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Behaviour of microchromosome-associated satellite DNA in the banded krait, *Bungarus fasciatus* (Ophidia, Elapidae)

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Abstract. Banded krait (*Bungarus fasciatus*) major satellite DNA ($\rho = 1{\cdot}700$ g/cm^3) is mainly localized in the C-band-positive regions of all the microchromosomes. Our study of the behaviour of this satellite DNA by *in situ* hybridization has revealed a striking polarization of this DNA in the follicular epithelial cells of the ovary during oogenesis and in the spermatids during spermiogenesis. The major satellite DNA is localized at the point of the subsequent protrusion of the acrosomal pole of the round spermatid nuclei and remains in close contact with the developing sperm tip during the process of spermiogenesis. There appears to be an attraction between a specific region of the nuclear membrane and satellite-rich chromatin of the microchromosomes that brings about their polarization. We discuss possible functions of such extreme polarization of microchromosomes in specific cell types during oogenesis and spermiogenesis.

Keywords. Satellite DNA; microchromosomes; oogenesis; spermiogenesis; acrosomal pole.

1. Introduction

Snakes and birds possess two size classes of chromosomes: microchromosomes and macrochromosomes. Brown and Jones (1972) demonstrated by *in situ* hybridization that GC-rich satellite DNA in the Japanese quail is predominantly localized in the microchromosomes. Stefos and Arrighi (1974) showed that in chicken microchromosomes appear to contain more repetitive DNA than the macrochromosomes. Singh *et al.* (1976a) reported the presence of a satellite DNA in the rat snake (*Ptyas mucosus*) which constitutes 5% of the haploid genome and is predominantly localized in the microchromosomes. They observed aggregation of those microchromosomes that are rich in satellite DNA and suggested that these microchromosomes are probably involved in nucleolus organization. This corroborates the hypothesis put forward by Brown and Jones (1972) that repetitive DNA in some way determines the clumping of this heterochromatin, thus bringing together the genes necessary for formation of a functional nucleolus. In this paper we report the presence of a major satellite DNA in another species of snake, the banded krait (*Bungarus fasciatus*). This DNA constitutes 8–9% of the genome and is mainly localized in the microchromosomes. This observation and other evidence suggest that microchromosomes in snakes and birds may contain a distinct type of satellite DNA. This is in agreement with the view that the microchromosomes of birds and snakes have common ancestry and belong to a line of karyotypic evolution distinct from other vertebrate lines (Ohno 1970).

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Singh *et al.* (1976b, 1979, 1980) showed the presence of a sex-specific minor satellite DNA, Bkm, in females of banded krait that is localized in high copy number in the W chromosomes of all snake families with differentiated sex chromosomes and is highly conserved and preferentially associated with the sex chromosomes of other organisms (Singh *et al.* 1980, 1981; Singh and Jones 1982). Singh *et al.* (1979), by making use of sex-chromosome-specific DNA probes, studied behaviour of the W chromosome during the cell cycle in somatic and germ cells, particularly in interphase and prophase stages in which chromosomes are normally unidentifiable. These stages have previously not been accessible to such scrutiny because of the lack of a probe specific for a single chromosome. These studies revealed that the W sex chromosome remains condensed in interphase nuclei of all somatic cells but decondenses extensively in growing oocytes. Another study of the behaviour of an autosomal satellite DNA (*B. fasciatus* major) that is mainly localized in the microchromosomes revealed that in growing oocytes chromatin rich in autosomal satellite DNA remains condensed while chromatin rich in Bkm decondenses completely. At later stages of oogenesis the situation is reversed: the W chromosome becomes condensed while the autosomal-satellite chromatin is decondensed (Singh *et al.* 1979). In the present investigation we found a striking polarization of chromatin rich in satellite DNA in the follicular epithelial cells of the ovary during oogenesis, suggestive of elimination and hence diminution of this type of chromatin. This prompted us to study the behaviour of this chromatin in somatic and germ cells during spermatogenesis also. The study revealed similar localization of satellite DNA during spermatogenesis. The results of these studies are reported here.

2. Materials and methods

2.1 *Chromosome preparation*

Mitotic chromosome preparations from two males and three females of banded krait were made from short-term leukocyte cultures by the usual air-drying procedure. Cultures were treated with colchicine (0·015 μg/ml) for 4 h and 0·075 M KCl for 8–10 min, and fixed in 1 : 3 acetic acid methanol. For meiotic chromosome preparations testis was dissected out in tissue culture medium (MEM) and cells were squeezed out with the help of toothed forceps. Slides were prepared by air-drying procedure after 10-min treatment of cells in hypotonic solution (0·075 M KCl) and three 10-min changes in acetic acid methanol (1 : 3). Slides were stored at 5°C.

2.2 *C-banding procedure*

To stain constitutive heterochromatin the procedure of Sumner (1972) was followed. Chromosomes were treated for 1 h in 0·2 N HCl at room temperature, 4–10 min in 5% aqueous solution of barium hydroxide octahydrate at 50°C, and 1·5 h in 2× SSC (1× SSC is 0·15 M NaCl, 0·015 M sodium citrate, pH 7·0) at 60°C, and stained for 1·5 h in buffered Giemsa (1 ml Giemsa, 50 ml 0·1 M phosphate buffer pH 6·8).

