Features of the *mammal mar1* transposons in the human, sheep, cow, and mouse genomes and implications for their evolution

Marie-Véronique Demattei,¹ Corrine Augé-Gouillou,¹ Nicolas Pollet,² Marie-Hélène Hamelin,¹ Michèle Meunier-Rotival,² Yves Bigot¹

¹I.R.B.I., Groupe d'Etude des Parasites Moléculaires, UPRESA CNRS 6035, Faculté des Sciences, Parc Grandmont, 37200 Tours, France

²INSERM U347 affiliée au CNRS, 80 Rue du Général Leclerc, 94276 Le Kremlin Bicêtre Cedex, France

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Abstract. Mariner-like elements (MLE) belong to the Tc1/*mariner* superfamily of class II transposons. We have analyzed the *mariner* related to the *cecropia* subfamily, and called *mammal mar1*, in four mammalian genomes, *Bos taurus* (Bovidae), *Homo sapiens* (Primata), *Mus musculus* (Rodentia), and *Ovis aries* (Ovidae). Three kinds of MLE sequences were found in all these species: full-length 1.3-kbp elements, shorter elements 80 bp–1.2 kbp, and single inverted terminal repeats (ITRs). All the 1.3-kbp genomic copies sequenced had an open reading frame encoding a transposase interrupted by stop codons or frame shifts. Phylogenetic analysis of the full-length elements suggested at least two distinct populations of *mammal mar1* elements in each species. This was confirmed by using a statistical method that allows defining populations. Finally, the evolutionary origin of the *mammal mar1* elements and the paradoxes are discussed.

Introduction

The mariner-like elements (MLEs) are members of a diverse and taxonomically widespread superfamily of eukaryotic transposons that are present in both vertebrates and invertebrates (for review, see Plasterk et al. 1999). They are small elements about 1280 bp long and are flanked by inverted terminal repeats (ITRs), each 28–30 bp long. These elements move about in genomes by a conservative cut-and-paste transposition, similar to that described for several bacterial insertion sequences (reviewed by Mahillon and Chandler 1998). This process occurs via a DNA intermediate and is catalyzed by a single transposase encoded by the transposon. Whereas MLEs are widespread in the eukaryotes, very few contain an effective open reading frame encoding the transposase, because of the presence of stop codons and frameshifts. All the functional natural elements found so far are related to the *Mos-1* element isolated from *Drosophila mauritiana* (Maruyama et al. 1991).

MLEs were found in mammalian genomes in 1995 when two MLE “species” related to the *cecropia* and *irritans* subfamilies were discovered in the human genome, and named *Hsmar1* and *Hsmar2*, respectively (Augé-Gouillou et al. 1995; Oosumi et al. 1995).

The *Hsmar1* consensus element (Acc# U52077) is 1287 bp long, with 30-bp perfect ITRs, and encodes a 343 amino acid transposase (Morgan 1995). About 200 copies of *Hsmar1*, 2400 copies of 80-bp paired ITR structures, and 4600 copies of single ITRs are believed to be present in the human genome based on the databank analyses. There are *mariner* elements closely related to *Hsmar1* elements in the genomes of primates, sheep, and cows, but they seem to be absent from Scandentia, Dermoptera and Rodentia. This suggested that these MLEs entered mammalian genomes a very long time ago, or on several occasions. Robertson and

Zumpano (1997) propose that the ancestor of the *Hsmar1* in the human genome gave rise to a single lineage of *Hsmar1* elements and was formed about 50 million years (Myr) ago.

The present study focuses on the *Hsmar1* subfamily in four mammalian genomes: *Homo sapiens* (human), *Mus musculus* (mouse), *Ovis aries* (sheep) and *Bos taurus* (cattle). They are called *Hsmar1* (human), *Mmmar1* (mouse), *Oamar1* (sheep) and *Btmarr1* (cattle), and this lineage of elements is referred to as *mammal mar1*. Their consensus sequences, diversity, numbers of MLEs and ITRs, and nucleotide divergence were all investigated by using sequences obtained by polymerase chain reaction (PCR) amplification or extracted from the databanks in order to improve our understanding of their evolution.

