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Banded krait minor-satellite (Bkm)-associated Y chromosome-specific repetitive DNA in mouse

Lalji Singh*, Shirly G.Panicker, Raghavender Nagaraj and Kshitish C.Majumdar
Centre for Cellular and Molecular Biology, Hyderabad 500007, India

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ABSTRACT

The mouse Y chromosome remains highly condensed in all somatic tissues but decondenses extensively in testis. We have isolated a mouse Y chromosome-specific repeat M34 (11.5 kb) and shown that this is distributed along the Y chromosome except the sex-determining region (the Y short arm) in which GATA repeats are predominantly concentrated. It has 32 copies of GATA repeats in a 2.7 kb fragment. About 200–300 copies of M34 on the Y chromosome are interspersed among other sequences. A 1.2 kb fragment (p3) of M34, containing GATA repeats, also has scaffold attachment region (SAR) motifs which bind to nuclear matrices. A strong affinity of histone H1 to SAR motifs is implicated in maintaining the condensed state of the Y chromosome in somatic tissues. The probable significance of molecular organization of the Y chromosome is discussed.

INTRODUCTION

The banded krait minor (Bkm) satellite DNA sequences are highly conserved and preferentially associated with the sex chromosomes (1–3). In mouse, Bkm sequences are concentrated at the paracentric region of the Y chromosome (3), now considered to be the Y short arm (4), which is necessary and sufficient to convert a chromosomally female XXSxr mouse into a male (3). The major conserved component of Bkm is a tetranucleotide repeat of GATA (5, 6). The consistent association of GATA repeats with the sex-determining chromosomes in many diverse species of eukaryotes, strongly suggests that Bkm may have a role in chromosomal sex-determination. To find out the functional significance of Bkm in sex-determination, it was decided to isolate and characterize mouse Y chromosome-specific DNAs associated with Bkm. Screening of a mouse genomic library with a Bkm probe led to the isolation of several clones, of which clone M34 was found to be repetitive and Y-chromosome specific. Molecular characterization of M34 has revealed a characteristic distribution of SAR motifs and GATA repeats along the length of the Y chromosome. This is contrary to the earlier report (3) that Bkm sequences are exclusively concentrated in the sex-determining region of the Y. The mouse Y chromosome remains condensed in all somatic tissues but decondenses extensively in testis. A strong affinity of histone H1 to SAR motifs, which are present along the length of the Y chromosome adjacent to GATA repeats, may be involved in maintaining the condensed state of the Y chromosome in somatic tissues. The probable significance of the characteristic distribution of GATA repeats along the mouse Y chromosome in its condensation/decondensation cycles is discussed.

MATERIALS AND METHODS

DNA isolation, restriction and gel electrophoresis

Male and female mouse DNA was isolated from pooled tissues and restriction digested DNA was size fractionated by agarose gel electrophoresis (3).

^{32}P-labelling of probes

The specific DNA probe used for filter hybridization was labelled by nick translation with ^{32}P-dATP (spec. act. 3000 Ci/mmol from Jonaki, BARC, India) by using Amersham nick translation kit.

Transfer and hybridization of DNA

Gel-fractionated DNA was transferred to Hybond nylon membrane (Amersham) by vacuum blotting (7). Blots were hybridized in 0.5 M PO_4 buffer (pH 7.4), containing 7% SDS, with $1-5\times10^6$ cpm/ml of single-stranded ^{32}P-labelled probe (spec. act. 4×10^8 cpm/μg) at 65°C for 12–14 h. Blots were washed at 65°C with 3 changes of 2×SSC [(20×SSC is 3 M NaCl in 0.3 M trisodium citrate (pH 7.0)] and 1% SDS for 10 min each, one change of 1×SSC and 1% SDS for 15 min, one change of 0.5×SSC and 1% SDS for 15 min, and one change of 0.1×SSC and 1% SDS for 15 min and exposed to X-ray film (Konica) at −70°C for 15–48 h.

In situ hybridization

The procedure described by Jones (8) and Singh *et al.* (9, 10) was used.

Isolation and subcloning of M34

A library of *Alu*I-digested male mouse liver DNA was constructed according to the method of Maniatis *et al.* (11) in Charon 4A vector using *Eco*RI linkers. The library was screened using a

*To whom correspondence should be addressed

nick-translated uncloned Bkm probe. Several positive clones were recovered, which we subsequently probed with 2(8) consisting largely of repeats of the tetranucleotide GATA. One of the strongly positive clones, M34, containing a 11.5 kb insert, produced 4 fragments upon *Eco*RI digestion. The *Eco*RI fragments were subcloned into the pUC18 through shotgun cloning and grown in *E.coli* DH5α host strain. These clones were designated p21, p17, p66 and p19 in decreasing order of size of inserts. The p17 was further subcloned by creating deletions using an erase-a-base system (12) from Promega.

DNA sequencing and analyses

DNA sequencing was carried out according to the method of Sanger *et al.* (13) following an improved strategy for rapid sequencing of both strands of DNA cloned in a plasmid using either Taq polymerase (14) or by sequenase reaction (15). The Taq polymerase kit was purchased from Promega and sequenase version 2 kit from United State Biochemical Corporation.

