CHAPTER IV

MOLECULAR CHARACTERIZATION OF MALE-SPECIFIC CLONE M34 OF MOUSE
INTRODUCTION

In the heterogametic sex, Y (in organisms with male heterogamety) and W (in organisms with female heterogamety) chromosomes, in general, are largely heterochromatic. The W chromosome in birds (Stefos and Arrighi, 1974), snakes (Singh et al., 1976), fish (Bertollo and Cavallaro, 1992), and the Y chromosome in mammals (Cooke, 1976), lizards (Olmo et al, 1987), amphibia (Schmid, 1983), fishes (Kallman, 1984), insects (Weith and Traut, 1980) and in various invertebrates including Drosophila (Peacock et al., 1978; Vogt and Henning, 1986) are invariably constitutively heterochromatic and rich in highly repetitive (satellite) DNA. Although chromosomal sex-determination has evolved independently at different evolutionary times in as diverse systems as insects, plants and vertebrates (Jones and Singh, 1985), in every case it has led to heterochromatinization of the entire sex-determining chromosome. This may probably have caused virtual loss of all its other genetic functions and led to specialization of the entire chromosome with a singular function of sex-determination. This indicates that chromosomal sex-determination has not been disadvantageous in evolutionary term irrespective of the probable loss of genetic functions and developmental monosomy. This raises the question of necessity for the evolution of a specialised sex-determining chromosome at the expense of most of its genes, not involved in sex-determination, particularly when sex ratio can be maintained by a pair of alleles as is the case in a large number of eukaryotes (Ohno, 1967). In order to answer this question and also to understand the molecular bases of sex-determination the earlier workers (Singh et al., 1980) isolated DNA contained in the W sex chromosome of a poisonous Indian
snake *Bungarus fasciatus* (Elapidae) and designated it as Bkm (banded krait minor satellite) DNA. The Bkm sequences are conserved in a wide variety of eukaryotes (Singh *et al*., 1981; Jones and Singh, 1981; Singh and Jones, 1982). In snakes these sequences are interspersed with other DNA sequences throughout the W chromosome in all the species sampled so far, except the primitive boid snakes with morphologically indistinguishable sex chromosomes (Singh *et al*., 1980). In the mouse these sequences are organised in a sex-specific pattern and are concentrated at the paracentric region of the Y chromosome (Singh *et al*., 1981; Jones and Singh, 1981) now considered to be the short arm (Bishop *et al*., 1988). This region of the mouse Y chromosome is sex-determining and is necessary and sufficient to convert a chromosomally female XXSxr mouse into a male (Singh and Jones, 1982). The major conserved component of Bkm is a tetranucleotide repeat of GATA (Epplen *et al*., 1982, Singh *et al*., 1984). Although GATA repeats are highly conserved, it seems improbable that it is itself sex-determiner. However, preferential association of these repeats with the sex-determining chromosomes in many diverse species of eukaryotes with male as well as with female heterogamety suggests that Bkm probably has a significant role in chromosomal sex-determination.

Singh *et al*. (1979) demonstrated that the snake W chromosome remains condensed in interphase nuclei of all female somatic cells but decondenses extensively in the growing oocytes. Based on the universal heterochromatic nature of the sex-determining chromosomes (Y in the case of male heterogamety and W in the case of female heterogamety) and implicated role of Bkm
sequences, which are distributed along the length of snake \( W \), in co­
ordinated decondensation of the \( W \) chromosome (Singh et al., 1979),
it is probable that \( Y \) chromosome is similarly organized at molecular
level. To confirm this, Singh et al. (1988) screened mouse genomic
library with Bkm probe and isolated a \( Y \) chromosome-specific
repetitive clone, M34 which is distributed along the length of the \( Y 
except the sex-determining region (short arm of \( Y \); Bishop et al.,1988). In order to understand the sequence organization of M34,
particularly the Bkm and its flanking sequences, the present study
was undertaken with the hope that it may reveal functional
significance of the organization of these repeats on the sex
chromosomes.

