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Unusual molecular characteristics of a repeat sequence island within a Giemsa-positive band on the mouse X chromosome

(CpG islands/long interspersed repeats)

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ABSTRACT **The mouse genome contains 50 copies of a long complex repeat unit localized as a repeat sequence island to the A3 Giemsa-positive (dark) band on the mouse X chromosome. The repeat units are not tandemly arranged but are juxtaposed and inserted by unrelated sequences of high repetition. The repeat sequence island possesses two notable features that have been suggested as diagnostic features of mammalian Giemsa-positive bands. First, the repeat sequence island encompasses a 1-megabase region devoid of CpG islands; second, it features a high concentration of L1 long interspersed repeat sequences.**

For some time, it has been recognized that there is a difference in base composition between the Giemsa (G)-positive and G-negative bands in mammalian chromosomes (1–3). The late-replicating G-positive bands appear to be overall A+T rich compared to the earlier replicating G-negative bands (4). *In situ* hybridization experiments demonstrate that the relatively G+C-rich *Alu* sequences are clustered to G-negative bands. In contrast, A+T-rich L1 long interspersed repeat element (LINE) sequences appear to be preferentially distributed in G-positive bands (5). That the genome may be divided into a G+C-rich component and an A+T-rich component is also supported by the observed distribution of CpG islands. CpG islands are short stretches of sequence [0.5–2 kilobases (kb)] highly enriched in unmethylated CpG dinucleotides that appear to be strongly associated with coding sequences (6, 7). Most housekeeping genes examined appear to be associated with a CpG island on the basis of sequence data (8). While some tissue-specific genes do not appear to possess characteristic CpG islands, others such as the α-globin gene of humans possess a characteristic island (9).

The number of CpG islands in the mammalian genome (30,000) indicates that on average they would occur every 100 kb. Indeed, for many clusters of genes mapped by pulsed-field gel electrophoresis (PFGE), CpG islands occur frequently, as recognized by clusters of restriction sites for enzymes that recognize unmethylated G+C-rich sequences. For example, the pulsed-field linkage map of the factor VIII (*Cf-8*), *G6pd*, and *P3* genes on the mouse X chromosome indicates a number of CpG islands spaced every 50–100 kb (10). In contrast, the human *DMD* gene appears to lie within an extensive genomic region [>1 megabase (Mb)] largely devoid of CpG islands (11), while preliminary observations on the mouse *Dmd* gene indicate a similar genomic organization lacking extensive CpG islands (N.B., unpublished data). The *Cf-8/G6pd/P3* gene cluster has been localized to the mouse X chromosome XB G-negative (light) band (12), whereas *Dmd* is localized within the mouse XC G-positive (dark) band (E.P. Evans, personal communication) or at the interface of bands XB and XC (12). Similarly, the human *DMD* gene lies within the Xp21 G-positive band on the X chromosome. A recent survey of gene localizations by *in situ* hybridization indicates that the bulk (74%) are located within the G-negative light bands (1, 12). The prevailing picture is that gene sequences, both housekeeping and tissue-specific, predominately lie within G-negative bands and that they are sparse within G-positive bands.

Whether G-positive bands are exclusively populated by a few exceptionally large genes such as *DMD* that are unassociated with CpG islands or whether they have a patchwork organization incorporating other sequence domains of unknown organization is unclear. We present evidence for the latter alternative in that we have identified a 1-Mb region of the mouse X chromosome devoid of CpG islands and located within the G-positive XA3 band. The region is a repeat sequence island in that it contains a large repeat unit exclusively located to this 1-Mb region. In addition, the region appears to be a molecular sump for L1 LINE sequences.

MATERIALS AND METHODS

Interspecific Cross and Genetic Analysis. An interspecific *Mus domesticus/Mus spretus* cross segregating for the coat-texture mutations *Hq*, *Ta*, and *Li* (13) was used to generate backcross progeny for the genetic analysis of DXSmh141 and other microclones and has been described in detail (14, 15). High molecular weight DNA was prepared from mouse tails (14).

