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Functional Significance of Molecular Organization of Sex Chromosomes

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Sex-determining chromosomes are neterochromatic, stain darkly with Giemsa stain, remain highly condensed and transcriptionally inactive in all somatic tissues, and are enriched in repetitive DNA. A sex chromosome-specific satellite DNA Bkm (banded krait minor satellite), which is highly conserved in a wide spectrum of eukaryotes, consists of tetranucleotide repeats of GATA. In the mouse, these sequences are predominantly concentrated in the short arm of the Y chromosome which is sex-determining. By screening the human and mouse genomic libraries with Bkm probe, we have recovered a human and a mouse Y chromosome-specific clones p102d(2) and M34 respectively which are species-specific and distributed along the Y chromosome except the sex-determining region. The conserved component of these repeats is tetranucleotide repeat GATA. The sex-determining chromosomes show cycles of condensation and decondensation in germ cells of the heterogametic sex in contrast to all somatic tissues, where it remains highly condensed and transcriptionally inactive. This led to the prediction of the existence of a factor which would bind specifically to Bkm for bringing about the coordinated decondensation and functional activation of the sex chromosome. Such a factor has been identified as sex and tissue-specific DNA-binding protein present in the snake ovary and mouse and human testis which is designated as Bkm-binding protein (BBP). We propose a novel role of Bkm in bringing about decondensation of the sex chromosomes (Y and W) in response to BBP. This explains the evolution, universal heterochromatic nature and functional significance of molecular organization of the sex determining chromosomes.

Key Words: Bkm-binding protein, Decondensation, GATA-binding protein, Heterochromatin, Y-specific repetitive DNA

Introduction

Development of embryonic bipotential gonadal primordium either in a testis or an ovary is probably under the control of a pair of alleles in a large number of species of vertebrates (fish, amphibia and reptiles). This pair of allele is inherited in heterozygous form by the male in species with male heterogamety and by the female in species with female heterogamety. However, in more advanced vertebrates one entire chromosome is assigned exclusively for this purpose. It is believed that the sex chromosomes are the specialised autosomes (Ohno 1967) referred to as X and Y in male heterogamety and Z and W in female heterogamety. The sex determining chromosomes—the Y and the W are largely heterochromatic and rich in repetitive DNA (see Singh & Majumdar 1993). This is of special significance considering the fact that chromosomal sex-determination (CSD) has evolved on many separate occasions during the course of evolution in higher eukaryotes (Jones & Singh 1985). But in every case it has led to the same consequence of heterochromatinization of the entire sex-determining chromosome and loss of its other genetic functions. This makes one wonder about the neces-

sity for the evolution of sex chromosomes at the expense of its other genes, which are rendered functionless, when sex ratio can be maintained by a pair of alleles (Ohno 1967). The fact that CSD exists in different groups strongly suggests that it has not been disadvantageous in evolutionary terms. In order to understand the mechanism involved and functional significance of this process, we have isolated and characterized repetitive DNA contained in the W chromosome of snake and Y chromosome of mouse and human. We have used these DNAs as probes for finding: when and where the sex determining chromosomes (Y and W) become functional; what are the nucleotide sequences in common between snake, mouse and human; whether there is any commonality in organization of these sequences on the W and Y chromosomes; and what is the significance of specific distribution pattern of such sequences on the sex determining chromosomes. We present evidences, which for the first time, explain the evolution, universal hetrochromatic nature, and functional significance of molecular organization of the sex-determining chromosomes (Y and W).

Snake W Chromosome-specific Satellite DNA

Singh et al. (1976, 1979) identified and isolated a W chromosome-specific satellite DNA by analytical ultracentrifugation of female genomic DNA of poisonous Indian snake *Bungarus fasciatus* (Elapidae) and designated it as Bkm (banded krait minor satellite DNA). The Bkm sequences are highly conserved and present in all eukaryotes studied so far but absent in prokaryotes. By using Bkm probe the W chromosome of any species of snake can be identified except the primitive snakes belonging to the family Boidae having undifferentiated sex chromosomes (Singh et al. 1980). In all the species of snakes studied so far, the Bkm sequences are distributed along the entire length of the W chromosome interspersed among other sequences. In *Drosophila melanogaster* Bkm sequences are predominantly located in the region of X chromosome (Singh et al. 1981) which is implicated in male sterility (Rahman & Lindsley 1981). In mouse, Bkm sequences are concentrated in the short arm of the Y chromosome (Singh et al. 1981, Jones & Singh 1981a, b) which is necessary and sufficient to convert a chromosomally female mouse into a male. This sex-determining region of the Y chromosome has been duplicated and translocated to the distal end of the long arm of the Y in the carrier mouse which regularly gets translocated to the X chromosome during spermatogenesis causing sex reversal (Singh & Jones 1982). The major conserved component of Bkm is a tetranucleotide repeat of GATA (Epplen et al. 1982, Singh et al. 1984). Although GATA sequences are preferentially associated with the sex-determining chromosomes in a wide range of species, it is very difficult to attribute a function to such a simple repeat.

The Physical State and Behaviour of W Chromosome in Somatic and Germ Cells of Female Snakes

In snakes, which have differentiated sex chromosomes, the W chromosome is entirely hetrochromatic and stains positively by C-banding technique (figure 1a). In situ hybridization using ^{3}H labelled Bkm probe, shows distribution of grains along the entire length of the W chromosome (figure 1b). In interphase nuclei of various somatic tissues a high concentration of grains is found exclusively in a single region corresponding to the W chromatin body (figure 1c) showing condensed and therefore, inactive state of the W chromosome. In the developing oocytes however, the W chromosome is extensively decondensed (figure 1d, e). This is in agreement with the earlier report of Singh et al. (1979).

