Chapter II

BANDED KRAIT MINOR SATELLITE (BKM) DNA SEQUENCE: DISTRIBUTION AND ROLE IN SEX CHROMOSOMAL ORGANIZATION
2.1 EVOLUTIONARY CONSERVATION AND FUNCTIONAL SIGNIFICANCE OF BKM SEQUENCES

Bkm is a female-specific minor satellite DNA, obtained by isopycnic density gradient centrifugation of the DNA from the poisonous Indian snake, Banded Krait, *Bungarus fasciatus* (Singh et al., 1976b, 1979).

Bkm sequences are highly conserved among eukaryotes and have been reported from yeast and from invertebrates like, dinoflagellates, coelenterates, echinoderms, insects. These sequences are present in most of the vertebrates such as amphibians; reptiles including snakes, crocodiles, alligators, gharials and turtles; birds; and mammals including mice and man (Singh et al., 1981, 1984; Singh and Jones, 1982; Jones and Singh, 1981, 1982; Jones, 1983, 1984, 1985; Jones et al., 1983; Alonso et al., 1983; Schafer et al., 1986a,b; Erickson et al., 1988; Kent et al., 1988; Nanda et al., 1988, 1990, 1992; Durbin et al., 1989; Demas et al., 1990; Simpson, 1990; Willhoeft and Traut, 1990; Demas and Wachtel, 1991; Li et al., 1991; Wachtel et al., 1991; Tiersh et al., 1992; Lang et al., 1993; Aggarwal et al., 1994; Kirchhoff, 1988; Nagaraju et al., 1995). However, Miklos et al. (1989) have reported the absence of these sequences from bovine and ovine species, under the hybridization conditions, that detects single copy DNA sequences. *In situ* hybridization studies show that Bkm sequences are preferentially located on sex chromosomes in *Drosophila* (Singh et al., 1981), snakes and birds(Singh et al., 1976b), mice (Jones and Singh, 1981), humans (Singh and Jones, 1986), thus suggesting the possibility of a conserved sex-specific role in the organization and functioning of the sex chromosomes (Singh et al., 1976b, 1981; Jones, 1983; Jones and Singh, 1985).

2.2 BKM IN SNAKES AND BIRDS

Both snakes and birds, having female heterogamety (ZZ male/ZW female), show hybridization on the W chromosome with Bkm probe (Singh et al., 1976a, 1980; Jones and Singh, 1981). Quantitative filter hybridization shows the presence of Bkm-related DNA even in males, though much lesser in amounts. In snakes, Bkm is present on the autosomes as well, but in lesser quantities, compared to the W chromosome (Jones, 1985).

Highly evolved snakes, belonging to family Viperidae and Elapidae, have morphologically distinct W chromosome having very high concentration of Bkm.
sequences. The primitive snakes of the family Boidae, however, neither show morphologically distinguishable sex chromosomes nor high amounts of Bkm in their genome. In some of the snakes of the family Colubridae, the sex chromosomes cannot be distinguished morphologically, but the W chromosome can be identified at DNA level by *in situ* hybridization with Bkm and by its allocyclic and giemsa-staining behaviour (Singh, 1972; Singh et al., 1980). Thus the increase in Bkm sequences precedes the morphological differentiation of sex chromosomes and correlates with the evolution of chromosomal sex determination (CSD) (Singh et al., 1980; Jones and Singh, 1981).

### 2.3 BKM IN MICE

Bkm sequences are predominantly concentrated in the paracentric region of the mouse Y chromosome (Singh et al., 1981; Jones and Singh, 1982; Epplen et al., 1982; Jones and Singh, 1981). This region was later confirmed to be the short arm of the Y chromosome (Bishop et al., 1988; McLaren et al., 1988; Roberts et al., 1988).