Materials and methods

Nucleic acid analysis. Genomic DNAs from *Homo sapiens* (white European), *Bos taurus* (Holstein race), and *Ovis aries* (Ile de France race) were obtained from sperm and lymphocyte samples of one male. *Mus musculus* DNA (Balb-c strain) was obtained from the lymphocytes of males. DNA purifications, digestions with restriction enzymes, agarose gel electrophoresis, and Southern blots were all performed as in Augé-Gouillou et al. (1995). The DNA probes were labeled with [α^{32} P] dATP (ICN products) by primer extension using the Klenow fragment of DNA polymerase I and the oligonucleotides with [γ^{32} P]-ATP and T4 polynucleotide kinase (ICN products and Promega). Hybridizations were performed in 0.5 M Na₂HPO₄-NaH₂PO₄, 7% SDS, incubated overnight at 65°C. The Southern blot membranes (NylonN+, ICN products) were washed with SSC (1× SSC = 0.15 M NaCl, 0.015 M trisodium citrate), at 65°C.

Dot blot estimation of the number of copies of mammal mar1 elements and ITR in genomes. Dot blots of genomic DNA and a mix of full-length *mammal mar1* clones linearized with *EcoRI* (Acc# AJ009227–AJ009236 for human, Acc# AJ009242–AJ009246 for sheep, Acc# AJ009223–AJ009226 plus AJ009247 for cow, Acc# AJ009237–AJ009241 for mouse) were performed on NylonN+ (ICN products). Genomic DNAs from *Escherichia coli* and *Drosophila melanogaster* were used as negative hybridization controls. The concentration of DNA was determined assuming that 1A₂₆₀ = 50 µg/ml. *Hsmar1*, *Oamar1*, *Btmarr1*, and *Mmmar1* clones were used as references for quantification. The amounts of *Hsmar1* elements were quantified by probing the membranes with a mix of five *Hsmar1* DNA fragments (Acc# X84285–X84289) for the human DNA sperm. Similarly, the probes for membranes containing sheep or cow sperm DNA were a mix of six *Oamar1* or *Btmarr1* DNA fragments (Acc# X84290–X84295 or Acc# 84296–X84301). The membranes containing DNA from mouse lymphocytes were probed with a mix of three probes. The amounts of *mammal mar1* ITRs in the mammalian genomes were quantified with a single-stranded oligonucleotide (5'-TATTAGTTGGTGCAAAAGT-3') as probe. Hybridizations were done in 0.5 M Na₂HPO₄-NaH₂PO₄, 7% SDS, overnight at 65°C or at 42°C. The membranes were washed with a final washing in 2× SSC, 0.1% SDS at 65°C or in 6× SSC, 0.1% SDS at 42°C with the oligonucleotide probe. Hybridized DNA was quantified with a Direct Imager (Packard).

Fig. 1. Amplification of *H. sapiens* (lanes 1–3), *M. musculus* (lanes 4–6), *O. aries* (lanes 7–9), and *B. taurus* (lanes 10–12) genomic DNA by using a 20-mer primer corresponding to the ITR of Robertson's *Hsmar1* consensus sequence. Genomic DNA was from sperm (lane 1–3 and 7–12) and from blood (lane 4–6). No differences were observed when DNA from blood was used for *H. sapiens*, *O. aries* and *B. taurus*. **(a)** Amplification products were separated by electrophoresis (1.5% agarose gel), stained with ethidium bromide, and photographed. **(b)** Southern blots of the agarose gel were probed with a mix of five fragments originating from *Hsmar1* clones (Acc# X84285–X84289; lanes 1–3), a mix of six DNA fragments purified from *Oamar1* clones (Acc# X84290–X84295; lanes 7–9) and a mix of six DNA fragments purified from *Btmarr1*-related clones (Acc# 84296–X84301; lanes 10–12). The 17 fragments were mixed to probe the mouse amplification products (lanes 4–6). MgCl_2 concentrations: 1.5 mM (lanes 1, 4, 7, and 10), 2.5 mM (lanes 2, 5, 8, and 11), and 3.5 mM (lanes 3, 6, 9, and 12). The molecular weight markers are in lanes M1 and M2, and the sizes (in kbp) are indicated in the left- and right-hand margins.