Southern Hybridization, Library Screening, and Clone Analysis. Progeny DNAs from the backcross were restriction digested and Southern blotted onto Hybond-N membranes (Amersham) by standard methods. Filters were hybridized as described (15) and washed with 2× NaCl/Cit/0.1% $NaDodSO_4$ at 65°C and autoradiographed overnight at −70°C with intensification (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate). Clone versus clone hybridizations were washed extensively in 0.1× NaCl/Cit/0.1% $NaDodSO_4$ at 65°C and autoradiographed at room temperature for <1 hr. Low-stringency hybridizations were carried out in 3× NaCl/Cit at 50°C and washed in 3× NaCl/Cit at 50°C. Probes were labeled by the random-priming method (16). A partial *Hae* III/*Alu* I Charon 4A BALB/c sperm DNA library (17) was screened with DXSmh141 as described (18). We have found that two clones possess relatively short genomic inserts (≈8 kb) due to retention of one of the two 7-kb stuffer fragments

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Abbreviations: G, Giemsa; LINE, long interspersed repeat element; PFGE, pulsed-field gel electrophoresis; Mb, megabase(s); LCRU, long complex repeat unit.

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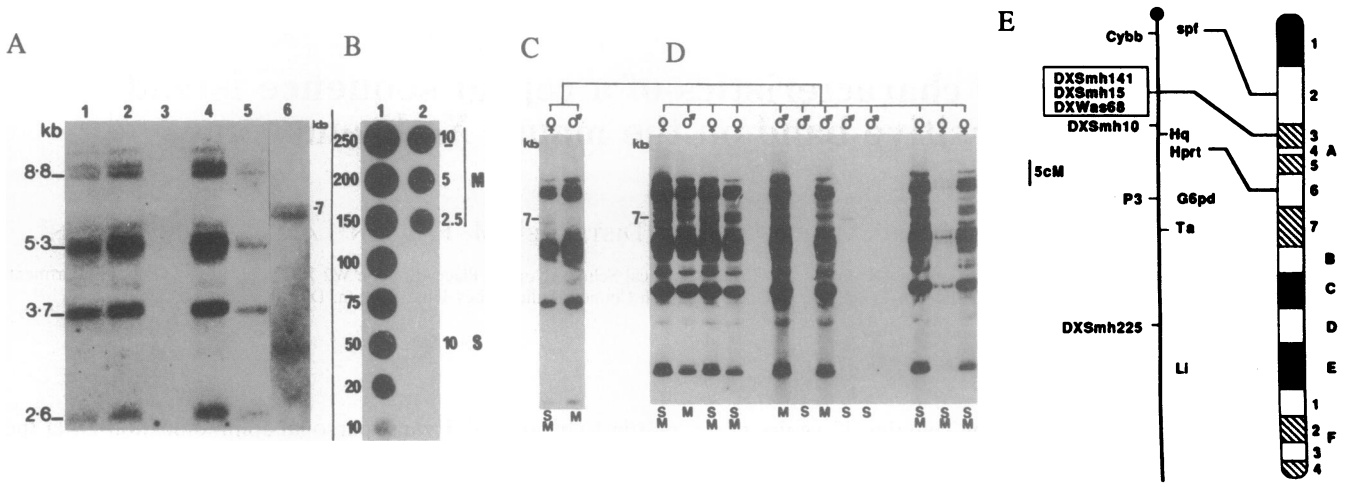