2.3 *Preparation of cells from ovary and other tissues*

Ovary, spleen, kidney and brain were dissected out in MEM. Cells were squeezed out using toothed forceps, centrifuged at 300 *g* for 5 min, and directly fixed in 3 : 1 methanol acetic acid with three changes of 10 min each without hypotonic pretreatment. One drop of cell suspension was air-dried on acid-cleaned slides and stored dry at 5°C.

2.4 *DNA extraction*

Four female and three male *B. fasciatus* and one male Indian cobra *Naja naja naja* (family Elapidae), and one male and one female *Ptyas mucosus* (family Colubridae) were obtained from West Bengal state, India. DNA was isolated from liver, kidney, heart, blood and gonads from each individual and processed separately by the method of Marmur (1961) with repeated phenol–chloroform, RNAase and Pronase treatments.

2.5 *Isolation of DNA from spermatozoa*

Vas deferens and epididymis were dissected out from two male banded kraits in the breeding season. Sperms were squeezed out in MEM using toothed forceps and pelleted by centrifugation (300 *g*). The pellet was resuspended in 0·1 M Tris-HCl buffer, pH 8·0, containing 0·5% sodium dodecyl sulphate (SDS) for 15 min to eliminate leukocyte or epithelial cell contamination. Sperm cells were recovered by centrifugation at 1000 *g* for 15 min at room temperature, resuspended in 5 ml of Tris buffer, pH 8·0, and incubated with shaking in the presence of 4·5 mM dithiothreitol for 15 min at room temperature. SDS was added to a final concentration of 0·25% and proteinase K to a final concentration of 100 µg/ml. The mixture was incubated at 37°C for 4 to 6 h or overnight. DNA was isolated by repeated phenol–chloroform–isoamyl alcohol treatment, and precipitated by adding 1/30 vol 3 M sodium acetate, pH 5·5, and one vol isopropanol. The precipitate was washed in 70% ethanol, lyophilized, and dissolved in 10 mM Tris-HCl, pH 7·5, and 1 mM EDTA.

2.6 *Isolation and purification of satellite DNA*

Satellite DNA was isolated from total DNA in Cs_2SO_4/Ag^+ gradients following the procedure of Jensen and Davidson (1966). Optimum separation was achieved at an Ag^+ to DNA ratio of 0·25. *B. fasciatus* major satellite DNA was isolated from the female and purified by successive Cs_2SO_4 and CsCl gradient centrifugation in a Beckman VTi-50 rotor for 17 h at 46,000 rpm at 25°C. The buoyant density was determined by centrifugation in neutral CsCl in a Spinco model E analytical centrifuge at 44,000 rpm for 20 h at 25°C using *Micrococcus lysodeikticus* DNA (ρ = 1·731 g/cm^3) as density marker. The buoyant density of the major satellite DNA was 1·700 g/cm^3.

2.7 *Transcription of satellite DNA*

Satellite DNA was transcribed using equimolar amounts of [^{3}H]ATP (sp. act. 29 Ci/mmol), [^{3}H]UTP (sp. act. 21 Ci/mmol), [^{3}H]GTP (sp. act. 15 Ci/mmol), and [^{3}H]CTP (sp. act. 18 Ci/mmol), 2 μg of satellite DNA, and 2 units of *E. coli* DNA-dependent RNA polymerase type I as described by Moar *et al.* (1975).

2.8 *Determination of* T_{opt}

The rate of formation of hybrids of the satellite DNA with complementary RNA (cRNA) was studied by filter hybridization in excess of cRNA (Gillespie and Spiegelman 1965) in 3× SSC (Birnstiel *et al.* 1972; Moar *et al.* 1975). The optimum temperature (T_{opt}) for hybrid formation in 3× SSC of *B. fasciatus* major satellite DNA was 58°C (data not shown).

2.9 *In situ hybridization*

The procedure described by Jones (1973) was followed, with minor modifications. Slides were heated to denature the DNA (Singh *et al.* 1977) and a hybridization reaction carried out with 5 μg/ml cRNA per slide at 58°C (T_{opt}) in 3× SSC for 3 h at a cRNA concentration of 0·6 μg/ml (sp. act. $1{\cdot}7 \times 10^7$ counts/min/μg). After hybridization slides were treated with RNAase (20 μg/ml) for 30 min at 37°C in 2× SSC, washed overnight in 2× SSC at 5°C, and dried through an alcohol series.

2.10 *Autoradiography*

After hybridization slides were dipped in Ilford K_2 nuclear emulsion diluted 1 : 1 with distilled water at 40°C. Exposure was for 3 to 8 weeks at 4°C. The slides were then developed in Kodak D19b developer as described by Singh *et al.* (1977) and stained in Giemsa (2 ml Giemsa, 50 ml 0·1 M phosphate buffer pH 6·8) for 10–40 min. Photographs were taken on Agfa Gevaert Copex Pan 35 mm film using a Zeiss photomicroscope.