Bkm-associated Y Chromosome-specific Repetitive DNA in Mouse

The DNA sequence analysis revealed that conserved component of Bkm is a tetranucle-

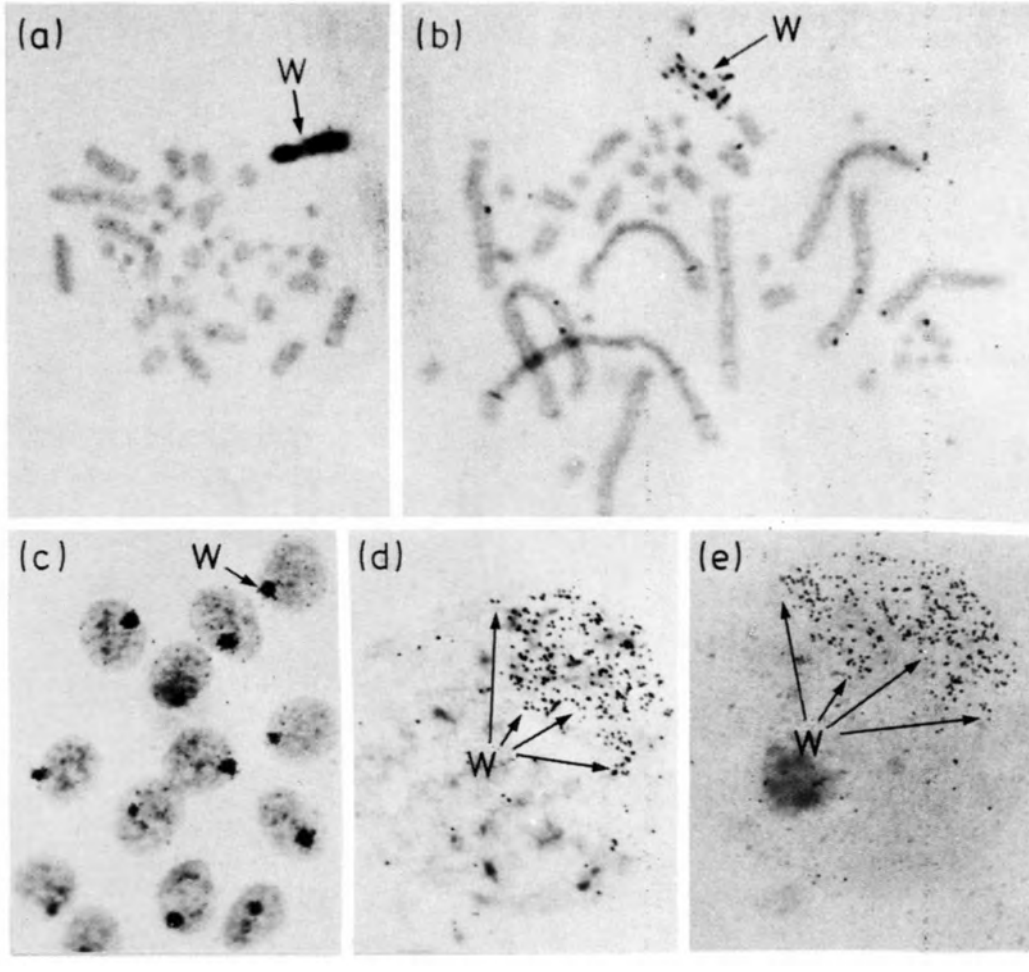

Figure 1 Heterochromatic nature of W chromosome and cross in situ hybridization of Bkm nick translated satellite DNA with the somatic cells and the developing oocytes in snake: (a) Metaphase chromosome of *Bungarus walliwall* showing entirely C-band positive and heterochromatic nature, typical of the functionally specialised W sex chromosome; (b) Cross hybridization of ^{3}H nick translated Bkm satellite DNA with metaphase chromosomes of *Natrix piscator* after 15 days exposure showing concentration of grains along the W chromosome indicating that Bkm sequence interspersion has occurred throughout the length of the W chromosome; (c) Interphase nuclei of blood cells of *Bungarus caeruleus* female hybridized in situ with ^{3}H nick translated Bkm satellite DNA showing concentration of grains in highly condensed W chromatin body resembling X chromosome sex chromatin in mammalian females;(d&e)Autoradiograph showing decondensation of the W chromosome (arrowed) in the oocytes of *Ptyas mucosus* female and *Bungarus caeruleus* female respectively by ^{3}H Bkm hybridization in situ

otide repeat of GATA. It became very difficult to imagine how such a simple repeat could be involved in sex determination. Considering the fact that statistical probability of occurrence of simple repeats on any chromosome is very high, many scientists felt that no special significance should be attached for the occurrence of GATA repeats on the sex chromosomes. But it is very difficult also to ignore the fact that Bkm sequences are predominantly concentrated along the W chromosome of all the species of snakes studied so far and in certain species of birds (Singh et al. 1980); in the sex determining region of the mouse Y chromosome (Singh & Jones 1982); in the Y chromosome of human (Singh & Jones 1986), and on the X chromosome of *Drosophila melanogaster* (Singh et al. 1981). So it is argued that since no other simple repeat is known to be so consistently associated with the sex chromosomes as is GATA repeat, it must have its funtional significance. Therefore, we decided to screen the mouse and human genomic libraries with Bkm probe to isolate and characterize the clones which are repetitive and Y-specific. One of the clones thus obtained in mouse is M34. Southern blot hybridization of male and female mouse genomic DNA with M34 revealed its sex-specificity (figure 2). These sequences are not present in the sex reversed XXSxr male mouse and, therefore, are absent from the sex determining region of the Y (Singh et al. 1994a). In brief, the 11.5 kb long sequence is the repeating unit which is distributed along the Y chromosome except the sex-determining region (the Y short arm). The M34 sequences are species-specific and are not present in rat and other mammals. There are 200-300 copies of M34 on the Y chromosome interspersed among other sequences. There are 32 copies of GATA repeats present on a 1.2 kb fragment of M34, which also has scaffold attachment region (SAR) motifs (ATATTT) which bind to nuclear matrices. Histone H1 strongly binds to such SAR motifs suggesting its role in condensation of the Y chromosome in somatic cells.

