigned to this group on the basis of genetic evidence. These include a homozygous-lethal a^j mutation and two homozygous-lethal *s*-locus mutations. [However, because there is as yet no positive proof that *s* is a nonvital locus, it cannot automatically be concluded—as it can for the other loci (8, 24)—that *s* lethals involve deletions of genes additional to the marker.] Two primary mutants ($se^{OR\text{-}1Mm}$ and $s^{OR\text{-}5Mf}$) died before test. A mottled male derived from matings made during week 7 after MLP treatment was mosaic for a *p*-locus mutation. Because the MLP-exposed cells were early-differentiating spermatogonia, which were still to undergo a number of cell divisions, the mosaic mutant (not shown in Table 1) was probably of spontaneous origin.

The last column of Table 1 summarizes the overall conclusions from the various lines of evidence (genetic, cytogenetic, and molecular) concerning the size of the genetic lesions involving the specific loci. Altogether, 15 of the 17 specific-locus mutations recovered from postspermatogonial groups were testable; of these, at least 12 (and possibly a 13th) give evidence of being deletions or other rearrangements involving a specific-locus marker. By contrast, only 1 of the 7 testable mutations in the groups derived from exposed spermatogonial stem cells and differentiating spermatogonia was a LL. The postspermatogonial groups, further, provided evidence for four rearrangements at miscellaneous positions in the genome, whereas there was no evidence for such rearrangements in the spermatogonial group.

Mutations Not Involving the Marker Loci. In addition to recessive mutations at the seven specific loci listed in Table 1, a number of dominant mutations were detected. Four of these dominants, derived from matings during weeks 1, 4, 5, and 7 after MLP treatment, were spotting mutations, two of which were found to involve the dominant-white-spotting (*W*) locus. Among progeny derived from 7.5 mg of MLP per kg treatment of spermatogonial stem cells was a female with striated fur that was transmitted as an autosomal dominant, and a white-mottled sterile male with an X0/XY/XYY mosaic constitution. An X-linked mottling mutation was discovered in a female derived from matings during week 7 after 7.5 mg of MLP per kg. A stock derived from one of the specific-locus mutations, $d^{opOR\text{-}4Mg}$ (Table 1), later independently segregated animals manifesting a recessive anemia.

DISCUSSION

With MLP, as with CHL, a high proportion of the mutations induced in postspermatogonial stages appear to be deletions or other rearrangements; positive evidence to this effect was obtained for 12 or 13 of 15 tested MLP mutations and for 10 of 13 tested CHL mutations. Only one of seven MLP mutations and none of four CHL mutations derived from exposed spermatogonial stages gave evidence of being a LL. These results support our earlier hypothesis (11) that the germ-cell stage in which a lesion is induced is probably the major determinant of the type of mutation recovered (as well as of the mutation rate): LLs are the norm for postspermatogonial stages but are only rarely recovered from exposures of spermatogonia.

Results of this investigation illustrate the capabilities of combining genetic, cytogenetic, and molecular analyses for characterizing the nature of specific-locus mutations. Fine-structure molecular mapping of long regions (tens of megabases) surrounding specific loci has been greatly facilitated by the availability of genetic reagents (particularly, sets of overlapping deletions) generated in specific-locus experiments over decades (8). Further, the genes corresponding to some of the specific-locus markers themselves have been cloned and partially sequenced. This high-density-structural information about the regions surrounding the SLT's marker loci, reciprocally, permits increasingly detailed characterization of the structural nature of lesions induced by given mutagenic exposures of germ cells.

In the current experiment, only a limited number of probes for three of the seven loci (*b*, *c*, and *d*) was used because the initial objective was merely to identify LLs. Mutations at these loci can be further analyzed with numerous additional probes already available, thus mapping and characterizing deletion breakpoints in some detail. Eventually, such analyses might provide information on the full size of the lesions and on whether mutational hotspots from certain treatments might exist. Probes are now also available for analysis of mutations involving *a*, *p*, and *se*. This constantly developing body of information adds great powers to the SLT for *qualitative* analysis of mutations. In the current study, other lines of investigation (cytogenetic and genetic) also provided evidence on lesion type, especially for loci at which mutations were not analyzed molecularly. For one mutation, $d^{opOR\text{-}3Mf}$, cytogenetic data showed a rearrangement that apparently is undetected by the rather low-resolution, single-site molecular analysis currently possible for the *d* locus with available probes.

