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Genetics

Insertion of long interspersed repeated elements at the *Igh* (immunoglobulin heavy chain) and *Mlvi-2* (Moloney leukemia virus integration 2) loci of rats

(polymorphism/repeated DNA/thymoma/provirus integration/transposable element)

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ABSTRACT **Restriction enzyme analysis of normal DNA derived from individual rats of the National Institutes of Health outbred Osborn–Mendel colony revealed that two independent single-copy loci, the *Igh* (immunoglobulin heavy chain) locus and the *Mlvi-2* (Moloney leukemia virus integration 2) locus, a putative oncogene, are polymorphic (i.e., exhibit allelic variation). The polymorphism at both loci was due to the presence or absence of a long interspersed repeated DNA element (LINE). The LINE insertion in the *Igh* locus occurred in the joining (*J*) region, which is involved in the physiological rearrangement of this locus. The LINE insertion in the *Mlvi-2* locus has occurred approximately 6 kilobases from the region of provirus integration in Moloney murine leukemia virus-induced rat thymomas. The two inserts are colinear with each other and with other randomly selected cloned copies of the rat LINE family, the general characteristics of which we also present. LINE insertion in the *Mlvi-2* locus was observed in several rat strains, established from independent rat colonies, suggesting that LINE-containing *Mlvi-2* alleles may be widespread in the rat population. LINE insertion in the *Igh* locus was observed in 1 of 27 rats. The detection of a LINE-related polymorphism at two nonselected loci indicates that LINEs are transposable. The presence or absence of these long (>5 kilobases), highly transcribed elements at single-copy loci could have profound effects on gene activity. Furthermore, LINE-containing single-copy loci could be affected by homologous interaction between the resident LINE and any of the other 50,000 or so copies of these elements in the rat genome.**

At least one-third of various mammalian genomes is composed of families of repeated DNA sequences, the function of almost all of which is not known (1, 2). Families of highly repeated (≥30,000 copies per family) "interspersed" sequences account for a substantial fraction of the repeated DNA of primate (human, monkey) and certain rodent genomes (hamster, mouse) (2). Although interspersion originally implied that the individual members of a given DNA family were scattered among the single-copy DNA (3), recent studies (ref. 4 and references therein) indicate that the members of interspersed families are usually contiguous with each other in clusters. The mammalian interspersed sequences are usually highly transcribed and appear to fall into two broad classes: Short interspersed repeated DNA families (SINEs) (2) contain short (typically <350 base pairs) repeated sequences; examples are the "*Alu*" family of humans or the B1 and B2 families of mice (5, 6). Long interspersed repeated DNA families (LINEs) (2) contain long [>4 kilobases (kb)] complex repeated DNA structures (7–13) and are responsible for the prominently stained electrophoretic bands produced by digesting total genomic DNA with appropriate restriction endonucleases (2).

Similar to the genomes of other mammalian species, the rat genome also contains a number of different, highly repeated, interspersed sequences (4). These sequences are highly transcribed and include representatives of both the SINE and LINE classes of repeated DNA, both of which resemble their counterparts in the mouse. Sequences of both of these classes are organized as permuted clusters in the rat, a result that probably explains why these highly repeated interspersed sequences were not detected as such in earlier reassociation studies on rat DNA (14).

In the present report, we show that two single-copy loci of rats—the *Mlvi-2* (Moloney leukemia virus integration 2) locus, a putative oncogene involved in the induction of thymic lymphomas (15–17), and the *Igh* (immunoglobulin heavy chain) locus—are polymorphic (i.e., exhibit allelic variation). The polymorphism is due to the presence or absence of a typical rat LINE, the general characteristics of which we also present here.

Our finding of a LINE-related polymorphism at two nonselected, genetically independent loci suggests that such polymorphisms may be common in the rat population. This could be caused either by a high rate of LINE transposition or by genetic selection. Whatever the explanation, the presence or absence of a LINE element could potentially exert profound effects on the contiguous DNA sequences, including effects on gene activity and all of the genetic rearrangements that occur when homologous DNA sequences interact.