Sequence homology search was done against EMBL nucleotide sequence database release 32 (1992), using the algorithm of Wilbur and Lipman (41) and the scan was done with a *K*-tuple value of 1. Using a computer programme of PC/gene package, search for direct and inverted repeat was also carried out.

Slide preparation of cells from various tissues

Cells from gonads and other tissues were prepared according to Singh *et al.* (17). Sertoli cells from new born mice testes were prepared as described by Hadley *et al.* (18) and were suspended in methanol−acetic acid (3:1). One drop of the cell suspension was air dried on each slide.

Testis section and chromosome preparation

Testes sections from normal adult male mouse and from an azoospermic (somatic mosaic of 10% XY and 90% XX cells) male mouse with small testis were prepared as described by Singh *et al.* (10). Chromosomes were prepared from short term bone marrow cultures by the usual air-drying procedure.

SAR-binding to nuclear scaffolds

The subclone p3 (1.2 kb) of p17 containing SAR was used for scaffold-binding studies following the procedure described by Luderus *et al.* (19). The *Hin*dIII/*Eco*RI double-digested restriction fragments of p3 were end labelled with ^{32}P-dATP using the Klenow fragment. Nuclear matrices (5×10^5 nuclear equivalents in 100 μl) isolated from mouse liver following the procedure described by Luderus *et al.* (19) were incubated overnight with end-labelled DNA fragments (40,000 cpm, 2.5 ng) and double digested (*Eco*RI and *Hin*dIII) *E.coli* DNA (10 μg), at 37°C, to allow exogenous SARs to bind to the mtrices by displacing endogenous SARs. Matrix-bound DNA was separated from unbound DNA by centrifugation in a microfuge for 5 min. Radioactive DNA fragments present in both fractions were purified, size fractionated on agarose gel, and visualized by autoradiography.

RESULTS

Genomic blots probed with M34

One of the several Bkm-positive clones isolated from the male mouse genomic library was M34. *Eco*RI genomic blots of T16, XSxr female, XYSxr male, XXSxr sex-reversed male, XY male and XX female mice, *Mus musculus domesticus*, when hybridized with M34 showed no hybridization in the female (Figure 1, track 5) but revealed a strong signal at approximately 3 kb and a relatively weaker signal at 8 kb and five faint bands (c, d, e, f and G) in the size range of 0.5−3 kb (Figure 1, tracks 4 and 2), in the XY and XYSxr males respectively. There was no

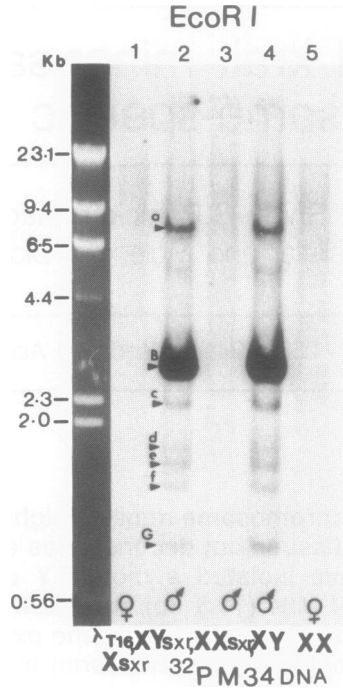


Figure 1. 10 μg of mouse DNA restricted with *Eco*RI, fractionated on agarose gel and hybridized with ^{32}P nick translated clone M34. Note male-specific hybridization (track 2 and 4) suggesting Y-chromosomal origin of the clone. Sex-reversed mice XXSxr (tracks 1 and 3) carrying the translocation of the sex-determining region of the Y chromosome, do not show any hybridization. These sequences are therefore, absent from the sex determining region of the Y chromosome.

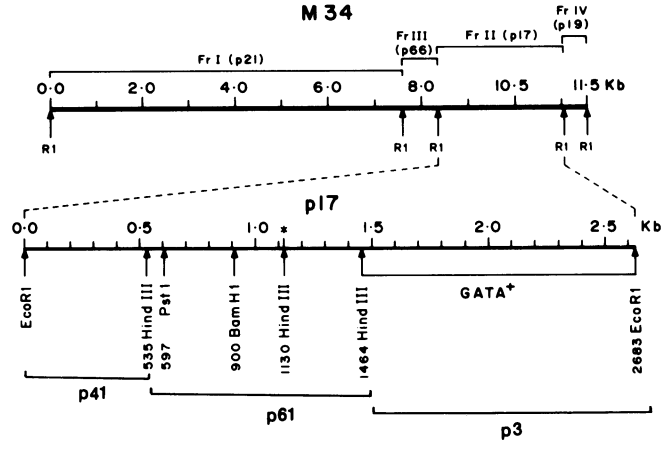


Figure 2. Restriction map of M34. The total insert is 11.5 kb which can be cleaved from the vector by *Eco*RI digestion in 4 fragments which are serially numbered in decreasing order of size and have been subcloned in pUC18 vector. Further restriction map of fragment II (subclone p17) showing the Bkm positive fragment as GATA$^+$ is highlighted. The entire fragment (p17) consisting of 2683 bp has been sequenced.

