ISOLATION AND CHARACTERIZATION OF A MOUSE Y CHROMOSOMAL REPETITIVE SEQUENCE

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ABSTRACT

The Y chromosome plays a dominant role in mammalian sex determination, and characterization of this chromosome is essential to understand the mechanism responsible for testicular differentiation. Male mouse genomic DNA fragments, cloned into pBR322, were screened for the presence of Bkm (a female snake satellite DNA)-related sequences, and we obtained a clone (AC11) having a DNA fragment from the mouse Y chromosome. In addition to a Bkm-related sequence, this fragment contained a Y chromosomal repetitive sequence. DNA isolated from the XX sex-reversed male genome produced a hybridization pattern indistinguishable to that obtained with normal female DNA, suggesting that the AC11 sequence is not contained within the Y chromosomal DNA present in the sex-reversed male genome. Based on the hybridization patterns against mouse Y chromosomal DNA, AC11 classified 16 inbred laboratory strains into two categories; those with the Mus musculus musculus type Y chromosome and those with the M.m. domesticus type Y chromosome. Three European subspecies of Mus musculus (M.m. brevirostris, M.m. poschiavinus and M.m. praeextus) possessed the M.m. domesticus type Y chromosome, whereas the Japanese mouse, M.m. molossinus, had the M.m. musculus type Y chromosome. The survey was also extended to six other species that belong to the genus Mus, of which M. spretus and M. hortulanus showed significant amounts of AC11-related sequences in their Y chromosomes. The male-specific accumulation of AC11-related sequences was not found in M. caroli, M. cookii, M. pahari or M. platythrix. This marked difference among Mus species indicates that the amplification of AC11-related sequences in the mouse Y chromosome was a recent evolutionary event.

THE XY mode of sex determination operates in mammals, and a responsible gene(s) on the Y chromosome acts as a dominant trait, i.e., individuals with the Y chromosome develop testes regardless of how many Y chromosomes are present. In human beings, the sex-determining gene(s) is assigned to the short arm of the Y chromosome (for a review, see Davis 1981). In mice, a male with XX karyotype has been identified. This trait is dominant, and the responsible gene is designated Sxr (sex-reversal) (Cattanach, Pollard and Hawkes 1971). Using a satellite DNA sequence (Bkm) isolated from a female snake, Singh and Jones (1982) demonstrated that in the XX Sxr mice, a small segment of the Y chromosome was transferred to the X chromosome. This
transfer occurs during male meiosis between the \(X\) and \(Y\) chromosome (Evans, Burtenshaw and Cattanach 1982). Since \(XX\) \(Sxr\) mice develop testes, the transferred segment should contain a gene(s) responsible for primary sex determination. Note, however, that the testes are small and functional sperm are absent (Cattanach, Pollard and Hawkes 1971). Eicher, Philips and Washburn (1983) described another mouse mutant in which a substantial amount (observable by light microscopy) of the \(Y\) chromosome is transferred to the \(X\) chromosome. This mutant develops testes containing functional sperm. It can then be speculated that the \(Y\) chromosomal region present in this mutant but absent in the \(XX\) \(Sxr\) mice contains loci necessary for sperm maturation.

Accumulating evidence indicates that, in addition to the \(Y\) chromosome, genes on other chromosomes are also involved in primary sex determination. For example, when the \(Y\) chromosome of \(M.m.\) domestica was introduced into \(C57BL\) background, \(XY\) offspring developed as true hermaphrodites or females (Eicher et al. 1982; Eicher and Washburn 1983). The formation of ovarian tissues in these \(XY\) animals could be interpreted as the result of a "miscommunication" between the \(M.m.\) domestica type \(Y\) chromosome and \(C57BL\) testes determining genes residing on other chromosomes. Such incompatibility creates a fertility barrier between two populations and could lead to species diversification. The study of the structure and function of the \(Y\) chromosome is therefore important in order to understand both the evolution of species and the mechanism of primary sex determination. One approach would be to isolate \(Y\) chromosomal DNA fragments and examine their molecular organization, location and conservation among related species.

