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Transposition of a long member of the L1 major interspersed DNA family into the mouse beta globin gene locus

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ABSTRACT

A long member of the highly repeated long interspersed DNA family L1Md (for L1 in Mus domesticus) has integrated by transposition into a target site which lies between the two adult beta globin genes of mouse. DNA hybridization and nucleotide sequence analysis show that this target site, which is part of the single copy DNA flanking the globin genes, is interrupted by the L1 element in one chromosome but is uninterrupted in both allelic and ancestral chromosomes. Other large DNA rearrangements of the region between the two adult beta globin genes are also associated with these allelic chromosomes, and include insertions or deletions of both single copy DNA and simple and complex repetitive DNA. This has caused extensive reorganization of this intergenic region. However, the distance between the two genes flanking this region remains conserved, suggesting that the spacing of the globin genes may be subject to conservative selection.

INTRODUCTION

A current problem of interest in genetics is to determine the mechanisms by which intergenic DNA rearrangements might alter the expression of nearby genes. In this regard we have examined the kinds of DNA rearrangements that have taken place within a typical gene cluster, the beta globin locus of the common laboratory mouse, Mus domesticus.

The molecular cloning and analysis of the globin gene cluster of two mouse haplotypes, Hbb^d and Hbb^s, has been described previously (1,2). Comparison of these allelic gene clusters has revealed that they are similar in structure over most of their length, with the exceptions of an insertion of a repeated sequence nearby an adult-type Hbb^d pseudogene (3), and a region of uncharacterized structural dissimilarities lying between the two adult globin gene loci (2). Here we report that the intergenic region between the two adult gene loci contains

evidence of extensive sequence reorganization. The events which comprise this reorganization include both a transposition of a highly repeated mouse sequence into the region, and separate insertions or deletions of large blocks of repetitive as well as single copy intergenic DNA. Interestingly, these events have left the intergenic distance between the adult genes conserved.

The transposition event we describe here involves a member of a major family of mouse long interspersed sequences called L1Md (for L1 in Mus domesticus) (4,5). L1, which is repeated roughly 100,000 times in mouse (6), is native not only to mouse but to all mammals (5). There is a large body of indirect evidence supporting the hypothesis that L1 and other highly repeated DNA families are capable of sequence transposition. Such evidence consists of the presence of short direct repeats surrounding the putative inserted element (7), or the presence of elements intercalated within other repetitive sequences (8,9,10,3,11) or within a subset of multiply-duplicated gene loci (12). However, direct evidence documenting a transposition event is supplied only by a comparison of allelic variants of a single target region which shows one of the allelic sites interrupted by the mobile element (13,14,15,16). By the comparison of allelic chromosomes this report presents direct evidence for transposition of a member of a long interspersed family in the mouse. Recently, a short Alu-like sequence has been documented to have inserted by DNA transposition into an allele of the rat prolactin gene locus by a similar analysis (13), and the L1 family has been shown to be mobile via transposition into the canine c-myc locus (17).

METHODS

Nomenclature

The adult globin genes in this study are designated by the nomenclature of Brown et al. (18), as modified by the Mouse Globin Nomenclature Meeting, Jackson Laboratory, Maine, 1984. The 5'-ward adult beta globin gene locus is Hbb-b1, and the 3'-ward adult beta globin gene locus is Hbb-b2. In Hbb^d, the 5'-ward gene is $Hbb\text{-}b1^d$, or βdmaj in the old nomenclature, while the 3'-ward gene is $Hbb\text{-}b2^d$, or βdmin in the old nomenclature (1). Their Hbb^s

haplotype alleles are Hbb-b1^s, or βs in the old nomenclature, and Hbb-b2^s, or βt in the old nomenclature (2).

The presence of L1Md sequences in the Hbb^d globin locus has been described elsewhere (19,3). In that study eight distinct L1Md sequences were identified and labeled L1Md-1 through L1Md-8. Of these sequences, L1Md-5 and L1Md-6 comprise a part of the long region of Hbb^d repetitive sequences which we show is missing from Hbb^s. The long L1Md element which has inserted via transposition into the Hbb^s adult globin region is designated here as L1Md-9.

Cloning and hybridization analysis

The genomic clones containing the adult beta globin genes of Hbb^d (CA11 and CE14) and Hbb^s (BA4 and BA1) have been described previously (1,2). An EcoRI-HindIII 6 kb subfragment of CE14 containing the Hbb-b2^d gene with 5'-ward flanking DNA was subcloned into pBR322 to give clone pHE117. An M13 subclone of the 5'-ward 1.35 kb EcoRI subfragment of CE14 was described previously (19). The EcoRI 10.7 kb subfragment of BA4 containing the Hbb-b1^s gene with flanking DNA was cloned into pBR322 to give clone pHE402. This clone was a gift of Dr. Stephen Hardies.