MATERIALS AND METHODS

Animals. The laboratory strains of rats described in this report were kindly provided by C. T. Hansen and D. Bolte (Veterinary Resources Branch, Division of Research Services, National Institutes of Health). The wild *Rattus norvegicus* was kindly provided by S. C. Frantz (New York State Department of Health, Troy, NY). The Moloney murine leukemia virus (Mo-MuLV)-induced thymomas were induced by intraperitoneal injection of approximately 50,000 XC plaque-forming units of Mo-MuLV in newborn Osborn–Mendel rats (15).

Southern Blot Analysis of Cellular and Cloned DNA. DNA was digested with restriction endonucleases and was electrophoresed in horizontal agarose gels. After transfer to nitrocellulose filters, it was hybridized to the appropriate ^{32}P-la-

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Abbreviations: *Igh*, immunoglobulin heavy chain locus; *Mlvi-2*, Moloney leukemia virus integration 2; SINE, short interspersed repeated DNA family; LINE, long interspersed repeated DNA family; *J*, joining region; kb, kilobase(s); Mo-MuLV, Moloney murine leukemia virus.

beled DNA probes. Hybridizations were at 42°C in 50% (vol/vol) formamide/0.75 M NaCl/75 mM sodium citrate, pH 7.0. After hybridization, the filters were washed first in 0.3 M NaCl/30 mM sodium citrate, pH 7.0/0.1% $NaDodSO_4$ at room temperature. This was followed by one or two 30-min washes at 65°C in 15 mM NaCl/1.5 mM sodium citrate, pH 7.0/0.1% $NaDodSO_4$.

Molecular Cloning. Cellular DNA (0.2–0.3 mg) was digested with either *Sac* I or *Bam*HI and was fractionated by preparative agarose gel electrophoresis. DNA from fractions enriched for the desired fragments were ligated to the arms of the appropriate bacteriophage λ vector [λ1059 (18) or a *Sac* I-modified λ1059 (16)]. The ligation product was packaged *in vitro*, and individual plaques of the resulting phage were screened with the appropriate cellular DNA probes.

RESULTS

Polymorphism in the *Igh* and *Mlvi-2* Loci of Osborn–Mendel Rats. Normal DNA from two different Osborn–Mendel rats was digested with *Sac* I or *Eco*RI and was hybridized to the J_{11} probe derived from the *J* (joining) region of the mouse *Igh* locus (19). This probe hybridized to two *Eco*RI restriction fragments in the DNA of one rat (Fig. 1*A*, lane d) but to only one *Eco*RI fragment in the DNA of the second rat (Fig. 1*A*, lane c). The *J* region probe also hybridized to two restriction fragments when the rat DNA used for lane d was digested with *Sac* I (Fig. 1*A*, lane b). The smaller, 11-kb fragment is partially obscured by a nonspecific autoradiographic signal on this blot, but the lack of the larger, 16.5-kb *Sac* I fragment is clearly evident in the *Sac* I digest of the DNA of the second rat (Fig. 1*A*, lane a). One of the rats (Fig. 1*A*, lanes a and c) was homozygous, since it carried only one of the two *Igh* alleles, whereas the other (Fig. 1*A*, lanes b and d) was heterozygous. The detection of the restriction frag-

