Chromatin ultrastructure of lower vertebrates[1]

Maria Luiza Beçak[2], K. Fukuda and Sylvia Mendes Carneiro

Serviço de Genética, Instituto Butantan, São Paulo (Brazil), 15 November 1976

Summary. Meiotic and mitotic chromosomes from amphibians and snakes were studied by electron microscopy. By using water spreading, preceded by a mild NaCl pretreatment, we showed: 1. 'Beads on a string' arrangement of the chromatin fibres; 2. The presence of loops at pachytene chromomeres as well as during metaphase of both mitosis and first meiosis; 3. Transcriptional activity for non-ribosomal RNA on peripheral loops during the middle pachytene.

The 'beads on a string' model for the eukaryote chromatin fibre is supported by several findings. Its most direct evidence, derived from electron microscopy studies, show that chromatin is organized as globular repeating units arranged along the fibre[3–5]. The repeats, designated 'nucleosomes', were described in the interphase chromatin of rat thymus, rat liver chicken and amphibian erythrocytes. The different values reported for the diameter of globules (70 Å, 100–300 Å, 128 Å) are probably due to differences in methods. The filaments connecting the nucleosomes are DNase-sensitive and are about 15 Å wide. Recently, nucleosomes were also reported in metaphase chromosomes[6] of L-929 cells from the American Type Tissue Collection. The conclusions obtained by nuclease chromatin digestion agree with those from electron microscopy. According to Hewish and Burgoyne[7], short segments of DNA, about 200 base pairs long, are inaccessible to nuclease digestion. It was suggested that these regions of DNA are associated with histones, in the beads. The 'beads on a string' model is also supported by X-ray diffraction and chemical analysis of histone interactions in solution[8]. Histone H3 associates with H4 and H2A associates with H2B. These findings led Kornberg[9] to postulate that the chromatin fibre is organized as a repeating unit of 2 each of the 4 main types of histones and about 200 base pairs.

On the other hand, most of the studies on the arrangement of the chromatin fibre into the architecture of the chromosome focalize on aspects of the chromomere and the interchromomere. Chromomeres are morphological units seen along the chromosome axes at prophase of meiosis and mitosis. Many chromomeres exhibit loops, especially at the oocyte diplotene stage of various animals. Loops are also present at meiotic prophase in mammalian and insect spermatocytes[10,11]. The classical loops of lamp-

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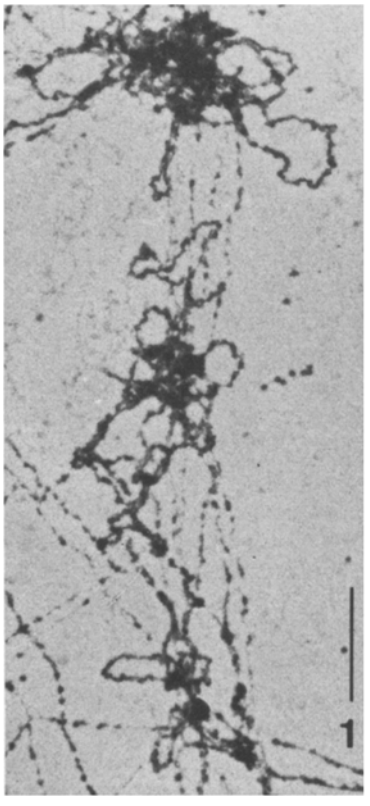