The globin gene-flanking DNA probes "400", "900" and "A" were prepared by purification of restriction fragments via agarose electrophoresis and butanol DNA extraction (20). "900" was further purified by subcloning into M13-mp18, and double-stranded insert DNA was used as probe. The repetitive element probes "3" and "5" were described elsewhere (5). Nick translations were done as described elsewhere (5). Restriction digestions, southern blotting, and high and low stringency hybridizations were done as described elsewhere (5). All work was carried out under the prevailing NIH guidelines for recombinant DNA research.

DNA sequence analysis

The HpaI-BamHI 980 bp subfragment of BA4 containing the 5' end of L1Md-9 was cloned into M13-mp11 and portions were sequenced on both strands by a modified Sanger procedure (21). Sequence analysis used homology search programs as described elsewhere (5). The time of divergence of the Hbb^s and Hbb^d sequences was determined by the method of Miyata (22). The small direct repeats flanking L1Md-9 in Hbb^s and their single

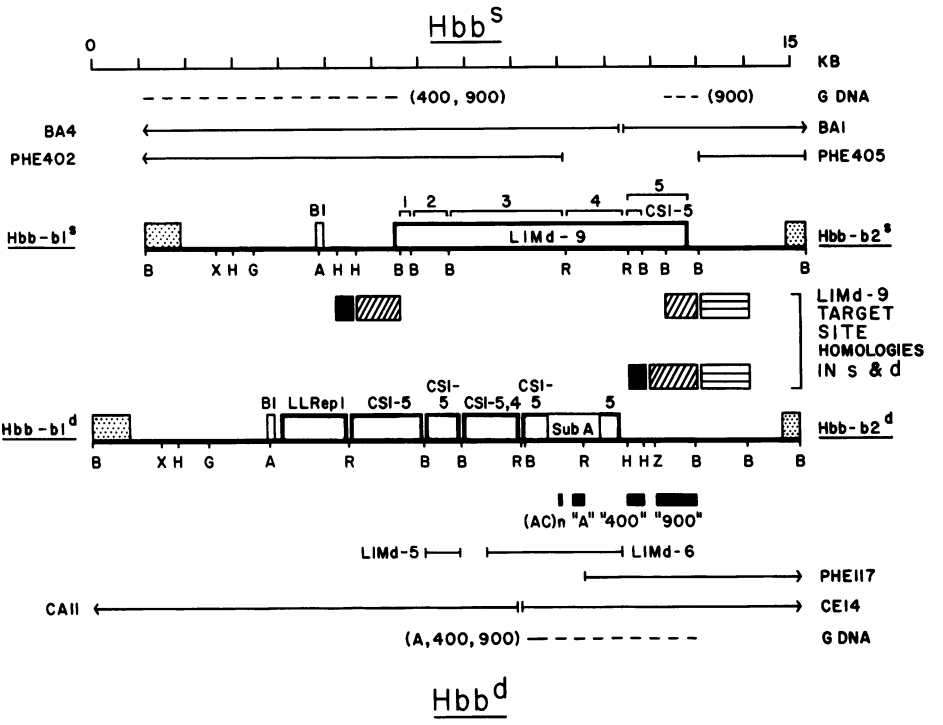

Figure 1. The intergenic DNA sequences between the Hbb-bl and Hbb-b2 adult beta globin genes. The region between the adult beta globin genes is shown for both the Hbb^d and Hbb^s haplotypes. The thickly outlined white boxes on both maps are repeated DNA sequences that are not shared between the haplotypes. The white box on the Hbb^s map ("L1Md-9") represents a long member of the repetitive L1Md family. Its subfragments are diagrammed "1", "2", "3", "4", "CS1-5", and "5", as defined elsewhere (5). The white boxes on the Hbb^d map represent portions of a different long repetitive region. Some of its subfragments are labeled "4", "CS1-5", or "5", which indicate to which portion of L1Md they hybridize. There are 3 to 4 separate repetitions of the small "CS1-5" sequence and thus 3 to 4 short L1Md elements in this structure. "L1Md-5" and "L1Md-6" are earlier designations (19) for two of them. The 5' portion of this region contains the repeated sequence LLrepl (24). Thick shaded boxes between the maps represent a subset of restriction fragments which hybridize between Hbb^s and Hbb^d. Like shading signifies homology between the haplotypes. Two of these regions (the black and diagonally striped boxes) were identified with the Hbb^d region probes "400" and "900" (shown below the Hbb^d map), while a third homologous region (the horizontally striped box) was determined previously (2). Probe "A" was prepared from a single copy sequence lying within the Hbb^d long repetitive region. This sequence, previously denoted "substitution A" ("Sub A"), is absent from Hbb^s at this position (2). "(AC)n" is a short stretch of alternating AC nucleotides identified with synthesized (AC)n probe (not shown).