FIG. 1. Chromosome X specificity, copy number estimate, and the genetic analysis of the repeat sequence island. (*A*) *Taq* I digests probed with DXSmh141. Lanes: 1, MAE28 cell line DNA (containing Chinese hamster chromosomes and mouse chromosomes X and 12); 2, MAE32 cell line DNA (containing Chinese hamster chromosomes and mouse chromosomes X and 16); 3, E-36, parent Chinese hamster cell line DNA; 4, SWR, mouse inbred line DNA; 5, C57BL/10, mouse inbred line DNA (underloaded); 6, *M. spretus* DNA (the 3-kb band in this lane represents some cross-hybridization between probe and plasmid included in the digest as a control for extent of DNA digestion). (*B*) Dot blots probed with DXSmh141. Lanes: 1, serial dilutions of DXSmh141 clone representing 10–250 copies of DXSmh141 sequence in 10 μg of male genomic DNA; 2, dots of 2.5–10 μg of male *M. domesticus* DNA and 10 μg of female *M. spretus* DNA. (*C*) *Taq* I digests of DNA from a F_1 female parent carrying *Hq* and DNA from the male to which it was backcrossed probed with DXSmh141. (*D*) *Taq* I digests of DNA from a variety of backcross progeny probed with DXSmh141. M indicates inheritance of *M. domesticus* allele; S indicates inheritance of *M. spretus* allele. (*E*) Genetic map position of the repeat sequence island. The repeat sequence island maps to the mouse X chromosome G-positive band A3. The genetic relationship of the repeat sequence island to a number of other markers on the mouse X chromosome (Table 1) is shown. The concordance between the genetic and physical maps (*Right*) is indicated in two other instances: *spf* and *Hprt* (see text).

of the Charon 4A vector (see Fig. 4). We presume that this arises due to incomplete removal of the stuffer fragment prior to library construction (17). λ clones were restriction mapped exactly according to published methods (19). A 1.5-kb *Eco*RI fragment from clone λban11 (designated DXSmh15) was gel purified and subcloned into pUC13 for further analysis.

PFGE Conditions. Agarose blocks of high molecular weight DNA for PFGE analysis were prepared from freshly extracted thymus of C57BL/10 young male mice (6–10 weeks old) according to established methods (20). Fragments in the 50- to 1000-kb range were separated on 0.8% agarose gels at 170 V over a period of 28 hr in 0.5× TBE buffer (1× TBE = 90 mM Tris·HCl/90 mM boric acid/1 mM EDTA, pH 8) using a clamped homogeneous electric field apparatus (LKB Pharmacia). A switching interval of 55 s was chosen and the buffer temperature was maintained at 13°C. *Saccharomyces cerevisiae* markers (Beckman) ranging in size from 260 to 1500 kb were used as molecular weight standards. Enhanced resolution of fragments below 500 kb was achieved by running a 1.5% agarose gel for 33 hr at 150 V with a 60-s switching interval at a constant 20°C buffer temperature, using an orthogonal field gel alteration apparatus (LKB Pharmacia). DNA fragments >1 Mb were separated on a 0.8% agarose gel at 75 V for 94 hr at 15°C, using a 1-hr switching interval with one change of buffer on the orthogonal field gel alternation system. *Hansenula wingeii* chromosome markers gave accurate sizing in the range of 1–3 Mb. All gels were depurinated for 20 min in 240 mM HCl to induce nicking prior to blotting.

RESULTS

DXSmh141 Is a Chromosome X-Specific Low Repeat Sequence. DXSmh141 is one of the 550 genomic clones isolated by microdissection and microcloning of a proximal region of the mouse X chromosome (21). Hybridization of DXSmh141 to *Taq* I digests of genomic DNA from mouse inbred strains C57BL/10 and SWR revealed an identical complex pattern of bands of widely varying intensity (Fig. 1*A*). Hybridization of DXSmh141 to *Taq* I-digested DNA of cell hybrids MAE28 and MAE32, which contain, respectively, mouse chromosomes X and 12 and mouse chromosomes X and 16 on a background of Chinese hamster chromosomes (22), revealed an identical pattern of bands in both cases to the genomic digests of inbred strains. No hybridization was seen to Chinese hamster DNA, indicating that all sequences detected by DXSmh141 are chromosome X specific. Dot blot analysis of DXSmh141 revealed that there are ≈50 copies of DXSmh141 per haploid genome of *M. domesticus* and far fewer copies (<10) of DXSmh141 in the *M. spretus* genome (Fig. 1*B*). Hybridization of DXSmh141 to *Taq* I digests of *M. spretus* DNA reflects this observation (Fig. 1*A*). Hybridization of DXSmh141 to genomic digests of *M. spretus* DNA under low-stringency conditions failed to reveal further bands (data not shown).