RESULTS

4.1 Restriction analysis, subcloning and restriction
mapping of M34 clone

4.1.1 Restriction analysis of M34 clone

Total insert size of M34 was 11.5 kb. When M34 DNA was restricted
with EcoRI four fragments of 7.6 kb (fragment 1), 2.7 kb (fragment 2),
0.7 kb (fragment 3) and 0.5 kb (fragment 4) were obtained.
Restriction analysis of M34 is shown in Fig. 4.1. Out of the 4
fragments only fragment 2 (2.7 kb) was found to be positive for
Bkm 2(8) probe (data not shown).
Fig. 4.1. Restriction analysis of M34 clone. M34 DNA cloned in charon4A lambda vector digested with EcoRI showing 4 fragments of 7.6 kb (fragment 1), 2.7 kb (fragment 2), 0.7 kb (fragment 3) and 0.5 kb (fragment 4).
4.1.2 Subcloning of M34 fragments

By shotgun cloning as described in section 2.19, four EcoRI fragments of M34 were subcloned into the EcoRI site of either pUC9 or pUC18 vector. Recombinants were identified either by colony hybridization as described in section 2.27 or by minipreparation of plasmid as described in section 2.11.1. Restriction analysis of subclones of fragments 1, 3 and 4 are shown in Fig. 4.2. The three smaller fragments of M34 (2.7 kb, 0.7 kb and 0.5 kb) were subcloned as such, whereas only a part of the large fragment I (7.6 kb) was subcloned. The various subclones are named as p21 (fragment 1), p11 (fragment 2), p66 (fragment 3) and p19 (fragment 4). When p21 (subclone containing only part of fragment 1) was restricted with EcoRI, two fragments were obtained (Fig. 4.2, lane 11). One fragment was of the size similar to that of fragment 4 (Fig. 4.2, lane 5) and the other fragment of the size of the vector (Fig. 4.2, lane 11). Since it was shotgun cloning, two fragments of M34 (Fragments 1 and 4) were ligated together and cloned into the same plasmid molecule. When p21 was double digested with EcoRI and HindIII, two fragments of 2.2 and 0.6 kb were obtained in addition to the 0.5 kb fragment 4 (Fig. 4.2, lane 12). This indicated that out of 7.6 kb of fragment 1 only 2.8 kb was subcloned. It appears that rest of the fragment was deleted while cloning.
Fig. 4.2. *Restriction analysis of M34 subclones.* Extreme left is lambda HindIII marker lane. Lanes 1-3: Undigested pUC9 vector, pUC9 digested with EcoRI, pUC9 double digested with EcoRI/HindIII respectively. 4-6: fragment 4 of M34 cloned in pUC9 (p19) undigested, p19 digested with EcoRI, p19 double digested with EcoRI/HindIII respectively; 7-9: fragment 3 of M34 cloned in pUC9 (p66) undigested, p66 digested with EcoRI, p66 double digested with EcoRI/HindIII respectively; 10-12: fragment 1 of M34 cloned in pUC9 (p21) undigested, p21 digested with EcoRI, p21 double digested with EcoRI/HindIII. Arrows indicate various inserts released from the vector. Asterisk indicates EcoRI/HindIII fragment containing the multiple cloning site of the vector.
4.1.3 Subcloning of Bkm 2(8) positive fragment 2 of M34

The second fragment of M34 which is 2.7 kb in length was found to be positive for Bkm 2(8) probe. This fragment was initially subcloned into the EcoRI site of pJRD158 and was designated as p17. Since it was not possible to do sequencing with this vector, this fragment was recloned into the EcoRI site of pUC18 and the clone is designated as p11 (both p17 and p11 refer to the same fragment 2 but the vectors are different). When p11 was double digested with EcoRI and HindIII, three fragments of 1.2 kb, 0.9 kb and 0.5 kb were obtained (Fig. 4.3, lane 1). Of these fragments only 1.2 kb fragment was positive for Bkm 2(8). The 1.2 kb and 0.5 kb fragments were EcoRI/HindIII fragments whereas, the 0.9 kb was the HindIII fragment. All these fragments were also subcloned into pUC18. Restriction analysis of p11 and its subclones are given in Fig. 4.3. Restriction maps of p11 and M34 are shown in Fig 4.4.