Dashed lines represent the sizes and locations of restriction fragments of total genomic DNA ("G DNA") which hybridize in Figures 2 and 3 with the probes indicated in parentheses beside each line. Solid lines below and above the maps designate the extent of cloned regions. The thinly outlined white boxes on both maps represent shared B1 repetitive elements. The stippled boxes on both maps represent the two adult globin gene loci. Restriction sites: B=BamHI, R=EcoRI, H=HpaI, A=AvaI, X=XbaI, G=BglII, Z=HaeIII. H, A, X, and G sites have been mapped for both alleles only in the first 4kb, thus are not shown elsewhere except for the H sites that define the "400" region and its Hbb^s homolog. The single Z site shown is the 5' endpoint of the "900" region.

counterpart in Hbb^d were not included in this analysis, due to their redundant alignment.

RESULTS

Alteration of the repetitive DNA profile in the region between the adult beta globin genes

Mapping and hybridization studies (19 and data not shown) indicated that both repetitive and single copy DNA sequences lay between Hbb-b1, the 5'-ward adult beta globin gene locus, and Hbb-b2, the 3'-ward adult beta globin gene locus (Figure 1). While the region between the two adult genes seemed in both haplotypes to consist of a stretch of single copy sequence within which a single complex of repetitive sequences resides the nature of the repetitive sequences in the two haplotypes appeared to tbe quite different (Figure 1). To determine the nature of these differences the s- and d- repetitive regions were compared to each other by DNA hybridization. Probes comprising most of the 5' half of the Hbb^s repetitive region fail to hybridize to the Hbb^d repetitive region (probe "3", Figure 2 and probe "2", data not shown), indicating that the repetitive sequences of the Hbb^s and Hbb^d adult globin region are different in structure and are unique to each haplotype.

The long Hbb^s repetitive region has previously been shown (5) to be a single member of the long repetitive element family called L1Md (19, 5). Here we call this member L1Md-9. Its length and structure is the same as that of the canonical long structure of the L1Md family (5). However, both long and short members of this family exist. The short elements are usually truncated at