The ability to generate large numbers of mutations and a high proportion of deletions and other rearrangements among these mutations makes a mutagen valuable for studies seeking to recover developmentally significant variants (at any locus throughout the genome) that are amenable to molecular access. We have earlier shown CHL to be such a mutagen (9, 13). The effects of MLP are generally similar to those of CHL in terms of both overall mutation rate on a per-mol basis (12) and the high frequency of deletions and other rearrangements among the mutations induced in postspermatogonial stages.

Although early spermatids yield the highest mutation rates for both chemicals, the percentage of all postspermatogonial mutations derived from that stage is not as high for MLP as for CHL. If one desired to produce large numbers of deletion mutations, it might be more practical to collect offspring from the first 4 weeks after MLP exposure, rather than from week 3 alone (as is sometimes done with CHL), thus reducing the total number of males that must be exposed. However, MLP is significantly more toxic to the exposed animals than is CHL; for practical purposes only about one-half the dose can be used for generating mutations.

We are grateful to Drs. L. J. Stubbs and M. D. Shelby for critical reading of the manuscript; to C. V. Cornett, L. D. Taylor, and E. L. Phipps for technical assistance; and to Ms. E. B. Matthews for secretarial help. This research was jointly sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, and by the National Institute of Environmental Health Sciences under Interagency Agreement 222Y01-ES-10067.

1. Russell, L. B., Selby, P. B., von Halle, E. W., Sheridan, W. & Valcovic, L. (1981) *Mutat. Res.* **86**, 329–354.
2. Russell, W. L. (1965) *Nucleonics* **23**, 53–56.
3. Russell, L. B. & Russell, W. L. (1960) *J. Cell. Comp. Physiol.* **56**, Suppl. 1, 169–188.
4. Russell, W. L. (1951) *Cold Spring Harbor Symp. Quant. Biol.* **16**, 317–336.
5. Russell, L. B. (1971) *Mutat. Res.* **11**, 107–123.
6. Russell, L. B., Montgomery, C. S. & Raymer, G. D. (1982) *Genetics* **100**, 427–453.
7. Rinchik, E. M. (1991) *Trends Genet.* **7**, 15–21.
8. Rinchik, E. M. & Russell, L. B. (1990) in *Genome Analysis*, eds. Davies, K. & Tilghman, S. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), Vol. 1, pp. 121–158.
9. Rinchik, E. M., Bangham, J. W., Hunsicker, P. R., Cacheiro, N. L. A., Kwon, B. S., Jackson, I. J. & Russell, L. B. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1416–1420.
10. Russell, L. B. (1986) *Genetic Toxicology of Environmental Chemicals, Part B: Genetic Effects and Applied Mutagenesis* (Liss, New York), pp. 437–447.
11. Russell, L. B., Russell, W. L., Rinchik, E. M. & Hunsicker, P. R. (1990) in *Biology of Mammalian Germ Cell Mutagenesis*, Banbury Report 34, eds. Allen, J. W., Bridges, B. A., Lyon, M. F., Moses, M. J. & Russell, L. B. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 271–289.
12. Russell, L. B., Hunsicker, P. R. & Shelby, M. D. (1992) *Mutat. Res.*, in press.
13. Russell, L. B., Hunsicker, P. R., Cacheiro, N. L. A., Bangham, J. W., Russell, W. L. & Shelby, M. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3704–3708.
14. Cacheiro, N. L. A. & Russell, L. B. (1975) *Genet. Res.* **25**, 193–195.
15. Evans, E. P., Breckon, G. & Ford, C. E. (1964) *Cytogenetics* **3**, 289–294.
16. Oakberg, E. F. (1984) in *Mutation, Cancer, and Malformation*, eds. Chu, E. H. Y. & Generoso, W. M. (Plenum, New York), pp. 549–590.
17. Kwon, B. S., Wakulchik, M., Haq, A. K., Halaban, R. & Kestler, D. (1988) *Biochem. Biophys. Res. Commun.* **153**, 1301–1309.
18. Jackson, I. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4392–4396.
19. Zdarsky, E., Favor, J. & Jackson, I. J. (1990) *Genetics* **126**, 443–449.
20. Rinchik, E. M., Russell, L. B., Copeland, N. G. & Jenkins, N. A. (1986) *Genetics* **112**, 321–342.
21. Shibahara, S., Tomita, Y., Sakakura, T., Nager, C., Chaudhuri, B. & Müller, R. M. (1986) *Nucleic Acids Res.* **14**, 2413–2427.
22. Copeland, N. G., Hutchison, K. W. & Jenkins, N. A. (1983) *Cell* **33**, 379–387.
23. Lyon, M. F. & Kirby, M. C. (1991) *Mouse Genome* **89** (1), 37–59.
24. Russell, L. B. (1989) *Mutat. Res.* **212**, 23–32.