2.11 *^{32}P labelling of probe*

Uncloned major satellite DNA of *B. fasciatus* was labelled with [^{32}P]dATP (sp. act. 3000 Ci/mmol) by nick translation using the Amersham nick translation kit. [^{32}P]dATP was obtained from Jonaki, Bhabha Atomic Research Centre, India.

2.12 *Southern blot hybridization*

Genomic DNAs of *B. fasciatus*, *B. caeruleus* (common Indian krait), *Naja naja naja* (cobra) and *Ptyas mucosus* (rat snake) were digested with *Hin*fI (4 units per μg DNA, at 37°C for 12 h), fractionated in 0·8% agarose gel in TPE buffer (15 mM Tris-HCl pH 7·8, 18 mM NaH_2PO_4, 0·5 mM EDTA) by horizontal gel

electrophoresis (2–3 V/cm^2) for 16 h, denatured, and transferred onto Hybond-N nylon membrane (Amersham) by vacuum blotting (Olszewska and Jones 1988). The blot was baked at 80°C for 2 h under vacuum. Prehybridization was done at 65°C for 3 h in 7% SDS and 0·5 M sodium phosphate buffer pH 7·5. Hybridization was carried out in the same but fresh buffer containing ^{32}P-labelled banded krait major satellite DNA probe (sp. act. 1×10^8 cpm/μg) at 65°C for 20 h. The blots were then washed at 65°C in 2× SSC and 0·1% SDS, two changes of 30 min each; in 1× SSC and 0·1% SDS for 30 min; in 0·5× SSC and 0.1% SDS for 20 min. X-ray film was exposed to the blots at −70°C for 24 h with an intensifying screen.

3. Results

3.1 *Analytical equilibrium density gradient centrifugation*

When DNA samples (70 μg) from males and females of *B. fasciatus* are centrifuged to equilibrium in Ag^+/Cs_2SO_4 gradients at an Ag^+ to DNA phosphate ratio of 0·25 in an analytical ultracentrifuge, a prominent satellite band is observed on the heavy side of the main DNA band (data not shown). In centrifuged female DNA, another, less prominent (minor) satellite DNA band appears. The minor satellite DNA is apparently restricted to the female sex (Singh *et al.* 1980). To obtain an approximate quantitative estimate of the major satellite DNA in the genome a smaller amount of male DNA (6 μg) was centrifuged to equilibrium in an Ag^+/Cs_2SO_4 gradient. The distinct satellite band that was obtained on the heavy side of the main band DNA (the major satellite DNA band) has been calculated by area measurements to amount to 8–9% of the genome (data not shown).

3.2 *C-banding*

The diploid number of chromosomes in this species is 38 in both the sexes (Singh 1974). Because of the gradual gradation in the size of the chromosomes the distinction between macrochromosomes and microchromosomes is not well defined. However, when chromosomes are arranged in decreasing order of size, seven pairs (pairs 1–6 and sex chromosomes) can be classified as macrochromosomes and the rest (pairs 7–18) as microchromosomes. All the 24 microchromosomes possess prominent terminal C-bands. The W chromosome is entirely C-band-positive. Both the homologues of pair 1 have faintly stained C-bands in their centromeric and telomeric regions and in the regions of secondary constriction in the long arm. Both the homologues of pairs 2, 3, 4 and 6 and the Z chromosome also have faintly stained C-bands in their centromeric and telomeric regions. The distribution of C-bands on the chromosomes is shown in figure 1.

3.3 *C-band polymorphism*

One of the homologues of macrochromosome pair 5 has a prominent C-band in the centromeric region whereas the other homologue lacks a C-band (figure 1). This phenomenon was observed in all three females whose chromosomes were subjected to C-banding.

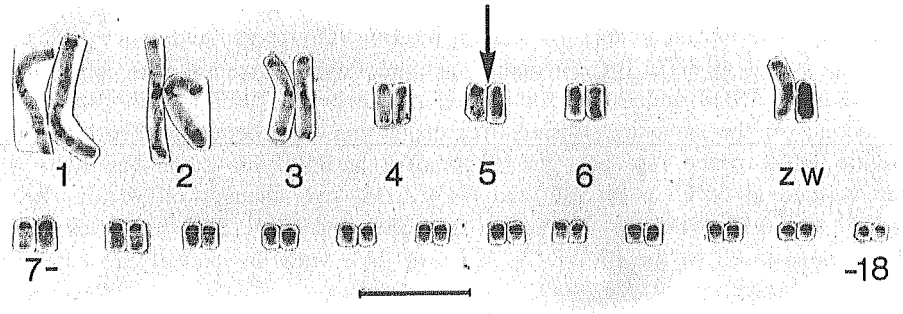

Figure 1. Female karyotype of *B. fasciatus* from leukocyte culture, showing C-banding. Note the presence of C-band in the centromeric region of one of the homologues of pair 5 and absence from the other. Also note the entirely darkly stained W chromosome and the prominent C-banding in the proximal region of the microchromosomes. Bar = 10 μm.