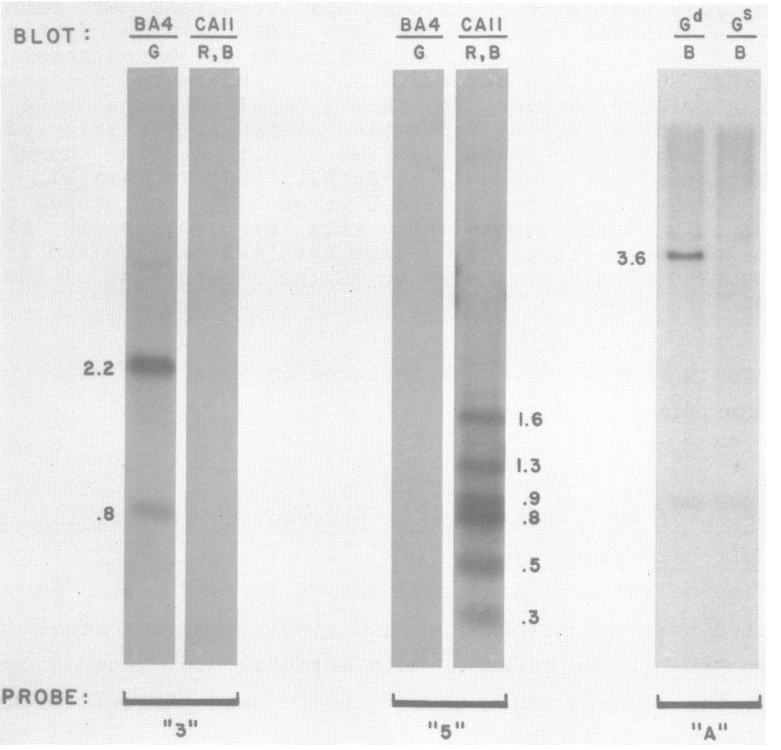

Figure 2. The s- and d- haplotype long repetitive sequences show dissimilar hybridization patterns. Shown are southern blot hybridizations. Sizes of bands are given in kilobases. "G", "R", and "B" indicate cleavage of DNA samples with BglII, EcoRI, or BamHI, respectively. Lanes 1 and 2 show the hybridization of the allelic Hbb^s clone BA4 (Figure 1) and Hbb^d clone CA11 (Figure 1) to a probe containing a region of L1Md-9 (region "3", Figure 1). Lanes 3 and 4 show the hybridization of BA4 and CA11 to a probe representing the 3' 1.3kb of the L1Md structure (region "5", Figure 1). Lanes 5 and 6 show the hybridization of Mus domesticus Hbb^d and Hbb^s total genomic DNA ("G^d" and "G^s") to the single copy probe "A" (Figure 1), which lies within the Hbb^d repetitive region. The band in lane 5 corresponds to the region shown by a dashed line below the Hbb^d map in Figure 1. The faint background smears and bands in lanes 5 and 6 are caused by contamination introduced during agarose gel purification of probe "A" from a clone containing both "A" and L1Md DNA fragments.

random distances from the 3' end of the canonical L1Md structure (23,19) and can also apparently comprise larger clustered and scrambled arrangements of repetitive DNA (6). The Hbb^d repetitive region, unlike L1Md-9, appears to contain a cluster of short and

multiply repeated L1Md members. For example, a probe with homology only to the 3' 1.3kb of L1Md-9 detects multiple restriction fragments (.8kb, 1.3kb, and 1.6kb) within the Hbb^d repetitive region (Figure 2, lanes 2-3; the .3kb, .5kb and .9kb bands lie elsewhere, within an L1Md member 5' to Hbb-b1). The exact number of L1Md elements in this region is not known, but since it contains three to four separate repetitions of a 300bp subfragment of the L1Md structure (region "CS1-5", Figure 1), it is likely that there are three to four distinct L1Md elements making up this cluster.

The terminal short L1Md element in the Hbb^d cluster of repetitive elements contains an apparent insertion of 1.1kb first identified by electron microscopy and called "substitution A" (see "Sub A", Figure 1) (2). A probe prepared from this inserted sequence (probe "A", Figure 1) hybridizes to a single restriction fragment in Hbb^d total genomic DNA (Figure 2, lane 5), indicating that "substitution A" contains single copy DNA. Most of the remainder of "substitution A" does not hybridize to nick-translated genomic DNA probe (data not shown), which suggests that it too is single copy. The genomic subfragment that hybridizes with probe "A" in Hbb^d is the same size as the "A"-homologous fragment in the cloned globin locus (Figure 1), indicating that the intercalation of this sequence within an L1Md member is not a cloning artifact. This single copy sequence is entirely absent from the Hbb^s genome (Figure 2, lane 6).

In addition to containing at least three truncated L1Md elements the Hbb^d repetitive cluster contains two other classes of repeated sequence. One is a simple (AC)n sequence (Figure 1), and the other (Figure 1) is a member of a second complex repetitive family called LLRep1 (24). The region containing the short LLRep1 sequence (Figure 1) does not hybridize to L1Md regions 2-5 (not shown). However, its homology (as well as that of LLRep1 itself) to the 5'-most 200bp of L1Md (region 1, Figure 1) has not been investigated.

Precise mapping of the locations of the repetitive sequences between the adult genes in the Hbb^d and Hbb^s haplotypes indicates that they are not in exactly the same position relative to the adult globin genes (Figure 1). The long L1Md-9 element in Hbb^s