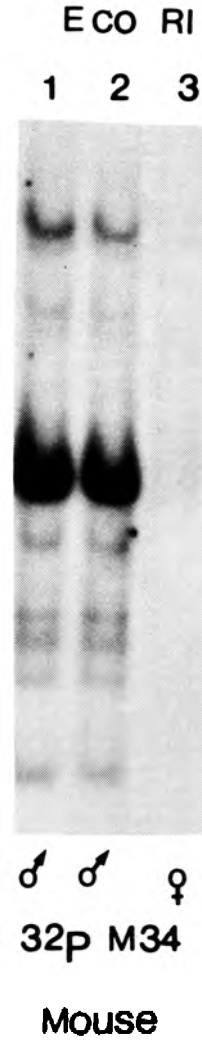

Figure 2 10 μg of mouse male and female genomic DNA restricted with EcoRI, fractionated on agarose gel and hybridized with ^{32}P nick translated clone M34. [Note male-specific hybridization (tracks 1 and 2) suggesting Y-chromosomal origin of the clone].

Since 32 copies of GATA repeats are the internal constituent of M34 repeats which are present in 200-300 copies distributed along the Y chromosome except the sex deter-

mining region, the GATA repeats are present along the Y chromosome including the sex-determining region. This shows astonishing similarity in molecular organization of the snake W chromosome and the Y chromosome of mouse. This is in contrast to our earlier report that Bkm is present exclusively in the sex-determining region of the mouse Y chromosome (Singh & Jones 1982).

The Physical State and Behaviour of the Mouse Y Chromosome in Somatic and Germ Cells

In situ hybridization using ^{3}H labelled M34 probe was done to find out its location on the Y chromosome and when and where the Y chromosome becomes functional (decondensed). This revealed that M34 sequences are distributed specifically along the mouse Y chromosome which remains highly condensed in all somatic tissues (figure 3b) but decondenes extensively in certain cell types of the testis (figure 3c). Guttenbach et al. (1989) reported that the mouse Y chromosome decondenses in the Sertoli cells of the mouse testis. However, our study of fractionated Sertoli cells of normal male mouse testis and the sections of adult mouse testis using ^{3}H labelled M34 as well as 2(8) probes revealed that contrary to the report of Guttenbach et al. (1989) the Y chromosome remains condensed in Sertoli cells of adult testis but decondenses extensively in the primary spermatocytes (Singh et al. 1994a).

Bkm-associated Y-Chromosome-specific Repetitive DNA in Human

The long arm of the human Y chromosome consists of two major repeats of which 3.4 kb and 2.1 kb Hae III fragments are the most predominant (Cooke 1976, Cooke & MaKay 1978, Kankel et al. 1979, Cooke et al. 1982, Kunkel & Smith 1982, Smith et al. 1987). Singh and Jones (1986) have shown higher concentration of Bkm sequences on the human Y chromosome. In order to understand

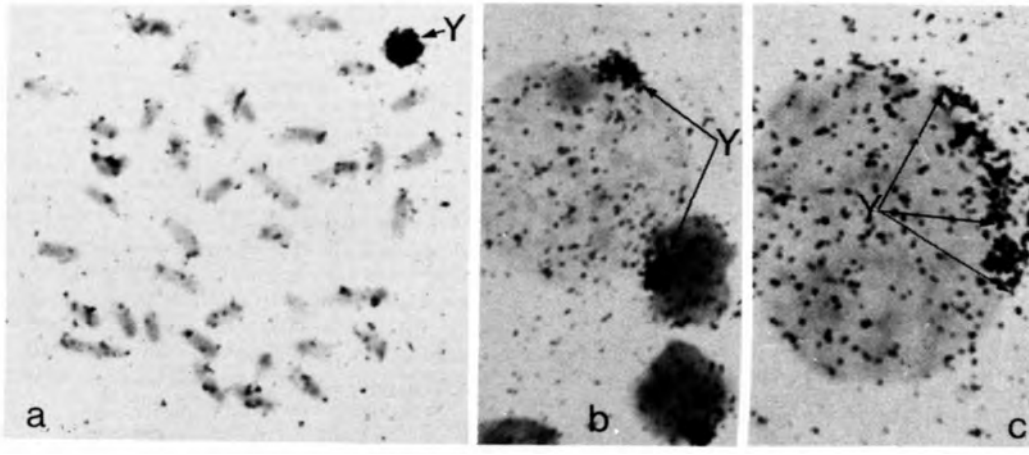

Figure 3 In situ hybridization of ^{3}H-labelled mouse Y chromosome-specific clone M34; (a) metaphase plate after 4 weeks exposure showing hybridization covering the entire Y chromosome except the sex-determining region; (b) Interphase nuclei of bonemarrow showing condensed state of Y chromosome; (c) Interphase nucleus of primary spermatocyte in which the Y chromosome is extensively decondensed.

the functional significance of molecular organization of human Y chromosome, the human Y chromosome enriched cosmid library has been screened with Bkm probe and several positive clones have been obtained. One of the clones recovered is C102. One of the Eco RI fragments of the clone C102 is further subcloned in a plasmid and designated as p102d(2). This gives male-specific pattern on Southern blot (figure 4) and shows distribution of these sequences along the entire long arm of the Y chromosome interspersed among other sequences (figure 5a, b). The p102d(2) sequences are absent in the centromeric region and the short arm of the Y chromosome which is sex-determining. This is remarkably similar to the distribution pattern of the M34 squences on the mouse Y chromosome, which is also absent in the sex--determining short arm of the the Y. The p102d(2) sequences are species-specific. These do not show significant hybridization even with the genomic DNA of other primates.