Table 1. Genetic mapping of the repeat sequence island

	Cybb	DXSmh10	*Hq*	P3	*Ta*	DXSmh225
DXSmh141	5/39	7/164	13/142	20/86	19/62	24/45
	(13 ± 5)	(4 ± 2)	(9 ± 2)	(23 ± 5)	(30 ± 6)	(53 ± 7)

Interspecific backcross progeny were scored for the inheritance of coat-texture mutations *Hq* and *Ta* and genomic DNA was analyzed for the inheritance of *M. domesticus* and *M. spretus* restriction fragment length variants for probes DXSmh141, Cybb, DXSmh10, P3, and DXSmh225. The number of recombinants observed out of the total number of progeny analyzed is indicated, and the calculated recombination distance (in centimorgans) ± SE is shown in parentheses.

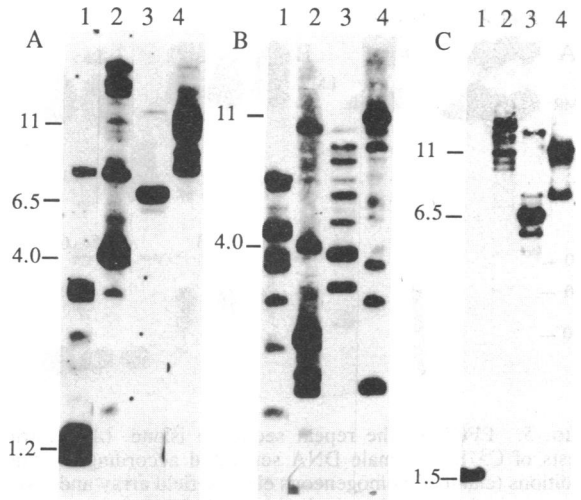

FIG. 2. Genomic organization of the LCRU. Three component sequences of the LCRU were hybridized to genomic digests of mouse DNA. (*A*) DXSmh141. (*B*) DXWas68. (*C*) DXSmh15. DXSmh141 was hybridized under low-stringency conditions. Lanes: 1, *Eco*RI; 2, *Pvu* II; 3, *Hin*dIII; 4, *Kpn* I. Sizes of fragments are in kb.

DXSmh141 Maps as a Single Mendelian Locus in Interspecific Crosses. The presence of restriction fragment length variation between the *M. domesticus* and *M. spretus* genomes for DXSmh141 has allowed us to map the DXSmh141 sequences with the use of an interspecific *M. domesticus* × *M. spretus* backcross segregating for the *Hq* and *Ta* coat-texture mutations (14). Progeny were scored for the coat-texture mutations and a variety of microclone and genic probes including DXSmh141. Surprisingly, the complex pattern of bands detected by DXSmh141 segregated as a single Mendelian locus (Fig. 1 *C* and *D*). All male progeny showed either the total *M. domesticus* pattern or the *M. spretus* pattern. No combination of *M. domesticus* or *M. spretus* bands was observed in 105 male progeny. Taking account of all the progeny scored for a variety of X chromosome probes, the DXSmh141 locus maps to a region of the X chromosome 9 centimorgans proximal to *Hq* (Fig. 1*E* and Table 1).

DXSmh141 and DXWas68 Are Part of a Long Complex Repeat Unit (LCRU). The bulk of DXSmh141 sequences are organized as 1.2-kb *Eco*RI fragments and contained within a 6.5-kb *Hin*dIII unit and a 4-kb *Pvu* II unit (Fig. 2*A*) that overlap to form a repeat unit some 9 kb long (see map in Fig. 4). In addition, DXSmh141 detects a major large *Kpn* I band of 11 kb. Another random genomic chromosome X-specific repeat sequence clone, DXWas68, isolated by flow-sorting of the mouse X chromosome (23), is also part of the LCRU. Like DXSmh141, DXWas68 is present in 50 copies of *M. domesticus* (23) and far fewer copies in *M. spretus* (data not shown). The DXWas68 locus was mapped in a different set of backcross progeny from a *M. spretus* × C57BL/6JRos interspecific cross (12) and was found to be nonrecombinant with DXSmh141 (V. Chapman, personal communication). DXWas68 detects both the major 11-kb *Kpn* I and 4-kb *Pvu* II fragments detected by DXSmh141 in genomic digests of mouse DNA linking it to the DXSmh141 sequence (Fig. 2*B*) and completing a genomic consensus restriction map of a LCRU that extends over 14 kb in total (see Fig. 4).