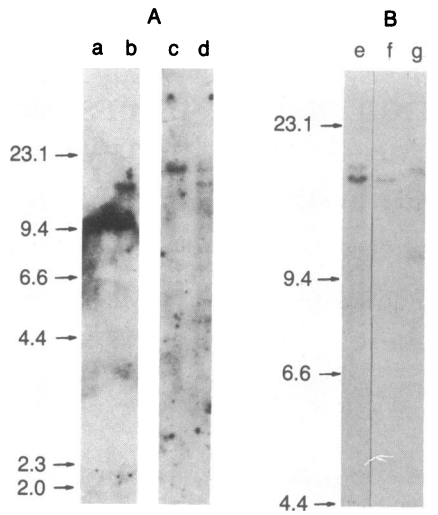

FIG. 1. Restriction fragment length polymorphism in the *Igh* and *Mlvi-2* loci of the rat. Molecular sizes in kb are indicated. (*A*) Southern blot analysis of cellular DNA from two normal Osborn–Mendel rats. The DNA was digested with either *Sac* I (lanes a and b) or *Eco*RI (lanes c and d) and was hybridized to the J_{11} probe derived from the *J* region of the mouse *Igh* locus (19). (*B*) Southern blot analysis of *Bam*HI-digested genomic DNA from two normal Osborn–Mendel rats (lanes e and f) and from the Mo-MuLV-induced rat thymoma A_2 (lane g). These DNAs were hybridized to the pTS6 probe derived from the rat *Mlvi-2* locus (refs. 16 and 17; see Fig. 2*D*).