4.2 Genomic organization of M34 DNA

In order to know the genomic organization of M34 in mouse, Southern analysis of DNA from male and female mice was done with $^{32}$P labelled probes of various fragments of M34. In all the blots, hybridization was observed only in male DNA and no convincing hybridization was detected with the female genomic DNA. 10 μg of EcoRI digested male and female mouse DNA was probed with either $^{32}$P labelled EcoRI fragment 1 of M34 eluted from the gel or subclone p21, containing part of fragment 1, showed
Fig. 4.3. *Restriction analysis of p17 DNA and its subclones*. Extreme left and right are lambda HindIII and lambda HindIII/EcoRI double digested molecular weight markers respectively. Lane 1: 2.7 kb fragment 2 of M34 subcloned into pUC18 double digested with EcoRI/HindIII (p11) showing three fragments; 2: 2.7 kb fragment 2 of M34 subcloned into pJRD158 restricted with EcoRI (p17); 3: 1.2 kb EcoRI/HindIII fragment of p11 cloned in pUC18 restricted with EcoRI/HindIII (p3); 4: 0.9 kb HindIII fragment of p11 cloned in HindIII site of pUC18 restricted with HindIII (p56); 5: 0.5 kb fragment of p11 cloned in pUC18 restricted with EcoRI/HindIII (p41).
Fig. 4.4. Restriction map of M34 and p11(p17). The total insert is 11.5 kb which can be cleaved from the vector by EcoRI digestion into 4 fragments which are serially numbered in decreasing order of size and have been subcloned in pUC9/18 vector. Further restriction map of fragment II (subclone p11/p17) showing Bkm positive fragment as GATA$^+$ is highlighted. This entire fragment (p11) consisting of 2683 bp has been sequenced. Asterisk indicates that HindIII site is modified.
two prominent bands of 8 kb (a) and 2.7 kb (b) and another band of 5.8 kb (*) as shown in Fig. 4.5, track 1. Genomic blots (EcoRI digested) from male and female mice probed with fragment 2 (p11) showed two prominent bands of 2.9 and 2.7 kb and one very faint band of 0.84 kb (Fig. 4.5, track 3). Hybridization of EcoRI digested mouse DNA blot with M34 fragment 3 (p66) (Fig. 4.5, track 5) also showed one prominent band of 8 kb (a), similar in size and intensity to band (a) in Fig. 4.5, track 1. Blot hybridization with M34 fragment 4 (p19) with the genomic DNA revealed 3 bands of 8 kb, 5.8 kb and 0.84 kb respectively (Fig. 4.5, track 7). From the hybridization pattern it was clear that band (a) was detected by fragment 1, 3 and 4; 5.8 kb band was detected by fragment 1 and 4; 2.7 kb band was detected by fragment 1 and 2; 1.3 kb band by fragment 3; and 0.8 kb was detected both by fragments 2 and 4.

EcoRI digested DNA of mouse male and female probed with subclones (p3, p61, p41) of fragment 2 (p11) individually, as expected, showed almost the same male-specific hybridization pattern detected by the p11 probe. Two prominent bands of 2.9 and 2.7 kb and one band of 0.84 kb were observed (Fig. 4.6).