A dominant mutation, known as Sxr, was shown to be the cause of sex reversal of chromosomally female (XX) individuals into sterile males (Cattanach et al., 1971). Singh and Jones (1982), through their ingenious experiments demonstrated that the Bkm-positive region of the mouse Y chromosome is necessary and sufficient for sex determination. When this region is transferred to the distal end of the X chromosome, it causes sex reversal in XX mice. They elegantly demonstrated that in the carrier mice (XYSxr), the sex determining Bkm-positive region of the Y chromosome is duplicated and transferred to the distal end of the long arm of the Y chromosome. The XYSxr males, during spermatogenesis, pass on this Bkm-rich region from the tip of one of the chromatids of the long arm of the Y chromosome to one of the chromatids of the X chromosome through meiotic crossover between the two chromosomes. This results in 25% of the progeny as sex-reversed XXSxr males, when crossed to normal females. These observations, not only explained the inheritance pattern of Sxr in these XYSxr males and narrowed down the male-determining region to the proximal region of the Y chromosome, but also associated Bkm intimately, if not specifically, with sex-determination.

A region on chromosome 17, rich in Bkm, was implicated in autosomal sex-reversal (Washburn and Eicher, 1983; Kiel-Metzger and Erickson, 1985) and was shown to contain genes expressed in the testis (Yoem et al., 1992). These
observations further strengthen the role of Bkm, as proposed by Singh and Jones (1982) in controlling the expression of genes involved in sex-determination and/or spermatogenesis.

A Y chromosome-specific clone, M34, was isolated by double-screening of a male mouse genomic library, first with uncloned Bkm and subsequently with cloned Bkm-2(8) containing long stretches of tetranucleotide repeats of GATA (Singh et al., 1988). Southern and in situ hybridization showed that M34 is distributed all along the Y chromosome, except for the sex determining and pseudoautosomal region (Singh et al., 1988, 1994a). Singh et al. (1994a) implicated these sequences in bringing about coordinated decondensation of the entire Y chromosome.

2.4 BKM IN HUMANS

Bkm sequences are distributed all over the genome and show very high rate of polymorphism in human DNA. However, Southern blot analysis of human male and female DNAs using Bkm-2(8), a clone rich in GATA repeats, and uncloned Bkm as probes, showed no sex-specific hybridization pattern (Singh and Jones, 1986). This is in contrast to the observation in mice (Singh et al., 1981; Jones and Singh, 1982). Intriguingly, in situ hybridization, with uncloned Bkm on human male chromosomes showed hybridization with most of the small acrocentric chromosomes, including the Y chromosome. The grains were concentrated more on the proximal region of the Y chromosome, the region associated with sex determination (Singh and Jones, 1986; Arnemann et al., 1986; Rasheed et al., 1991).

Screening of a human Y chromosomal DNA library with uncloned Bkm yielded C102, among many positive clones. Genomic DNA hybridization with p102d(2), a subclone of C102, gave male-specific pattern, suggesting its origin from the Y chromosome. In situ hybridization showed its distribution along the length of the Y chromosome, except for the short arm of the Y chromosome. This distribution of p102d(2) is astonishingly similar to the distribution pattern of M34 on the mouse Y chromosome (Singh and Majumdar, 1993).
2.5 Bkm in *Drosophila*

*In situ* hybridization with the polytene chromosomes of the larval salivary glands of *Drosophila melanogaster* localized Bkm to a small, well-defined, euchromatic region, 19F-20AB, near the base of the X chromosome. Southern blot analysis showed no difference in the hybridization pattern of Bkm with adult male and female DNAs.

Bkm-positive genomic clones, recovered from *Drosophila* genomic library, showed hybridization, as expected, to the base of the X chromosome, in the region 19F-20AB (Singh et al., 1981). Two GATA-rich clones, Bkm-2(8) (a subclone of *Drosophila* clone, CS314) and M3.1 (a subclone of Mouse clone, M41) hybridized to the base of the X chromosome as well as to all the Bkm-positive genomic clones. However, one *Drosophila* clone, CS325, did not hybridize to the base of the X chromosome. It was found by hybridization that this clone does not contain GATA sequences (Singh et al., 1984). This strongly suggests the existence of other sequences in Bkm, which are common between the snake and *Drosophila*.

2.6 Sequence Analysis and Transcription Pattern of Bkm

Sequencing of Bkm-positive fragments isolated from *Drosophila*, snake, mouse and human clones showed simple repeats of tetranucleotide GATA as the major conserved component (Epplen et al., 1982; Singh et al., 1984, 1994a; Schafer et al., 1986a; Rasheed et al., 1991; Panicker, 1993). Simple quadruplet repeats of GACA are also present in certain clones sequenced from mouse and snakes (Epplen et al., 1982; Schafer et al., 1986a; Panicker, 1993), but have not been found in clones of other species sequenced so far. In most of the clones, the repeats (GATA or GACA) are arranged in the form of long, contiguous stretch(es) in addition to dispersed monomers.