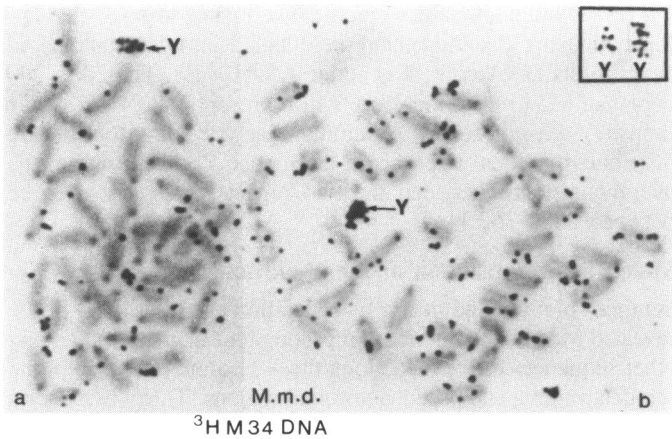


Figure 3. *In situ* hybridization of male mouse metaphase chromosomes with ^{3}H-labelled clone M34. (**a**) After 2 weeks exposure; (**b**) After 3 weeks exposure: In the inset are Y chromosomes from two different metaphases, showing hybridization covering the entire Y chromosome except the sex-determining region.

hybridization with the DNA of T16, Sxr female and sex-reversed XXSxr male carrying only the sex-determining region of the Y chromosome (Figure 1, tracks 1 and 3). There was no enhanced signal with the DNA of XYSxr male carrying the additional sex-determining region (Sxr) apart from the normal Y chromosome, compared to XY male (Figure 1, tracks 2 and 4). The M34 sequences, therefore, are absent from the sex-determining region of the mouse Y chromosome. M34 sequences were not found in females and would be thus absent in the pseudoautosomal region involved in the crossing over between X and Y chromosomes. Singh *et al.* (20) had earlier shown male-specific hybridization of *M.spretus Eco*RI genomic blots with M34. These sequences were not detectable in the genomes of rat and human males and females (data not shown). M34 sequences are, therefore, mouse-specific.

Organization of M34 DNA repeats

A restriction map of M34 is given in Figure 2. The total insert size is 11.5 kb which upon digestion with *Eco*RI produced 4 fragments. These fragments were cloned in pUC18 vector. One of the subclones p17 was found to be positive for Bkm. *Eco*RI-digested mouse genomic DNA probed with ^{32}P labelled *Eco*RI fragment I of M34 (p21) eluted from the gel showed two prominent bands of 8 kb (a) and 2.7 kb (B) and a faint band of 5.8 kb. The band 'a' which was very faint with M34 probe (Figure 1, track 2) was very intense with p21 probe (data not shown). Hybridization with M34 fragment II (p17), however, revealed two prominent bands of 2.9 and 2.7 kb and one very faint band of 0.84 kb (data not shown) similar to bands 'B' and 'G' respectively (Figure 1, tracks 2 and 4). While there were two very close bands detected with p17 probe, only one broad band, 'B', was detected with M34. Perhaps the broad band 'B' represents both bands fused together. Hybridisation with M34 fragment III (p66) showed one prominent band 'a' much stronger in intensity and a very faint band, 'f' of similar intensity as in Figure 1, tracks 2 and 4 respectively (data not shown). Similarly, blot hybridized with M34 fragment IV (p19) revealed two low intensity bands (8 kb and 5.8 kb) similar in intensity and size and a very prominent band G (0.84 kb) much stronger in intensity to that detected by M34 respectively (Figure 1, tracks 2 and 4). No detectable hybridization was seen with the female genomic DNA. Hybridization of *Eco*RI-digested M34 DNA with ^{32}P-labelled eluted DNA probes of fragments I, II, III and IV (Figure 2) did not show any cross hybridization of one fragment with the other (data not shown) suggesting that each insert was unique. The intensity of hybridization of male genomic DNA with different fragments of M34 strongly suggested the repetitive nature of each fragment. The M34 (11.5 kb), therefore, seemed to be a repeating unit or a part of a larger repeating unit.

In situ hybridization using ^{3}H-labelled M34 probe showed distribution of grains along the length of the Y chromosome (Figure 3a and b). This was unexpected, as the Y chromosome in mammals is generally constitutively heterochromatic and rich in highly repetitive (satellite) DNA but the mouse Y chromosome has been considered to be an exception because of its being C-band negative (21). *In situ* hybridization using one of the subclones p17 (*Eco*RI fragment II of M34) revealed a hybridization pattern similar to that obtained by M34 (data not shown), suggesting p17 as a part of the repeating unit.