3.4 *Chromosomal localization of satellite DNA*

The major satellite DNA is localized in C-band-positive regions of all the microchromosomes. Very few grains are observed on the macrochromosomes, except on pair 5 (figure 2). This observation was regardless of exposure time (three weeks to two months).

3.5 *Satellite DNA polymorphism*

One of the chromosomes of pair 5 (numbered on size basis) shows a distinct polymorphism in the distribution pattern of satellite DNA. In one homologue, the satellite DNA is localized in the centromeric region (figure 2), which stains

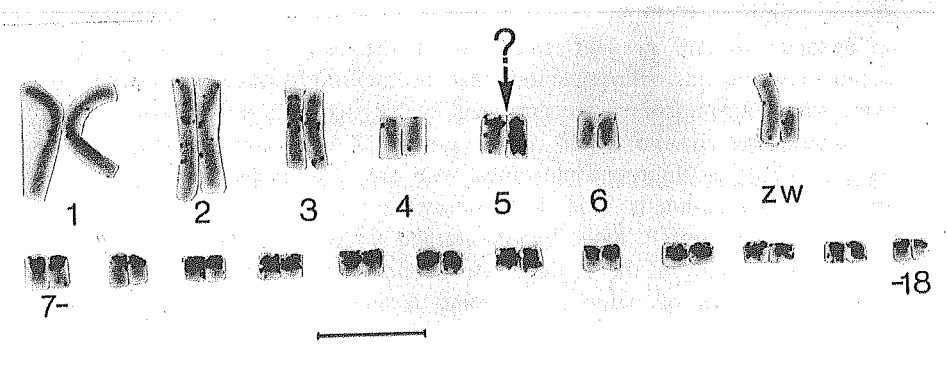

Figure 2. Female karyotype of *B. fasciatus* from leukocyte culture, showing hybridization with the cRNA of major satellite DNA. The satellite cRNA has predominantly hybridized to the C-band-positive regions of all the microchromosomes (pairs 7–18) and to only one pair of macrochromosomes (pair 5), where it is localized in the C-band-positive centromeric region of one homologue and is distributed along the entire length of the other. Identification of chromosome pair 5 from the W is not unequivocal unless the chromosomes have previously been identified by C-banding. In this karyotype it has been ranked on size basis (arrow). There is no hybridization with the rest of the macrochromosomes. Exposure was for three weeks. Bar = 10 μm.

prominently by the C-banding procedure (figure 1). However, the satellite DNA in the other homologue is distributed along its entire length (figure 2). All of more than 100 metaphases examined after *in situ* hybridization invariably revealed the same pattern of hybridization. Perhaps it is this particular chromosome 5 homologue that is C-band-negative (figure 1, pair 5). A similar pattern of hybridization was obtained on chromosome preparations from another female individual. In this particular case a small distal region of the chromosome 5 homologue that shows more than only centromeric hybridization is occasionally free from grains (figure 3, pair 5). In contrast to this situation in the females, in both the males examined grains are localized invariably only in the centromeric region of both homologues of pair 5 (figure 4). The distribution of grains in the rest of the chromosomes is exactly the same as in the females. Though the number of individuals examined in the present investigation is small, the findings suggest that the polymorphism

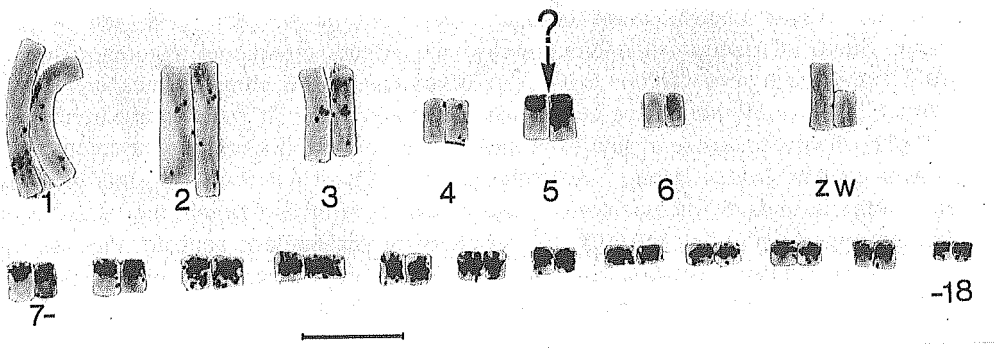

Figure 3. Female karyotype of *B. fasciatus* from leukocyte culture from an individual different from the one represented in figures 1 and 2, showing hybridization with major satellite cRNA. The hybridization pattern is exactly the same as in figure 2 except for pair 5 (ranked on size basis), in which one homologue, as in figure 1, has hybridization in the centromeric region and the other throughout its length but for a small distal region. Exposure was for two months. Bar = 10 μm.