4.3 Northern analysis of M34 sequence

Total RNA (20 μg each) from liver, testis and kidney was run on agarose gel, transferred onto nitrocellulose membrane and hybridized with $^{32}$P labelled nick translated M34 probe. No hybridization signal was detected in any of the samples. When the same blot was reprobed with 28S RNA, hybridization was observed, indicating good quality of RNA (data not shown).
Fig. 4.5. Southern analysis of mouse DNA with M34 fragments. Male and female mouse genomic DNA (10 µg each) digested with EcoRI, size fractionated on agarose gel and hybridized with $^{32}$P labelled probes p21 (Fragment I), p17 (Fragment II), p66 (Fragment III), and p19 (Fragment IV), tracks 1 and 2, 3 and 4, 5 and 6, and 7 and 8 respectively, showing male-specific hybridization.
Fig. 4.6. Southern analysis of mouse DNA with p11(p17) and its subclones. Southern blot hybridization of male and female mouse genomic DNA (10μg each) digested with EcoRI, and hybridized with $^{32}$P labelled probes p11, p3, p61 and p41, tracks 1 and 2, 3 and 4, 5 and 6, 7 and 8 respectively, showing male specific hybridization. p11 is EcoRI fragment II of M34. p3; p61 and p41 are 533 bp, 929 bp, and 1219 bp fragments respectively of EcoRI/HindIII double digestion of p11.
4.4 Chromosomal localization of M34 sequence

*In situ* hybridization of male mouse chromosomes with $^{3}$H labelled M34 probe revealed the distribution of grains along the length of the Y chromosome except the sex determining region (Fig. 4.7). *In situ* hybridization with p11 also revealed similar pattern of grain distribution (data not shown) suggesting that p11 is indeed a part of the repeating unit of M34.

4.5 Nucleotide sequencing of Bkm 2(8) positive fragment of M34 (p11-M34 EcoRI fragment 2)

4.5.1 Generation of nested deletion clones of p11

The nucleotide sequence of the p11 (p17) DNA was determined using the dideoxy chain termination method. The maximum length of the target DNA that can be sequenced in a single set of sequencing reaction is only up to 400 bases. Therefore, to sequence it, nested deletions were generated in the p11 clone using exonuclease III as detailed in section 2.32.

The subcloning of 2.7 kb insert into multiple cloning site of pUC18 enabled the selection of two unique restriction sites which are between the end of the insert to be deleted and the sequencing primer binding site. In order to facilitate the unidirectional deletion of 2.7 kb insert in clone p11, the plasmid was double digested with SphI and SalI, which cleaved within the polylinker region (multiple cloning site). Digestion with SalI enzyme left a four-base 3'
Fig. 4.7. *In situ* hybridization of male mouse chromosomes with $^3$H labelled M34 probe.

a. After 2 weeks exposure. Note distribution of grains along the length of the Y chromosome at a regular interval.

b. After 3 weeks exposure. Note high concentration of grains on the Y chromosome. In the inset are Y chromosomes from two different metaphases showing hybridization at a regular interval covering the entire Y chromosome except the sex-determining region.
protrusion that protected the remainder of the vector from exonuclease III attack, whereas, 5' protruding end left by SphI digestion allowed the unidirectional digestion of the insert sequence from the 5' protruding end. The schematic diagram of deletion cloning is given in Fig. 4.8. In order to determine the extent of digestion, aliquots of DNA were taken at different time points during digestion with exonuclease III and were analyzed by gel electrophoresis. The plasmid DNA from several deletion clones spanning the entire length of the insert (Fig. 4.9) was isolated and used for sequencing. The complete sequencing of pl11 was done by using deletion clones as well as various subclones of pl11. For sequencing the deletion clones, universal M13 forward primer was used whereas, for sequencing subclones both forward and reverse primers were used.

The strategy adopted for sequencing the deletion clones and subclones and the primers used for sequencing are shown in Fig. 4.10. The nucleotide sequence obtained from different deletion clones and subclones was collated to get the complete nucleotide sequence of the pl11.