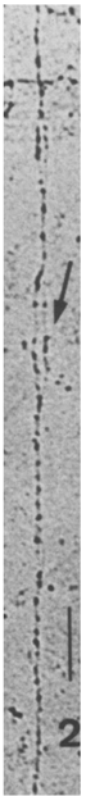

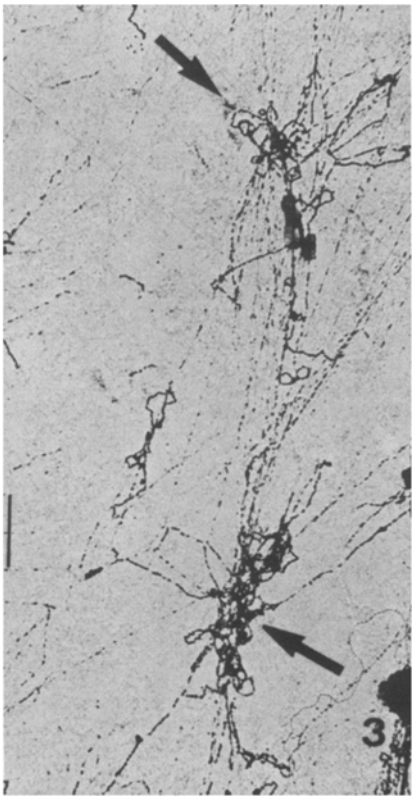

Fig. 1. Middle portion of a pachytene bivalent of the frog O. americanus, showing chromomeric loops attached to longitudinal fibrils; 0.6% NaCl treatment, water spreading, aqueous PTA stained; bar = 0.5 μm.

Fig. 2. Segment of a pachytene loop of O. americanus, showing double fibrils (arrow) and arrays of PTA stained globules; 0.6% NaCl treatment, water spreading, aqueous PTA stained; bar = 0.5 μm.

Fig. 3. Xenodon neuwiedii: snake pachytene showing microchromosome bivalent (arrows) with loops and banded chromatin fibres; 0.6% NaCl treatment, water spreading, aqueous PTA stained; bar = 1 μm.

brush chromosomes of amphibian oocytes have been shown to be involved in active RNA transcription[12]. Transcription of both ribosomal and non-ribosomal RNA were evidenced for pachytene loops of mouse and dipteran spermatocytes[10,13]. In this paper we describe the nucleosomes and the lampbrush pattern in snake microchromosomes; and transcriptional activity for non-ribosomal RNA in pachytene loops of spermatocytes. Pairing by either quadrivalents or bivalents has been previously shown in the 4 n species of Odontophrynus americanus[14]. Our findings on the 4 n are preliminary data of a comparative investigation of the loops in the 2 n and 4 n species.

Material and methods. Whole mount preparations were obtained from males of the snake Xenodon neuwiedii, 2 n=36 (Colubridae), and of the frog Odontophrynus americanus, 4 n=44 (Ceratophrydidae). The seminiferous tubules were placed in 0.6% or 0.7% NaCl solution for 5–35 min, and squashed on a slide. The cell suspension obtained was spread over distilled water (pH 7.0) in a plastic tray with Teflon bars and collected on Parlodium (1.5%) covered grids. The specimen was stained in aqueous PTA (1%, 5 min at room temperature), or aqueous uranyl acetate (1%, 10 min) then washed in distilled water and air-dried. The electron micrographs were obtained in a Siemens Elmiskop I, 60 KV, at low magnifications. Fixative and critical point drying were not employed. The following enzymatic treatment were used before staining: 1. DNase (Worthington), electrophoretically purified deoxyribonuclease (DPFF) RNase free, 50 µg/ml acetate buffer with 1 mM $MgCl_2$, pH 7.0, for 30–60 sec at 37 °C; 2. RNase A, protease free, type XII-A, Sigma Chem. Company, 100 µg/ml in distilled water, heated to 80 °C for 10 min. The grids were placed in a drop of this enzyme at 37 °C for 30–60 sec; 3. Trypsin-BDH Chemicals Ltd. Pools, England, 0.01% in phosphate buffer, pH 6.8, at 37 °C, 30–60 sec.

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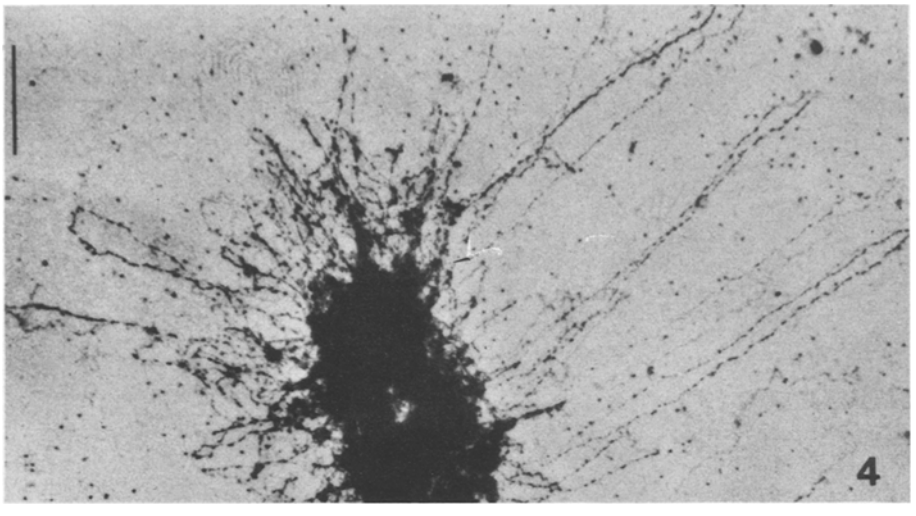