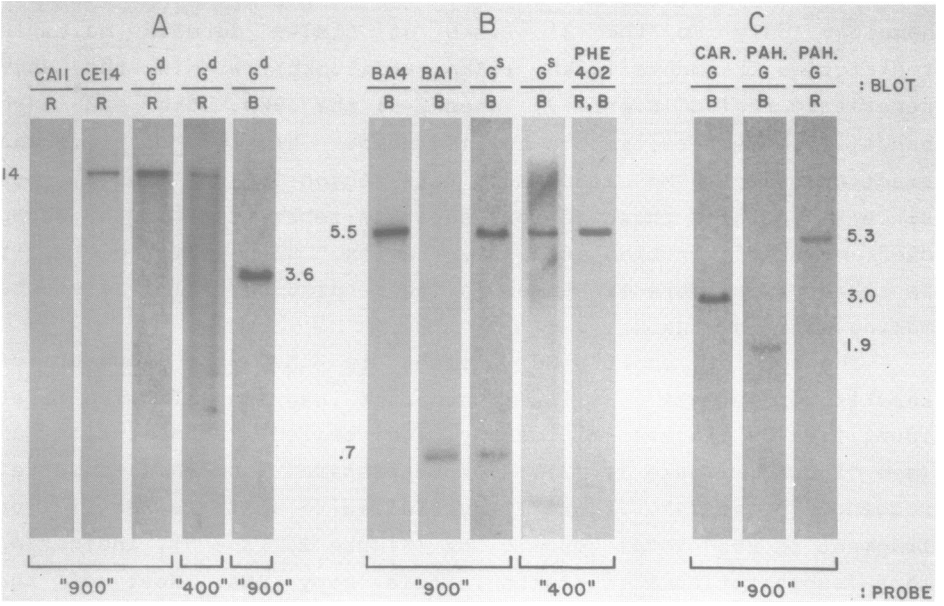

Figure 3. A single Hbb^d sequence hybridizes to an interrupted Hbb^s counterpart which flanks L1Md-9. Shown are southern blot hybridizations. Band sizes are in kilobases. "R" and "B" designate cleavage of the DNA samples with EcoRI or BamHI, respectively. The probes "400" and "900" represent regions containing the 5' flank and target site of the uninterrupted Hbb^d target site region, respectively (Figure 1). These probes were hybridized to either cloned DNA or total genomic DNA or both, and they detect the target site region in Hbb^d (Panel A), in Hbb^s (Panel B), and in Mus caroli and Mus pahari (panel C). The locations of the Hbb^d clones CA11 and CE14 and their orthologous Hbb^s clones BA4 and BA1 are shown in Figure 1. The pHE402 digest in lane 10 is equivalent to that of BA4 cut with BamHI (Figure 1). "G^d" and "G^s" signify total Hbb^d or Hbb^s genomic DNA, respectively. "CAR. G" and "PAH. G" signify total genomic DNA of Mus caroli and Mus pahari, respectively. The genomic and cloned DNA fragments shown for Hbb^d and Hbb^s correspond to the regions shown by dashed lines in Figure 1. Probe "400" is contaminated as described for probe "A" (Figure 2), thus causing faint repetitive DNA signals in lanes 4 and 9. All the genomic DNA lanes shown used 4 micrograms DNA. Because the cross-species genomic probings (panel C) gave weaker signal strengths, these probings were repeated with 8 micrograms of DNA per lane (not shown) to confirm that only one signal was present in each lane.

lies within 2.1 kb of Hbb-b2^s, but is missing from the corresponding region in the Hbb^d chromosome. In turn, the 7.2kb of repetitive sequence in Hbb^d is located roughly 3.6kb 5'-ward

of Hbb-b2^d and is missing from the corresponding region in Hbb^s.

Interruption of an intergenic sequence by the L1Md-9 element

The region where L1Md-9 lies in Hbb^s is present in Hbb^d as a 900 bp long restriction fragment located roughly 1.8 kb 5'-ward of the Hbb-b2^d gene (Figure 1). When this 900 bp region is used as a probe ("900", Figure 1) to total Hbb^d genomic DNA or globin region clones, it hybridizes (Figure 3, panel A) only to the single Hbb^d sequence (3.6kb) from which the probe originated (Figure 1). However, it hybridizes to two regions within total Hbb^s genomic DNA or globin region clones (Figure 3, panel B). One of these regions (.7kb) is located the same distance from the adult b2 gene in Hbb^s as is the 900bp fragment from the b2 gene in Hbb^d, while the other (5.5kb) is displaced 6.3kb 5'-ward of the location of the 900bp sequence (Figure 1). Also, a second Hbb^d probe taken from just 5' to the 900bp sequence, called "400", hybridizes to a single sequence in either haplotype (Figure 3, panels A and B), but in Hbb^s this sequence is also displaced 6.3kb 5'-ward (Figure 1). The 5'-ward displaced "400"- and "900"- homologous sequences abut the 5' boundary of the 6.3 kb long L1Md-9 element (Figure 1), which suggests that L1Md-9 has simply interrupted the "900"-homologous sequence by inserting into it, thus displacing its 5' half.

That this apparent insertion of L1Md-9 into Hbb^s is not rather a precise deletion of L1Md-9 from Hbb^d can be tested by determining which arrangement is ancestral to the other. This was done by examining whether the target site is interrupted by L1Md-9 or uninterrupted in animals, such as Mus caroli or Mus pahari, which diverged from Mus domesticus (25,26) before our estimation of the time of the L1Md-9 rearrangement (see DISCUSSION). When the "900" probe representing the uninterrupted target site region is hybridized to various restriction digests of Mus caroli or Mus pahari total genomic DNA (Figure 3, panel C) just one homologous genomic fragment is seen rather than two, and the size of the fragment in each case (1.9kb, 3.0kb, or 5.9kb) is too small to contain the 6.3kb L1Md-9 element. This indicates that the ancestral target site region contained no L1Md-9 sequence and therefore that the L1Md-9 sequence seen in Hbb^s resulted from an insertion. The poorer homology that these

