identical with that of DNA polymerase β, comparing the results shown in figures 1 and 2, it is suggested that the differential sensitivity to EtBr in replicative DNA synthesis and UDS was due mainly to the difference in the DNA polymerases involved and the differential sensitivity to EtBr of DNA polymerases α and β. The difference in the IC_{50} values between DNA synthesis in permeable cells and DNA synthesis in the DNA polymerase-activated DNA system may be due to the difference in the physical properties between chromatin DNA and activated DNA[20].

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Chromosome stabilizing structures in mitotic Indian muntjac *(Muntiacus muntjak)* cells[1]

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Summary. A new technique which removes all membranes, cytoskeletal elements, organelles, but preserves intact metaphase, anaphase and telophase configurations is combined with scanning electron microscopy (SEM) as an approach for direct visualization of chromosomal behavior in late mitosis. With this approach we are able to confirm the presence of a centromeric ring which stabilizes the centromeres during the cell cycle and present evidence for a lattice-like sheet of interchromatidic fibers in late mitosis.

The radial arrangement of chromosomes on the mitotic spindle of plant and animal cells has led to the agreement that the telophase arrangement of chromosomes is maintained throughout interphase[2-6]. The radial arrangement of the metaphase chromosomes of the Indian muntjac and the low diploid number (7 in the male) of this species is ideal for investigation of the centromere clustering. By using a cell synchrony procedure, large numbers of metaphase, anaphase and telophase configurations were obtained for analysis without the use of spindle-disrupting drugs. The configurations, collected at various stages of mitosis were stripped of their membranes, organelles and cytoskeletal elements by a newly developed technique which permitted direct visualization of the intact mitotic structures. A large percentage of the mitotic configurations retain the radial array of chromosomes in spite of the treatment with hypotonic solution, fixation with methanol:acetic acid, spreading onto glass coverslides, hot acetic acid treatment and critical point drying. The radial arrangement of metaphase chromosomes is maintained because all chromosomes are associated at the centromere region, by a filamentous ring which interconnects the adjacent centromeres.

Also in this study it was revealed by 3-dimensional visualization of critically point-dried, organelle-free anaphase and telophase configurations that a lattice-like network of fibers bind the chromatids and their telomeres into a late mitotic configuration. It is with these data and that obtained from the the arrangement of chromatin in interphase cells[2,4-7] that we support the concept of centromere clustering throughout the muntjac cell cycle.

Materials and methods. Fibroblast-like cells of the Indian muntjac *(Muntiacus muntjak)* were obtained from the American Type Tissue Culture Collection. Cells were cultured in Ham's F-12 media supplemented with 20% fetal calf serum. Cells were synchronized using a double-thymidine block[8] and mitotic cells were removed by selective detachment.

Collected cells were hypotonically shocked with 0.075 M KCl for 6 min at 37 °C. Cells were recollected and fixed in 3:1/methanol:acetic acid for 24 h. After several washings in 3:1 fixative cells were concentrated in fixative. One drop of cell concentrate was dropped on a 22-mm diameter No. 2 coverglass and permitted to dry for 30 sec. The coverglass was then immersed in 50% acetic acid at 101 °C for 1–2 sec. Rapidly, so as to prevent drying, the coverglass was transferred to 50% ethanol at room temperature. The coverglasses were then transferred through graded alcohols to acetone for dehydration. The cells were critically point-dried in liquid-gaseous CO_2 and sputter-coated with gold palladium, about 50 Å thickness. An AMR-1000A scanning electron microscope was used for observation of the surface topography of the pre-nuclear structures and records of selective configuations were collected on Polaroid N/P 55 film.

Results. Cells harvested after mitotic selection yielded largely metaphase and anaphase with the remaining telophase and interphase cells. Metaphase were difficult to find because the ma-