Recent advances in recombinant DNA technology have made it possible to isolate \(Y\) chromosomal DNA. Human \(Y\) chromosomal DNA fragments were isolated by several groups (Bishop et al. 1983; Burk, Ma and Smith 1985; Cooke, Brown and Rappold 1984). It is of particular interest that \(Y\) chromosomal material has been reported in human \(XX\) males (Guellaen et al. 1984; Page, de la Chapelle and Weissenbach 1985). Cloning of mouse \(Y\) chromosomal DNA was reported by Eicher, Philips and Washburn (1983), Lamar and Palmer (1984) and Bishop et al. (1985). These clones should provide a powerful tool to study the structure, function and evolution of the \(Y\) mouse chromosome.

In mice, Bkm-related sequences are more abundantly present in the male genome than in the female genome (Singh and Jones 1982; Singh, Purdon and Jones 1981). This observation suggested a possibility that Bkm sequences could be used as probes to isolate mouse \(Y\) chromosomal DNA. We randomly cloned male genomic DNA into pBR322 (Boliver et al. 1977), and transformed \(E.\ coli\) colonies were screened for the presence of Bkm-related sequences. Among 600 clones, two positive clones were identified. In this report, one of them, designated AC11, is described. In addition to a Bkm-related sequence, AC11 contains a mouse \(Y\) chromosomal repetitive sequence with which we studied the structure and evolution of the mouse \(Y\) chromosome.

**MATERIALS AND METHODS**

**Identification of clones:** Male mouse (\(C57BL/6J\)) DNA isolated from liver was digested with \(BamHI\) (Boehringer Mannheim Canada, Dorval, Quebec), ligated to a plas-
mid pBR322 (Boliver et al. 1977) and introduced into a strain of E. coli LE 392 by a CaCl₂ method (Dagert and Ehrlich 1979). Transformed bacterial colonies were grown on a filter (Gene Screen, New England Nuclear Canada, Lachine, Quebec) and were screened for the presence of Bkm-related sequences using a cloned Drosophila Bkm sequence CS 316 (Singh and Jones 1982). The hybridization conditions were the same as described below, except that the hybridization mixture contained E. coli DNA that did not cross-hybridize with Bkm-related sequences. Filters were washed in 1 × SSC at 50°.

**DNA blot hybridization:** Mouse genomic DNA was digested with appropriate restriction enzymes under the conditions recommended by suppliers (Bethesda Research Laboratories, Bethesda, Maryland and Boehringer Mannheim Canada) and was electrophoresed on 0.8% agarose gels using a buffer containing 40 mM Tris-acetate, pH 8.0, and 2 mM EDTA. DNA fragments were transferred to membrane filters (Gene Screen, New England Nuclear Canada) as described by Southern (1975). Filters were pretreated with 50 μg/ml of denatured herring sperm DNA in a solution containing 5 × SSC (1 × SSC is 0.15 N NaCl and 0.015 N Na-citrate), 50% formamide, 1 × Denhardt’s solution (Denhardt 1966) and 0.1% SDS for 12–16 hr at 41°. Filters were then hybridized to ³²P-labeled probes in the same solution for 16 hr, washed in 500 ml of 0.1 × SSC at 50° and exposed to Kodak XAR-5 films for 10–18 h unless otherwise indicated.

**Preparation of probes:** To make radioactive probes, DNA was labeled with [α⁻³²P]-dCTP (3000 cpm/mmol, Amersham Canada Ltd., Oakville, Ontario) using a nick translation kit (Bethesda Research Laboratories). When necessary, DNA fragments were eluted from agarose gels using DEAE coated membrane filters (Schleicher and Schuell, Inc., Keene, New Hampshire) before nick translation.

**Estimation of copy number:** The copy number of AC11 was estimated by dot-blot analysis. Various amounts of AC11 (fragment 1) were mixed with herring sperm DNA, denatured with alkali (0.3 N NaOH) and dot-blotted on a membrane filter. Prehybridization and hybridization conditions were as described above. Various autoradiographic exposures permitted estimation of the copy number by comparing the intensity of hybridization with that obtained with various amounts of male and female mouse DNAs.