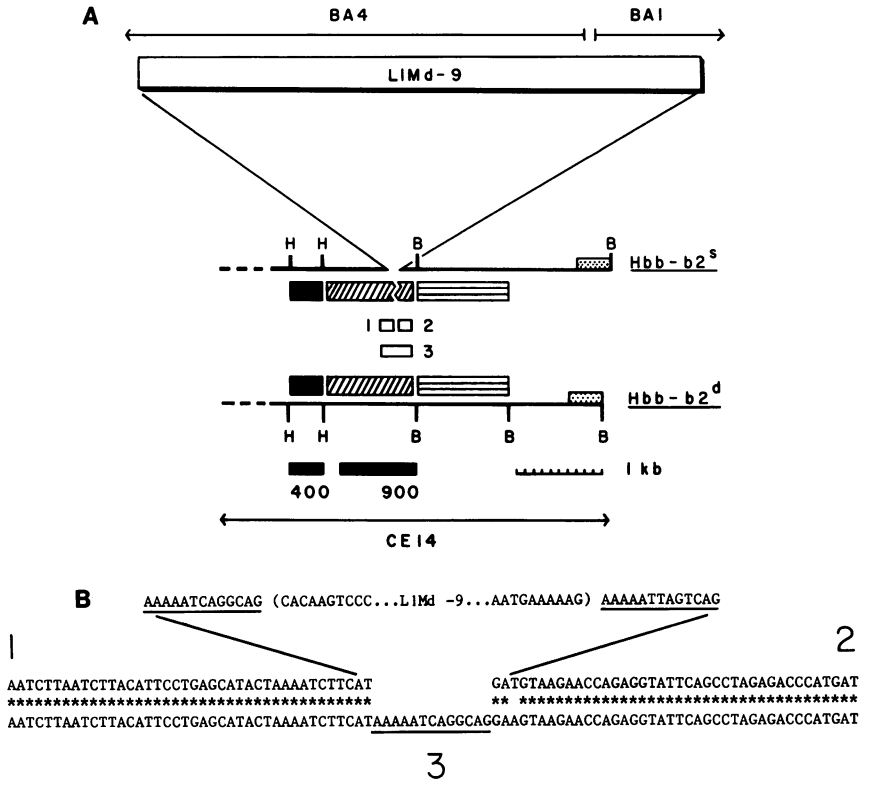

Figure 4. DNA transposition of the long L1Md-9 element. Panel A diagrams the globin-region target site for both s- and d-haplotype chromosomes, the inserted Hbb^s L1Md-9 element, and the areas ("1", "2", and "3") for which nucleotide sequence is shown in Panel B. The thick boxes which diagram homologous restriction fragments are shown as in Figure 1. Locations of probes are shown as in Figure 1. Sequence "1" is from positions 90 bp to 153 bp 5'-ward of the 3' BamHI site of the HpaI-BamHI 980 fragment in the Hbb^s clone BA4 (Figure 1). The location of sequence "2" is from positions 182 bp to 245 bp 5'-ward of the 5' BamHI site of the 2.35 kb BamHI fragment shown in Hbb^s clone pHE405 (Figure 1). The location of sequence "3" is from positions 185 bp to 278 bp 5'-ward of the 5' BamHI site of the 5'-ward 1.1 kb BamHI fragment shown in Hbb^d clone pHE117 (Figure 1).

Insertion of the L1Md element has led to the generation of a 13 nucleotide imperfect direct repeat of the Hbb^s target sequence, whereas this sequence is seen only once in Hbb^d. Panel B shows these allelic target sequences (which are underlined) as well as 40 nucleotides of both 5'-ward and 3'-ward flanking sequence (labeled "1", "2", and "3" as in panel A). Also shown are 10 nucleotides of both the 5' and 3' ends of the L1Md-9 element in Hbb^s (in parentheses). Sequence "1" was generated as described in the methods section. Sequences "2" and "3" were generated by Shyman et al. (32).

species' genomic DNA samples exhibit to the 900bp target site probe than that shown by genomic DNA from either domesticus haplotype (Figure 3) is consistent with the divergence of Mus caroli and Mus pahari from Mus domesticus prior to the divergence of Hbb^s from Hbb^d.

Transposition of the L1Md-9 element

There are distinct restriction site similarities between the allelic counterparts of the target region for L1Md-9 (Figure 4, panel A). This suggested that these allelic sequences were quite homologous and would thus be easy to align at the nucleotide level in order to trace the effects of the L1Md-9 insertion. Subsequent alignment of the nucleotide sequences bordering L1Md-9 with their homologous sequences in Hbb^d documents that L1Md-9 is surrounded by a 13bp imperfect direct repeat of a sequence present only once in the Hbb^d target region (Figure 4, panel B). The presence of this direct repeat, apparently generated by duplication of the single allelic sequence, is consistent with the hypothesis that L1Md-9 entered the globin locus via transposition. Currently the 13 base pair direct repeat is imperfect due to the presence of two nucleotide substitutions, which were presumably introduced during or after the transposition event.