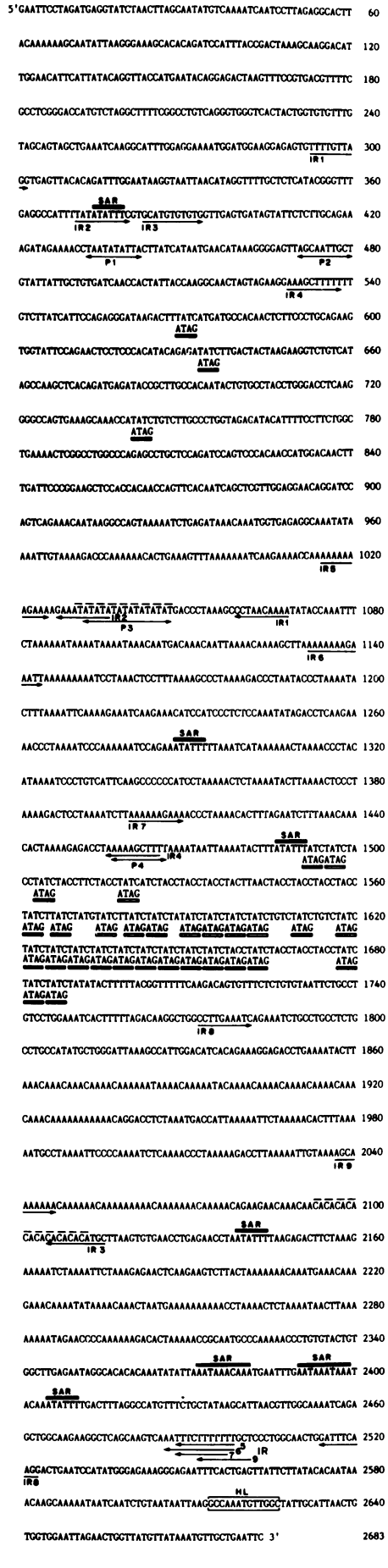
Deletion cloning and sequencing of p17 (M34 *Eco*RI fragment II)

The p17 was double digested with *Eco*RI and *Hin*dIII producing three fragments of 535 bp (p41), 929 bp (p61) and 1219 bp (p3). The restriction map of p17 is given in Figure 2. Repeated sequencing of p17 revealed an additional *Hin*dIII site at 1130 bp, shown by a star (Figure 2). This site however, was not recognised by *Hin*dIII. A possibility of site-specific modification could not be ruled out. An *Eco*RI digest of mouse male and female genomic DNA probed with various fragments of p17 individually, as expected, showed male-specific pattern of hybridization as detected by the p17 probe except the 1219 bp fragment (p3) which failed to detect a 0.84 kb band detected by other fragments (data not shown).

Deletion-subcloning of p17 was done by using an erase-a-base system. The nucleotide sequence of p17 thus determined is given in Figure 4. The insert was 2683 base pairs long. The main feature of the DNA sequence of p17 is the presence of 32 copies of GATA repeat of which 12 copies are contiguous between the base pairs 1617 and 1664, and the presence of several copies of SAR recognition sequences. Potential small open-reading frames (ORFs) and polyadenylation sites are also present in the sequence. However, we failed to detect transcripts of these sequences in any of the adult tissues (liver, kidney, spleen and testis) tested so far. A potential hairpin loop was detected between base pairs 2615–2625. A number of palindromic sequences, repeats and inverted repeats with their positions are shown in Figure 4. The core consensus sequence TGACG for ATF binding (22) was present in the region corresponding to base pairs 171–175. Ten copies of two base pair CA repeats, having the potential of acquiring a Z-DNA conformation, were present between base pairs 2092 and 2112. Apart from GATA repeats and short stretches of CA, AT, A and T (Figure 4), no apparent repeating unit was obvious in the 2683 base pair sequence of p17.

Estimation of copy number

The copy number of p17 and M34 fragment I in male mouse genome was estimated by slot blot analysis. Approximately 200–300 copies of p17 and M34 fragment I sequences were estimated to be present in the mouse Y chromosome. This further confirms the entire M34 (11.5 kb) being the repeating unit.



The haploid mammalian genome is estimated to be 3×10^9 bp. Since mouse Y chromosome represents 2.5% of the total haploid genome, its DNA content is about 7.5×10^7 bp. The 200–300 copies of M34 repeat therefore will represent 3.1–4.6% of the mouse Y chromosome. Assuming that the M34 repeats are distributed at equal intervals in the mouse Y chromosome, the average distance between the two repeats is estimated to be between 238–363 kb.

Molecular organization of the Y chromosome

Southern blotting and *in situ* hybridization using the M34 probe revealed M34 as the minimum repeating unit interspersed among other sequences distributed along the Y chromosome except the sex-determining and pseudoautosomal regions. Thirty two copies of GATA repeats are integral constituents of the M34 repeats in the Y chromosome. This was further substantiated by *in situ* hybridization using the Bkm 2(8) probe (predominantly containing GATA repeats) which showed distribution of grains along the Y chromosome including the sex-determining region except the pseudoautosomal region. This however, was obtained under reduced stringency of hybridization and much longer exposure (data not shown). When high stringency was used and shorter exposure given, hybridization was predominantly confined to the short arm which is sex-determining, as reported earlier (2, 3). Because of the high concentration of GATA sequences in the sex-determining region compared to other regions of the Y and elsewhere in the genome, usually only the sex-determining region of Y is detected by *in situ* hybridization using Bkm 2(8) probe. M34 sequences were not detected in the sex determining region of the Y chromosome in spite of the fact that GATA repeats form part of the M34 repeating unit. This might be due to the fact that GATA repeats form a very small proportion of the 2.7 kb repeat and, therefore, hybridization is driven by DNA sequences other than the GATA repeats of p17.