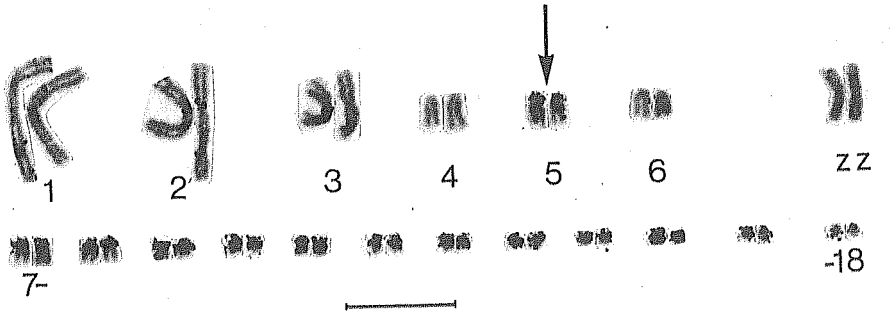

Figure 4. Male karyotype of *B. fasciatus* from leukocyte culture, showing hybridization with major satellite cRNA. Grains are predominantly localized in the proximal region of all the microchromosomes and in the centromeric regions of both homologues of pair 5 (arrow). Note absence of the autosome (chromosome 5) showing distribution of grains along its entire length in the female. Exposure was for three weeks. Bar = 10 μm.

observed may be sex-limited. Studies on more individuals of both sexes from the same as well as different populations are needed for an understanding of the significance of this polymorphism.

3.6 *Behaviour of microchromosomes during spermatogenesis*

In situ hybridization of the major satellite DNA (cRNA) to cells of various somatic tissues showed a high concentration of grains in two to five chromocentre regions of interphase nuclei. Figure 5a shows hybridization in leukocyte nuclei. In spermatocytes, however, grains are concentrated in a single small region (figure 5b). A similar concentration of grains in a single region is observed in oocytes (Singh *et al.* 1979). In spermatocytes that had just entered the leptotene stage of meiosis, the grains, instead of being restricted to a single central spot, are distributed in a larger, peripheral area (figure 6a). In spermatocytes fixed at later stages of leptotene (figure 6,b&c), grains occupy almost half of the peripheral area of the nuclei. Such polarization of microchromosomes persists throughout zygotene (figure 6,d–f) until pachytene (figure 6,g–j). At pachytene, when chromosomes are more distinct because of their more condensed and paired state, it is quite apparent that the microchromosomes segregate from the macrochromosomes into one, or sometimes two, prominent groups at one side of the nucleus (figure 6,h–j). During this process the telomeric ends of macrochromosomes orient themselves towards the centre and the centromeric portions towards the periphery of the nucleus opposite the site of segregation of the microchromosomes. An attraction of some sort between a specific region of the nuclear membrane and satellite-rich chromatin of the microchromosomes appears to bring about such a polarization. The polarized orientation of the chromosomes starts disappearing at late pachytene and early diplotene stages, when longitudinal separation of the paired chromosomes is initiated (figure 7,a&b).

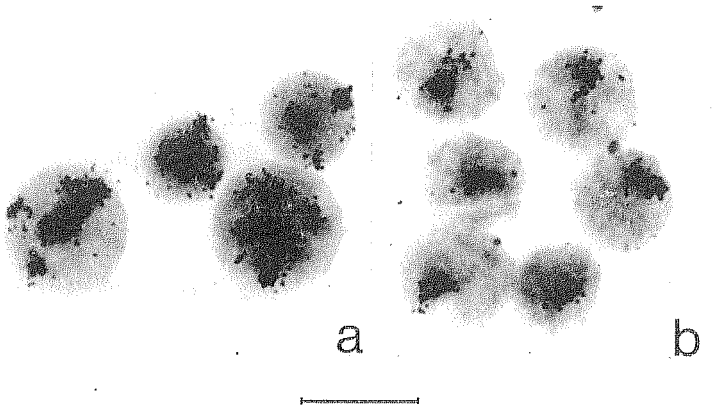

Figure 5. Male interphase nuclei of *B. fasciatus* from (a) leukocyte culture and (b) spermatocytes from testis, showing hybridization with major satellite cRNA. Note that, in (a), grains are localized in regions of chromocentres, which vary in number from two to five, and, in (b), grains are concentrated in a single region. Exposure was for three weeks. Bar = 10 μm.