PCR amplifications and fragment cloning. Polymerase chain reactions (PCR) were performed on 50 ng genomic DNA. PCR reactions were done in 10 mM Tris-HCl, pH 9/1.5 mM MgCl_2 /50 mM KCl/0.1% TritonX100/150 mM each dNTP/0.1 mM of the oligonucleotide (5'-TATTAGGTTGGTGCAAAAGT-3') in a 100- μl reaction volume with 2 units Taq DNA Polymerase (Promega). Each PCR was carried out in a programmable thermal controller (Perkin-Elmer) for 30 cycles. The cycle was: denaturation at 94°C for 1 min, annealing at 53°C for 1 min, and extension at 72°C for 1 min. At the end of the 30th cycle, the heat denaturation step was omitted, and extension was allowed to proceed at 72°C for 10 min. PCR fragments were eluted from agarose gels with a Gene Clean Kit (BIO 101) and inserted into the pGEM-T vector (Promega).

DNA sequencing reactions. Plasmids were purified and sequenced as previously described (Rabouan et al. 1999). The 25 sequences reported in this paper appear in the DDBJ/EMBL/GenBank sequence database under accession numbers AJ009223–AJ009247. The four consensus sequences reported in this paper, ConsHs, ConsBt, ConsOa, and ConsMm, are deposited in the DDBJ/EMBL/GenBank sequence database with the complete alignments under accession number DS34749.

Sequence analyses. The Infobiogen facilities (Dessen et al. 1990) were used for GenBank searches, extractions, sequence alignments and calculations. GenBank release 114 was used for searches and extractions of MLE sequences. The CpG dinucleotide frequencies were checked by calculating the ratio of observed CpG frequency:expected CpG frequency (CpG obs/exp). For each investigated sequence, the expected CpG frequency was calculated from the G and C contents. Thus, the expected CpG frequency should be: $21 \times 15 = 3.1\%$ for a DNA having a G content of 21% and a C content of 15%.

Statistical treatment. The density ellipses reflecting the features of each MLE population in the mammalian genomes were defined, computed, and drawn from the distribution of CpG obs/exp related to the similarity percentage of each MLE sequence with their consensus. Their shape and area described the distribution in each defined MLE population with a probability equivalent to 95% (black line) and 99% (grey line) confidence limit. Before computing the density ellipse, the bivariate normal distribution was tested for normality (Shapiro-Wilk W test at 0.05 significance; SAS Institute 1995).

Results

Size and number of copies of mammal *mar1* elements. Previous studies on *mammal mar1* elements have revealed a 10–20% di-

vergence between the sequences of mammal species (Robertson and Zumpato 1997). This sequence variation, which also alters restriction enzyme cleavage sites, prevents any study of the pattern of full-length and deleted elements in genomes by Southern blot/hybridization experiments with genomic DNA digests. This was, therefore, done by PCR with the ITR sequence of the consensus sequence, AccN# U52077, to design a primer.

Several PCR products, 0.43–1.3 kbp long, were detected on agarose gels in the four species (Fig. 1a). However, most of them did not hybridize with the *mammal mar1* probes. Their presence in the final PCR products was not owing to a setting deficiency of the optimal amplification conditions. They probably resulted from the presence nearby of single ITRs, which were used as annealing sites during PCR, thus allowing the amplification of these non-MLE fragments. Similar PCR results were obtained with DNA from sperm or lymphocytes from human, cow, and sheep, thus suggesting that there were no differences between these two tissues. This was confirmed with Southern blot/hybridization investigations by using restriction enzymes insensitive to methylated cytosine (data not shown).

Three main forms of *mammal mar1* fragments were identified in the human, sheep, and cow samples: 1.3 kbp, 1.2 kbp, and 0.6 kbp (Fig. 1b). Only the 1.3-kbp form was found in the mouse samples (Fig. 1b, lanes 4 and 5). Sequencing 22 elements (AccN# AJ009223–AJ009233 and AJ009237–AJ009247) confirmed that the 1.3-kbp fragments were full-length transposons. The three 1.2-kbp fragments sequenced from the human genome contained a small deletion (AccN# AJ009234–AJ009236). This suggested that the 0.6-kbp fragments were *Hsmar1* elements with internal deletions.

The numbers of *mammal mar1* elements were obtained by dot blot/hybridization developed with the *mammal mar1* probe. There were about 1000 *mammal mar1* elements in humans, 20–50 in the mouse, 3000 in the sheep, and 3500 in the cow. Similarly, hybridizations performed with the ITR probe indicated that there were 7000, 1000, 10,000, and 10,000 ITRs in the human, mouse, sheep, and cow genomes. As each MLE had two ITRs, there are over 5000 free ITRs in the human genome. This agrees with the presence of 80-bp paired ITR structures and single ITRs (Morgan 1995; Robertson and Zumpato 1997). The excesses of about 900 ITRs in the mouse, 3500 ITRs in the sheep, and 3000 ITRs in the cow suggest that there are also many single ITRs.