DISCUSSION

We have shown that the majority of L1 sequences in mouse (19) and other mammals (5) are short pseudogene copies of an as yet unisolated protein-encoding gene (27,5). It has been suggested that this highly repeated family undergoes concerted evolution at least in part by periodic transposition of new short or long L1 sequences into the genome (25,28). In addition we have argued that there must be an ongoing deletion mechanism which removes old sequences (28). Recently a short L1 member was shown to have undergone transposition (17). Here we show that a long and potentially full-length L1 member has also undergone transposition, and we have identified other short L1 DNA sequences whose absence in mouse from the Hbb^s haplotype globin locus may well be due to the deletion mechanism mentioned above.

It is apparent from evolutionary analysis of the aligned target site DNA of the s- and d- chromosomes (see METHODS) that

the divergence of the s- and d- target sequences, and thus the sequence rearrangement involving L1Md-9, happened quite recently in evolutionary history. The total mismatch of these sequences between the s- and d- haplotypes is only 1 out of 80, or 1.2%. This corresponds to a time of divergence roughly 1.2 million years (Myr) ago. The L1Md-9 rearrangement has occurred since then if we assume that gene conversion cannot have homologized these target sequences after their interruption by L1Md-9. There are two models we have considered which explain the absence of L1Md-9 in the d-haplotype and its presence in the s-haplotype. The first simply presumes that L1Md-9 transposed into the s-haplotype globin locus after the s/d split. The other model we have considered also presumes the L1Md-9 transposition, but dates it earlier -- before the s/d split and after the split of *domesticus* from the other mouse species studied here. In this model both s- and d- haplotypes would have originally possessed the L1Md-9 element. In such a model, the L1Md-9 sequence would have precisely deleted from Hbb^d via homologous recombination within its flanking 13bp direct repeats. There is some precedence for such deletions, since in *E. coli* deletions have been shown to occur between directly repeated sequences of five or more base pairs (29), and a similar association of short direct repeats with deletion endpoints has been proposed for eukaryotic cells (30).

The relatively recent time of the L1Md-9 rearrangement corresponds well with the estimated 1.7 Myr half-life for particular L1 sequences during their concerted evolution (28). This is consistent with the model that the rapid rate of L1 sequence turnover involves actual transpositions and deletions of these sequences, not just sequence conversion.

It seems likely that the periodic transposition and deletion of members of this highly repeated sequence family is capable of modulating gene expression. This could occur in two ways. One would be by introducing or removing L1-specific regulatory sequences that activate or suppress the transcription of nearby genes. An unusual feature of L1 in this regard is that it can transpose different structural subsets of its full-length sequence, which presumably could cause different regulatory effects. As an example of this model, it has been proposed that a

truncated dog L1 sequence may have enhanced the transcription of a nearby c-myc gene (17). Regarding the mouse beta globin locus, the s- and d- alleles of the adult genes appear to be regulated in a similar fashion (31) even though markedly different L1Md structures lie within their vicinity.

The second way in which L1 transpositions or deletions could affect gene expression would be by altering the distance between these genes and other regulatory elements. Interestingly, the overall distance between Hbb-b1 and Hbb-b2 is nearly the same for both the s- and d- haplotypes even though nearly 13 kilobases of DNA has been inserted or deleted within the intergenic region, involving four or five L1Md elements as well as other repeated and single copy DNA sequences. In fact, given the estimated rate of L1 sequence turnover (28) and assuming this to be totally due to transposition and deletion events one would expect the spacing between the adult genes to be different. This raises the possibility of natural selection for a particular gene distance. The examination of the intergenic distance between the adult beta globin genes of related mouse species should reveal whether or not the retention of the 14-15 kb distance seen in this study is a consistent feature of rodent beta globin loci.

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REFERENCES

1. Jahn, C.L., Hutchison, C.A. III, Phillips, S.J., Weaver, S., Haigwood, N.L., Voliva, C.F. and Edgell, M.H. (1980) Cell 21, 159-168.
2. Weaver, S., Comer, M.B., Jahn, C.J., Hutchison, C.A. III and Edgell, M.H. (1981) Cell 24, 403-411.
3. Voliva, C.F., Martin, S.L., Hutchison, C.A. III and Edgell, M.H. (1984) J. Mol. Biol. 178, 795-813.
4. Burton, F.H., Voliva, C.F., Edgell, M.H. and Hutchison, C.A. III (1983) DNA 2, 82.
5. Burton, F.H., Loeb, D.D., Voliva, C.F., Martin, S.L., Edgell, M.H. and Hutchison, C.A. III (1985), manuscript submitted.
6. Gebhard, W. and Zachau, H.G. (1983) J. Mol. Biol. 170, 255-270.
7. Jagadeeswaran, P., Forget, B.G. and Weissman, S.M. (1981) Cell 26, 141-142.
8. Grimaldi, G. and Singer, M.F. (1982) Proc. Natl. Acad. Sci.