Organization and Extent of the LCRU in Genomic Clones. DXSmh141 was used to screen a partial *Hae* III/*Alu* I Charon 4A BALB/c sperm DNA library (17) to analyze in detail the organization of the LCRU. Five genomic clones were isolated (λban11, -13, -14, -16, and -19), restriction mapped, and hybridized to a variety of probes (Fig. 3). Four of the five clones, λban 11, -13, -14, and -16 hybridize to DXWas68 (Fig. 3*B*) and the relative positions of DXSmh141 and DXWas68 accurately reflect their positions on the genomic consensus map (Fig. 4). To further investigate the extent of the LCRU in each clone a 1.5-kb *Eco*RI fragment was subcloned from λban11 and named DXSmh15. Hybridization of DXSmh15 to genomic digests (Fig. 2*C*) indicates that it detects the 6.5-kb *Hin*dIII unit and 11-kb *Kpn* I unit revealed by DXSmh141. All genomic clones, except λban13, contain DXSmh15 (Fig. 3*C*) and, apart from λban19, in the same relative position with respect to DXSmh141 and DXWas68 (Fig. 4). Hybridization of a 6.5-kb *Kpn* I/*Hin*dIII restriction fragment from clone 14 (14KH) demonstrates that all clones, except λban13, show extensive hybridization to 14KH (Fig. 3*D*) and enables us to determine the exact extent of this region of the LCRU on the restriction maps of the five clones (Fig. 4). Comparison of the restriction maps of the five clones indicates that the LCRU does not extend beyond the left-hand *Kpn* I site delimiting the genomic consensus restriction map. In clones λban14 and -16, restriction fragments beyond this leftward *Kpn* I site are highly repetitious as revealed by their hybridization to total mouse DNA (data not shown). The presence of a highly repetitious fragment at the right-hand end of λban13 suggests that the LCRU does not extend much to the right of the DXWas68 sequence (Fig. 4). Moreover, DXSmh15 also detects a diverse range of *Pvu* II large fragments in genomic blots (Fig. 2*C*) that presumably arise from the LCRU internal *Pvu* II site and a variety of other sequences at the left-hand end of the repeat. Overall, the data indicate that there is no simple tandem repetition of the LCRU but rather it is dispersed amongst a variety of other sequences of high repetition.

Insertion of High Copy Sequences into the LCRU. The restriction site pattern of the high copy sequences in λban16 and λban19 (data not shown) suggested they may be members of the mouse LINE family L1. Digests of each clone were hybridized to two probes derived from different regions of the mouse L1 repeat (Fig. 3 *E* and *F* and Fig. 4): first, a 900-bp *Pst* probe from the 5′ end of the L1 repeat and present in some 10,000 copies in the mouse haploid genome (24); second, an

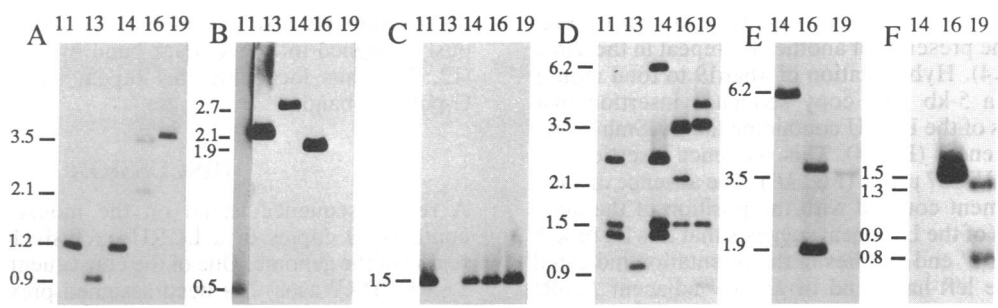

FIG. 3. Sequence organization of genomic clones containing the LCRU. Restriction digests of clones λban11, -13, -14, -16, and -19 were hybridized to a variety of DNA probes: DXSmh141 (*A*), DXWas68 (*B*), DXSmh15 (*C*), 14KH (*D*), *Pst* (*E*), MIFC37 (*F*). (*A–E*) *Eco*RI digests. (*F*) *Eco*RI/*Hin*dIII digests. Sizes of fragments are in kb.