Features of mammal *mar1* consensus sequences. The 25 sequences of the full-length and 1.2-kbp mammal *mar1* elements (Acc# AJ009223-AJ009247) from the four species were aligned (the alignment is available under DS34739 from FTP.EBI.AC.UK in directory pub/databases/embl/align). The consensus sequence in each species was deduced, taking into account the nature of the most frequent base at each position. No full-length ORF encoding a transposase was found, because there were 6–8 stop codons at the same positions in the four consensus sequences. These stop codons were due to cytosine-thymidine (C-T) transitions occurring in six CpG dinucleotides (positions 227, 470, 663, 670, 715, and 740), one CpT dinucleotide (position 874), and one CpC dinucleotide (position 989). The four consensus sequences were modified to obtain a putative ORF encoding the transposase, then called *ConsOa*, *ConsMm*, *ConsBt*, and *ConsHs*.

Within-species phylogenies of mammal *mar1* elements. The phylogenetic relationships between mammal *mar1* elements were first analyzed within each species. Two files were set up for each species in order to use the maximum data in the databank. The first contained full-length nucleotide sequences, whereas the second contained nucleotide sequences (about 510 bp long) corresponding to the region encoding the transposase between the two conserved amino acid domains, WVPHEL and YSPDLAP. We used an invertebrate MLE sequence from the entomoparasitic nematode *Heterorhabditis bacteriophora* as outgroup (Grenier et al. 1999). This MLE has a mean nucleotide similarity of 70.5% with the complete U52077 sequence and appeared to be the full-length element most closely related to the mammal *mar1* element in Blast searches performed with the databanks.

Twelve full-length *Hsma1* elements extracted from the human sequences in the Genbank, (called hsG1 to hsG12), and the Robertson's *Hsma1* consensus sequence (Acc# U52077) were included in the first file with the sequences of our seven full-length elements and the *ConsHs* sequence. The two consensus trees obtained were similar, the differences being in the number of bootstrap replications supporting nodes. The second file combined the same elements plus five sequences in the databank (Acc# X85285–X84289, Augé-Gouillou et al. 1995). The two consensus trees obtained with this file had many architectural differences and node values lower than those obtained with the first file. This indicates that the nucleotide sequences corresponding to the region encoding the transposase between the WVPHEL and YSPDLAP domains did not contain enough information to reveal the phylogeny of *Hsma1* elements. A similar result was obtained with *Oama1* sequences, whereas there were no differences between the trees developed from the two files with the *Btma1* sequences.

The architecture and the node values in the tree (Fig. 2a) suggested that *Hsma1* elements formed at least two groups. All the elements obtained in the present work by PCR amplification except for the hs18 element were in the first group, in which the values at nodes indicated that the relationships were well defined (Fig. 2a, elements in the black square). All the sequences extracted from the databank (hsG1 to hsG12) plus the hs18 elements were in the second group (Fig. 2a, elements in the grey square). The low values at nodes indicated that the relationships between these elements were poorly defined. Their location close to the root of the tree, the branch lengths, and the width of their distribution in the tree indicated that they were more divergent than those of the first group. This indicates that the second group of elements is probably older than the first.

Analysis of the tree developed with the *Oama1* elements also suggested two groups (Fig. 2a, elements in the black and grey squares). But one of these group contained only one element, oa1. Two groups might be defined by the phylogenetic analysis of the *Btma1* elements (Fig. 2b, elements in the black and grey squares).

Fig. 2. Consensus trees of the phylogenetic relationships between mammal *mar1* elements from the human (a), cow (b), sheep (c), mouse (d) genomes and resulting from neighbor-joining computations. The number for each hs, bt, oa, and mm sequence corresponds to those in the alignment available under the accession number DS34739 from FTP.EBI.AC.UK in directory pub/databases/embl/align. hsG1 to hsG12 elements were extracted from 12 sequences (Acc# Z95124, AC002460, AF029308, Z81009, AJ239318, AC005090, AC006148, AC004063, AC006199, AC004385, AC006143, and Z98746). *Hbma1* is the *H. bacteriophora* MLE outgroup (Acc# U68392). The trees in a, b and d were developed from the full-length nucleotide sequences of the mammal *mar1* elements. Nucleotide sequences corresponding to the region encoding the transposase between the WVPHEL and YSPDLAP domains were used in c. The groups of related elements in each tree are boxed in black and grey. The values in italics at nodes are the number of times that the branch was present among the 1000 bootstrapped trees. Only nodes having bootstrap values over 20% were kept.