Behaviour of Y Chromosome in Somatic and Germ Cells of Human Male

The short arm of the Y is euchromatic. A number of genes including the primary sex determination locus (SRY) and ZFY are assigned to the short arm of the Y (see Whiscnant et al. 1991). The long arm (Yq) is predominantly heterochromatic. The p102d(2) sequences are concentrated in this heterochromatic region. In situ hybridization of p102d(2) in interphase nuclei of various somatic tissues shows a high concentration of grains in a small peripheral region showing highly condensed state of the Y chromosome (figure 5c). Speed et al. (1993) have shown that Y chromosome becomes greatly decondensed in the Sertoli cells and at zygotene stage of adult testis. Thus like the W chromosome in snakes, and Y chromosome in mouse, the Y chromosome in human undergoes cycles of condensation and decondensation in a sex and tissue-specific manner.

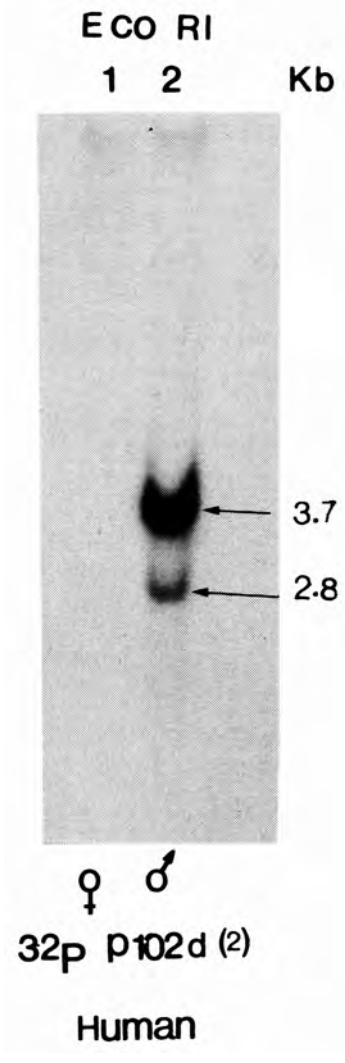

Figure 4 Human male and female DNA (10 μg each) was digested with Eco RI, fractionated on 0.8% agarose gel, transferred onto Hybond nylon membrane and hybridized wth ^{32}P nick translated p102d(2) DNA. After 14 hours of exposure one very strong hybridization to a restriction fragment of 3.7 kb and one comparatively less intense hybridization to a 2.8 kb fragment are observed specifically in the male (track 2).

Sex and Tissue-specific Bkm (GATA)-Binding Protein (BBP) in Snakes

The sex-determining chromosomes (W and Y) remain highly condensed in all somatic tissues but decondense extensively in the

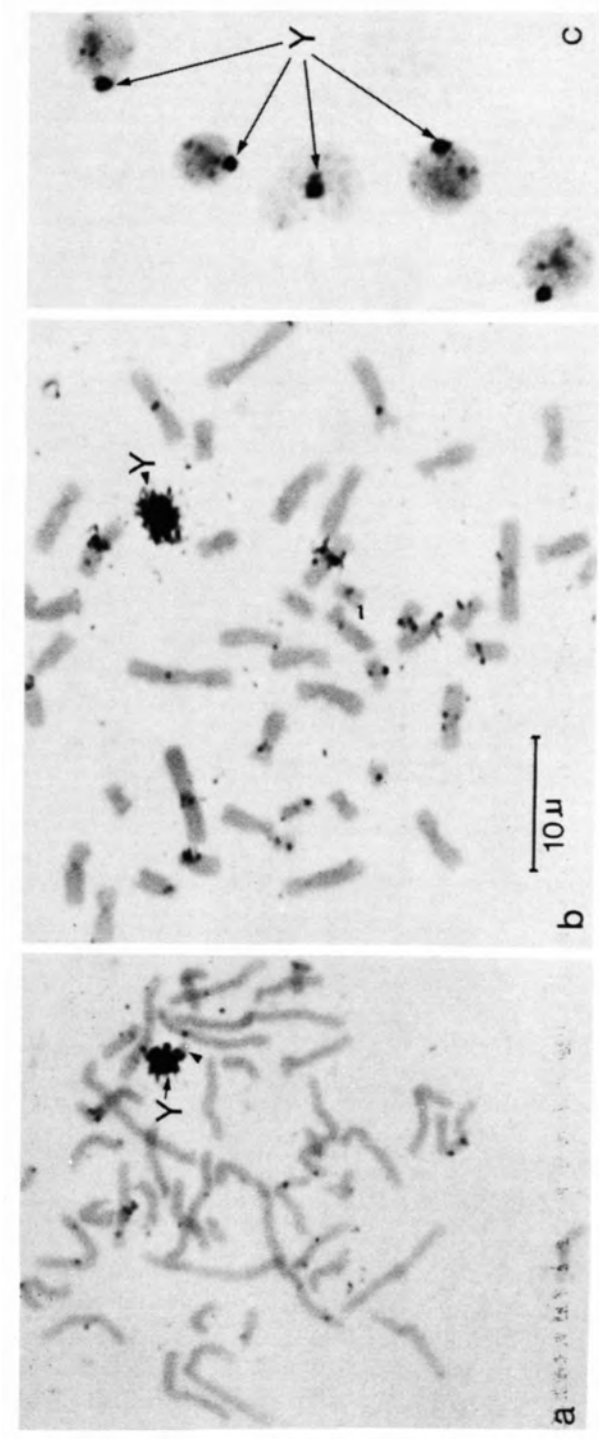

Figure 5 In situ hybridization of normal human male chromosomes prepared from short term leukocyte culture with ^{3}H labelled Y specific clone p102d(2) which is a subclone of Eco RI fragment 2 of a cosmid clone 102D isolated by screening Y chromosome enriched library with Bkm; (a) A prometaphase showing elongated Y chromosome exposed for 3 weeks. Note distribution of grains covering almost entire long arm of the Y chromosome. Short arm of the Y chromosome (small arrows) and centromeric region is completely devoid of grains; (b) Metaphase chromosomes after 3 weeks exposure showing concentration of grains on the Y and also some cross hybridization; (c) Interphase nuclei of blood cells of human male showing concentration of grains in highly condensed Y chromosome.

