Evidence for mobility of a new family of mouse middle repetitive DNA elements (LTR-IS)

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ABSTRACT

Locus variation and sequence conservation of mouse LTR-IS elements, a new family of middle repetitive DNA sequences was studied. It is shown that LTR-IS sequences are present in all the inbred strains and subspecies of *M. musculus* tested and in *M. cooki* and *M. caroli*. Their arrangement in mouse genomes is polymorphic. Southern blot analysis and DNA sequencing revealed the existence of homologous DNA sequences with and without LTR-IS element insertion. LTR-IS sequences therefore appear to have arisen in early mouse ancestors and have, at least at some point, been mobile.

INTRODUCTION

In a previous paper (1) we have identified a novel family of middle repetitive sequences called LTR-IS. They show structural characteristics of both, insertion elements (IS) and solitary retroviral long terminal repeats (LTR). LTR-IS elements are about 500 bp long, have 11 bp inverted repeats at their termini and contain signals implicated in RNA polymerase II transcription and regulation. The elements, which number about 500 per mouse haploid genome, are interspersed among variable flanking regions of mouse DNA.

Genetic evidence has long suggested that deletion and rearrangement of sequences at particular eukaryotic loci are due to the mobilization of transposable DNA elements (2). This view has been supported at the molecular level by recent evidence that transposable elements are directly adjacent to chromosomal rearrangements in yeast and Drosophila (3, 4). Transposable element-like structures in mammals have not yet been rigorously identified. However, it has been shown that retroviral sequences can function directly as insertion mutagens (5, 6, 7), and it has been proposed that a reverse transcriptase mediated re-

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arrangement via an RNA intermediate, might be a further mechanism of transposition (8, 9). Structural features of LTR-IS elements indicate that they could be mobile in the genome and function as insertion mutagens. It was therefore important to study whether during the evolution of the mouse species or during the development of the individual LTR-IS sequences rearrange in the genome. For this reason we have examined the locus variation and sequence conservation of LTR-IS elements in different tissues and in various strains of mice and other animal species.

MATERIALS AND METHODS

Animals

All M. musculus subspecies and M. species were generous gift of Dr. Jan Klein, Max-Planck-Institut, Tübingen.

DNA preparation

The isolation of high m. w. DNA from animal tissues was carried out according to Maniatis et al. (10) avoiding any ethanol precipitation step. DNA used for all described Southern blot analysis was from male animals. No sex-based differences were found between liver DNAs of 129/J inbred strain.

Cloning

DNA fragments were identified by hybridization, eluted from an agarose gel and cloned in the lambda phage NM 641 vector (11).

Hybridization analysis

DNA separations on agarose gels and Southern transfers were performed as described (12). All hybridizations were carried out in 50 % formamide, 5 x SSPE, 5 x Denhard's reagent, 100 μg/ml denatured salmon sperm DNA (10). Stringent conditions were defined as following: Hybridization at 42° 36 hrs and washing in 0.2 x SSC at 60°; non-stringent: hybridization at 37° 36 hrs and washing in 2 x SSC at 60°.

Hybridization probes

An LTR-IS specific probe (pB8S) was a subcloned Bgl II/Hind III fragment of the clone pB8 (1) into pUC8. pB8F3 probe was an Xba I/Xba I fragment of the clone pB8 in pUC8 (see Fig. 1).

DNA sequence analysis

DNA fragments were end labelled and analyzed according to the method of Maxam and Gilbert (13).

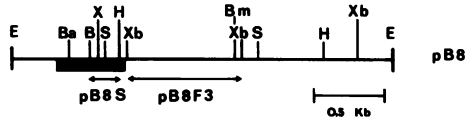

Fig. 1
Partial restriction map of the LTR-IS clone pB 8
Thick bar indicates the LTR-IS specific sequence. The fragments that were subcloned and used as hybridization probes are indicated with arrows. Vector sequence is not shown. B, Bgl II; Ba, Bal I; Bm, Bam H 1; E, Eco RI; H, Hind III; S/Sst I; X, Xho I; Xb, Xba I.

RESULTS

Southern blot hybridizations were used to study the organization of LTR-IS elements in genomic DNA. Fig. 1 shows the restriction map of LTR-IS clone pB8 and the fragments of this clone which were used as hybridization probes. One probe represents the 3' end of the LTR-IS specific sequence (pB8S), the other probe was derived from 3' end flanking mouse sequence (pB8F3). Isolated DNAs were digested with restriction endonuclease Eco RI, electrophoresed in a 1 % agarose gel, transfered to nitrocellulose, and hybridized to the labelled LTR-IS specific probe. Since no Eco RI site is present within the 500 bp LTR-IS element, the fragments detected by hybridization display locations of LTR-IS sequence within the cellular genome.