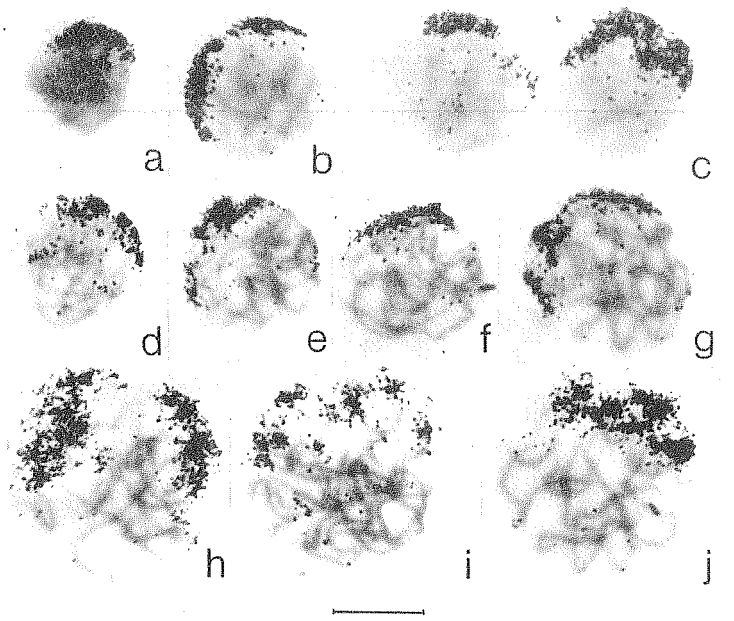

Figure 6. Meiotic preparations from *B. fasciatus* testis, showing *in situ* hybridization with major satellite cRNA. Note the characteristic polarization of microchromosomes revealed by the peripheral distribution of grains. a, Early leptotene stage of meiotic prophase; b&c, late leptotene; d–f, zygotene; g–j, pachytene. Exposure was for three weeks. Bar = 10 µm.

Polarized orientation of microchromosomes and macrochromosomes is no longer visible at diakinesis and metaphase I. Bivalents are easily identifiable because of shortening of the chromosomes and terminalization of chiasmata. There are large unlabelled bivalents representing the macrochromosomes and small bivalents showing concentration of grains on both the ends representing the terminal centromeric regions of both homologues of the microchromosomes (figure 7,c&d). Metaphase II (figure 7e) shows the haploid number of chromosomes after reduction division; grains are mainly concentrated on the microchromosomes. At anaphase II (figure 7f) each anaphase group is made up of the haploid number of chromosomes. The microchromosomes, as revealed by grain distribution, occupy a central position in each anaphase group.

3.7 *Extreme polarization of satellite DNA suggests its elimination during spermiogenesis*

A remarkable behaviour of satellite DNA is revealed in post-meiotic cells during spermiogenesis. In early spermatids grains are localized in high concentration in close proximity to the nuclear membrane at the region of elongation of the nuclear protuberance (figure 8,b–d). This indicates that in the nuclei of spermatids the major satellite DNA (perhaps all of the microchromosomes) occupies a specific position. At later stages of spermiogenesis localization of grains is observed outside

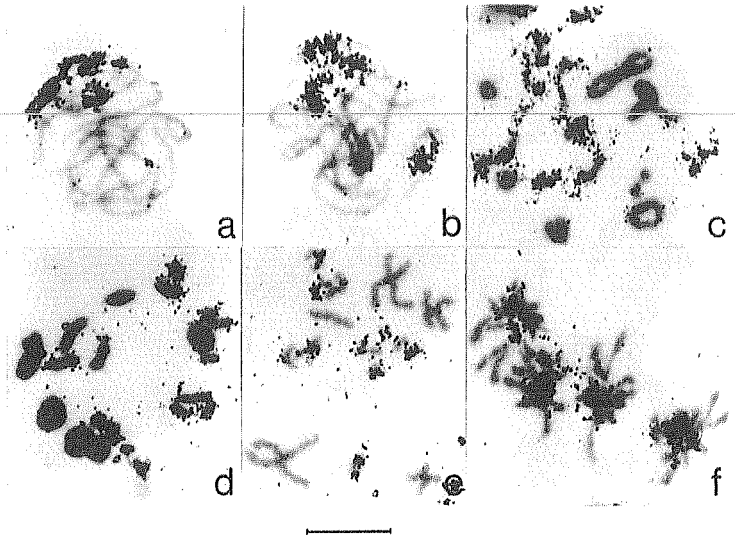

Figure 7. Meiotic preparations from *B. fasciatus* testis, showing hybridization with major satellite cRNA. a&b, Late pachytene or early diplotene stages. Note the residual polarized orientation of microchromosomes revealed by grain distribution. c, Diakinesis stage, showing terminalization of chiasmata. Note the localization of grains on both the ends of microchromosome bivalents showing the position of the respective centromeres. Macrochromosome bivalents are essentially unlabelled. d, Metaphase I, showing distribution of grains in microchromosome bivalents. e, Metaphase II, showing haploid number of chromosomes after reduction division. Grains are localized in the microchromosomes. f, Anaphase II, showing presence of satellite DNA in microchromosomes, which occupy a central position in each anaphase group. Exposure was for three weeks. Bar = 10 μm.

of the nuclear protuberance. This raises the possibility that some of the major satellite DNA may be expelled from the nuclei of spermatids (figure 8,a&e–g).

3.8 *Is major satellite DNA eliminated during spermiogenesis?*

To find out if satellite DNA is indeed eliminated during spermiogenesis, we isolated DNA from spermatozoa collected from vas deferens and epididymis of banded krait males in the breeding season, from somatic tissues of banded krait females, and from somatic tissues of rat snake, cobra and the common Indian krait. Southern hybridization of equal amounts of genomic DNA (5 μg each, restricted with *Hin*fI and fractionated on agarose) with the ^{32}P-labelled uncloned Bkm probe revealed equal intensity of hybridization with sperm DNA and DNA from blood (figure 9, lanes 3 and 4). This rules out the possibility of elimination of satellite DNA during spermiogenesis. Had there been elimination one would have expected no hybridization or drastically reduced hybridization with sperm DNA. Absence of any detectable hybridization with genomic DNA of *P. mucosus* (figure 9, lanes 1 and 2), *B. caeruleus* (lane 5) and *N. naja naja* (lane 6) shows that banded krait major satellite DNA is species-specific.