**Animals:** Mouse inbred strains C57BL/6J, C3H/HeJ, DBA/2J, SWV (Virgo and Miller 1974) and a stock containing a Robertsonian translocation Rb(5.19)1Wh (White and Tjio 1967) were obtained from D. Trasler of this department. Strain 129/J was from A. Peterson, Montreal General Hospital. CD1, a randomly bred stock, was purchased from Charles River Canada Inc. (St. Constant, Quebec). Other strains were purchased from the Jackson Laboratory (Bar Harbor, Maine). *Mus caroli* was obtained from A. Peterson. Other wild mice were obtained from Litton Bionetics (Kensington, Maryland) through M. Potter of the National Cancer Institute; they included *M. cookii*, *M. hortulamus*, *M. pahari*, *M. platythrix*, *M. spreus*, *M.m. domesticus*, *M.m. praetextus*, *M.m. breviostris*, *M.m. poschiavinus*, *M.m. musculus* and *M.m. molossinus*.

It should be noted that the taxonomy of the genus Mus is incomplete and in debate (Marshall and Sage 1981; Thaler, Bonhomme and Britton-Davidian 1981). The relationship between *M.m. domesticus* (European house mouse) and *M.m. poschiavinus* (Tobacco mouse) is especially confusing. They are chromosomally distinct (Gropp et al. 1972) but morphologically and biochemically indistinguishable from each other (Marshall and Sage 1981)

**RESULTS**

*E. coli* colonies carrying hybrid plasmids were screened for the presence of a Bkm-related sequence, and the identification of AC11 is shown in Figure 1A. The use of a less-stringent washing condition, necessary to overcome the species barrier, was responsible for the high background seen in the figure. The insert was a 3.8-kb-BamHI fragment. When AC11 was hybridized to
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Figure 1.—Identification of ACII: A, DNA isolated from male (C57BL) spleen was digested with BamHI and cloned into pBR322. Transformed E. coli colonies were screened for the presence of Bkm-related sequences using a cloned Drosophila Bkm sequence as the probe. The arrow indicates ACII. The relatively high background was a consequence of a less-stringent washing condition (1 × SSC, 50°C), necessary to overcome the species barrier. B, male or female C57BL DNA was cleaved with EcoRI, transferred to a membrane filter and hybridized to ACII in the presence of herring sperm DNA (50 μg/ml), which absorbed Bkm-related sequence in ACII, leaving male-specific hybridization bands. M: male; F: female.

Mouse genomic blots, smeared patterns of hybridization were obtained because of the presence of Bkm-related sequences in the mouse genome (Singh, Purdom and Jones 1980, 1981; our data with ACII not shown). The smeared background could be significantly reduced when hybridization was carried out in a solution containing 50 μg/ml of herring sperm DNA that was also rich in Bkm-related sequences and absorbed the Bkm-related sequences in ACII (Figure 1B). There were at least eight male-specific bands, ranging from 3.2 kb to 12.0 kb with a strong hybridization at around 9.0 Kbp, that actually consisted of two bands at 9.0 kb and 8.5 kb. This hybridization pattern suggests the presence of a Y chromosomal repetitive sequence within ACII. The 1.3-kb band was common to both sexes and also corresponded to the position of an evolutionarily conserved repetitive sequence known as MIF-1 (Bennett et al. 1984; Brown and Dover 1981; Heller and Arnheim 1980; Meunier-Rotival et al. 1982; Meunier-Rotival and Bernardi 1984). The presence of a MIF-1-like sequence in ACII was confirmed by its cross-hybridization to a clone (designated H-1; Y. Nishioka, unpublished results) containing a mouse MIF-1 sequence (data not shown). However, the homology is not strong, and extensive washing significantly reduced the intensity of the 1.3-kb band.

A restriction map of ACII is shown in Figure 2A. The Bkm-related sequence is present exclusively in subfragment 2 (data not shown). Each subfragment (1–5) was eluted from the gel, nick translated and hybridized to male
Figure 2.—Southern blots of AC11 subfragments. A. A restriction map of AC11. B: BamHI; H: HaeIII; T: TaqI; solid line: AC11 insert; dotted line: pBR322. B, Each fragment was eluted from agarose gel, nick translated and hybridized to EcoRI-digested male C57BL DNA transferred to membrane filters. Their hybridization patterns were compared to that obtained with AC11 (a 3.8-kb BamHI fragment). The dot indicates the 4.2-kb band and the arrow points to the 3-kb band. C, About 10 μg of male and female C57BL DNAs digested with EcoRI were transferred to a membrane filter and hybridized to the nick-translated subfragment 1. The female lane showed weak hybridization in comparison with the male lane. D, Each subfragment was eluted from agarose gel, nick translated to approximately the same specific activity (5 × 10⁷ cpm/μg DNA) and hybridized to EcoRI-digested female C57BL DNA. The filter was exposed to an x-ray film for 7 days at -70° with an intensifying screen. The subfragments 4 and 5 produced very faint bands. The subfragment 2 contained a Bkm-related sequence and produced a smear (not shown).