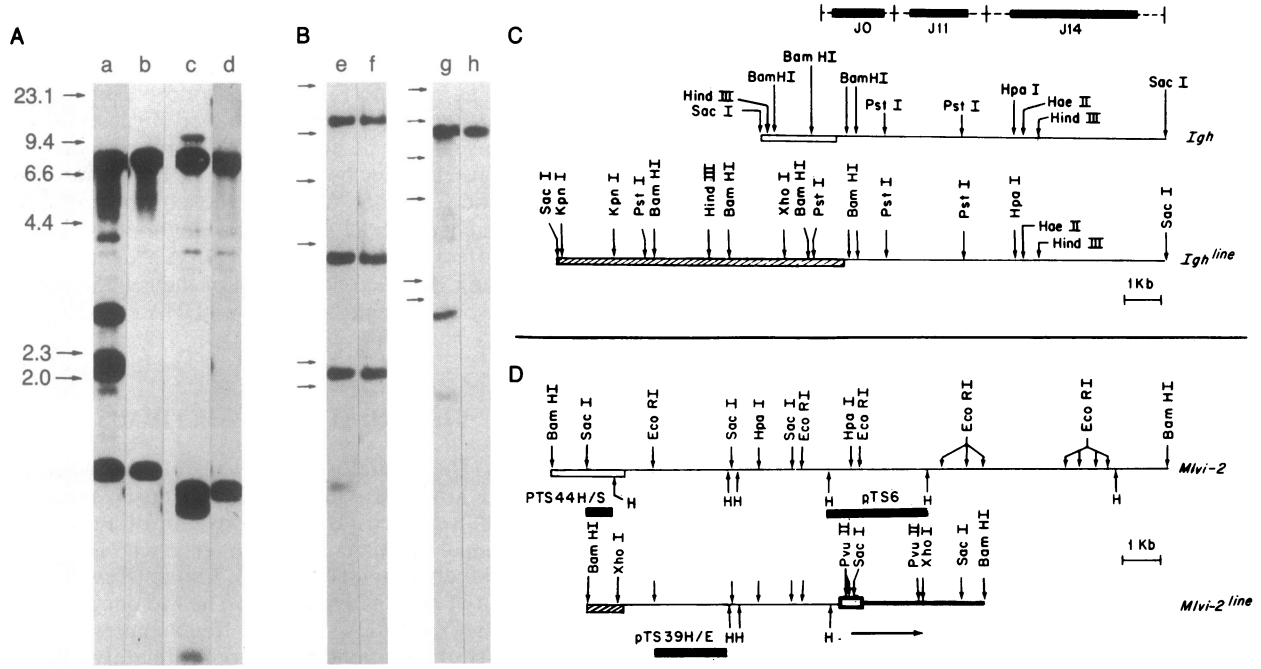

FIG. 2. Nature of the polymorphism in the *Igh* and *Mlvi-2* loci. (*A*) Southern blot analysis of *Bam*HI digests of the *Sac* I inserts from the Igh^{line} clone (lanes a and b) and the *Igh* clone (lanes c and d). The Igh^{line} clone was hybridized with itself (lane a) or with the *Igh* clone (lane b). The *Igh* clone was hybridized with itself (lane c) or with the Igh^{line} clone (lane d). Arrows indicate positions of molecular size (kb) markers. (*B*) Southern blot analysis of the *Sac* I-digested *Bam*HI insert from the *Mlvi-2* clone (lanes e and f) and the *Xho* I-digested *Bam*HI insert of the $Mlvi\text{-}2^{line}$ clone (lanes g and h). The *Mlvi-2* clone was hybridized with itself (lane e) or with the $Mlvi\text{-}2^{line}$ clone (lane f). The $Mlvi\text{-}2^{line}$ clone was hybridized with itself (lane g) or with the *Mlvi-2* clone (lane h). Arrows indicate same size markers as in *A*. (*C*) Restriction endonuclease map of the cloned sequences of the two *Igh* alleles (*Igh* and Igh^{line}). Solid black bars above the map indicate the various functional domains of the *Igh* locus as determined by hybridization to probes derived from clones of the mouse *Igh* locus. Open and hatched bars indicate the approximate position of the heterologous sequences between the two alleles. (*D*) Restriction endonuclease map of the cloned sequences of the two *Mlvi-2* alleles (*Mlvi-2* and $Mlvi\text{-}2^{line}$). Solid black bars below the map indicate the DNA probes used in this work. Open and hatched bars indicate the heterologous sequences between the two alleles. The heavy line at the 3′ end of the $Mlvi\text{-}2^{line}$ clone represents the integrated Mo-MuLV proviral DNA. The arrow indicates the orientation of the provirus, and H indicates the positions of the *Hin*dIII restriction sites.

ment polymorphism with both *Eco*RI and *Sac* I indicated that the alteration at this locus is not the result of a single base change.

Polymorphism was also detected in the *Mlvi-2* locus. Normal DNA from two different Osborn–Mendel rats was digested with *Bam*HI and was hybridized to the *Mlvi-2* probe pTS6 (refs. 16 and 17 and Fig. 2*D*). Two distinguishable *Mlvi-2* alleles were observed (Fig. 1*B*, lanes e and f). One rat (Fig. 1*B*, lane e) was heterozygous at this locus, and the other (Fig. 1*B*, lane f) was homozygous.

Mlvi-2 represents a putative oncogene identified as a common region for provirus DNA integration in Mo-MuLV-induced rat thymic lymphomas. Therefore, an additional polymorphism due to the insertion of a Mo-MuLV provirus was observed in tumor cell DNA from approximately 50% of the thymomas examined (ref. 16 and unpublished data). An example of this somatic polymorphism observed in tumor cell DNA is shown in Fig. 1*B*, lane g. Tumor cell DNA from the rat thymoma A_2, derived from a *Mlvi-2* heterozygous rat, was digested with *Bam*HI and hybridized to the pTS6 probe. One of the *Mlvi-2* alleles is altered in this tumor due to the insertion of a Mo-MuLV provirus at this site.

To study the nature of the polymorphisms at the rat *Igh* and *Mlvi-2* loci, the following genomic DNA fragments were cloned in a λ1059 vector: the 16.5-kb and 11-kb *Sac* I fragments, representing the rat *Igh* locus (see lane b, Fig. 1*A*), and the 16.5-kb and 12-kb *Bam*HI fragments from tumor A_2, representing the *Mlvi-2* locus (see lane g, Fig. 1*B*). The clones of the 16.5-kb and 11-kb *Sac* I fragments are referred to as Igh^{line} and *Igh*, respectively, and the clones of the 16.5-kb and 12-kb *Bam*HI fragments are referred to as *Mlvi-2* and $Mlvi\text{-}2^{line}$, respectively.

To compare the DNA sequences between the two *Igh* alleles and between the two *Mlvi-2* alleles, cross-hybridizations were carried out between the cloned DNAs of the respective pairs (Fig. 2 *A* and *B*, respectively). The results are similar for each locus in that one allele contained DNA sequences not present in its counterpart. Restriction maps of the cloned sequences of the two alleles of the *Igh* and of the *Mlvi-2* loci are shown in Fig. 2 *C* and *D*. The maps show the heterologous sequences between the two clones of each pair (hatched bar versus open bar) and indicate the approximate position of the recombination event that generated the polymorphism at each locus. It is interesting that the recombination event in the *Igh* locus occurred in the *J* region, which is involved in the physiological rearrangement of this locus (20, 21). The rearrangement of the *Mlvi-2* locus was the result of a recombination event that occurred about 6 kb from the region of provirus DNA integration in Mo-MuLV-induced rat thymomas.