Frequent rearrangement of LTR-IS elements during ontogenesis, if occurs, could be detected by comparing DNAs from different organs of an individual. However, the above described analysis did not reveal any differences in the hybridization patterns between DNAs from several organs of a 129/J mouse (data not shown).

A similar analysis was therefore performed with DNAs isolated from livers of several animal species. Comparison of the hybridization patterns (Fig. 2) shows that all *M. musculus subspecies* and inbred mouse strains tested contain multiple copies of LTR-IS hybridizing fragments in approximately equal amounts. Despite the complexity of the banding pattern some differences indicating polymorphism are visible between inbred strains and *M. m. subspecies* and become more distinct between

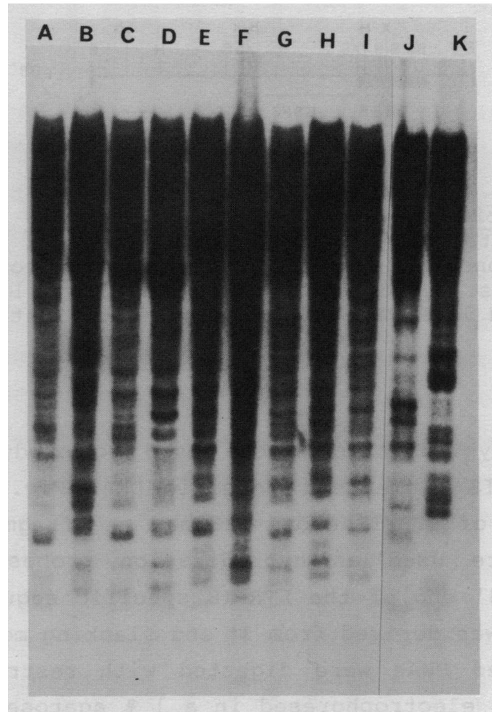

Fig. 2
Organization of LTR-IS sequences in various mouse strains. Liver DNAs digested with Eco RI were analyzed by Southern blotting and hybridized to ^{32}P labelled LTR-IS specific probe. A) DBA, B) C67Bl/6, C) Balb/C, D) C3H, E) 129/J, F) *M. m. molossinus,* G) *M. m. castaneus,* H) *M. m. spicelegus,* I) *M. m. musculus*, J) *M. caroli,* K) *M. cooki.*

M. species.

In order to determine the range of LTR-IS sequence conservation several other animal DNAs were tested by hybridization to LTR-IS specific probe. Under non-stringent conditions only DNA from *M. platytrix* showed weak hybridization. No hybridization was detected for *M. pahari,* *R. rattus.* *M. aureus,* *G. gerbillus* and *D. melanogaster.*

The observed differences in the Southern blot banding pattern could reflect simple restriction site polymorphism, due to single base changes, or could reflect different genomic locations. To distinguish between these two possibilities we have studied the organization of DNA at a specific locus de-

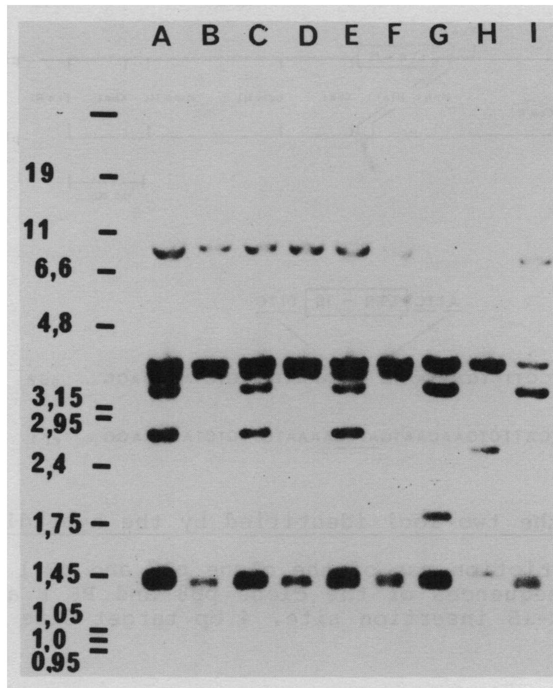

Fig. 3
Polymorphism of the B8F3 locus
Liver DNAs digested with *Eco* RI were analyzed by Southern blotting and hybridized to ^{32}P labeled pB8F3 DNA. A) 129/J, B) C57Bl/6, C) DBA, D) Balb/C, E) C3H, F) *M. m. musculus*, G) *M. m. spicelegus*, H) *M. m. molossinus*, I) *M. m. castaneus.*

fined by a DNA fragment located in the 3' end flanking region of the LTR-IS clone pB8 (Fig. 1).