germ cells of the heterogametic sex (ovary in snakes and testis in mouse and human) in such a way as if the entire chromosome is behaving like a supergene. In order to explain the phenomenon of decondensation, we anticipated the existence of a factor which can specifically bind to sex chromosome-specific repeats. Such a factor was predicted to be a sequence-specific DNA-binding protein which may recognise sex-chromosome-specific repeats. Since GATA repeats are the only the conserved component of the sex chromosome-specific repeats, we predicted that such a DNA binding protein would specifically recognize GATA repeats and would be not only sex-specific but tissue and developmental stage-specific also. Such a sex and tissue-specific factor indeed is present in snake ovary (figure 6). This highly sex and tissue-specific factor is purified as a polypeptide of 57.5 kd from the rat snake ovary and has been named as Bkm-binding protein (BBP) by virtue of its binding to GATA repeats of Bkm (Singh et al. 1994b).

Sex and Tissue-specific Bkm (GATA)-Binding Protein (BBP) in Mouse Testis

Based on the analogy of snakes, as predicted, such a sex- and tissue-specific protein which binds GATA component of M34 sequences distributed along the Y chromosome was detected in mouse testis by using the electrophoretic mobility shift assay. The ^{32}P end labelled GATA (16 mer) probe (figure 7) detects two specific DNA protein complexes (C1 and C2) with testis nuclear extract, which are not detected with the nuclear extracts of other tissues (liver, kidney) of the male and female (figure 8).

Sex and Tissue-Specific Bkm (GATA)-Binding Protein in Human Testis

A sex and tissue-specific protein which binds GATA repeats is detected in human adult testis by using electrophoretic mobility shift assay. Two specific DNA protein complexes (C1 and C2) are detected with ^{32}P-labelled GATA (16 mer) probe and testis nuclear extract (Singh et al. 1994b).

Discussion

The Molecular Organization of the W Chromosome of Snake and Y Chromosomes of Mouse and Human is Strikingly very Similar

Studies of Singh and his collaborators have revealed the following remarkable similarities in molecular organization of the W chromosome of snake and Y chromosome of mouse and man: (1) All of them are heterochromatic, C-band positive and synthesize their DNA out of phase during S period of the cell cycle (Kofman et al. 1970, Ray-Chaudhuri & Singh 1972, Gartler & Andina 1976); (2) They have conserved Bkm sequences (GATA repeats) distributed along the length interspersed among the other sequences (Singh et al. 1980) as a part of species-specific and sex chromosome-specific repeats [p 19 in banded krait *Bungarus fasciatus* (Panicker & Singh 1994), M34 in mouse and p102d(2) in human (Singh & Majumdar 1993)]; (3) The sex chromosome-specific repeats which are species-specific (M34 in mouse and p102d(2) in human) are absent in the sex determining region of the mouse and human Y chromosome respectively; (4) Contrary to sex and species-specific repeats, Bkm sequences (GATA repeats) are present in the sex-determining region of the mouse (Singh & Jones 1982) and human Y chromosomes (Singh & Jones 1986). The sex-determining region of the snake W chromosome is not yet defined; (5) In female snake, the W chromosome remains condensed in somatic tissues but decondenses extensively during oogenesis (Singh et al. 1979). Similarly, the mouse and human Y chromosomes remain condensed in all somatic tissues but decondense in specific cell types in testis. This is the stage at which the X chromosome in all male heterogametic organisms gets inactivated and Y chromosome becomes functional.