In situ hybridization with ^{3}H-labelled 2(8) and M34 probes strongly suggests p17 (containing GATA repeats) as part of the M34 repeating unit. One would then expect to see much reduced hybridization intensity of each band on Southern blot in a male mouse having partial deletion of the Y chromosome. We digested 10 μg DNA of each of the male mutant mouse B10-BR-Ydel/Ms (23), having reduced size of the Y chromosome due to partial deletion, and the normal male mouse (with normal Y chromosome) with *Eco*RI and hybridized with ^{32}P-labelled p17 probe. As predicted, each band of Y del mouse showed reduced intensity by one-third in comparison to the normal male mouse on Southern blot (data not shown). This was in agreement with the reduction in size of the Y chromosome due to deletion (23).

Figure 4. Nucleotide sequence of a Bkm-positive subclone p17 of mouse Y chromosome-specific clone M34, which is 2683 bp long. There are 32 copies of GATA repeats shown by open bars in the complementary strand; 9 copies of AT repeats shown by broken lines above the sequence, 7 copies of SARs shown by solid bars above the sequence; 9 inverted repeats numbered IR1–9 shown by arrows under the sequence, direction of arrow indicating the direction of the repeat; 4 palindromic sequences, p1–4, shown by double-headed opposing arrows under the sequence; one potential hairpin loop forming sequence HL underlined on both the sides; and10 copies of dinucleotide repeat, CA, having the potential to adopt a left-handed DNA conformation (Z-DNA), shown by broken lines above the sequence. Nucleotide sequence of p17 has been submitted to the EMBL Data Library and has been assigned the following accession number: X 71670 *Mus musculus* p17 sequence.

This further confirms that p17 is an integral part of the repeating unit distributed along the Y chromosome.

Decondensation of the Y chromosome in germ cells

In situ hybridization using a ^{3}H-labelled M34 probe revealed a condensed state of the Y chromosome in somatic cells of various tissues (data not shown) and in Sertoli cells of the sections of small testis from a male azoospermic (somatic mosaic of 10% XY and 90% XX cells) mouse, which were devoid of germ cells and contained predominantly Sertoli cells (Figure 5a). Isolated Sertoli cells of normal mouse testis hybridized with ^{3}H-labelled M34 as well as 2(8) probes and adult mouse testis sections hybridized with ^{3}H-labelled M34 probe also confirmed that the mouse Y chromosome remains condensed in Sertoli cells (Figure 5b) but decondenses extensively in certain cell types of germ cells (Figure 5c–e). These results are contrary to the report of Guttenbach *et al.* (24) who showed that the mouse Y chromosome decondenses in the Sertoli cells.

High-affinity-binding of histone H1 to Y-chromosome-specific sequence p3

Nuclear extracts prepared from different tissues of male and female mice were subjected to 10% SDS–polyacrylamide gel electrophoresis and electroblotting onto a nitrocellulose membrane. The membrane was subjected to protein–DNA-binding assay in the presence of a 1000–2000-fold excess of *E.coli* DNA and ^{32}P-labelled mouse Y chromosome-specific p3 insert. The ^{32}P-labelled probe bound very strongly to a protein present in all the tissues of male (Figure 6) as well as female mouse (data not shown). This protein corresponded in size to histone H1 in the marker lane (data not shown) which also showed strong binding. When ^{32}P-labelled 545 bp-long, AT-rich insert of clone 2(8) containing 66 copies of GATA repeats (6) was used under identical binding conditions, no binding was detected. This ruled out GATA repeats as the recognition sequence of histone H1 in the p3 clone. The remaining sequences of p3 which also include the SAR sequence ATATTT, therefore, must include the sequences required for DNA conformation that would allow a strong binding of histone H1. From these results we conclude that p3 insert contains the DNA sequence for which histone H1 shows a very strong affinity. Considering the number of copies of these sequences on the Y chromosome (200–300), it is possible that histone H1 may be responsible for maintaining the condensed state of the entire Y-chromosome in various somatic tissues.

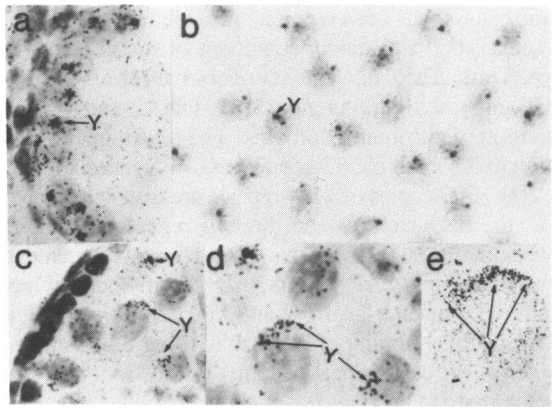


Figure 5. *In situ* hybridization of ^{3}H-labelled mouse Y chromosome-specific clone M34. (**a**) Frozen testis section of ST mouse (a somatic mosaic of 10% XY and 90% XX cells, devoid of germ cells) showing condensed state of the Y chromosome in Sertoli cells, shown by arrow; (**b**) Separated Sertoli cells from the mouse testis, obtained as described in Materials and method, showing condensed state of the Y chromosome; (**c**) Frozen section of testis of normal male mouse, showing the decondensed state of the Y chromosome in primary spermatocytes (long arrows). Other cells show condensed Y chromosome (short arrow); (**d**) Part of 'c' shown at higher magnification; (**e**) One spermatocyte showing extensive decondensation of the Y chromosome as revealed by grain distribution.