Nature of the DNA Alteration at the *Mlvi-2* and *Igh* Loci. To study the alteration of the *Mlvi-2* locus, DNA from a homozygous or a heterozygous rat was digested with *Sac* I and hybridized to the *Mlvi-2* probes pTS39H/E (Fig. 3*A*, lanes a and b) and pTS44H/S (Fig. 3*A*, lanes c and d). Both probes detected 3.5-kb and 10-kb DNA fragments in DNA from the heterozygote, a result that suggests that the *Mlvi-2* alleles differ by the presence of an approximately 6.5 kb long DNA element. The site of insertion is represented by the point where the *Mlvi-2* clone diverges from the $Mlvi\text{-}2^{line}$ clone (Fig. 2*D*). This was confirmed by cloning the 10-kb *Sac* I fragment to produce another *Mlvi-2* clone, λCl228 A_2^{line}. A restriction endonuclease map of this clone, showing the DNA regions that are homologous to the pTS39H/E and pTS44H/S probes, is shown in Fig. 3*C*.

A similar DNA element is also present in one of the *Igh* alleles. A blot of *Pst* I-digested DNA from the Igh^{line} clone (diagrammed on the lower map, Fig. 2*C*) was hybridized to the *Mlvi-2* clone shown in Fig. 3*C*. As Fig. 3*B* shows, there is strong homology between the DNA element inserted in the

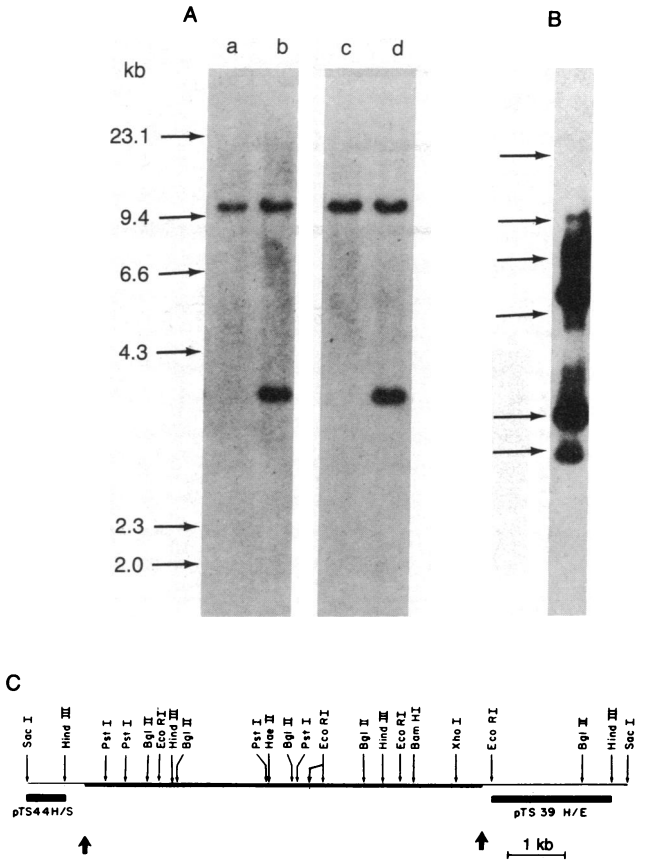

FIG. 3. Nature of the DNA alteration in the *Igh* and *Mlvi-2* loci. (*A*) Southern blot analysis of *Sac* I-digested normal rat DNA from a homozygous (lanes a and c) and a heterozygous (lanes b and d) rat. Digests were hybridized with the pTS39H/E (lanes a and b) or pTS44H/S (lanes c and d) probe. (*B*) Southern blot analysis of *Pst* I-digested λL_1c (Igh^{line}) DNA hybridized with the λCl228 A_2^{line} ($Mlvi\text{-}2^{line}$) probe. (*C*) Restriction endonuclease map of the λCl228 A_2^{line} clone. Solid black bars indicate the sequences that are homologous to the pTS39H/E and pTS44H/S probes.