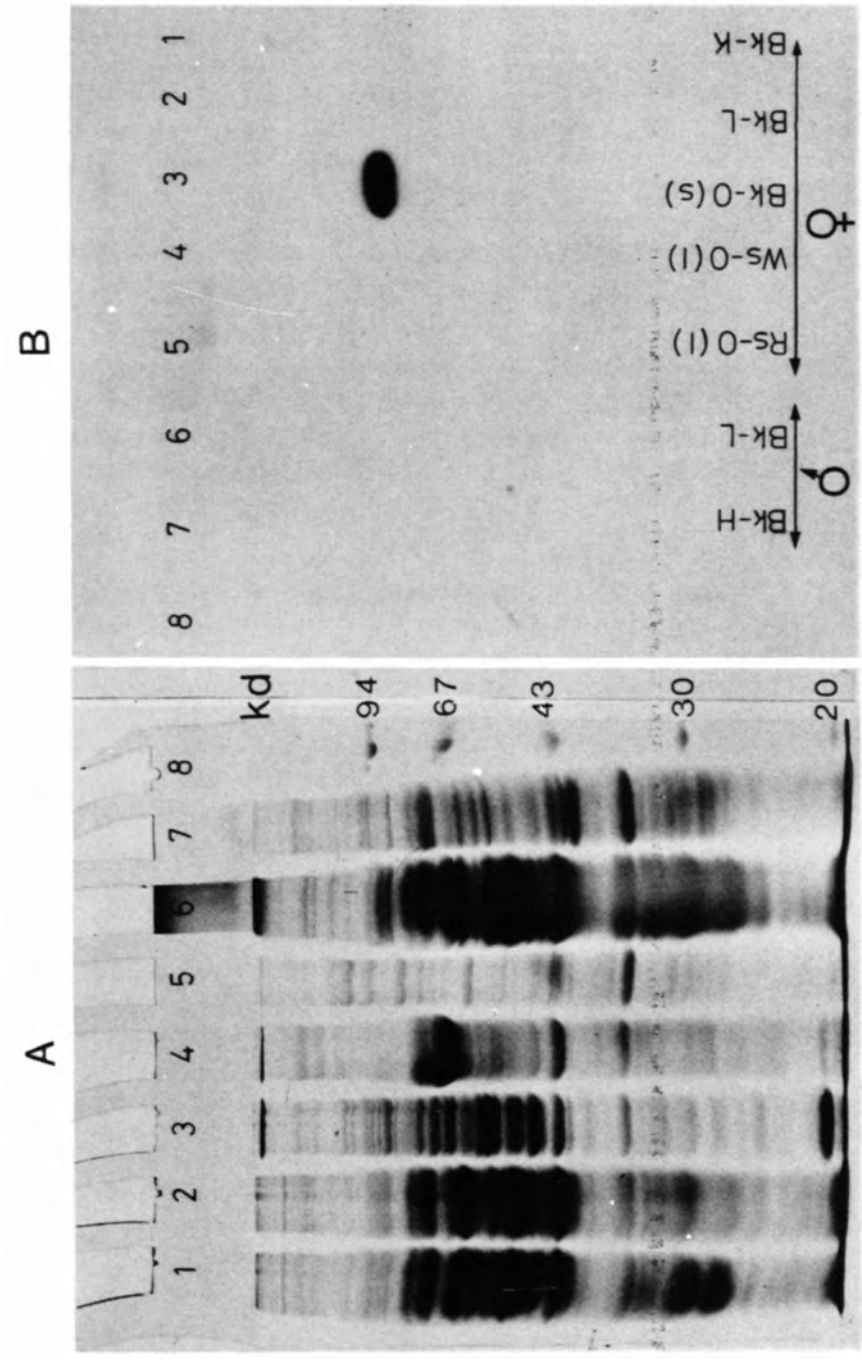

Figure 6 Sex and tissue specificity of BBP. A: Coomassie brilliant blue stained SDS PAGE protein gel; B: Southwestern blotting of the other half of the same gel having identical tracks, with ^{32}P end labelled 2(8) probe mainly containing GATA repeats. Tracks 1-5: Female banded krait kidney, liver, ovary small; water snake ovary large; rat snake ovary large, respectively. Tracks 6-7: Male banded krait liver and heart respectively. Track 8: Molecular weight marker. BBP is detected only in ovary at an early stage of developing oocytes.

