Translocation and amplification of an X-chromosome DNA repeat in inbred strains of mice

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ABSTRACT

A 9-kb repetitive DNA fragment (70-38) located near the centromere of the mouse X chromosome is amplified and translocated to an autosome in different inbred strains of mice. *In situ* hybridization and hybrid cell studies showed that probe 70-38 is located only on the X chromosome in mouse strains A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J and SWR/J. However, in four other mouse strains the DNA sequence is found near the centromere of an autosome in addition to the X chromosome. This autosome differs among the mouse strains (chromosome 11 in C57BL/10J or ScSn, chromosome 13 in NZB/B1NJ and chromosome 17 in SJL/J and P0). In those strains where the repeated sequence is located on an autosome, it has been amplified to about 100 copies. Restriction enzyme digestion patterns suggest a common structure for 70-38 sequences in the different strains. The changes in copy number, restriction enzyme digestion patterns, and chromosomal location of 70-38 reflect a rapid genomic evolution in inbred mouse strains.

INTRODUCTION

Repeated and satellite DNA sequences represent a large proportion of the eukaryotic genome and are likely to play an important role in chromosomal organization and function. Repeated DNA sequences can be interspersed in the genome, like the primate Alu sequences, or located at specific chromosomal sites. A number of chromosome-specific repeats have been isolated from both mice and humans (1-14). Repeated DNA sequences confined to the mammalian X chromosome are interesting with respect to the conservation of linkage of genes on the X chromosome (1-3,13). We recently reported a repeated element located at the centromeric region of the mouse X chromosome (13). This 9-kb DNA fragment is repeated about twenty times in the mouse genome. Here we demonstrate that this repeated element is amplified and present on an autosome, in addition to the X chromosome, in certain inbred mouse strains. The general term "translocation" is used throughout the present paper to describe our findings without connotation to mechanisms which are presented in the discussion. The translocation and amplification events have occurred in a

region close to the highly repeated DNA of centromeric heterochromatin and this may have resulted from exchanges in this region.

MATERIALS AND METHODS

Mice

Mice from strains A/J, AKR/J, BALB/cJ, C57BL/6J, C57BL/10J, C3H/HeJ, DBA/2J, NZB/B1NJ, SJL/J and SWR/J were obtained from the Jackson Laboratory. Mice from strain C57BL/10ScSn mdx were obtained from K. Moore (University of California, Berkeley). Mice from Pathology Oxford (PO) were from G. Martin (University of Washington, Seattle).

Cell Lines

Hybrid cell lines EBS-5Ag and EBS-13Ag were provided by P. Lalley (Institute for Medical Research, Bennington, VT) and are described in Lalley et al. (15).

DNA probes

The isolation and characterization of probe 70-38 were described previously (13,16). A probe for the metallothionein gene (mMT-1) located on chromosome 8 (17) was provided by R. Palmiter (University of Washington, Seattle). Retroviruses M720 (18) and Maloney-like murine leukemia virus were from E. Birkenmeier (Jackson Laboratory, Bar Harbor, Maine). The probe pBS18 for the LINE1 element (19) was from B. Schmeckpeper (Johns Hopkins University, Baltimore, MD).

DNA Blot Analysis

Methods for DNA preparation from cell lines and mouse liver or spleen and conditions of Southern blotting and DNA hybridization were described in Disteche et al. (13).

In Situ Hybridization

Metaphase chromosome preparations were obtained from mouse embryo cultures or from mouse bone marrow after injection of yeast culture by the method of Lee and Elder (20). In situ hybridization to probe 70-38 was as described in Disteche et al. (13).

RESULTS

Probe 70-38 is a 9-kb repeated DNA sequence that is located primarily on the mouse X chromosome (13). Hybridization of probe 70-38 to Southern blots containing BclI digests of equal amounts of mouse DNA from 12 different mouse strains showed that approximately 20 copies of 70-38 were present in each of the mouse strains A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, DBA/2J, SWR/J and

Figure 1. Autoradiography of a Southern blot containing BclI-digested DNAs from mouse strains A/J (lane 1), AKR/J (lane 2), BALB/cJ (lane 3), CBA/J (lane 4), C3H/HeJ (lane 5), DBA/2J (lane 6), SWR/J (lane 7), C57BL/6J (lane 8), C57BL/10J (lane 9), NZB/B1NJ (lane 10), SJL/J (lane 11) and C57BL/10ScSn mdx (lane 12). Arrows indicate variant bands. The blot was hybridized to ^{32}P -labeled probe 70-38.