Sequence p3 containing SAR motif ('ATATTT') binds to nuclear matrices

DNA sequence analysis revealed that the 1219 bp DNA fragment (p3) of p17 contains several SAR motifs (Figure 6) indicating

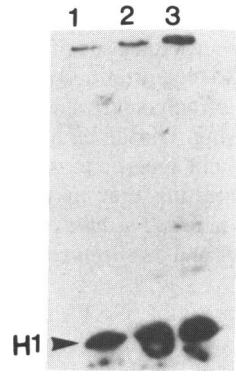


Figure 6. DNA-binding assay of protein of nuclear extracts from the testis, kidney and liver (tracks 1, 2 and 3 respectively) fractionated on 10% polyacrylamide gel and electroblotted onto a nitrocellulose membrane in the presence of a 1000–2000 fold excess of unlabelled *E.coli* DNA, using ^{32}P-labelled mouse Y chromosome-specific p3 insert (1219 bp) between base pairs 1464–2683 of p17 (Fig. 4) containing SAR motifs and GATA repeats. Note strong binding to histone H1 present in all the tissues.

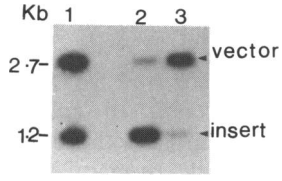


Figure 7. Specific SAR binding to nuclear matrices. Nuclear matrices were isolated from mouse liver following the procedure of Luderus *et al.* (19). A plasmid pUC18 containing 1.2 kb insert with SAR motiffs (p3) was double-digested with *Eco*RI and *Hin*dIII. Restriction fragments were end-labelled with [α^{32}P]dATP and incubated with nuclear matrices in the presence of *E.coli* competitor DNA (10 μg per assay); 3 ng of radiolabelled restriction fragments was added to 5×10^{6} nuclear equivalents of nuclear matrices in a volume of 100 μl. Incubation was carried out overnight at 37°C. The matrices and shells were pelleted, radioactive restriction fragments present in the pellet and the supernatant were purified, size separated on a 1.2% agarose gel, and visualized by autoradiographyhy. Track 1: *Eco*RI and *Hin*dIII double-digested and end-labelled clone p3 showing 2.7 kb vector and 1.2 kb insert bands of equal intensity as a control. 2: DNA isolated from the pellet showing prominent insert band and a very faint vector band, indicating that the insert containing SAR is predominantly bound to nuclear matrices. 3: DNA isolated from the supernatant showing unbound predominant vector band and a very faint residual insert band.

that it may provide binding sites for the nuclear matrix. In order to confirm this, nuclear matrices were isolated from mouse liver, and scaffold-binding studies were carried out. As expected, the insert containing the SAR motifs was predominantly bound to nuclear matrices and therefore, detected prominently in the pellet fraction (Figure 7, track 2), whereas the vector DNA remained mostly unbound and was detected predominantly in the supernatant fraction (Fig. 7, track 3).

DISCUSSION

Repetitive sequence families specific to the mouse Y chromosome have been reported by several groups (20, 25–31). There are several features of M34 which are in common with some of the sequences described earlier by others. Since M34 is a 11.5 kb repeat, it seems probable that others have cloned random small fragments of the same M34 repeat. As expected, these repeats show hybridization patterns different from each other but most of them are mouse specific and specific to the Y chromosome but absent from the sex-determining region. The present study strongly suggests that a fragmentary study of repetitive sequences can at times be confusing. Our clone M34 containing a 11.5 kb insert is the repeating unit specific to the mouse Y chromosome (the length of the repeating unit may be even larger). M34 sequences are present in *Mus musculus musculus*, *M.m.domesticus*, *M.m.poschiavinus* and *M.spretus* (20) but absent in rat and human.

We have earlier reported that GATA repeats are predominantly concentrated in the sex-determining region (Y short arm) of the mouse Y chromosome (6). We have now found that these repeats are also distributed along the length of the Y chromosome interspersed amongst other sequences as a part of Y-specific repeat M34 (200–300 copies). This is similar to the distribution of Bkm along the length of the snake W chromosome (1). Absence of M34 repeats in the sex-determining region of the Y chromosome testifies that it is differently organised than the rest of the Y chromosome. The available evidence suggests that the human Y chromosome may also be similarly organised (our unpublished data). This similarity in molecular organization of the Y and W chromosomes is perhaps a reflection of a common mechanism involved in bringing about specialization of the sex-determining chromosomes.