Genomic DNA digested with EcoRI. As seen in Figure 2B, all the subfragments produced hybridization patterns very similar to that obtained with AC11. These results justify the use of AC11 as a probe to study the Y chromosome without eliminating Bkm-related sequences that appeared to be efficiently absorbed by herring sperm DNA. In addition, the following observations deserve special mention. (1) The 4.2-kb band (indicated by a dot in Figure 2B) is unique to subfragment 4. (2) The sequence sharing homology with M1F-1 (indicated by an arrow in Figure 2B) is present only in subfragment 2. (3) A Y chromosomal single-cope sequence was not found in AC11. Using subfragment 1, we estimated the copy number of AC11-related sequences in the Y chromosome. Dot blot hybridization against male and female DNAs indicated that AC11-related sequences were repeated about 300 times in the male genome (data not shown). This estimation roughly agrees with the frequency (13/10⁵ plaques) of finding AC11 positive plaques in a male mouse genomic library.

Although AC11-related sequences are repeated several hundred times in the
Y chromosome, they are not male-specific (Figure 2C). A prolonged exposure clearly revealed several hybridization bands in female lanes that share homologies with the AC11 subfragments (Figure 2D). We constantly observed extremely weak hybridization signals against subfragments 4 and 5. Upon prolonged exposure, necessary to detect bands in female lanes, subfragment 2 produced a smear because of the Bkm-related sequences (data not shown). Against BamHI-digested male genomic DNA, AC11 showed multiple bands ranging from 3.0 kb to 9.6 kb, with a strong signal at 3.8 kb, this size corresponding to the insert itself (Figure 3). The female lane showed one band (7.0 kb), and no hybridization was observed at 3.8 kb, indicating that AC11 was indeed derived from the Y chromosome. DNA isolated from an XX Sxr mouse showed a hybridization pattern indistinguishable to that obtained from normal female DNA (Figure 4). The absence of male-specific bands in the XX Sxr genome indicates that the concentration of AC11-related sequences must be located in regions of the Y chromosome not involved in sex determination.

It is generally accepted that biologically active DNA sequences are less methylated (for a review, see Doerfler 1983). To estimate the extent of DNA methylation in and around AC11-related sequences, DNAs isolated from adult male brain, liver, spleen, kidney and testes were digested with HpaII or MspI and hybridized to AC11 (Figure 5). MspI digestion defined multiple bands with a major signal at 6.8 kb. HpaII digestion, on the other hand, showed a smear at the top of each lane. This marked difference in hybridization patterns between MspI and HpaII digests indicated that, in the adult tissues we exam-
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FIGURE 4.—Southern blots of XX Sxr male DNA. DNAs were isolated from spleen of an XX Sxr male and its sibs, cleaved with EcoRI and hybridized to AC11 (A), the AC11 subfragment 5 (B) or the AC11 subfragment 1 (C). Male and female DNA isolated from C57BL were used as the controls. Section (C) was obtained after exposing the filter to an X-ray film for 7 days at −70° with an intensifying screen. M: male; F: female.

FIGURE 5.—Extent of DNA methylation in the Y chromosome. About 10 μg of DNAs isolated from testes (T), brain (B), spleen (S) and liver (L) of an adult male C57BL were digested with 40 units (a) or 80 units (b) of HpaII (H) or MspI (M) for 1 hr at 37°, transferred to a membrane filter and probed with the AC11 subfragment 1.

ined, the Y chromosome is highly methylated in the vicinity of AC11-related sequences.

To know if the sequence in AC11 is evolutionarily conserved, subfragment 1 was hybridized to DNAs isolated from human lymphocytes and rat liver. No
Figure 6.—Species-specific hybridization of AC11. Genomic DNAs were isolated from different Mus species, cleaned with EcoRI and hybridized to AC11. Geographically, the *M. caroli* originate from Chonburi Province, Thailand; *M. spretus* from Puerto Real, Spain; *M. pahari* from Tak Province, Thailand; *M. hortulanus* from Pancevo, Yugoslavia; *M. platythrix* from Misare, India; and *M. cookii* from Tak Province, Thailand. M: male DNA; F: female DNA.