Mlvi-2 locus and the novel sequences identified at the *Igh* locus (hatched bar, Fig. 2*C*). Therefore, the allelic forms of both the *Mlvi-2* and the *Igh* loci are due to the presence or absence of homologous DNA elements. Both elements are highly repeated in the rat genome as determined by hybridization of nitrocellulose filter-bound cloned DNA to radiolabeled genomic rat cell DNA (results not shown).

The Repetitive DNA Elements at the *Mlvi-2* and *Igh* Alleles are LINEs. Both the repetition frequency and the length of the repeated DNA present in the *Mlvi-2* and *Igh* loci suggested that these elements are rat LINEs. Furthermore, the $Mlvi\text{-}2^{line}$ clone hybridizes strongly to the 1.35-kb and 2.4-kb *Eco*RI bands or the 5-kb *Bam*HI band that are prominent above the background on stained electrophoretic gels of *Eco*RI or *Bam*HI digests of total genomic rat DNA (data not shown). These bands are derived from rat LINEs (ref. 4 and unpublished observations). The identity of the repeated DNA elements at the *Mlvi-2* and *Igh* loci was confirmed by hybridization of the $Mlvi\text{-}2^{line}$ and Igh^{line} clones to radiolabeled fragments of rat LINE DNA that were subcloned from randomly selected λ genomic clones of rat LINEs (4) (see below).

Fig. 4*A* (top diagram) shows a typical rat LINE which is at least 6 kb long and contains three internal *Eco*RI sites that define 2.4-kb and 1.35-kb segments (designated B and D, respectively) that are present in almost all of the 50,000 or so genomic copies of this structure. An *Eco*RI site is present at