4.5.2 Sequence analysis of pl11(pl17)

The complete nucleotide sequence of pl11(pl17) is given in Fig. 4.11. The actual size of the insert was 2683 base pairs. The main features of DNA sequence of pl11 was the presence of 32 copies of GATA repeats of which 12 copies were continuous between the basepairs 1617 to 1664 in the sequence, and presence of several copies of scaffold associated region (SAR) recognition sequences.
Fig. 4.8. Schematic diagram of deletion cloning
Fig. 4.9. Deletion clones of p11 obtained with exonuclease III. Lane 1: p11 DNA without deletion; 2-12: clones with varying degrees of deletions; 13: vector DNA alone.
Fig. 4.10. Sequencing strategy used for p11. The arrows and number show the direction and the clone used respectively for sequencing. 's' indicates subclones, 'd' indicates deletion clones and 'c' indicates p11 clone. pUC/M13 primer was used for sequencing all the clones. The number along the unbroken line indicates the total nucleotide sequence of p11 DNA.
Fig. 4.11. Nucleotide sequence of p11. Nucleotide sequence of Bkm-positive subclone p11(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 complimentary 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 IR 1-9 shown by arrows under the sequence, direction of arrow indicates the direction of the repeat; 4 palindromic sequences p1-4 shown by double headed arrows under the sequence; and one potential hairpin loop forming sequence(HL)underlined on both the sides.
Potential open reading frames (ORFs) and polyadenylation sites are shown in Fig. 4.12. The sequence analysis revealed 11 ORFs of ≥ 60 aminoacids (Fig4.12). One of the largest ORFs corresponding to base pairs 574 to 1059 of the GATA strand had the potential to code for 162 aminoacids (Fig4.13). However, there was no polyadenylation site for this ORF in the sequence. Characteristic sequences for intron-exon junction were also absent. A potential hairpin loop was detected between base pair 1615-2625. The conformation of the loop is shown in Fig. 4.14. A number of palindromic sequences, direct repeats and inverted repeats with their positions are also shown in Fig 4.11. The core consensus sequence for ATF (activating transcription factor) binding sequence TGACG (Dworetzky et al., 1992) was present between the basepairs 171 to 175. Apart from GATA repeats and CA repeats this sequence has short stretches of AT, A and T sequences. Sequence homology search was done using the programme fastscan of the PC/gene package and fastN of the pronuc against EMBL sequence data library. No significant homology was found with any of the sequences in the database.

**DISCUSSION**

Repetitive sequences specific to the mouse Y chromosome have been reported by several groups (Nallaseth et al., 1983; Lamar and Palmar, 1984; Bishop et al., 1985, Nallaseth and Deway, 1986; Nishioka and Lamothe, 1986; Nishioka, 1987, 1988; Singh et al., 1988). The Y chromosome-specific clones isolated by various groups are the members of Y chromosome specific multisequence families which are mouse-specific and detect restriction fragment length polymorphism in the Y chromosome. There are several features of
Fig. 4.12. ORF analysis of p11. Location of open reading frames in all the three possible reading frames (a, b and c) for one of the strands are shown at the top and for the GATA strand at the bottom (d, e, f). One of the largest possible ORFs having the potential to code for 162 amino acids corresponding to base pairs 574 to 1059 of the GATA strand is shown. This ORF, however does not have polyadenylation site in the sequence.
LOCATIONS OF ORFs AND POLYADENYLATION SIGNALS IN THE NUCLEOTIDE SEQUENCE OF p17

- **ORF 10**: 626 nucleotides
- **ORF 6**: 896 nucleotides
- **ORF 3**: 1156 nucleotides
- **ORF 9**: 846 nucleotides
- **ORF 5**: 956 nucleotides
- **ORF 11**: 1126 nucleotides

- Polyadenylation signals
- OR: Open reading frames
- aa: Amino acids

**Fig. 4.12**
Fig. 4.13. Largest ORF of p11 showing aminoacid sequences of the resulting protein
Fig. 4.14. Potential hairpin structure of p11(p17). A potential hairpin loop formation between base pairs 2615-2625 of p11 sequence.
M34 which are common with some of the sequences described by others. M34 sequences are present in *Mus musculus musculus*, *Mus musculus domesticus*, *Mus musculus poschiavinus* and *Mus spretus* (Singh *et al.*, 1988) but absent in *Mus caroli* (Singh, unpublished data). *Mus caroli* is diverged from *Mus musculus* 5-7 x 10^6 yrs ago (Martin *et al.*, 1985). So it seems that origin and amplification of M34 sequences in the Y chromosome had occurred after the divergence of *Mus caroli* nearly 5.7 million years ago.