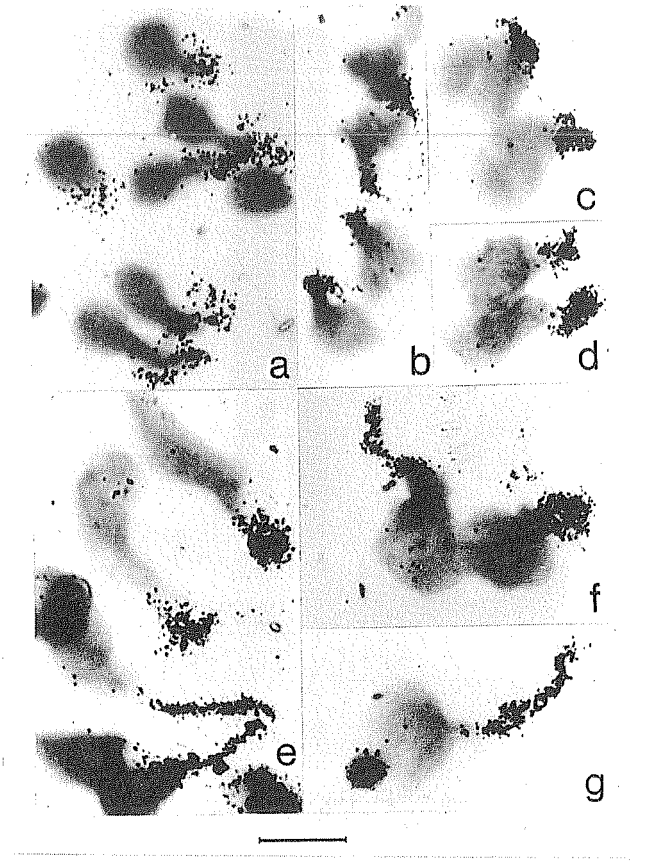

Figure 8. a–g, Various developmental stages of *B. fasciatus* spermatids, showing *in situ* hybridization with major satellite cRNA. a and e–g, Spermatids assumed to represent later stages of development, showing hybridization outside the nuclear protuberances. Exposure was for three weeks. Bar = 10 µm.

3.9 *Extreme polarization of major satellite DNA during oogenesis*

A similar remarkable behaviour of major satellite DNA is observed in the female, but the cells involved here are not oocytes but follicular epithelial cells. *In situ* hybridization with major satellite DNA (cRNA) shows hybridization outside of the nuclei of the follicular epithelial cells, again suggesting that these sequences may be eliminated from the nucleus (figure 10). These cells generally surround and remain in close association with the oocyte.

4. Discussion

The main finding described in this paper is the extreme polarization of the heterochromatin rich in microautosomal satellite DNA in male germ cells during spermiogenesis and in follicular epithelial cells during oogenesis. We have also

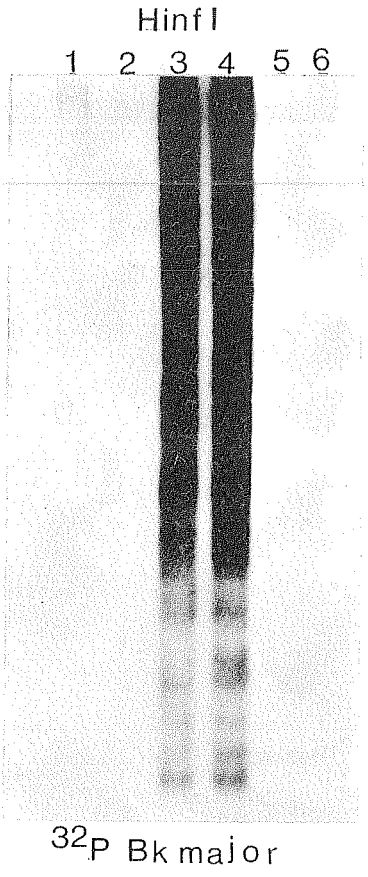

Figure 9. Autoradiograph showing Southern blot hybridization of genomic DNA of various species of snake with ^{32}P-labelled uncloned banded krait major satellite DNA probe. 1, *P. mucosus* (rat snake) female blood DNA; 2, *P. mucosus* male blood DNA; 3, *B. fasciatus* (banded krait) female blood DNA; 4, *B. fasciatus* male sperm DNA; 5, *B. caeruleus* (common Indian krait) male blood DNA; 6, *N. naja naja* (cobra) male blood DNA. Five µg of DNA digested to completion with *Hin*fI was loaded in each slot.

described an apparently sex-specific polymorphism in the distribution pattern of the satellite DNA, and its possible elimination from follicular epithelial cells of the ovary.