Figure 7.—Dimorphism in Y chromosomal DNA. Male genomic DNAs isolated from five strains (C57BL/6J, BALB/cJ, DBA/2J, C3H/HeJ, SWV) and a stock CD1 were cleaved with EcoRI and hybridized to AC11.
hybridization was observed even under less stringent washing conditions (data not shown). Similarly, no male-specific hybridization was found in DNAs isolated from *M. caroli*, *M. pahari*, *M. platythrix* or *M. cookii* (Figure 6). However, significant amounts of AC11-related sequences were present in the *Y* chromosome of *M. spretus* and *M. hortulanus*. This observation suggests that the amplification of AC11-related sequences in the *Y* chromosome took place after a common ancestor of *M. musculus*, *M. spretus* and *M. hortulanus* diverged from the lineages that led to *M. caroli*, *M. cookii*, *M. pahari* and *M. platythrix*.

Using three *Y* chromosome-specific clones, Lamar and Palmer (1984) studied restriction fragment length polymorphisms in nine inbred mouse strains and found two variations. We applied AC11 as the probe and extended the survey to 16 inbred laboratory mouse strains. Results are shown in Figures 7 and 8. AC11 detected only two variations. One group consisted of C57BL, BALB/c, DBA, C3H, CBA, HRS, NZB, SEC, CE, A and P, and SWV, SWR, RF and AKR belonged to the other group. Both outbred stocks CD1 and Rb(5.19)Wh possessed SWV type *Y* chromosome (Figures 7 and 8), whereas the *Y* chromosome found in a sib of an Sxr male was C57BL type (see Figure 4). Five strains (C57BL, BALB/c, C3H, DBA and SWV) were further studied with HindIII, PvuII, BamHI and XbaI. SWV always produced a different hybridization pattern than the other four strains, which were indistinguishable from each other (data not shown). Thus, we confirmed the observation of Lamar and Palmer (1984) that the *Y* chromosome exists in only two forms in classical inbred strains. Recently, using another *Y* chromosomal repetitive sequence PY353/B, Bishop et al. (1985) showed that the *Y* chromosomes found in inbred strains A, BALB/c, C57BL, CBA, C3H, DBA and 129 were of the *M. m. musculus* type, whereas SJL had the *M. m. domesticus* type *Y* chromosome.
Figure 9.—Dimorphism in Y chromosomal DNA. Male genomic DNAs isolated from *M. m. domesticus* and *M. m. musculus* male mice were cleaved with EcoRI and hybridized to AC11. The *M. m. domesticus* subspecies include the following substrains: (1) Centreville Light, Maryland; (2) Lewes, Delaware; (3) J. J. Downs, Ridgely, Maryland; (4) Upper Marlboro, Maryland; (5) Haven’s farm, Davidsonville, Maryland; (6) Watkin’s Star, Centreville, Maryland; (7) Sanner’s farm, Davidsonville, Maryland; and (8) California Boquet, Boquet Canyon, California. The *M. m. musculus* subspecies include the following substrains: (1) Czech I from Morovia, Czechoslovakia; (2) Czech II, Slovakia, Czechoslovakia; (3) Skive, Denmark; and (4) Vegrumbro, Denmark. C57BL and CD1 were used as controls.

Figure 10.—Comparison of Y chromosomal DNA from *Mus musculus* subspecies. Male and female genomic DNA isolated from *Mus musculus* subspecies were cleaved with EcoRI and hybridized to AC11. The *M. m. brevirostris* mice originated from Azrou, Morocco; *poschiavinus* 1 from Tirano, Italy; *praetextus* from Erfond, Morocco; and *poschiavinus* 2 from Zalende, Switzerland. These subspecies are biochemically very similar to *Mus musculus domesticus*. THALER, BONHOMME and BRITTON-DAVIDIAN (1981) classified European mice into four taxonomic units (*Mus* 1–4). All the above subspecies belong to *Mus* 1. C57BL and CD1 were used as controls. M: male; F: female.
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We isolated DNAs from *M. m. musculus* and *M. m. domesticus* and compared their hybridization patterns with those of C57BL and CD1 (Figure 9). We found that C57BL had the *M. m. musculus* type Y chromosome, whereas CD1 had the *M. m. domesticus* type Y chromosome. No variations were found among eight *M. m. musculus* and four *M. m. domesticus* mice trapped at the different locations specified in the legend to Figure 9. Our survey was further extended to three other European *M. m. musculus* subspecies, *M. m. brevirostris*, *M. m. poschiavinus* and *M. m. praetextus*, and to one Japanese subspecies, *M. m. molossinus*. These European subspecies possessed the *M. m. domesticus* type Y chromosome (Figure 10), whereas the Japanese species showed the *M. m. musculus* type Y chromosome (Figure 11). The results of our survey using AC11 are summarized in Table 1.