There may be a third group in this species, but the evidence for it was poor because it consisted of only one element, bt12. Finally, phylogenetic analysis of the murine elements also suggested two groups (Fig. 2c), which were well defined by the topology of the tree and the values at nodes.

Between-species phylogeny of mammal *mar1* elements. Analysis of the consensus trees (Fig. 3) confirmed that the *Hsma1* and *Mmma1* elements were divided into two groups. The locations of the oa1 and bt12 elements among the hsG1–hsG12 elements also suggested that there were two groups of *Oama1* elements, and maybe three groups of *Btma1* elements. This consensus tree also revealed that all the elements obtained in the present work by PCR

Fig. 3. Consensus tree of the phylogenetic relationships between mammal *mar1* elements from the human (hs), cow (bt), sheep (oa), mouse (mm) genomes. The numbers for each hs, bt, oa, and mm sequence correspond to those in the alignment already used in Fig. 2. Files containing full-length sequences of mammal *mar1* elements were used for *Hsmar1*, *Oamar1*, and *Mmmar1* elements. For *Btmr1* elements, sequences in the region encoding the transposase between the WVPHEL and YSPDLAP domains were used. The *Hsmar1* and *Mmmar1* elements obtained in the present work by PCR amplification are in black boxes and typed in white, whereas the *Btmr1* are in light grey and *Oamar1* in dark grey boxes. *Hbmar1* is the *H. bacteriophora* MLE outgroup. hsG1 to hsG12 are full-length elements extracted from human sequences found in databanks and typed in grey. The values in italics at nodes indicate the number of times that the branch was present among the 1000 bootstrapped trees. Only nodes with bootstrap values over 20% were kept.

amplification from the human, sheep, cow, and mouse genomes were in the same part of the tree (Fig. 3, elements in the different squares). The values at nodes distributing these elements suggested that the relationships between at least two populations were well defined in each species.

The *Hsmar1* populations in the human genome. Our phylogenetic results suggested that there were at least two populations of mammal *mar1* elements in the four mammalian genomes. However, while the phylogenetic analyses are a good way to represent genetic distances between elements, they are not appropriate for defining populations. Their analysis only allows us to suggest the presence of population. The mammal *mar1* sequences were, therefore, analyzed by mean sequence similarities, GC contents, and dinucleotide frequencies in graph analyses. They confirmed, statistically, the real presence of mammal *mar1* populations in these species. The best criteria for defining populations of mammal *mar1* elements were their CpG obs/exp ratios and their percentage of sequence similarity with the consensus of their own species.

Three sets of data were checked for *Hsmar1* populations in the human genome. The first was in the 10 *Hsmar1* elements obtained in the present work (Acc# AJ009223–AJ009247), the 12 hsG1–hsG12 elements, and the five shorter nucleotide sequences (Acc# X85285–X84289). Two groups of *Hsmar1* elements were distin-

guishable in the graph (Fig. 4a), showing the relationships between the percentages of nucleic acid similarity of each of these *Hsmar1* elements with the *ConsHs* sequence (vertical axis), and their CpG exp/obs ratios (horizontal axis). The first contained the hs16, hs17, and hs19–hs22 elements, whereas the 22 others were in the second. These groups had already been observed in our phylogenetic analyses (Fig. 2a). The density ellipses reflecting the features of each of these two groups were drawn with a probability equivalent to 95% (Fig. 4a, black line) and 99% (Fig. 4a, grey line) confidence limits. The absence of overlapping regions between the ellipses of the two groups confirmed that there were two distinct *Hsmar1* populations.

In total, 39 sequences of 600–1200 bp and corresponding to truncated *Hsmar1* elements were extracted from GenBank and placed in the second data set. A similar graphical analysis was performed (Fig. 4b), which defined only one population of *Hsmar1* elements. The features of the density ellipses for this population were similar to those of the second *Hsmar1* population defined in our first analysis (Fig. 4a).