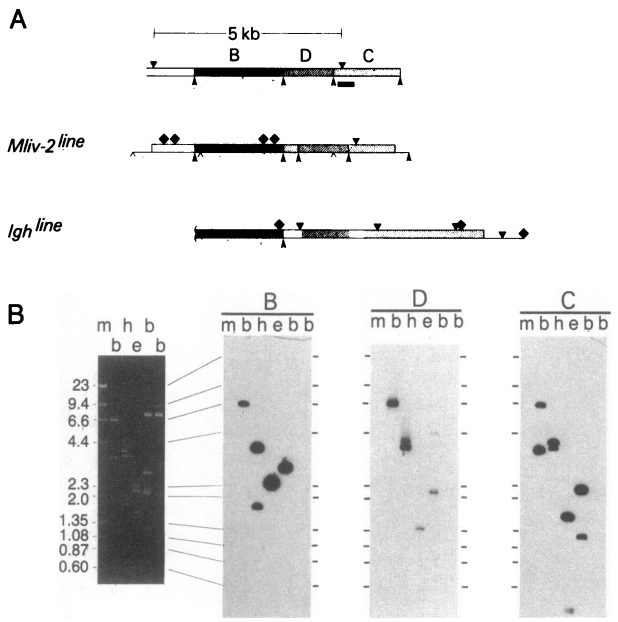

FIG. 4. Structure of several rat LINEs. (*A*) The top diagram is a generalized version of a rat LINE and shows only the position of the *Eco*RI and *Bam*HI restriction enzyme sites. The solid black bar below segment C corresponds to about 500 base pairs, represented in two previously sequenced repeat DNA clones (4) which contain a highly conserved *Bam*HI site. The LINE structures present at the *Mlvi-2* and *Igh* loci were determined by blot hybridization experiments such as those illustrated in *B*. The unshaded portions indicate regions that could not be unambiguously accounted for by the hybridization probes, and the nature of the sequences to the left of segment B in the *Igh* line has not been determined. ◆, ∧, ▲, and ▼ indicate *Pst* I, *Hin*dIII, *Eco*RI, and *Bam*HI sites, respectively. (*B*) Plasmid clones containing B, D, or C segments were radiolabeled and hybridized to the indicated nitrocellulose blots as described earlier (4). The letter over each lane, m, b, h, or e, indicate markers, *Bam*HI, *Hin*dIII, or *Eco*RI, respectively. The second, third, and fourth lanes of each blot (b, h, and e) contain endonuclease digests of the *Mlvi-2*^{line} clone (see Fig. 3*C*). The last two lanes b contain *Bam*HI digests of either the *Igh*^{line} clone or the *Igh* clone, respectively (see Fig. 2*C*). Sizes of markers (in kb) are at left.

a variable distance from the right end of the D segment but rarely closer than 1.35 kb. One of these variably sized fragments is designated C. The B-, D-, and C-type segments are present in roughly equimolar amounts in the genome. The DNA sequences contained in the B-, D- and C-type segments are highly represented in nuclear RNA. An additional segment (open bar) to the left of segment B hybridized to total rat DNA as if it also contained low-repeat DNA.

Essentially all of the genomic copies of the LINE contain the right-hand *Bam*HI site which resides in a region of the LINE (solid black bar) that is highly conserved. (The evidence for these and other characteristics of rat LINEs, as well as the DNA sequence of these structures, will be presented elsewhere.)

Fig. 4*A* also shows the structures of the LINEs at the *Mlvi-2* and the *Igh* loci, which were determined by experiments such as those whose results are shown in Fig. 4*B*. Both structures are generally colinear with the typical LINE and and demonstrate the variability in the length of the C-type segments. Furthermore, the *Mlvi-2* LINE also contains a left-hand segment of apparently low-repeat DNA, as judged by its hybridization to total rat DNA (results not shown). As Fig. 4*B* (autoradiogram C, lane e) shows, the C segment probe hybridized to two *Eco*RI fragments (≈1.5 and 0.4 kb) from the *Mlvi-2*^{line} clone, the smaller of which is between the B and D segments. None of the highly repeated LINE segments hybridized to DNA from either the *Igh* allele that lacks the LINE (Fig. 4*B*) or the *Mlvi-2* allele that lacks the LINE (results not shown).

Table 1. Distribution of the LINE insertion in the *Mlvi-2* locus among strains of rats

Strain	No. of animals tested	LINE insertion
Inbred		
Fischer 344	1	–
M520/N	1	–
BN/SsN	1	–
ALB/N	1	–
RCS/N	1	+*
Buffalo/N	1	+*
Outbred		
Osborn–Mendel	16	+†
Wistar–Furth	2	+*
Sprague–Dawley	2	+‡
R. norvegicus (wild)	1	–

*Homozygous.
†All 16 Osborn–Mendel rats were positive. Three of 16 were heterozygous, and the rest were homozygous.
‡One of the 2 Sprague–Dawley rats was positive (heterozygous).

***Mlvi-2*^{line} or *Igh*^{line} Alleles in Individual Rats.** All current laboratory strains of rats were derived from the single species *Rattus norvegicus* (22). Individual members of three outbred (Osborn–Mendel, Wistar–Furth, and Sprague–Dawley) and five inbred rat strains (Fischer 344, M520/N, BN/SsN, ALB/N, RCS/N, and Buffalo/N) were examined to determine whether they carried the LINE insertion in the *Mlvi-2* locus. An individual *R. norvegicus* captured in the wild was tested similarly. The results of this analysis, which involved hybridization of *Sac* I-digested DNA to the pTS39H/E probe, are shown in Table 1. DNA from the same individual rats was also examined to determine the possible insertion of LINE sequences in the *Igh* locus. This analysis revealed only one Osborn–Mendel rat that was heterozygous for this trait. All other individual rats were negative.

Three rat strains, Buffalo, Wistar–Furth, and Sprague–Dawley, are related to the original Wistar stock of rats (22). All of the other strains that we tested were derived from independent rat colonies established from American or European wild rats between 1900 and 1940 (22). Therefore, the *Mlvi-2* alleles seem to be widespread in rats. This is not true of the *Igh* alleles (see *Discussion*).