Chromosome diminution is known in other species, for example in ascarids (Boveri 1887; Bonnevie 1902) and in many copepods (Beermann 1959, 1966, 1977; Akifjev 1974), in which heterochromatic chromosomal segments are eliminated early in embryogenesis irrespective of their location in the chromosomes (interstitial as well as terminal) from the presumptive soma, but not from the germ cells.

4.1 *Possible significance of satellite DNA polarization in male germ cells during spermiogenesis*

Investigations in newts and salamanders and in chicken have revealed that a distinct heterochromatic chromosome segment is localized precisely at the point of subsequent

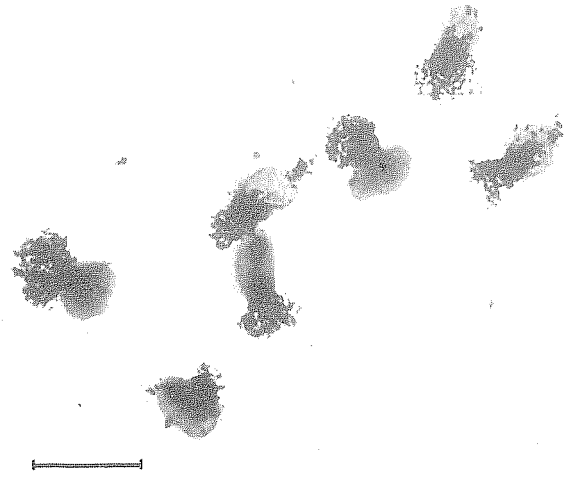

Figure 10. Follicular epithelial cells (granulosa cells) from *B. fasciatus* ovary, showing hybridization with major satellite cRNA. Note the extreme polarization of major satellite DNA suggesting its elimination from the nuclei. Exposure was for three weeks. Bar = 10 μm.

protrusion of the acrosomal pole of the round spermatid nuclei (Schmid and Krone 1975, 1976; Dressler and Schmid 1976). This heterochromatic block is called the acrosomal chromocentre. It remains in close contact with the developing sperm tip throughout spermiogenesis. It has been suggested that the acrosomal chromocentre contains genetic information necessary for development and organization of the sperm tip, including evagination of the nuclear membrane and elongation of the spermatid nucleus. Mutations affecting the acrosomal chromocentre cause abnormal spermiogenesis, suggesting that it has a morphogenetic function during spermiogenesis. It has also been postulated that genes for synthesis of acrosomal enzymes are localized in this chromosomal region, and the presence of constitutive heterochromatin in this region perhaps has a regulatory effect on the genetically important euchromatic segments of the acrosomal thread. Association of the heterochromatin rich in satellite DNA with the region of elongation of the nuclear protuberance of the spermatid nucleus in *B. fasciatus* shows that perhaps all of the microchromosomes are polarized into this region. Polarization of the microchromosomes is also observed during leptotene, zygotene and pachytene stages of spermatogenesis. Whether specific arrangement of the microchromosomes in the nuclei of spermatids helps in sperm morphogenesis is not clear.

4.2 *Significance of extreme polarization of satellite DNA in follicular epithelial cells during oogenesis*

The extreme polarization of microchromosomal satellite DNA in follicular epithelial cells of *B. fasciatus* oocytes gives appearance of its elimination, although we have as yet no definitive evidence in this regard. This phenomenon appears to be connected with oogenesis and may be more akin to nurse-cell activity. The fact

that ribosomal genes (rDNA) are localized in the heterochromatic regions of some of the microchromosomes of this species (unpublished data) and that there is no significant amplification of rDNA in the ovary (Singh *et al.* 1979) suggest that microchromosomes in snakes have an oogenic function, perhaps connected with supplying extra ribosomal templates (and other, possibly sarc genes and yolk protein, templates?) to the developing egg. This interpretation is supported by the fact that follicular epithelial cells in the diamond-backed water snake (*Natrix rhombifera*) become enlarged in size and inject most of their cellular contents into the cytoplasm of the growing oocyte (Betz 1963). This activity is partly analogous to the rDNA amplification that occurs in amphibians, which also produce yolky and relatively large eggs. Kanatani (1972) has demonstrated that the nucleic acid base 1-methyladenine triggers breakdown of the germinal vesicle and the subsequent process of meiotic maturation in starfish. In this light, if autosomal satellite DNA from follicular epithelial cells in snakes is eliminated, breakdown of it perhaps provides metabolic precursors of nucleic acids to developing oocytes, and these precursors, under the influence of pituitary gonadotrophins, bring about oocyte meiotic maturation and ovulation.

Cytological and histological studies have documented cell-type and tissue-type-specific changes in nuclear architecture; however, the functional significance of why nuclear morphology often varies radically and reproducibly with cell type is largely unexplained. It is possible that nuclei may undergo organizational changes that reduce accessibility of specific gene sets to factors that stimulate transcription. The precedence for this is the nucleolus in man, in which ribosomal RNA (rRNA) genes from five different chromosome pairs are brought together during interphase for rRNA transcription and subunit assembly. Polarization of microchromosomes during spermatogenesis and oogenesis in banded krait, as observed in the present study, may similarly serve the purpose of coordinated expression of the genes present in microchromosomes.

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