C57BL/6J (Fig. 1, lanes 1-8). In contrast, mouse strains C57BL/10J, NZB/B1NJ, SJL/J, C57BL/10ScSn mdx, and PO (not shown) showed a 4- or 5-fold amplification (80-100 copies) of the repeat (Fig. 1, lanes 9-12). The extent of amplification was determined by comparing the degree of hybridization of 70-38 to DNA from C57BL/6J and to dilutions of DNA from C57BL/10J.

The restriction enzyme patterns were found to be largely conserved between mouse strains A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, DBA/2J, SWR/J and C57BL/6J (using 12 different enzymes), except for a minor, faint, variant band that distinguished A/J and BALB/cJ from the other strains (Fig. 1, band at about 4 kb). In contrast, several variant bands were found with restriction enzymes MspI, HindIII, BglII, TaqI, BglI, BclI, MboI and EcoRI in the four strains (C57BL/10J or ScSn mdx, NZB/B1NJ, SJL/J and PO) showing amplification. The variant bands (Fig. 1, bands at about 8.6, 5.7, 3.0 and 2.1 kb) were similar in all four strains; in addition, minor, faint bands distinguished C57BL/10J or ScSn mdx, from SJL/J and PO and from NZB/B1NJ (Fig. 1, band at about 2.5 kb). A clear dosage difference between the intensity of hybridiza-

Figure 2. Autoradiography of a Southern blot containing BclI-digested male and female mouse DNA from strain C57BL/6J (lanes 1 and 2) and from strain C57BL/10J (lanes 3 and 4) hybridized to ^{32}P -hybridized probe 70-38.

tion of probe 70-38 to male and female mouse DNA was observed for strains A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, DBA/2J, SWR/J and C57BL/6J, as expected for an X-linked DNA sequence (Fig. 2, lanes 1-2). A less marked dosage difference between male and female mouse DNA was seen in mouse strains C57BL/10J or ScSn mdx, NZB/B1NJ, SJL/J and PO (Fig. 2, lanes 3-4). This was confirmed by densitometric measurements along the lanes. Male to female dosage differences of 1:2 and about 1:1.4 were obtained for strains C57BL/6J and C57BL/10J, respectively, suggesting autosomal as well as X-chromosome linkage of 70-38 in the latter strain. The DNA sequence 70-38 did not cross hybridize to retroviral sequences corresponding to two common mouse retroviruses. Similarly there was no cross hybridization to a probe for the LINE1 repeats (data not shown).

In situ hybridization to metaphase chromosomes from a male and a female mouse of each strain showed that DNA sequences homologous to probe 70-38 were located on the mouse X chromosome alone in mouse strains A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J and SWR/J. In contrast, in all of the mouse

Figure 3. In situ hybridization of ^3H -labeled probe 70-38 to mouse metaphase chromosomes. The Q-banded metaphase cells are on the left and the same cells photographed after increasing the background light for grain visualization are on the right. Metaphase chromosomes are from (a) a female mouse from strain C57BL/10ScSn mdx; (b) a male mouse from strain P0; (c) a male mouse from strain NZB/B1NJ.

Figure 4. Autoradiography of a Southern blot containing EcoRI digests of DNA from female mouse C57BL/6J (lane 1), male mouse C57BL/6J (lane 2), from Chinese hamster (CHO) (lane 3), from hybrid cells EBS-5Ag (lane 4) and EBS-13Ag (lane 5). (a) Hybridization to ^{32}P -labeled probe 70-38 (28-hr exposure); (a 2-week exposure of the blot did not show any further hybridization on lanes 3, 4, and 5); (b) hybridization to ^{32}P -labeled probe mMT-1 (1-week exposure). A size marker is on the left.

strains that showed amplification of 70-38 by Southern blot hybridization (see above), probe 70-38 showed an additional site of hybridization on one of three autosomes (chromosomes 11, 13, or 17). Previous data had shown that in mouse strain C57BL/10ScSn mdx, probe 70-38 hybridized to the centromeric regions of both the X chromosome and chromosome 11 (13). A similar distribution was seen in another C57BL/10 strain (C57BL/10J) (Fig. 3a). In mouse strains SJL/J and PO, sequences homologous to probe 70-38 were present at the centromeric region of both the X chromosome and of chromosome 17 (Fig. 3b), while in mouse strain NZB/B1NJ, probe 70-38 mapped to the centromeric region of the X chromosome and of chromosome 13 (Fig. 3c).