DISCUSSION

Highly repeated interspersed DNA sequences of the rat include both short and long interspersed families (4). The rat long interspersed family or LINE is related to the *Bam*HI (MIF-1) family of mice (9, 12, 13) and the *Kpn* I family of primates (7, 8, 10, 11).

This report shows that rat LINE family members cause polymorphisms at two independent, single-copy loci: *Igh* and *Mlvi-2*. This result indicates that LINEs are transposable. Although transposition may not be unexpected for a highly repeated family of dispersed DNA sequences, the present work shows that single-copy loci differ due to the presence or absence of an entire LINE. A LINE-related polymorphism could be the result of either an insertion or a deletion. However, analysis of the *Igh* locus suggests an insertion. LINE sequences were detected in the *J* region of the rat *Igh* locus in only 1 of 27 animals; that animal was heterozygous. Furthermore, at least two additional species [mouse and man (20, 21)] carry no repeated sequences in the *J* region. We conclude that it is unlikely that the rat *Igh* locus evolved interrupted by a LINE sequence that was subsequently excised. Since the regions of insertion at the *Igh* or

Mlvi-2 loci did not hybridize to rat LINE sequences, homologous recombination is not likely to account for insertion of LINEs on these loci. Since restriction enzyme analysis indicates that the single-copy DNA that flanks the LINE in either the *Mlvi-2* or *Igh* locus is the same as that of the alleles without the LINE (Figs. 2 and 3), LINE excision from these loci, if it occurs, must be rather precise.

In addition to the LINE-related polymorphism at the *Mlvi-2* and *Igh* loci, we have recently detected a LINE-related polymorphism at the insulin 1 locus (unpublished results). Therefore, LINE-containing alleles of single-copy loci are quite common in the rat population. Two explanations can account for this. Either LINE-containing alleles confer a selective advantage to the animals and are therefore efficiently fixed in the population or LINE transposition is actively occurring in present-day rats. If the latter is so, the rate of transposition might be high due to the high copy number of the rat LINE family. However, the LINE-related polymorphism of at least two of these loci (*Igh* and *Mlvi-2*) may be a consequence of the known recombinogenic properties of these loci (15, 20, 21).

Regardless of the means by which LINE-containing single-copy alleles have become prevalent in rat populations, the introduction (or loss) of these long, highly repeated, highly transcribed DNA elements at a particular single-copy locus could have significant biological consequences. For example, in addition to simply inactivating genes by direct insertion, LINEs might alter the regulation of genes by introducing their own regulatory sequences in the gene's vicinity. Finally, by virtue of their homology to 50,000 other such sequences, LINEs could promote all of the phenomena known to occur when homologous DNA sequences interact, including deletion, duplication, and other DNA rearrangements thought to be involved in genome evolution (23).

We have not yet studied the effect of the LINE insertions at the *Igh* and the *Mlvi-2* loci. However, it may be relevant that the Buffalo rat, an inbred strain which is homozygous for the LINE insertion in the *Mlvi-2* locus, has a high incidence of spontaneous thymomas (24). Since susceptibility to neoplasia may depend on cooperation of several genetic traits (25), the insertion of the LINE in *Mlvi-2* may be one, but not the only, genetic change required.

Mammalian genomes contain many DNA sequences that appear to move (5, 26–30), including subsegments of the *Kpn* I primate LINE family (31, 32). These sequences include SINEs (5), which are also highly repeated in the genome. It is interesting that we recently detected a polymorphism in the human homolog of the *Mlvi-2* locus due to the presence or absence of an *Alu* SINE (unpublished results).

In summary, we have presented evidence showing that transposition of LINEs causes polymorphism in single-copy regions of rat DNA. The significance of LINE insertion at a single-copy locus can be more fully appreciated when the LINE's structure and function(s) are understood. On the other hand, the natural proximity of LINEs to well-understood single-copy genes may, in turn, help to clarify the biological functions of LINEs.

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