In the present investigation we have shown that the mouse Y chromosome remains condensed and, therefore, largely transcriptionally inactive in the somatic cells but decondenses specifically in the germ cells (primary spermatocytes). This is the stage at which the X chromosome in all male heterogametic organisms gets inactivated and the Y chromosome becomes functional. This is in agreement with the recent findings (32) that Y-linked genes Zfy-1 (33); Spy (34); Sry (35); A1s9Y-1 and A1s9Y-2 (36), and Sby (37) are expressed specifically in the testis. A Y chromosomal DNA sequence 145SC5 which has a potential open reading frame coding for a protein consisting of 227 amino acids, exists in 200 copies along the length of the Y chromosome including the sex determining region and is specifically expressed in the testis (38). Unlike this sequence, M34 sequences have failed to detect any transcript in adult mouse liver, kidney and testes, and are absent in the sex-determining region of the Y chromosome. However, transcription of M34 sequence during embryonic development cannot be ruled out. Sequence comparison has not revealed any sequence homology between p17 and 145SC5. A mutation (Y del) in mice, caused by partial deletion of the Y chromosome, causes increased number of abnormal spermatozoa with flat acrosomal caps. This suggests that some sequences present in the long arm of the Y chromosome are involved in the control of acrosome development (23). Recently Xian *et al.* (39) reported that not only the morphology but also the fertilizing ability of spermatozoa is directly related to partial deletion of the Y chromosome. Phenotypic effect of these genes, therefore, will be detectable only after the effective number of copies of such genes is reduced below a threshold level due to deletion. This also indicates the necessity for coordinated activation of these gene-families in a specific tissue at a specific time. Probably, sex-determining chromosomes are endowed with such genes that require coordinated timely activation. In *Drosophila hydei* and many other *Drosophila* species, the Y-chromosome, during the primary spermatocyte stage, develops characteristic lampbrush loops of distinct morphology which are the sites of transcription. An ay1 sequence family is distributed along the lampbrush loop and is known to enhance the transcription of flanking DNA via the binding of specific nuclear proteins (40).

Condensation and decondensation of Y chromatin

In the present study we have shown that Y chromosome of mouse undergoes the cycles of condensation and decondensation in testis in contrast to all somatic tissues where it remains highly condensed and transcriptionally inactive. A W protein, which specifically recognises the chicken W-specific *Xho*I family of repetitive sequences, has been implicated in maintaining the stable condensed state of the W chromatin in somatic tissues (41, 42). No such Y-specific protein has so far been reported in mouse. A non-histone heterochromatin-associated protein, Hp1, encoded by the suppressor of position-effect variegation gene Su (var) 205, has been reported in *Drosophila* (43). In the present study, a strong preferential binding of histone H1 to the p3 fragment containing the SAR sequence motif (ATATTT) may be responsible for the highly condensed state of the Y chromosome in somatic cells. It has been reported that histone H1 preferentially binds to eukaryotic SAR under conditions of strong co-operativity (44–46). Thus, SARs nucleate co-operative H1 assembly along the SAR into the flanking non-SAR DNA, and control the conformation of chromatin domains. The p3 fragment contains, in addition to SAR motifs, 32 copies of GATA repeats. Assuming that the 200–300 copies of M34 repeats are distributed at regular intervals, the distance between the two repeats is estimated to be between 238–363 kb DNA. These regions flanked by p3 fragment-containing SAR motifs, may form loops which are anchored to the nuclear scaffold at SAR, delimiting the ends of active chromatin. Looping of DNA may bring SARs and GATA repeats of p3, in each repeating unit, in close vicinity of each other at the matrix. Histone H1 may bring about compaction by preferentially binding to SAR motifs and also to mini bands, resulting into highly condensed chromatin in interphase nuclei. It is interesting that a sex-specific DNA binding protein which recognises GATA repeats of Bkm, is predominantly present in the testis, the tissue in which the Y chromosome is decondensed and transcriptionally active (our manuscript submitted elsewhere). The co-existence of the BBP and the decondensed state of the Y chromosome in testis suggests that perhaps ordered organization of M34 repeats throughout the length of the Y chromosome plays an important role in condensation and decondensation cycles of the Y chromosome.