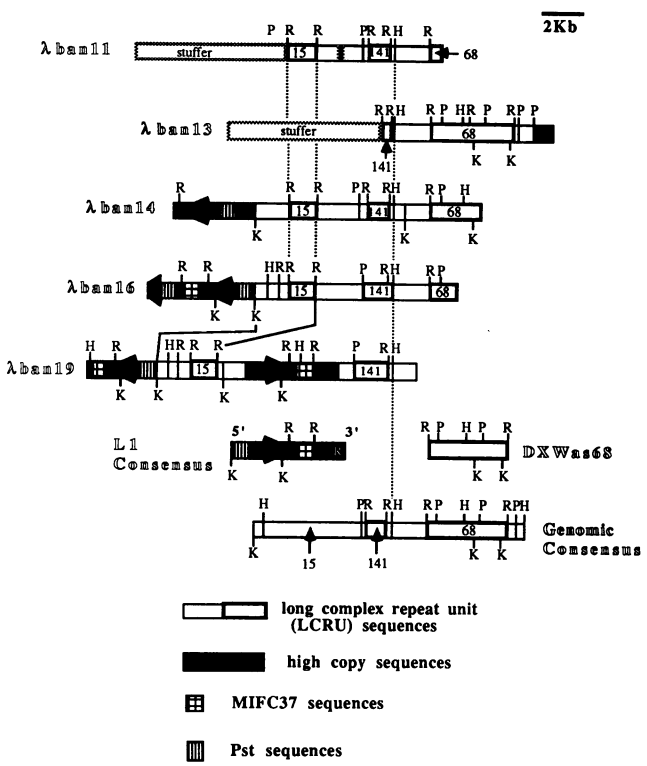

FIG. 4. Restriction maps of genomic clones containing the LCRU. R, *Eco*RI; K, *Kpn* I; H, *Hin*dIII; P, *Pvu* II. Open bars indicate LCRU sequences and open bars outlined in boldface are fragments hybridizing to individual LCRU probes. Solid bars represent restriction fragments containing high copy repeat sequences. Within these high copy repeat sequences the relative positions of the MIFC37 probe (hatched box) and the *Pst* probe (vertically lined box) are demonstrated. In addition, the restriction map of the DXWas68 probe, the consensus structure of the L1 repeat, and the genomic consensus structure of the LCRU are indicated. The maps are aligned at a common *Hin*dIII site at the right-hand end of the DXSmh141 sequence. λban11 and λban13 have relatively short genomic inserts due to the retention of a portion of a Charon 4A stuffer fragment.

850-bp MIFC37 probe 3′ to the *Pst* probe in the L1 repeat and present in some 20,000 copies in the mouse haploid genome (25). λban11 and λban13 failed to hybridize to either probe, but homologous sequences were found in λban14, -16, and -19. λban14 hybridized only to the *Pst* probe (Fig. 3*E*); the absence of MIFC37 suggests the orientation of the L1 repeat indicated in Fig. 4 and the MIFC37 is absent due to truncation at the left-hand Charon 4A cloning site. In λban16, closely adjacent sequences hybridized to both the *Pst* and MIFC37 probes (Fig. 3 *E* and *F*), indicating the presence of an L1 repeat in similar orientation to λban14 and closely juxtaposed to the LCRU (Fig. 4). In addition, a second *Pst* homologous fragment is detected at the very left-hand end of λban16 (Fig. 3*E*), indicating the presence of another L1 repeat in the same orientation (Fig. 4). Hybridization of λban19 to total mouse DNA indicated a 5-kb high copy sequence insertion that separates regions of the LCRU containing the DXSmh15 and DXSmh141 sequences (Fig. 4). This sequence insertion hybridized to the MIFC37 probe (Fig. 3*F*). The absence of a *Pst* hybridizing fragment coupled with the position of the diagnostic *Kpn* I sites of the L1 repeat suggests that this L1 repeat is truncated at its 5′ end and lies in the orientation indicated in Fig. 4. At the left-hand end of λban19 adjacent *Eco*RI restriction fragments hybridize to the MIFC37 and *Pst* probes, demonstrating the presence of a further L1 repeat juxtaposed at the left-hand end of the LCRU but in opposite orientation to that inserted in the LCRU. Another unknown high copy repeat sequence is also inserted in λban11. However, the relative positions of the flanking LCRU sequences (DXSmh15 and DXSmh141) are unaltered, suggesting that the extent of the repeat sequence insertion is small.