**Striking similarity and biological significance of the molecular organization of the snake W and the mouse Y chromosomes**

Singh and Jones (1982), demonstrated by *in situ* hybridization that Bkm sequences are predominantly concentrated in the sex-determining region (short arm) of mouse Y chromosome, giving an impression that rest of the Y chromosome is devoid of these sequences. In the present study, it has been shown that Bkm sequences constitute a part of the repeating unit of M34 which is distributed along the length of the mouse Y chromosome at a regular interval interspersed amongst other sequences. There are approximately 200-300 copies of M34 on the Y chromosome (Singh *et al.*, 1993a). This is similar to the distribution of Bkm along the length of the snake W chromosomes (Singh *et al.*, 1980). Absence of M34 in the XXSxr male and in the female mouse confirms that it is absent in sex-determining and pseudoautosomal region of the Y chromosome (Singh *et al.*, 1993a). Thus sex-determining region is differently organised than the rest of the Y chromosome (Singh *et al.*, 1993a). Human Y chromosome may also be similarly organised
(Singh and Majumdar, in press). Such an astonishing 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 snake, the W chromosome remains condensed in all somatic cells and decondenses extensively in developing oocytes (Singh et al., 1979, 1993b). Similarly in mouse the Y chromosome remains condensed and, therefore, largely transcriptionally inactive in the somatic cells but decondenses specifically in the primary spermatocytes (Singh et al., 1993a). This is the stage at which the X chromosome in all male heterogametic organisms gets inactivated and Y chromosome becomes functional. This is in agreement with the recent findings (Koopman et al., 1989) that a Y-linked Zfy-1 gene in mice, which encodes a Zinc finger protein (Page et al., 1987), a Y chromosomal gene (Spy) needed for normal spermatogenesis (Burgoyne, 1987), recently discovered gene Sry (Gubbay et al., 1990), a candidate spermatogenesis gene on the mouse Y chromosome (A1s9Y-1 and A1s9Y-2, Kay et al., 1991), and a testis-specific gene, Sby, mapping in the Sxr region of the mouse Y chromosome (Mitchel et al., 1991) are expressed specifically in the testis. Prado et al. (1992) have recently reported the nucleotide sequences of a Y chromosomal repetitive sequence 145SC5 from a Balb/c mouse which detects poly (A) transcripts in the testis and there are about 200 copies of this sequence distributed over the entire length of the Y chromosome including the sex-determining region. Sequence comparison has not revealed any homology between p11 and 145SC5. Besides, 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 sequences cannot be ruled out during embryonic development.