Southern blots of Eco RI digested DNA hybridized to B8F3 probe revealed differences in the size and number of fragments between different mouse strains (Fig. 3). One explanation for the observed differences in the fragment number could be the presence or absence of LTR-IS elements in the loci defined by B8F3 probe. To verify this explanation the Eco RI 3.3 kb fragment identified by the B8F3 probe in 129/J mouse DNA (the locus displaying the most variation) was cloned into lambda phage NM 641 vector and the resulting clone (pF1) was characterized by restriction mapping and sequencing. This sequence was then compared to the sequence of the original clone pB8 already known to contain LTR-IS element (Fig. 4).

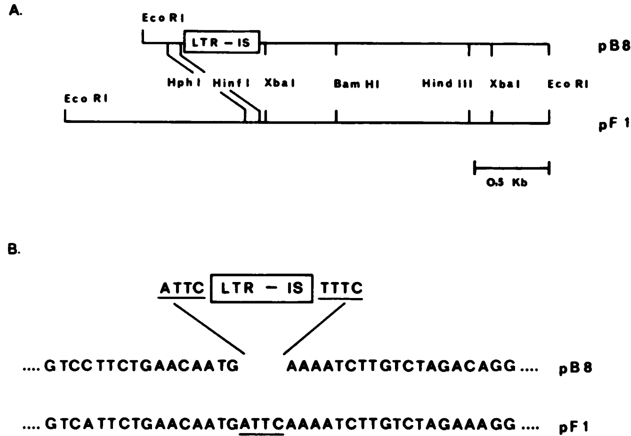

Fig. 4
Comparison of the two loci identified by the hybridization to the pB8F3 DNA
A) Partial restriction map of the clone pB8 and pF 1
B) Nucleotide sequences of the clone pB8 and PF 1 around the presumptive LTR-IS insertion site. 4 bp target site is underlined.

It is well established that transposable elements and retroviral proviruses generate short direct repeats of the host cell sequences at the insertion site of the element (14). Such target site duplications of 4 bp are characteristic for all sequenced LTR-IS clones (1). For the pB8 clone is this sequence ATTC-(LTR-IS)-TTTC (the one bp difference due probably to a point mutation). Sequence analysis allowed us to identify the ATTC sequence insertion site in the clone pF 1 (Fig. 4b). The sequence homology of the two clones starts 100 bp upstream from the LTR-IS insertion site and continues for at least 400 bp 3' direction (sequencing data, not shown). Restriction sites conservation (Fig. 4a) suggests that the homology encompasses the whole 3' ends of the clones. We conclude that the B8 locus was generated by an insertion of LTR-IS element into the sequence defined by the B8F3 probe.

DISCUSSION

LTR-IS elements are present in multiple copies in the genomes of various inbred strains, feral subspecies of *M. musculus* and

two other mouse species (*M. cooki* and *M. caroli*) of diverse geographic origin. This wide distribution of LTR-IS sequences suggests that they were present early in the evolution of the mouse probably before speciation and argues against recent insertions in the germ lines.

Sequence analysis (1) provided as yet inconclusive evidence that LTR-IS elements are mobile in the genome. Our results which describe the insertion of LTR-IS element into the B8F3 locus (Fig. 4) is the most convincing evidence to date to support this mobility. The conservation of the majority of the insertion sites among *M. musculus subspecies* (Fig. 2 and unpublished data) implies, however, that the LTR-IS loci are relatively stable within the genome. Thus, if LTR-IS elements are mobile they do not rearrange frequently, or alternatively they have lost their mobility during evolution.

Nothing is known about the mechanism of LTR-IS element rearrangement. Direct DNA transposition has not yet been proven in vertebrates but another possibility is that they might have arisen from complete provirus-like structures and were mobile through RNA-intermediates.

The data presented in this paper support further the notion that the large family of LTR-IS elements should be considered as source of genetic variability in the mouse genome and as evolutionary intermediate in retroviral evolution.

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