Fig. 4. O. americanus (4 n) mitotic metaphase chromosome showing loops and PTA banded chromatin; 0.6% NaCl treatment, water spreading, aqueous PTA stained; bar = 1 µm.

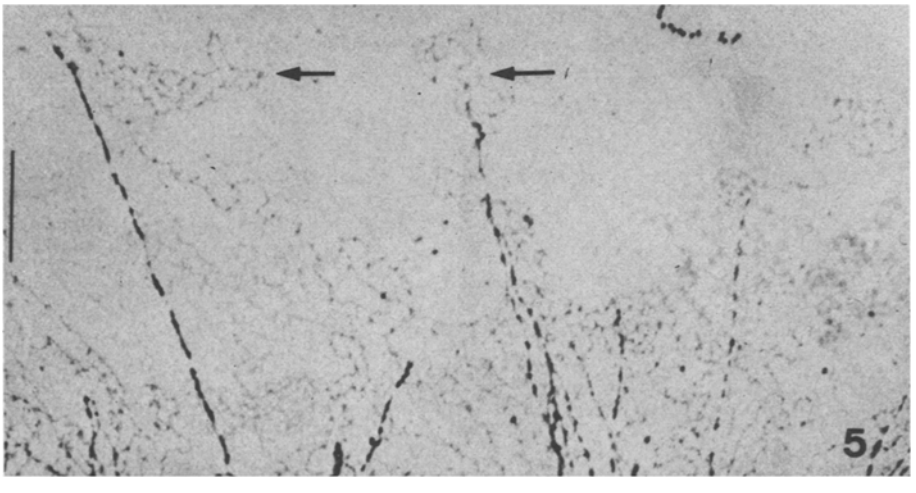

Fig. 5. O. americanus (4 n) pachytene: unstained peripheral loops (arrows); 0.6% NaCl treatment, water spreading, aqueous PTA stained; bar = 0.5 µm.

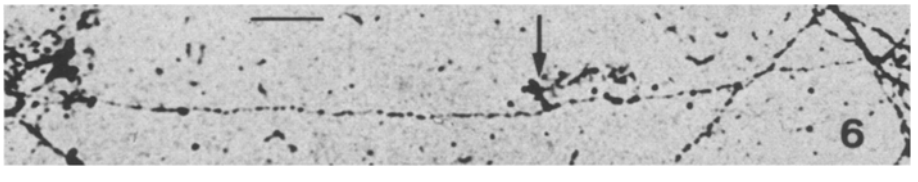

Fig. 6. O. americanus (4 n) pachytene: chromatin loop showing the 'beads on a string' pattern and a lateral RNP fibril (arrow); 0.7% NaCl treatment, water spreading, aqueous PTA stained bar = 0.5 µm.

Results and discussion. During the early and middle pachytene of our amphibian, the bivalents exhibit distinct chromomeres with protruding loops. These loops are attached to axial fibres which run along the chromosome length (figure 1). The axial fibres presented globules about 80 Å in diameter. The globules' diameter in the fibres of the loops was greater, about 160 Å. Sometimes, these loop fibrils showed a double structure (figure 2). From the point of view of chromosome ultrastructure, snakes provide an important advantage: the small size of the microchromosomes, with few chromomeres, facilitates the morphological analysis (figure 3). In the snake bivalents, the globules also measured 80 Å and 160 Å, in the axial and loop fibres, respectively.

Metaphase chromosomes, somatic as well as of first meiotic division, also show loops with 170 Å globules (figure 4). The enzymatic treatments showed that the globules of the chromatin fibre were resistant to trypsin and DNase I, but the interglobule fibres were removed by DNase I. Some pachytene peripheral loops showed unfolded segments. These segments were thinner, tortuous and unstained, except for discrete globules about 80 Å, widely spaced (figure 5). During the middle pachytene, bushes of lateral fibrils attached to the loops were observed. These fibrils were sensitive to trypsin and RNase treatments (figure 6).