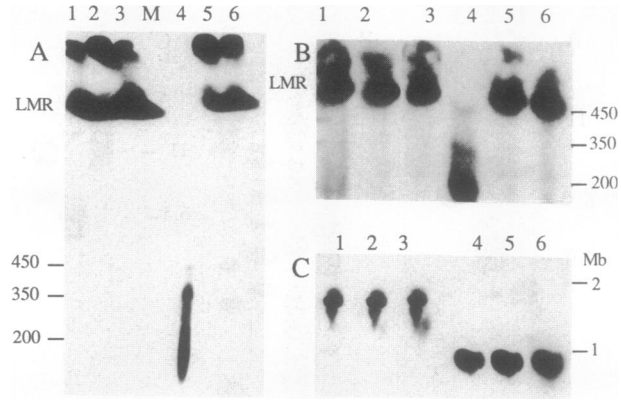

FIG. 5. PFGE of the repeat sequence island. (*A*) Restriction digests of C57BL/10 male DNA separated according to standard conditions (clamped homogeneous electric field array and a switching interval of 55 s) and probed with DXSmh141. Lanes: 1, *Sac* II; 2, *Not* I; 3, *Mlu* I; M, marker; 4, *Sfi* I; 5, *Bss*HII; 6, *Nru* I. LMR, limiting mobility region. Sizes of fragments are in kb. (*B*) Restriction digests of C57BL/10 male DNA separated according to modified conditions (orthogonal field array and 60-s switching interval) and probed with DXSmh141. Lanes: 1, *Bss*HII; 2, *Not* I; 3, *Nru* I; 4, *Sfi* I; 5, *Mlu* I; 6, *Sac* II. Sizes of fragments are in kb. (*C*) Separation of very large fragments under orthogonal field conditions (switching interval of 1 hr). Lanes: 1–3, *Mlu* I digests; 4–6, *Sac* II digests. The same *Mlu* I and *Sac* II digests were probed with DXSmh141 (lanes 1 and 4), DXWas68 (lanes 2 and 5), and DXSmh15 (lanes 3 and 6). Sizes of fragments are in Mb.

LCRUs Encompass a Region of the Mouse X Chromosome Devoid of CpG Islands. We have examined by PFGE the long-range physical organization of the LCRUs (Fig. 5). Digests of high molecular weight DNA from male C57BL/10 mice with rare cutter restriction enzymes were separated by PFGE and probed with DXSmh141. Under conditions that separate fragments of 1 Mb or less only *Sfi* I produced discrete bands (Fig. 5*A*); with other enzymes, hybridization occurred to the limiting mobility fragments of 1 Mb or greater. Three *Sfi* I fragments of 200, 350, and 450 kb are more clearly resolved under conditions that separate smaller fragments (Fig. 5*B*). Under electrophoresis conditions that separate fragments of 1 Mb or greater, DXSmh141 detects discrete bands with the rare cutter restriction enzymes *Mlu* I (1.5 Mb) and *Sac* II (0.8 Mb) (Fig. 5*C*). As would be expected given the linkage of DXSmh15 and DXWas68 with DXSmh141 in the LCRU, hybridization of the same filter with DXSmh15 and DXWas68 detected identical bands (Fig. 5*C*). The LCRUs appear to be physically confined as a repeat sequence island to a 1-Mb region of the mouse X chromosome largely devoid of unmethylated rare cutter enzyme sites and presumably also of CpG islands. DXWas68 was previously assigned to the A3 dark band by *in situ* hybridization (12, 23), thus localizing this repeat sequence island to a G-positive band.