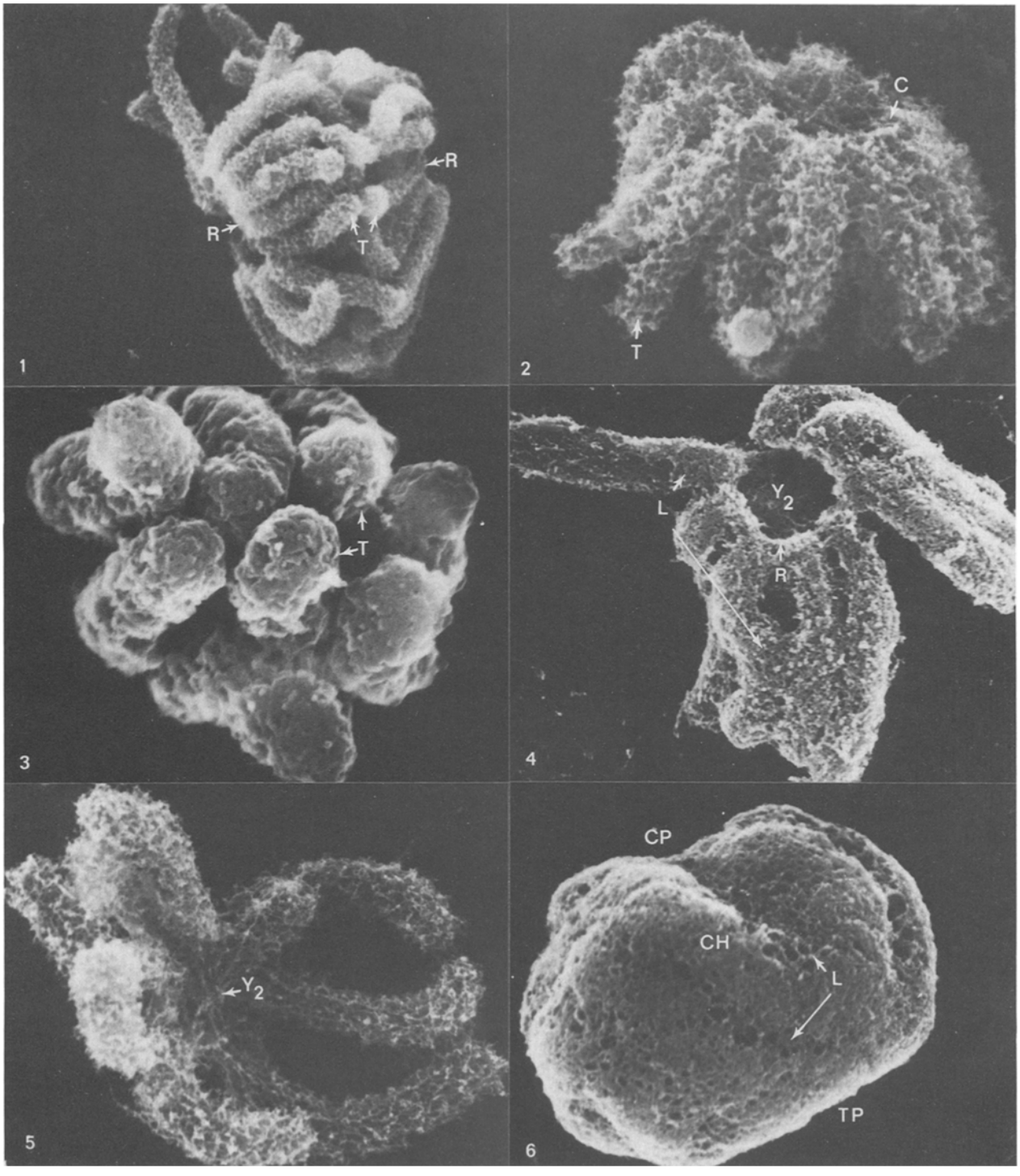

Figure 1. Lateral view of an Indian muntjac early anaphase with the centromeres and ring (R) leading the way in initial separation of the sister chromatids. The telomeres (T) of most of the sister chromatids retain their close association facilitating meridional orientation of the prenuclear configuration. Figure 2. Lateral view of a mid-anaphase configuration demonstrating meridionally arranged chromosomes. At this stage the chromosomes have developed incomplete polarity with the centromeres (C) clustered above and the telomeres (T) below. Figure 3. Polar view of the eight telomeres (T) which cluster to form the telomeric pole in late anaphase. Figure 4. Incomplete meridional arrangement of anaphase chromosomes resulting from preparation of material. This view from the centriole pole clearly demonstrates the circular centromeric ring (R) interconnecting the chromosomes in a clear circle. Note that the arms are flexed near the centromeres recessing the centromeric ring slightly at this pole. The fibrous material within the ring is the Y_2 chromosome with fibrous attachments suspending it in this position. The partially assembled lattice (L) of interwoven fibers can be seen partially covering one of the chromosomes while the remaining are completely covered and interconnected. Figure 5. An inside view of an early anaphase demonstrating the fibrous network that is suspending the Y_2 chromosome deep to the centromeric ring. Figure 6. A late telophase configuration in which chromosome detail is obscured by the interwoven lattice (L) of fibers covering and interconnecting the chromosomes. This lateral view exhibits meridional arrangement of the chromosomes (CH) with the centromere pole (CP) retaining a slight indentation and the amorphous telomeric pole (TP). The larger openings in the lattice between adjacent chromosomes are possibly a stretching phenomenon resulting from hypotonic swelling in preparation.

jority would progress to anaphase in the time required for cellection, and hypotonic shock. The metaphase configuration demonstrated the radial arrangement of chromosomes with the centromeres clustered in a clear circle at the center. The number 1 pair and the X chromosome are flexed at the centromere at an angle smaller than 90° so that the long and short arms are adjacent to each other, while pair number 2 and the Y, are unflexed. The Y_2 chromosome is located in the center of the ring which is formed by fibers which interconnect the centromeres.