Southern hybridization pattern with GATA-rich clone, Bkm-2(8), was similar to the pattern with uncloned Bkm in mouse and man, though in humans, the band intensity was higher with uncloned Bkm (Singh et al., 1981, 1984; Singh and Jones, 1982, 1986). This confirmed GATA repeats as the major conserved component of Bkm.

Comparative studies between cloned and uncloned Bkm in yeast and *Drosophila* (Singh et al., 1984), snakes (Singh et al., 1981) and man (Singh and Jones, 1986) hinted at the existence of other conserved component(s) in Bkm, in addition to
GATA repeats. Interestingly, uncloned Bkm, as a probe, showed certain bands specific to Australian aborigines in Southern hybridization which were absent in Caucasian population (Singh and Jones, 1986). Cloning of Bkm (from snakes) and double-screening of the resultant library with Bkm-2(8) and CS325 probes (GATA-rich and GATA-negative clones, respectively), yielded a clone, which could identify Australian aborigines-specific band pattern similar to that obtained with uncloned Bkm. The sequence analysis of this clone showed poly CA/GT repeats as the second conserved component of Bkm (Panicker, 1993).

Developmental stage-specific decondensation of the W chromosome in snakes and existence of potential open reading frames in GATA-rich clones (Singh et al., 1979, 1984) indicated that the Bkm sequences may be transcribed. Northern hybridization studies using poly-A+ RNA from different tissues of Drosophila, blow fly, mouse and rat (Epplen et al., 1982; Singh et al., 1984; Schafer et al., 1986b; Kirchhoff, 1988; Miklos et al., 1989) showed the existence of transcripts containing tracts of GATA. Some of these transcripts were developmental stage specific (Singh et al., 1984; Kirchhoff, 1988).

2.7 **Bkm is a structural and functional component of sex chromosomal chromatin**

The Bkm sequences are distributed along the length of the W chromosome in snakes. Similarly, Bkm-related, sex and species specific sequences of mouse and man i.e. M34 and p102d(2), respectively, are distributed all along the length of the Y chromosome except the sex determining region. Thus, the structural organization of the W chromosome of snakes and the Y chromosome of mammals seems to be similar, with respect to Bkm sequences.

The W chromosome of snakes and the Y chromosome of mammals are heterochromatic, transcriptionally inactive, show allocycly (in DNA replication), remain highly condensed in the somatic tissues (Ray Chaudhuri et al., 1970, 1971; Singh and Ray-Chaudhuri, 1975; Eicher and Washburn, 1986). *In situ* hybridization with Bkm shows a high concentration of grains, exclusively, in a single region of the interphase nuclei the somatic cells of snakes, thus confirming the condensed state of the W chromosome in the form of a chromatin body (Singh et al., 1979). The W chromosome of snake and the Y chromosome of mouse remain highly condensed in the various somatic tissues (Singh et al., 1979; Guttenbach et al., 1989; Nagaraj, 1994), but decondense extensively in the germ
cells, developing oocytes in the ovary of snakes and the testis of mouse (Singh et al., 1994b). Similarly, in Drosophila, extensive decondensation of Bkm-positive region is seen in the germ cells, while this region remains highly condensed in the somatic (follicle) cells of the ovary (Singh, L.- unpublished results; Fig 2.1).

A sex- and tissue-specific protein that binds to the conserved component of Bkm, i.e. GATA repeats and is present in the ovaries of various species of snake and testis of mouse and human has been reported (Singh et al., 1994b). This protein, designated as Bkm-binding protein or BBP has been implicated in coordinated decondensation and consequent activation of the genes present on the W and the Y chromosomes in snakes and mice, respectively.
Fig 2.1: *In situ* hybridization of squash preparation of *Drosophila* ovary with $^3$H labeled *Bkm-2*(8) probe. (A) Somatic cells showing condensed state of the base of the X chromosome, (B) A germ cell showing highly decondensed state of the base of the X chromosome.

Note that the magnification of both (A) and (B) is the same.