A mutation (Y del) in mice, caused by partial deletion of the Y chromosome causes increased number of abnormal spermatozoa with flat acrosomal caps suggesting that some sequences present in the long arm of the Y chromosome are involved in the control of acrosome development (Styrna et al. 1991). Recently Xian et al. (1992) reported that partial deletion of the Y chromosome affects not only the morphology but also the fertilizing ability of spermatozoa. Burgoyne et al. (1992) also have demonstrated that the mouse Y chromosome long arm is essential for the normal development of the sperm head. Contrary to the prediction of Ohno (1983) that the mammalian Y contains only a few genes concerned exclusively with primary sex-determination, the above results strongly indicate the presence of specific gene(s), probably in multiple copies, in the heterochromatic region 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 to below a threshold level due to deletion. This would explain the variation in the length of the Y chromosome in the population. This also indicates the necessity for co-ordinated activation of these gene-families in a specific tissue at a specific time. Probably sex-determining chromosomes are endowed with such genes which require co-ordinated timely activation. Genetic studies of mice having variable deletion of the Y chromosome may confirm this prediction.
It is known that in *Drosophila hydei* and many other *Drosophila* species the Y chromosome during primary spermatocyte stage develops characteristic lampbrush loops of distinct morphology which are active in transcription. A ayl sequence family is distributed along the lampbrush loop and are known to enhance the transcription of flanking DNA via the binding of specific nuclear proteins. Since total length of the transcription unit is a functional parameter for the fertility of the male germ cell, any disruption of the chromatin folding code in the transcribed Y heterochromatin is deleterious (Vogt and Hennig, 1986). In this context it is extremely interesting that a sex-specific DNA binding protein which specifically recognises Bkm repeats (GATA) is exclusively present in the testis, the tissue in which the Y chromosome is decondensed and transcriptionally active (Singh *et al.*, 1993a). Probably the ordered organization of Bkm sequences, which are distributed along with the species-specific repeat M34 throughout the length of the Y chromosome, may be responsible for co-ordinated decondensation of the Y chromosome in response to such a protein.

**M34 and condensation and decondensation of Y chromatin.**

Chromatin of interphase nuclei appears to be organised into topological domains or loops, which are constrained by a residual nuclear frame work (Benyajati and Worcel, 1976; Cook and Brazell, 1978; Igokemenes and Zachan, 1978, Lebkowski and Laemmli, 1982a, b). This loop model of chromatin organization predicts, specific attachment sites spaced along the DNA for scaffolding proteins that cross-tie the chromatin fibers (Laemmli *et al.*, 1978; Marsden and Laemmli, 1979). Such sites of attachment of DNA to
the nuclear matrix structure have been defined as SAR or MAR sites (scaffold attached regions or matrix attached region) and these are presumed to be the bases of chromatin loops which have been identified in a number of eukaryotic loci (Gasser and Laemmli, 1987; Gasser et al., 1989; Phi-van and Startling, 1988).

In the present study a number of SAR/MAR motifs have been found on p3 fragment of p11. It is known that histone H1 preferentially binds to SAR under the conditions of strong cooperativity (Sevall, 1988; Izaurralde et al., 1989; Kas et al. 1989). Singh et al. (1993a) have shown strong preferential binding of histone H1 to this fragment containing SAR sequence motif (ATATTT), which is a part of the repeating unit of M34 distributed along the length of the Y chromosome. The SARs nucleate cooperative H1 assembly along the SAR into the flanking non-SAR DNA and control the conformation of chromatin domains. 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. It is possible that 238 to 363 kb DNA flanked by p3 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 (Getzenberg et al., 1991). Looping of DNA may bring SARs and GATA repeats of p3 of each repeating unit in close vicinity of each other at the matrix. Histone H1 may then 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 the nucleus.

Singh et al. (1979) have shown that the W chromosome decondenses extensively in the oocytes in snakes. Similarly in the case of mouse the Y chromosome also gets decondensed in the spermatocytes (Singh et al., 1993a). It is also known that a sequence-specific DNA binding protein called Bkm binding protein (BBP) which specifically recognises GATA sequence repeats, is present in the developing oocytes of snakes. The molecular weight of such a protein is 57.5 kd. This is a conserved protein found in many species of snakes where W chromosome is differentiated from the Z chromosome both morphologically and at molecular level. Presence of such a protein has also been confirmed in the mouse testes, the tissue in which the Y chromosome is decondensed and transcriptionally active. Presence of such a BBP probably indicates its role in decondensation of the Y or W in the respective species.

It is possible that the BBP binds to the GATA repeats of the Y, which may cause replacement of histone H1 from the histone binding site (SAR), and thus bring about co-operative decondensation of the entire Y chromosome and make 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 the appropriate sequences 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 and Laemmli, 1987). Condensation/heterochromatinization thus represents an epigenetic regulatory switch establishing and maintaining the repression of certain genes or an entire chromosome.