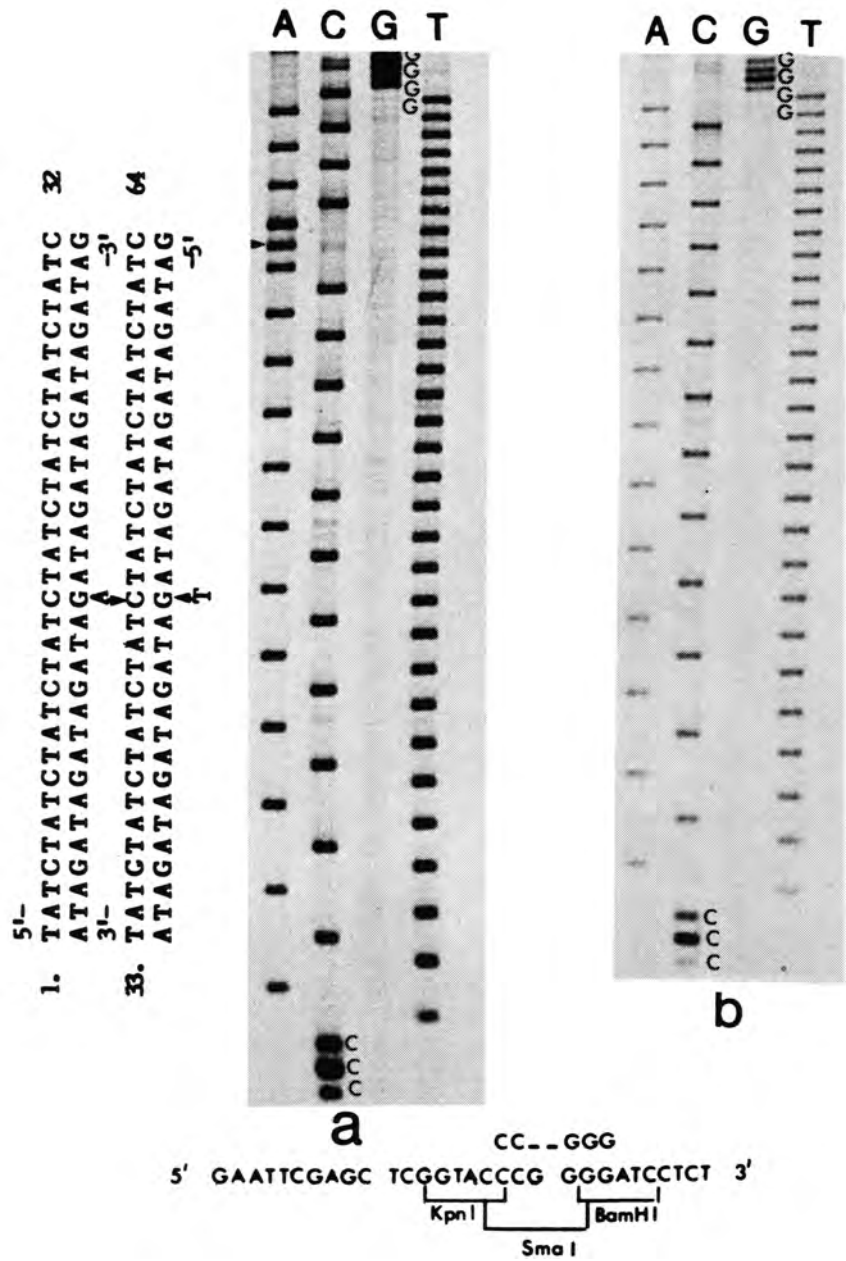

Figure 7 Confirmation of nucleotide sequence of two different clones containing synthetic oligonucleotide sequence GATA by DNA sequencing (Sanger et al. 1977). GATA and CTAT oligonucleotides (10 mers) were synthesized using Pharmacia gene assembler. Synthetic oligonucleotides were annealed, kinased, ligated and cloned into Sma I site of pUC18. To remove the insert from the vector for retardation experiment, adjacent restriction sites Bam HI and Kpn I in the polylinker region were used: (a) Nucleotide sequence of a clone containing GATA (16 mer) showing one C substituted by A (shown by arrow head). Therefore, this clone was not used for gel retardation assay; (b) Nucleotide sequence of another clone containing GATA (16 mer) without any base alteration. This clone was used for gel retardation assay.

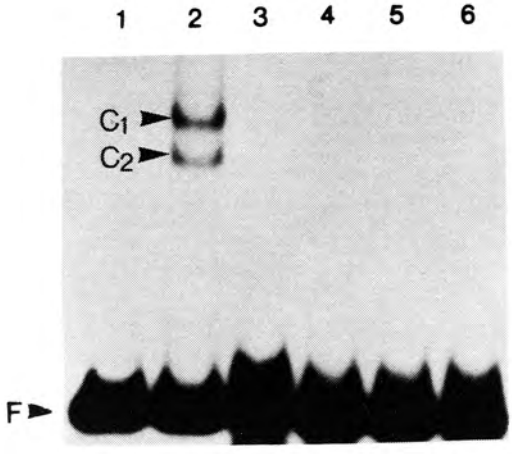

Figure 8 Autoradiograph of a gel retardation assay with ^{32}P end-labelled double-stranded oligonucleotide GATA (16 mer) fragment, in the presence of 20 μg nuclear extract of mouse testis, 3 μg *E. coli* non-specific competitor DNA and 1 ng of (30,000 cpm) probe.

Lane 1 shows radiolabelled GATA (16 mer) probe only. Lanes 2-6 represent the binding assay; lanes 2-4, nuclear extract from male mouse testis, liver and kidney, respectively; lanes 5 and 6, female mouse liver and kidney, respectively. F: free probe.

BBP and Decondensation of the W and Y Chromosomes

A sex and tissue-specific DNA-binding protein which recognises GATA repeats of Bkm is predominantly present in the snake ovary, and mouse and human testis, the tissue in which the W and the Y chromosomes respectively are decondensed and transcriptionally active. No matter how one interprets these results, the conclusion that ordered organization of Bkm along the W chromosome, and M34 and p102d(2) repeats throughout the length of the Y chromosome of mouse and human respectively, may be responsible for coordinated decondensation of the Y chromosome in response to BBP, is inescapable.

Recently a W-protein with high affinity for the W chromosome-specific Xho I family of repetitive squences in chicken (Tone et al. 1984) has been reported to be involved in maintaining stable condensed state of the W chromatin in the somatic tissues (Harata et al. 1988). The W prote in however, is not sex-specific as it also exists in the liver nuclei of the male chicken (Harata et al. 1988) and does not bind to the Bkm sequences. Saitoh et al. (1989) have recently shown that W-protein, unlike many sequence-specific DNA binding proteins, seems to recognise structural features of DNA for binding over as much as 300 bp stretch of DNA double helix and may be involved in the formation of higher order structure of chromatin rather than having specific regulatory functions in the transcription of genes.

It has been shown that the reglatory regions of globin and other erythroid-specific genes contain GATA motifs (Evans et al. 1988, Mignotte et al. 1989). A family of DNA-binding proteins which are thought to be involved in the regulation of erythroid-specific genes has been identified and called the GATA family of proteins (Romeo et al. 1990, Yamamoto et al. 1990). The members of this family are conserved in the DNA-binding domain which recognises the GATA motif (Yamamoto et al. 1990, Romeo et al. 1990, Morten et al. 1990).

In plants a nuclear factor, GA-1, bound to the GATA element of the chlorophyl a/b promoter cab-E has been reported. In cab-E promoter GA-1, specifically recognised and interacted with the three GATA motifs. Although a single GATA-box is sufficient for this interaction, the affinity increased dramatically when all three motifs are present (Schindler & Cashmore 1990). The sex and tissue-specific GATA (Bkm)-binding protein in snakes, mouse and humans reported in the present study is the first report describing sequence-specific DNA-binding protein which is implicated in bringing about decondensation of the entire W and Y chromosomes and thus rendering them accessible to other transcription factors and, therefore, more likely to be transcribed. In this respect it is novel and appears to be very different from

GATA-1, -2, -3 in animals and GA-1 in plants reported so far. Recently, in addition to its role in erythroid cell development, GATA-1 has been shown to be expressed in mouse testis also (Ito et al. 1993). Since the expression of GATA-1 is much higher in 2-week testis than in 5 or 10 week tissue (Ito et al. 1993), whereas expression of BBP is maximum in 4 week and adult testis and much less in 1-3 week testis (Singh et al. 1994b), the BBP is clearly distinct from GATA-1.