These data suggested that DNA sequences homologous to probe 70-38 were translocated and amplified at different autosomal sites in different inbred mouse strains. Alternatively, single copies of sequences homologous to probe 70-38 might be located on several mouse autosomes in all strains, and have been selectively amplified on a different autosome in different mouse strains. To further distinguish between these two possibilities, probe 70-38 was hy-

bridized to EcoRI-digested DNA from hybrid cell lines that lack the X chromosome but retain various mouse autosomes from strain BALB/cJ. Two mouse x Chinese hamster hybrid cell lines, EBS-5Ag (which retains mouse chromosomes 1, 2, 3, 4, 6, 7, 8, 10, 12, 13, 15, 16, 17 and 19) and EBS-13Ag (which retains mouse chromosomes 2, 7, 12, 14, 15, 17 and 19) were examined. No hybridization of probe 70-38 was observed to DNA from these hybrid cells (Fig. 4a). As a control, the same blots were hybridized with a single copy DNA sequence, mMT-1, located on mouse chromosome 8 (17). The mMT-1 probe showed clear hybridization to DNA of hybrid EBS-5Ag, which retains chromosome 8, but not to DNA of hybrid EBS-13Ag, which lacks chromosome 8 (Fig. 4b). This Southern blot analysis showed that single copies of 70-38 were not detectable in hybrid cells containing chromosomes 13 and 17 from strain BALB/cJ. Thus, it does not appear that the presence of multiple copies of sequence 70-38 on an autosome of some of the mouse strains results from the amplification of a single preexisting copy of the 70-38 sequence.

DISCUSSION

A low-repeat DNA sequence was shown to reside at the centromeric region of the mouse X chromosome. In the majority of inbred mouse strains, this repeated element was located exclusively on the X chromosome. However, in four mouse strains, our data suggest that sequences homologous to probe 70-38 were translocated from the X chromosome to an autosome and were amplified during or following the translocation event. The mechanisms of translocation and amplification are unclear. The structure of 70-38 appears to have been largely conserved during translocation and amplification. This is indicated by similarities in the major bands of the restriction enzyme digestion patterns in the different inbred mouse strains. Minor differences in the restriction enzyme digestion patterns seen in the strains with an autosomal site of 70-38, as compared to the other strains, were consistent, regardless of translocation to chromosome 11, 13, or 17. This similarity in the structure of the autosomal sequence homologous to 70-38 indicates that the mechanisms of translocation and amplification might be the same on the different autosomes.

Transposition and amplification of DNA sequences may be mediated by retroviruses. The repeat described here did not show homology to common retroviral sequences of mouse. In addition, retrovirus-mediated transposition is usually random throughout the genome while sequence 70-38 was translocated only to the centromeric region of autosomes. Chromosomal insertion of DNA sequences has also been reported to occur at the position of LINE repeats (21). However,

probe 70-38 did not show homology to a probe for a LINE repeat element that has a similar molecular structure on the X chromosome and on autosomes in human (19).

Another possibility is that 70-38 has been translocated by unequal crossing over due to proximity to highly repetitive DNA located at the vicinity of the centromere (22). The proximity of the centromeric regions in interphase nuclei could favor such exchanges; however, preliminary data of *in situ* hybridization of 70-38 to interphase nuclei indicate that DNA homologous to 70-38 is located at distinct, nonadjacent sites. Other DNA sequences that show quantitative and polymorphic variation between mouse strains are the ribosomal RNA genes located in the centromeric region of autosomes (23). Analyses using restriction enzyme polymorphic markers suggest that the ribosomal RNA genes located on different autosomes evolve independently (24). This correlates with the apparent lack of mouse chromosome association at the nucleolus formation. In contrast, in man where DNA exchanges were shown to take place between ribosomal RNA genes on different chromosomes, associations between nucleolar organizing regions frequently occur (25).

That centromeric regions may undergo frequent rearrangements is suggested in two recent reports. Jabs et al. (3) described a centromeric repeat, located both on the human X chromosome and on autosomes. This repeat was highly polymorphic between individuals, thus indicating a high degree of rearrangement. The plasticity of centromeric sequences was also evident in Butner and Lo's (26) report of a high frequency of DNA rearrangements in a thymidine kinase gene inserted close to centromeric mouse satellite DNA.

Translocation and amplification of 70-38 demonstrated here appears to be an example of rapid genomic evolution in different inbred mouse strains. C57BL/10J or ScSn mdx and C57BL/6J both were derived from the same black subline around 1940 (27); yet C57BL/10J or ScSn mdx showed hybridization of 70-38 to the X chromosome and chromosome 11, while C57BL/6J showed hybridization to the X chromosome alone. This indicates either rapid evolution in the sublines 10 and 6, or possibly the contamination of one of the sublines. The other mouse strains with a single autosome containing sequences homologous to 70-38 are not closely related. Both SJL/J and NZB/B1NJ may have different origins than most of the other mouse strains (27). Further analysis of the repeat 70-38 and of related sequences in different mouse species may provide new information on rate and mechanisms of genomic evolution in mice.

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