As anaphase begins, initial separation of the chromatids is at the ring (centromeres) with the telomeres of sister chromatids remaining attached (fig. 1). The centromeric ring is not the leading structure in mid-anaphase because the chromosomes flex near the centromere, slightly recessing the ring (fig. 2). The Y_2 chromosome is recessed even more in its central location deep to the ring (figs 4 and 5). At the telomeric pole (fig. 3) of the late anaphase and early telophase eight telomeres cluster to close the cylindrical-shaped configuration into a hollow structure. The eight telomeres present at this pole are those of the arms of number 1 (4 telomeres), number 2 (2 telomeres), Y_1 (1 telomere) and X (1 telomere).

From early anaphase the surface appearance of the chromosome is changing from an individual fibrillar appearance to a coarse-interwoven lattice of fibers which first appears near the centromere and progressively interconnects the adjacent chromosomes to their telomeres. There is some hesitation in referring to this lattice of interconnecting fibers as interchromosomal fibers, since it not only interconnects the chromosomes but also covers them (fig. 4). The origin of this lattice is unknown, but it closely resembles that outer fibrous network that completely surrounds the late telophase and interphase nucleus, presumably the dense lamina.

Discussions. These results suggest that the metaphase interchromosomal connections at the centromere (centromeric ring) preserve, in the dividing Indian muntjac fibroblasts, the chromosomal position from metaphase through telophase. This centromeric clustering is retained throughout the G_1, S, G_2 and prophase as demonstrated by several investigators[2,4-7,9]. Our 3-dimensional analysis of intact mitotic configuations with the centromeric ring and a lattice of interwoven fibers, covering and interconnecting the chromosomes from ring to telomere, provides evidence for structures which prevent chromosomal rearrangement in the cell cycle. We strongly feel that it is not the role of the nucleolus which holds parts of the chromosomes together in the cell cycle as proposed by Hsu et al.[7] because in Indian muntjac cells the nucleolus organizer regions (NORs) are located near the telomere on No. 1 and on the long arms of X and Y with the meridional orientation of the chromosomes in anaphase and telophase the NORs would be located at points apart from each other preventing strong structural interconnections. Therefore, a clustering of the NORs with nucleolar interconnections seems unlikely as a force factor in interphase chromosome alignment.

Lewis and Laemmli[12] and Hancock and Hughes[13] proposed that interphase chromatin is kept in an ordered state by association with the nuclear lamina. Gerace and Blobel[14] found that changes occur in the distribution of lamin proteins during cell division in populations of CHO cells when examined by immunofluorescence staining. Gerace and Blobel[14] postulated a disassembly of the lamins in prophase because the proteins were dispersed throughout the cytoplasm and reassembled into lamins in late anaphase and telophase on the surface of the daughter chromosomes.

In our metaphase configurations there are no interwoven fibers on the surface of, or between, adjacent chromosomes except at the centromeric ring. In our earliest anaphase preparations the assembly of the interwoven fiber complex begins near the centromere and by late anaphase or early telophase has progressed to the telomere. By late telophase all chromosomal resemblance has disappeared and the surface of the reforming nucleus is a syncytium of this dense lamina-like network of fibers (fig. 6).

The authenticity of the network of interconnecting fibers between adjacent chromatids is questionable because of the fixation process and slide preparation procedure. We admit that the brief hot acetic acid rinse appears devistating and could produce artifacts, but it is our interpretation, based on comparative preparative studies using the neutral pH isolation procedure of Wray and Stubblefield[15] and the non-ionic detergent isolation procedure of Paulson[16], that this interwoven network of fibers is not fabricated during preparation. Preparation of material with the previously mentioned isolation procedures produces mitotic configuration with the same basic shapes and with relative amounts of fibers interwoven between adjacent chromatids at metaphase, anaphase and telophase. The acid procedure was selected over the other 2 procedures because the mitotic configurations, retained excellent detail and were completely clean of cytoplasmic structures and debris, while the other two methods retained spindle and cytoplasmic structures and nuclear membrane fragments in later mitotic configurations. These residual structures partially obscured visualization of chromatid activity in late mitosis, but these procedures were useful in that they confirmed that the chromatid alignment and fiber network was the same when mitotic configuration were prepared by any of these methods.

The polarized organization of chromosomes throughout the cell cycle was described as early as 1885 by Rabl[10]. However, in recent years diverse approaches with new techniques and concepts have provided conflicting results on chromosomal organization in this field (for reviews, see Avivi and Feldman[2] and Comings[11]. As an example, this polarized orientation appears to be the case for cells with a low chromosome number such as muntjac and CHO (personal observation) cells, but not the case for cells with a high chromosome number such as HeLa (Welter and Hodge, in preparation). The conclusions derived by Sperling and Ludtke[9] that muntjac chromosome orientation and positions seem to be representative for mammals in general is only partially correct. It is conceivable that the centromeric ring and the interwoven lattice of fibers observed developing in anaphase, may be structural entities which stabilize chromosomal relationships throughout the entire cell cycle.

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