A third set of 46 *Hsmar1* sequences 460–800 bp long was extracted from the GenBank. All these human sequences were referenced as transcribed (messenger RNA and ESTs). Analysis of the graph representing their features (Fig. 4c) revealed a single *Hsmar1* population containing most of the sequences (45/46). Its features indicated that it was the same as the common population in the first (Fig. 4a) and second (Fig. 4b) analyses. Only one sequence (Acc# AI866805) did not meet the features of this population in this third analysis. Its similarity to the *ConsHs* sequence (90%) suggested that it was related to the population containing the hs16, hs17, and hs19–hs22 elements in the first analysis (Fig. 4a, 90–100%).

In conclusion, our analysis of sequences from different sources (108 in total) showed that there are at least two populations of *Hsmar1* elements. The amounts of elements in each of these populations cannot be defined. This is owing to the biases linked to our two sources of *Hsmar1* sequences (euchromatic sequences in GenBank and PCR fragments). The means of nucleotide sequence similarity within each of these populations were $81\% \pm 6$ for the population common to the three analyses and $95.5\% \pm 2$ for the population containing the hs16, hs17, and hs19–hs22 elements. This difference showed that the two *Hsmar1* populations have different ages. Thus, we called the older of these populations *Hsmar1-1* and the more recent population *Hsmar1-2* (Fig. 4d).

Mammal *mar1* populations in the sheep, cow, and mouse genomes. The results for the sheep, cow, and mouse mammal *mar1* elements (Fig. 5a, b, and c) were obtained by using our present data and published sequences (Augé-Gouillou et al. 1995). There may be two populations of mammal *mar1* elements in the sheep genome (Fig. 5a). In agreement with the phylogenetic tree (Fig. 3), one of these populations contained only one sequence (Oa1). The position of this *Oamar1* element in the general phylogenetic tree (Fig. 3) suggested that it originated from a population older than that containing the other *Oamar1* elements. These two groups were called *Oamar1-1* (Oa1) and *Oamar1-2* (Oa2–Oa5 and Oa2202–Oa2213).

Phylogenetic analyses of the *Btmr1* elements (Figs. 2c, 3) suggested that there were three populations in the cow genome. Our graphic analysis showed only two populations with very similar sequence features (Fig. 5b). The first population contained the Bt2231–Bt2239 elements, while the second contained the Bt11–Bt15 elements. Although they are probably very similar in age, we called them *Btmr1-1* and *Btmr1-2*.

Few sequences are available for the mouse genome, but the results clearly showed that there were at least two groups. The group containing mm6 and mm10 elements was called *Mmmar1-1*

Fig. 4. Graphs of the relationships between the percentage of nucleic acid sequence similarity of each *Hsmar1* sequence to the consensus sequence *ConsHs* (vertical axes), and their CpG exp/obs ratio (horizontal axes). **(a)** uses the 10 *Hsmar1* elements obtained in the present work (Acc# AJ009223–AJ009247), Robertson's consensus sequence U52077, the full-length elements *hsG1*–*hsG12*, and the five nucleotide sequences corresponding to the region encoding the transposase between the *WVPHEL* and *YSPDLAP* domains (Acc# X85285–X84289). **(b)** uses all the sequences of truncated *Hsmar1* elements extracted from Genbank. **(c)** uses all the human sequences corresponding to transcribed sequences and extracted from Genbank. **(d)** contains all the data used in **(a)**, **(b)** and **(c)**. The black and grey ellipses locate the groups of mammal *mar1* elements discriminated in these analyses with a confidence of 95% and 99%, respectively. The names given to each mammal *mar1* population are indicated.

elements, and the one containing mm7, mm8, and mm9 elements was called *Mmmar1-2* elements.

Discussion

The number of mammal *mar1* elements in the human, mouse, sheep, and cow genomes varies from about 20–50 to 1000–3500. The number of ITRs related to these elements varied from 900 in the mouse, 7000 in the human, to 10,000 in the sheep and cow genomes. As observed for SINES (B1 and B2) and L1 LINEs (Duret et al. 1995), they are much less numerous in the mouse genome, and only few full-length elements are detected. Mammal *mar1* elements are also not all the same size in the human, sheep, and cow. There are variable amounts of full-length and internally deleted elements in each species. The internal deletions are not the only source of length variations. Analyses of the databank sequences reveal that many are 5' or 3' truncated copies.