**DISCUSSION**

From mouse Y chromosomal libraries, LAMAR and PALMER (1984) isolated three male-specific sequences (PY1, PY2, PY3), and BISHOP et al. (1985) described one male-specific clone (pY353/B). These four DNA fragments (1) are members of small Y-chromosome-specific multisequence families, (2) are mouse-specific and (3) detect restriction fragments length polymorphism in the Y chromosome. Although our clone AC11 shares virtually identical properties with these clones, AC11 is clearly different from them on the following grounds. (1) against EcoRI-digested genomic DNA, AC11 recognizes two types of Y chromosomes, whereas pY353/B requires *MspI* or *HindIII* digestion; (2) against BamHI-digested genomic DNA, AC11 shows a pattern of hybridization different from those obtained with PY1, PY2 or PY3. Therefore, it seems that AC11 represents a member of a new small family of mouse Y chromosomal
TABLE 1
Distribution of AC11 repetitive sequences in the Y chromosome of the genus Mus

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographical origin of specimen examined</th>
<th>AC11-related repetitive sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mus musculus</em> species</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. caroli</em></td>
<td>Thailand</td>
<td>−</td>
</tr>
<tr>
<td><em>M. cookii</em></td>
<td>Thailand</td>
<td>−</td>
</tr>
<tr>
<td><em>M. hortulanus</em></td>
<td>Yugoslavia</td>
<td>+</td>
</tr>
<tr>
<td><em>M. pahari</em></td>
<td>Thailand</td>
<td>−</td>
</tr>
<tr>
<td><em>M. platythrix</em></td>
<td>India</td>
<td>−</td>
</tr>
<tr>
<td><em>M. spretus</em></td>
<td>Spain</td>
<td>+</td>
</tr>
<tr>
<td><em>Mus musculus</em> subspecies</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M.m. musculus</em></td>
<td>Czechoslovakia, Denmark</td>
<td>+(^a)</td>
</tr>
<tr>
<td><em>M.m. domesticus</em></td>
<td>Maryland, Delaware, California</td>
<td>+(^b)</td>
</tr>
<tr>
<td><em>M.m. poschiavinus</em></td>
<td>Switzerland, Italy</td>
<td>+(^b)</td>
</tr>
<tr>
<td><em>M.m. breviostris</em></td>
<td>Morocco</td>
<td>+(^b)</td>
</tr>
<tr>
<td><em>M.m. praetextus</em></td>
<td>Morocco</td>
<td>+(^b)</td>
</tr>
<tr>
<td><em>M.m. molossinus</em></td>
<td>Japan</td>
<td>+(^b)</td>
</tr>
<tr>
<td>Laboratory strains/stocks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL, BALB/c, DBA, C3H, CBA, HRS, NZB, SEC, CE, A, 129, P; AKR, SWR, RF, SWV, CD1,(^c)</td>
<td>+(^b)</td>
<td></td>
</tr>
<tr>
<td>Rb(5.19)1Wh,(^c) SJL(^d)</td>
<td></td>
<td></td>
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</tbody>
</table>

\(^a\) *M.m. musculus* type.
\(^b\) *M.m. domesticus* type.
\(^c\) Stocks.
\(^d\) Taken from Bishop et al. (1985).

repetitive sequences. The isolation of these sequences indicates that the mouse Y chromosome is, like the human Y chromosome, rich in repetitive sequences.