USA 79, 1497-1500.
9. Kominami, R., Muramatsu, M. and Morikawi, K. (1983) Nature 301, 87-89.
10. Thayer, R.E. and Singer, M.F. (1983) Mol. Cell. Biol. 3, 967-973.
11. Nomiyama, H., Tsuzuki, T., Wakasugi, S., Fukuda, M. and Shimada, K. (1984) Nucl. Acids Res. 12, 5225-5234.
12. Kress, M., Barra, Y., Seidman, J.G., Khoury, G. and Jay, G. (1984) Science 226, 974-977.
13. Schuler, L.A., Weber, J.L. and Gorski, J. (1983) Nature 305, 159-160.
14. Dunsmuir, P., Brorien, W.J., Simon, M.A. and Rubin, G.M. (1980) Cell 21, 576-579.
15. Hishinuma, F., DeBona, P.J., Astrin, S. and Skalka, A.M. (1981) Cell 23, 155-164.
16. Majors, J.E., Swanstrom, R., DeLorbe, W.J., Payne, G.S., Hughes, S.H., Ortiz, S., Quintrel, N., Bishop, J.M. and Varmus, H.E. (1980) Cold Spring Harbor Symp. Quant. Biol. 45, 731-738.
17. Katzir, N., Rechavi, G., Cohen, J.B., Unger, T., Simoni, F., Segal, S., Cohen, J. and Givol, D. (1985) Proc. Natl. Acad. Sci. USA 82, 1054-1058.
18. Brown, B.A., Padgett, R.W., Hardies, S.C., Hutchison, C.A. III and Edgell, M.H. (1982) Proc. Natl. Acad. Sci. USA 79, 2753-2757.
19. Voliva, C.F., Jahn, C.L., Comer, M.B., Edgell, M.H. and Hutchison, C.A. III (1983) Nucl. Acids Res. 11, 8847-8859.
20. Langridge, J., Langridge, P. and Berquist, P.L. (1980) Anal. Biochem. 103, 264-271.
21. Bankier, A.T. and Barrell, B.G. (1983) in Techniques in Nucleic Acid Biochemistry, Elsevier Press, Ireland.
22. Miyata, T., Yasunaga, T. and Nishida, T. (1980) Proc. Natl. Acad. Sci. USA 77, 7328-7332.
23. Fanning, T.G. (1983) Nucl. Acids Res. 11, 5073-5091.
24. Heller, D., Jackson, M. and Leinwand, L. (1984) J. Mol. Biol. 173, 419-436.
25. Martin, S.L., Voliva, C.F., Hardies, S.C., Edgell, M.H. and Hutchison, C.A. III (1985) Mol. Biol. Evol. 2, 127-140.
26. Foster, H.L. (1981) in The Mouse in Biomedical Research, Small, J.D. and Fox, J.G. Eds., Vol I, pp 18-22, Academic Press, New York.
27. Martin, S.L., Voliva, C.F., Burton, F.H., Edgell, M.H. and Hutchison, C.A. III (1984) Proc. Natl. Acad. Sci. USA 81, 2308-2312.
28. Hardies, S.C., Martin, S.L., Voliva, C.F., Hutchison, C.A. III and Edgell, M.H. (1985), manuscript submitted.
29. Farabaugh, P.J., Schmeissner, U., Hofer, M. and Miller, J.H. (1978) J. Mol. Biol. 126, 847-863.
30. Efstratiadis, A., Posakony, J.W., Maniatis, T., Lawn, R.M., O'Connell, C. , Spritz, R.A., DeRiel, J.K., Forget, B.G., Weissman, S.M., Slightom, J.L., Blechl, A.E., Smithies, O., Baralle, F.E., Shoulders, C.C. and Proudfoot, N.J. (1980) Cell 21, 653-668.
31. Wawrzyniak, C.J., Lewis, S.E. and Popp, R.A. (1985) in Fourth Conference on Hemoglobin Switching, Stamatoyannopoulos, G. and Nienhuis, A.W. Eds., Alan R. Liss, in press.
32. Shyman, S. and Weaver, S. (1985) Nucl. Acids Res., accompanying manuscript.