DISCUSSION

A repeat sequence island on the mouse X chromosome contains 50 copies of a LCRU exclusively located in this region of the genome. One of the constituent sequences of the LCRU (DXWas68) has been assigned previously to the A3 G-positive band of the mouse X chromosome (12, 23), in agreement with our genetic localization of the island as determined by interspecific crosses (14, 15, 23). The repeat sequence island lies between the *spf* locus, localized at band

A2 (26), and the *Hprt* locus, localized at A6 (27). PFGE analysis of the repeat sequence island indicates that it spans a 1-Mb region devoid of CpG islands. The rare cutter enzymes *Sac* II and *Bss*HII fail to cut within the repeat sequence island. Yet, *Sac* II and *Bss*HII sites are highly diagnostic of CpG islands. On average, for each of these enzymes, there are 1.2 sites per CpG island (7).

Analysis of five genomic clones encompassing some 75 kb (10%) of the repeat sequence island indicated a high concentration of L1 repeat sequences. Five different and separable segments of an L1 repeat were identified in three of the genomic clones, representing an average distribution of one L1 repeat every 15 kb in the repeat sequence island. L1 repeats are thought to transpose by reverse transcription and, as a consequence, many L1 repeats are truncated (28): sequences at the 3′ end of the repeat unit—e.g., the *R* sequences—are present more frequently than 5′ sequences. *R* sequences are present in 10^5 copies per genome, the more 5′ MIFC37 sequences are present in 20,000 copies per genome, and sequences represented by the most 5′ elements of the L1 repeat (e.g., the *Pst* probe used here) are present in 10,000 copies per genome, giving an expected average distribution for MIFC37 of around one every 150 kb and for *Pst* around one every 300 kb and for *R* sequences as frequently as every 30 kb. Three MIFC elements were detected in 75 kb of the repeat sequence island and four *Pst* elements. For both *MIFC* and *Pst* elements, the genomic density within the repeat sequence island is much higher than expected.

A similar departure from the expected average distribution of L1 elements is seen in the mouse β-globin gene cluster, which contains seven dispersed MIFC-homologous elements (28). Equally, the human β-globin gene cluster is characterized by a high density of L1 elements (29). The β-globin gene cluster in both mouse and human is not associated with a CpG island (9). In humans it has been located in a G-negative band 11p15.5 (30). For other tissue-specific genes that lie in G-positive bands and are devoid of CpG islands, such as *DMD*, it will be interesting to determine whether they also demonstrate high densities of LINE sequences. The finding of a repeat sequence island in a G-positive band that is densely packed with LINE sequences supports the prevailing notion that LINE sequences are preferentially associated with dark G-positive bands (5). We have previously reported a number of other repeat sequence clusters on the mouse X chromosome (14). Whether they share similar properties to the repeat sequence island described here remains to be determined. The demonstration that the A3 dark band of the mouse X chromosome contains a large array of localized repeat sequences, densely packed with LINE sequences, indicates that G-positive bands are not populated solely with large genes devoid of CpG islands. Rather, the evidence presented suggests that G-positive bands are probably a matrix of coding and noncoding sequences devoid of CpG islands that are somehow predisposed to accepting LINE sequences.

The large difference in copy number of the island sequences DXSmh141 and DXWas68 between *M. domesticus* and *M. spretus* indicates a gross difference in the long-range organization of this chromosomal region in the respective genomes. The equivalent chromosome region in *M. spretus* may now largely be devoid of a repeat sequence island of the type and characteristics identified here in *M. domesticus*. Alternatively, *M. spretus* may possess a repeat sequence island at the equivalent location but composed of a repeat unit unrelated to the LCRU identified in *M. domesticus*. Either way, the evolution of this G-positive chromosomal region appears particularly rapid.

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