Lamar and Palmer (1984) examined nine mouse strains and classified the Y chromosome into two types. Subsequently, Bishop et al. (1985) demonstrated that these two types corresponded to the *M.m. musculus* and *M.m. domesticus* type Y chromosomes. We surveyed 16 inbred strains and two stocks and confirmed that their Y chromosomes exist in only two forms, and most of the classical inbred strains have the *M.m. musculus* type Y chromosome. Among those with the *M.m. domesticus* type Y chromosome, two separate genealogical lines can be recognized. One is a Swiss line to which SWR, SWV, SJL, RF and CD1 belong and can be traced back to Lausanne, Switzerland, in 1924 (Morse III 1978; Rice and O'Brien 1980). The genetic background of Rb(5.19)1Wh was not identified when the mutant was found in 1967 at the National Institutes of Health (White and Tjio 1967). It is tempting to speculate that it also originated from the National Institutes of Health Swiss stock. The other line is AKR, which can be traced back to a Pennsylvania dealer in 1928 (Furth 1978). It is widely believed that Asian mice contributed to the establishment of classical inbred strains (Festing and Lovell 1981; Keeler 1931; Potter 1978). The fact that most classical inbred strains have the *M.m.*
Musculus type Y chromosome naturally led to a speculation that the Japanese mouse M.m. molossinus might have the M.m. musculus type Y chromosome (Bishop et al. 1985). We examined two M.m. molossinus specimens obtained from Litton Bionetics and found the M.m. musculus type Y chromosome. This observation suggests that M.m. musculus and M.m. molossinus are genetically closer to one another than either one is to M.m. domesticus and agrees with the phylogenetic relationship deduced from an analysis of mitochondrial DNAs (Bonhomme et al. 1984; Ferris et al. 1983; Moriwaki et al. 1982; Sage 1981). It is, however, important to point out that the origin of the M.m. musculus type Y chromosome in inbred strains might be further traced back to Western Chinese mice introduced into Japan (Yonekawa et al. 1982). A survey of Asian mice, including Chinese mice, is in progress. Since AC11 classifies the mouse Y chromosome into only two forms and the Y chromosome is transmitted only through males, its contribution to the elucidation of the origins of laboratory mice is limited. In this regard, DNA probes that define subspecies-specific hybridization bands would provide very useful molecular tools.

The human Y chromosome is highly heterochromatic, and at least 50% of its DNA is composed of repetitive sequences, of which the 3.4-kb HaeIII band is the most prominent species (Cooke, 1976; Kunkel, Smith and Boyer 1979). This fragment has been studied in some detail at the molecular level (Cooke and McKay 1978; Cooke, Schmidtke and Gosden 1982; Kunkel, Smith and Boyer 1979; Kunkel and Smith 1982). This repeat may have an autosomal origin as shown by the homology with autosomal sequences (Cooke and McKay 1978; Kunkel and Smith 1982; Szabo et al. 1980). It has been suggested that transfer of a few autosomal copies to the Y chromosome was rapidly followed by amplification mediated by unequal crossing over and/or duplication (Erickson and Goodfellow 1984). Such a mechanism can also account for the accumulation of AC11-related sequences in the mouse Y chromosome and is, in fact, strongly supported by the following observations. (1) AC11-related sequences are present in the female genome, and (2) among Mus species examined, M. spretus and M. hortulanus contain significant amounts of AC11-related sequences, whereas the other four species (M. caroli, M. cookii, M. pahari and M. platythrix) do not. The latter observation also suggests that the amplification of AC11-related sequences in the Y chromosome was an evolutionarily recent event. According to one estimation, M. caroli diverged from M. musculus 5–7 × 10⁶ yr ago, whereas M. platythrix diverged from M. caroli and M. musculus 10–12 × 10⁶ yr ago (Martin et al. 1985). It follows, then, that the amplification of AC11-related sequences in the mouse Y chromosome must have occurred within the last 5–7 × 10⁶ yr.

At present, we have no information on the function of AC11-related sequences except that, in various adult tissues, they are present in highly methylated forms. It is possible that AC11 contains sequences that are functionally significant. At least Bkm-related sequences are known to be transcribed in a quantitatively sex-specific manner (Epplen et al. 1982; Singh, Phillips and Jones 1984). If AC11-related sequences have a functional role, they may have contributed to the establishment of new species.
Finally, the $Y$ chromosomal repetitive sequences could be useful for a number of studies. For example, they would offer an easy means to identify the sex of mouse tissues. They could also serve as markers to identify male cells in male-female chimeras. Some of these applications are now being explored in our laboratory.

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