Our assumption that the PTA stained globules (80 Å) connected by thin fibres are similar to the 'nucleosomes' arrangement is supported by the morphology and the enzymatic results. The bigger diameter (160 Å) found in pachytene loops, as well as the double segments seen in some loops, may be explained by Henderson's model[15]. According to this investigator, during pachytene the arrangement of the double loops of each replicated homologue is one-sided. Later in diplotene, rotation occurs, displaying the single loops symmetrically at both sides. Therefore, replication during interphase would explain the double loops of pachytene. One question remains about the increased diameter (170 Å) found for single loops of mitotic and first meiotic metaphase. In these perhaps condensation by assembly of globules could play a role.

Regarding chromosome structure, the chromomeric loops we found in amphibian and snake spermatocytes are similar to the ones described for mammalian spermatocytes[11]. We could not elucidate how the loops protrude from the insertion point of the longitudinal fibril. The lateral fibrils attached to pachytene loops were presumed to be precursors of non-ribosomal RNA, as already known in other systems[10,12]. However, this eventual transcriptional activity of the loops needs further investigation. Concerning the degree of chromatin dispersion obtained by longer mild NaCl treatment, we believe it may be due to histone H1 extraction[3]. Perhaps this could explain the 15 Å of the connecting filaments in our preparations.

Interactions among beans in neighboring Faraday cages[1]

F. A. Brown, Jr, and Carol S. Chow

Department of Biological Sciences, Northwestern University, Evanston (Illinois 60201, USA), 28 March 1977

Summary. Bean seeds, during their initial 4 h of absorption of water while in a Faraday cage, are able to interact mutually with similar absorbing beans in nearby Faraday cages. The interaction effects complementarity of response between adjacent cages to a common, fluctuating environmental factor affecting water uptake.

Day to day termite food intake[2,3] and bean-seed water uptake[4,5] reflect variation in unknown subtle atmospheric factors. The bean-seeds can adopt either of 2 complementary states, displaying either +- or --correlations with it. Placed in water in separate vessels in close proximity 2 groups of seeds can mutually induce the 2 complementary states, their concurrent rates of water uptake correlating negatively with one another. Such interaction can have great potential significance for many facets of biology. The present experiment was designed to determine whether the fields involved in these actions can pervade 0.41 mm copper Faraday cages.

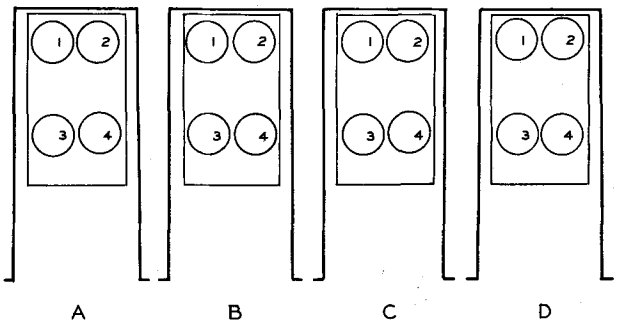

Fig. 1. The arrangement of 4 Faraday cages (*A–D*), and contained vessels (1–4) each with 20 bean-seeds in water.

4 cylindrical Faraday cages were lined up in a row with 35 cm between centers (figure 1). 16 20-bean (Phaseolus vulgaris) samples were weighed to the nearest centigram in flat (6×6 cm) aluminium-screen baskets[4]. 4 were submerged in vessels and placed in each of the successive cages at 4-min-intervals. Aluminium covers were clamped to the openings. After exactly 4 h the beans were rapidly blotted and wet-weighed, similarly at 4-min-intervals, and discarded. Water uptake, the weight difference between final wet weight and dry weight + 15 cg (= wetting) was expressed as percent weight increase of the original dry beans. Although cages A and B were grounded and C and D were not, no difference between them was noted. This experiment was performed on 73 days between 25 February and 7 June 1974 repeated using a new stock supply of beans on 49 days during 10 June through 16 August and repeated again on 42 days during 29 August through 28 October 1974.

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