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REFERENCES

1. Singh, L., Purdom, I.F. and Jones, K.W. (1980) *Chromosoma* **79**, 137–157.
2. Singh, L., Purdom, I.F. and Jones, K.W. (1981) *Cold Spring Harb. Symp. Quant. Biol.* **45**, 805–813.
3. Singh, L. and Jones, K.W. (1982) *Cell* **28**, 205–216.
4. Bishop, C.E., Weith, A., Mattei, M.G. and Roberts, C. (1988) *Phil. Trans. R. Soc. Lond.* **B 322**, 119–124.
5. Epplen, J.T., McCarrey, J.R., Sutou, S. and Ohno, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3798–3802.
6. Singh, L., Phillips, C and Jones, K.W. (1984) *Cell* **36**, 111–120.
7. Olszewska, E. and Jones, K.W. (1988) *Trends Genet.* **4**, 92–94.
8. Jones, K.W. (1973) Pain, R.H. and Smith, B.J. (eds). In *Biophysics and Cell Biology*. John Wiley and Sons, London, Vol. **1**, pp. 29–66.
9. Singh, L., Purdom, I.F. and Jones, K.W. (1977) *Chromosoma* **60**, 377–389.
10. Singh, L., Matsukuma, S. and Jones, K.W. (1987) *Devl. Biol.* **122**, 287–290.
11. Maniatis, T., Hardison, R.C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G.K. and Efstratiadis, A. (1978) *Cell* **15**, 687–701.
12. Henikoff, S. (1984) *Gene* **28**, 351–359.
13. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
14. Innes, M.A., Myanbo, K.B., Gelfand, D.H. and Brow, M.A.D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 19436–19440.
15. Tabor, S. and Richardson, C.C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4767–4771.
16. Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 726–730.
17. Singh, L., Purdom, I.F. and Jones, K.W. (1979) *Chromosoma* **71**, 167–181.
18. Hadley, M.A., Byers, S.W., Suarez-Quian, A., Kleinman, H.K. and Dym, M. (1985) *J. Cell Biol.* **101**, 1511–1522.
19. Luderus, M.E.E., de Graaf, A., Mattia, E., den Blaauwen, J.L., Grande, M.A., de Jong, L. and Driel, R.V. (1992) *Cell* **70**, 949–959.
20. Singh, L., Winking, H., Jones, K.W and Gropp, A. (1988) *Mol. Gen. Genet.* **212**, 440–449.
21. Dev, V.G., Miller, D.A., Charen, J. and Miller, O.J. (1974) *Cytogenet. Cell Genet.* **13**, 256–267.
22. Dworetzky, S.I., Wright, K.L., Fey, E.G., Penman, S., Lian, J.B., Stein, J.L. and Stein, G.S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4178–4182.
23. Styrna, J., Klag, J. and Moriwaki, K. (1991) *J. Reprod. Fert.* **92**, 187–195.
24. Guttenbach, M., Schmid, M., Jauch, A. and Vogt, P. (1989) *Chromosoma* **97**, 429–433.
25. Nallaseth, F.S., Lawther, R.P., Stallcup, M.R. and Dewey, M.J. (1983) *Mol. Gen. Genet.* **190**, 80–84.
26. Lamar, E. and Palmer, E. (1984) *Cell* **37**, 171–177.
27. Bishop, C.E., Boursot, P., Baron, B., Bonhomme, F. and Hatat, D. (1985) *Nature* **315**, 70–72.
28. Nallaseth, F.S. and Dewey, M.J. (1986) *Nucleic Acids Res.* **14**, 5295–5307.
29. Nishioka, Y. and Lamothe, E. (1986) *Genetics* **113**, 417–432.
30. Nishioka, Y. (1987) *Genet. Res. Camb.* **5**, 69–72.
31. Nishioka, Y. (1988) *Genet. Res. Camb.* **52**, 145–150.
32. Koopman, P., Gubbay, J., Collignon, J. and Lovell-Badge, R. (1989) *Nature* **342**, 940–942.
33. Page, D.C., Mosher, R., Simpson, E.M., Fisher, E.M.C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A. and Brown, L.G. (1987) *Cell* **51**, 1091–1104.
34. Burgoyne, P.S. (1987) *Development* **101** (supplement), 133–141.
35. Gubbay, J., Collignon, J., Koopman, P., Capel, B., Economou, A., Munsterberg, A., Vivian, N., Goodfellow, P. and Lovell-Badge, R. (1990) *Nature* **346**, 245–250.
36. Kay, G.F., Ashworth, A., Penny, G.D., Dunlop, M., Swift, S., Brockdorff, N. and Rastan, S. (1991) *Nature* **354**, 486–489
37. Mitchell, M.J., Woods, D.R., Tucker, P.K., Opp, J.S. and Bishop, C.E. (1991) *Nature* **354**, 483–486.
38. Prado, V.F., Lee, C.H., Zahed, L., Vekemans, M. and Nishioka, Y. (1992) *Cytogenet. Cell Genet.* **61**, 87–90.
39. Xian, M., Azuma, S., Naito, K., Kunieda, T., Moriwaki, K. and Toyoda, Y. (1992) *Biol. Reprod.* **47**, 549–553.
40. Vogt, P. and Hennig, W. (1986) *Chromosoma* **94**, 449–458.
41. Tone, M., Sakaki, Y., Hashiguchi, T. and Mizuno, S. (1984) *Chromoso*ma **89**, 228–237.
42. Harata, M. Ouchi, K., Ohata, S., Kikuchi, A. and Mizuno, S. (1988) *J. Biol. Chem.* **263**, 13952–13961.
43. Paro, R. (1990) *Trends Genet.* **6**, 416–421.
44. Sevall, J.S. (1988) *Biochemistry* **27**, 5038–5044.
45. Izaurralde, E., Kas, E. and Laemmli, U.K. (1989) *J. Mol. Biol.* **210**, 573–585.
46. Kas, E., Izaurralde, E. and Laemmli, U.K. (1989) *J. Mol. Biol.* **210**, 587–599.