A Possible Mechanism of Evolution of Switching on and off an Entire Sex-determining Chromosome

Bkm-related sequences are scattered throughout the genome of all of the species studied so far. It is possible that these are also involved in some aspect of gene control. If this is the case, then it is possible to envisage that chromosomal sex-determination may have evolved by the extension and amplification of such individual gene controls to the point when they have become capable of switching on and off an entire chromosome. From this point of view the sex-determining chromosome is a sort of "supergene" endowed with an enormously amplifed array of those sequences which normally control the expression of certain kinds of individual genes required in large quantities in a short duration at a specific stage of development and differentiation. Most obviously, the genes concerned would be those involved in differentiation rather than in "house keeping". This is substantiated by the fact that a Y chromosomal transcribed DNA sequence 145SC5 which has a potential open reading frame coding of a protein consisting of 227 amino acids exists in 200 copies along the length of the Y chromosome and is specifically expressed in the testis (Prado et al. 1992). Although its precise function is not yet known, its role in spermatogenesis is contemplated. If there is any merit in this hypothesis, then it is obvious that the study of the DNA of sex chromosomes has much to reveal about the control of gene expression in development.

Does BBP Preferentially Recognise the Y Chromosome?

The fact that male and female mouse DNA can be distinguished on Southern blot by Bkm probe indicates that Bkm sequences are differently organised on the sex chromosome. There is also indication that phages containing mouse-Y chromosomal fragments have longer stretches of GATA repeats (upto 120 base pairs) compared to the phages with non Y chromosomal DNA fragments of mouse (Schafer et al. 1986). It is also possible that the packaging of Y-DNA in chromatin, due to characteristic distribution of GATA repeats adjacent to SARs along the length of Y chromosome may bring GATA repeats of different repeating units together on the nuclear matrix as a large continuous stretch, which is recognised by the BBP. This aspect of molecular organization may be unique to the Y. This is substantiated by the fact that SAR motifs of M34 bind to nuclear matrix (Singh et al., 1994b). The fact that specific binding of BBP to ^{32}P labelled double stranded male mouse DNA is observed when thousand fold excess of female mouse DNA is used as a competitor, indicates that BBP preferentially recognises the Y chromosome. However, Bkm sequences have also been localized in the proximal region of chromosome 17 of mouse containing the major histocompatiability complex (Kiel-Metzger & Erickson 1984, Durbin et al. 1989). Study of this region containing the inversion of t complex has revealed an impressively large number of genes, virtually all of which are expressed in testis (Washburn & Eicher 1983, Sarvetnick et al. 1989, Yeom et al. 1992). These findings have led to the suggestion that the proximal region of mouse chromosome 17 plays a central role in male germ cell differentiation. It is tempting to suggest that BBP present in the testis causing decondensation of the Y chromosome may also recognise GATA repeats

present in this region of the chromosome and bring about coordinated activation of all the genes in this region. This is substantiated by the fact that virtually all the genes present in this region are expressed in the testis and are involved in male germ cell differentiation. Thus BBP may bind to the sex chromosome as well as other autosomal sites containing large stretches of GATA repeats and bring about activation of those domains probably containing genes whose products are required during spermatogenesis.

A strong and preferential binding of histone H1 to the fragment containing SAR sequence motif (ATATTT) of M34 may be responsible for the highly condensed state of the Y chromosome. It has been reported that histone H1 preferentially binds to eukaryotic SAR under the conditions of strong co-operativity (Sevall 1988, Izaurralde et al. 1989, Kas et al. 1989). Thus, SARs nucleate co-operative H1 assembly along the SAR into the flanking non-SAR DNA and control the conformation of chromatin domains. Probably H1 recognizes some alternative non-B structures in DNA formed at biased nucleotide sequences under torsional stress (Yaneva & Zlatanova 1992). Matrix attachment regions (MARs) or SARs (Gasser & Laemmli 1986) are 70% A + T rich and are characterized by their strong potential to extensive unwinding under superhelical strain which has been shown to be important for binding to the nuclear matrix (Kohwi-Shigenetsu & Kohwi 1990, Bode et al. 1992). The fragment of M34 which has SAR motifs also contains 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 to 363 kb DNA. These regions flanked by fragment containing SAR motifs, form loops which are anchored to the nuclear scaffold at SAR, delimiting the ends of active chromatin. The loops are then wound into the 18 radial loops that form a miniband unit or 1 turn on the chromatid. The minibands are continuously wound and stacked along a central axis to form each chromatid (Getenberg et al. 1991). Looping of DNA may bring SARs and GATA repeats of each repeating unit in close vicinity of each other at the matrix. Histone H1 may bring about compaction of the radial loops and minibands by preferentially binding to SAR motifs and also to mini bands resulting into highly condensed chromatin in interphase nuclei. The BBP present in germ cells may replace histone H1 by binding to GATA repeats adjacent to H1 binding site by changing the DNA conformation and thus bringing about cooperative decondensation of the entire Y chromosome and making it transcriptionally competent. This alteration of chromatin to a more open structure in committed cells is likely to be a necessary prerequisite for gene expression, allowing transacting factors, which actually activate the gene, access to appropriate sequnces within it. Thus, the dynamic changes of chromosomes (condensation, decondensation, puffing etc.) can be brought about by dynamic changes in the scaffold that would drag along the associated chromatin loops (Gasser & Laemmli 1987). Condensation/heterochromatinization thus represents an epigenetic regulatory switch establishing and maintaining the repression of certain genes or an entire chromosome.

It would be exciting to find out whether BBP is expressed in the gonadal ridge of 10.5-11 day old mouse embryos in the precursors of Sertoli cells, the stage at which sex of the embryo is determined. The presence of BBP at this stage would strongly suggest that decondensation of the Y in presence of the BBP may be the first step in the whole cascade of events in the complex pathway of sex-determination.

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