The analyses of the elements in each species indicate that there are at least two populations of mammal *mar1* elements in human (*Hsmar1-1* and *Hsmar1-2*), mouse (*Mmmar1-1* and *Mmmar1-2*), sheep (*Oamar1-1* and *Oamar1-2*) and cow (*Btmari1-1* and *Btmari1-2*). The location of the elements related to the *Hsmar1-1* and *Oamar1-1* populations in the phylogenetic tree and their great variations in sequence indicate that these two groups are more ancient than are the *Hsmar1-2* and *Oamar1-2*, respectively. Our results indicate that there are two populations of mammal *mar1* elements of mouse or cow origins. The presence of several populations of mammal *mar1* elements in these mammalian species suggests that they came from several expansions of one or a few elements at different times during the evolution of their respective host ancestors. This indicates that the MLE in mammalian genomes have a life and death cycle similar to those of the *AluI* SINES (Shen et al. 1991). We find mammal *mar1* elements in

rodents, primates, and artiodactyls. This indicates that the mammal *mar1* elements in the genome of the common ancestor of all these species might be older than 50 Myr and arose at least more than 100 million years ago (Springer 1997). We detected no mammal *mar1* elements older than those related to the *Hsmar1-1*, *Oamar1-1*, *Btmari1-1*, and *Mmmar1-1* populations in our studies. However, if such elements are present in mammalian genomes, their sequences are probably less than 60–70% similar. Such similarities prevent their detection in Fasta or Blast searches because they are identical to the mean nucleotide sequence similarity between *Hsmar1* and *Hsmar2* elements (52–68%).

Our explanation for the presence of several mammal *mar1* populations in the mammalian genomes appears to be parsimonious and minimizes horizontal transfers as previously recommended in the literature (Capy et al. 1996; Hartl et al. 1997). However, it does not answer a controversial observation obtained by comparing the invertebrate and mammalian MLEs related to the *cecropia* sub-family. Our analysis reveals that the older *Hsmar1* in the common ancestor of the rodents, primates, and artiodactyls is less than 60–70% identical to the *Hsmar1-1* and *Hsmar1-2* elements. Moreover, *Hsmar1-1* elements are only 75–85% similar to the *Hsmar1-2* elements. Robertson and Zumpano (1997) reported that the invertebrate MLEs in *Carpelius* sp., *Hydra littoralis*, and *H. bacteriophaga* genomes are 70–85% similar to the *Hsmar1-2* elements. These observations do not necessarily raise any question about the pattern that we propose here for the development of mammal *mar1* in the mammalian genomes. However, we cannot reasonably explain the processes producing such conservation between so different organisms without invoking horizontal transfers, at least once from vertebrates to invertebrates.

Several features described here for *Hsmar1* differ from those previously published and resulting from only databank analyses (Robertson and Zumpano 1997). A recent study of the distribution

Fig. 5. Graphs of the relationships between the percentage of nucleic acid sequence similarity of each mammal *mar1* sequence to its consensus sequence, ConsOa (a), ConsBt (b), and ConsMm (c) respectively (vertical axes), and their CpG exp/obs ratio (horizontal axes). The sequences of the region encoding the transposase between the WVPHEL and YSPDLAP domains were used in (a) and (b). They were extracted from the sequences (Acc# AJ009242–AJ009246 + X84290–X84295) in (a) and (Acc# AJ009223–AJ009226 + AJ009247 + X84296–X84301) in b. (c) uses the full-length sequences, AJ009237–AJ009241. The black and grey ellipses locate the groups of mammal *mar1* elements identified in these analyses with a confidence of 95% and 99%, respectively. The names given to each mammal *mar1* population are indicated.

and frequency of repeated sequences (Schug et al. 1998) found that the DNA sequences deposited in databanks are biased towards the euchromatic regions expressed. It may thus be inappropriate to estimate the frequencies of repeated sequence families in the total genome of a eukaryotic species by dividing the number of repeated

sequences identified by the total amount of sequence surveyed. Thus, if a family of transposable elements is preferentially interspersed in the heterochromatin or in weakly expressed euchromatic regions, the sequence analyses may be greatly biased owing to the kind of sequences in the databanks (our data suggest that this is the case for *Hsmar1* elements). This may have consequences for detecting transposable elements, establishing a consensus sequence, and estimating their number and diversity and for understanding their evolution.

Finally, we obtained these conclusions only because we compared the results of databank analyses and PCR amplification experiments. Thus, the difference in the number of the *Hsmar1* elements in the human genome (Robertson and Zuppano: 200; ours: 1000) reflects the fact that these transposons may not be randomly distributed in the human genome, as are other human transposable elements (Chen and Manuelidis 1989).

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