

THE CHROMATOID BODY IN MALE RAT GERM CELLS: ULTRASTRUCTURE, MORPHOGENESIS AND INTRACELLULAR MOTION

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Chromatoid body (CB) is an essential organelle of mammalian spermatocytes and spermatids. Although it is discovered about 100 years ago and recently studied rather intensively by means of the electron microscope, there are still problems related to the ultrastructure, morphogenesis and CB intracellular transport which are not completely clarified yet. It is not precisely established which intracellular structures are involved in CB motion, its formation (by which structures and how), its relation with other organelles, etc.

The object of the present study presents certain unclear aspects of the ultrastructure, morphogenesis and CB intracellular motion in germinative cells (spermatocytes and spermatids) of rat testis.

Material and methods

The study covers white male Wistar rats of different age (30, 40, 50, and 60 days old). Material for electron microscopic examination is taken immediately after killing (dekapitating) the animals. A routine fixation technique is applied (3 per cent glutaraldehyde, 1 per cent osmium tetroxide diluted in 0.1 M phosphate buffer, pH 7.4 at 4 °C; ethanols, propylene oxide, embedding in durcupan ACM). Ultrathin sections are done and then contrasted with uranyl acetate and lead citrate. Observations are performed under the electron microscope JEM 7A.

Results and discussion

C B u l t r a s t r u c t u r e

CB in the cells studied shows ultrastructural features described by other investigators (4—7, 9). It is composed by 2 main components: a) a membrane-free large aggregate of electron dense material, and b) numerous associated vesicles (fig. 1). Besides CB with characteristic structure and size there are also CB with smaller in size dense substance (fig. 1, 2) with fragmentations (fig. 3). We call these CB miniature (immature, still forming). Therefore, we suppose the possibility for them to aggregate their «fragments» and/or to co-migrate towards each other and then to fuse into larger, mature CB that is illustrated for instance on fig. 2.

Present results add the following new ultrastructural features to the known CB morphology (4—7, 9): microtubules (MT) are associated with CB (fig. 1); fine-filamentous structures irradiating from the surface of the dense substance of CB (fig. 2); cisternae of the smooth endoplasmic reticulum associated with CB (fig. 1).

CB morphogenesis

Some of the results presented confirm the statement that dense substance can originate from chromatin material (5, 9) entering the cytoplasm from the nucleus, most probably passing through nuclear pores (fig. 2). CB accompanying vesicles originate probably from the outer nuclear membrane resulting from its evagination and following break away in the form of vesicles (fig. 2). We do not exclude the possibility for some vesicles to possess a Golgi origin (1). We presume that CB associated cisterns of smooth endoplasmic reticulum originate (a) from the fusion of vesicles and/or (b) rough endoplasmic reticulum (after dropping off of its ribosomes). We further on assume that this endoplasmic reticulum can control local Ca^{2+} concentration — within and around CB — as it does in other

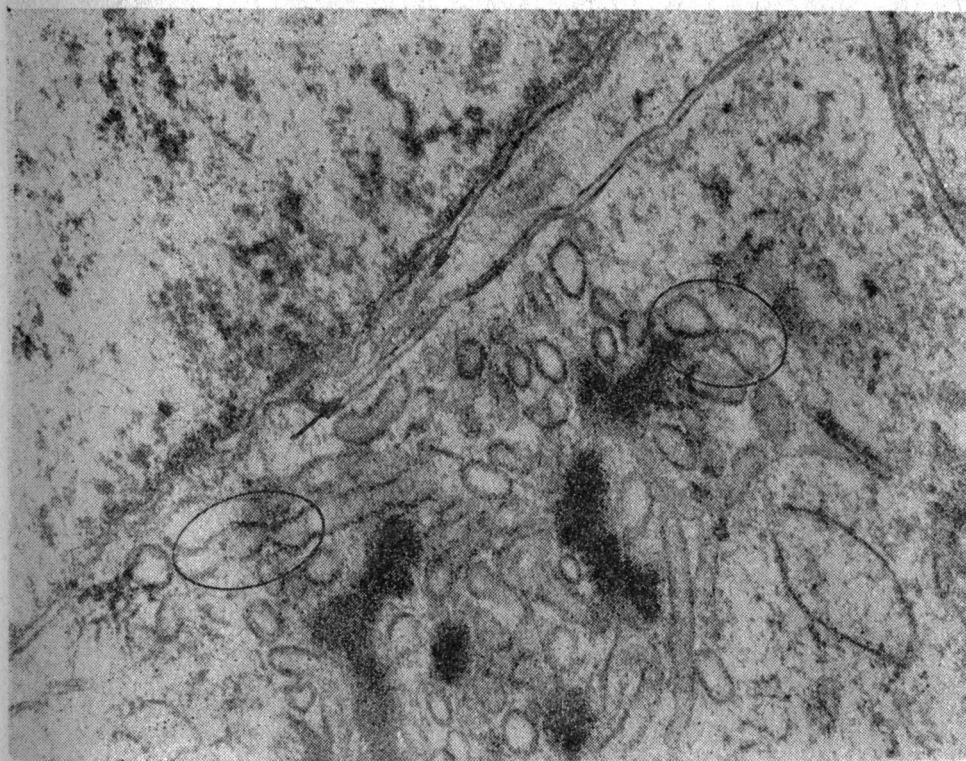


Fig. 1. Spermatid. CB as electron dense material surrounded by numerous vesicles and cisterns. In a circle vesicles are indicated more probable at fusion stage than at separation one. Microtubules indicated by arrows are located near CB. Magn. x 20000.

cells, too (8, 10). On the other hand, that can participate in the process of «assembly-disassembly» of CB-associated MT. It is known that this process is very sensitive to Ca^{2+} concentration changes (10).

Intracellular CB motion

We suppose the following possibility for CB translocation — from the perinuclear area to the caudal pole of the spermatid. We consider CB-associated MT, filamentous network (irradiating from the surface of the dense substance) and smooth membranes (vesicles and cisternes) structures directly engaged in CB motion (a CB motor apparatus?) (3). After reaching the caudal pole of the spermatid, CB disintegrates (fig. 4). We assume that lysosomal structures located near to CB (1), can take part in this process.

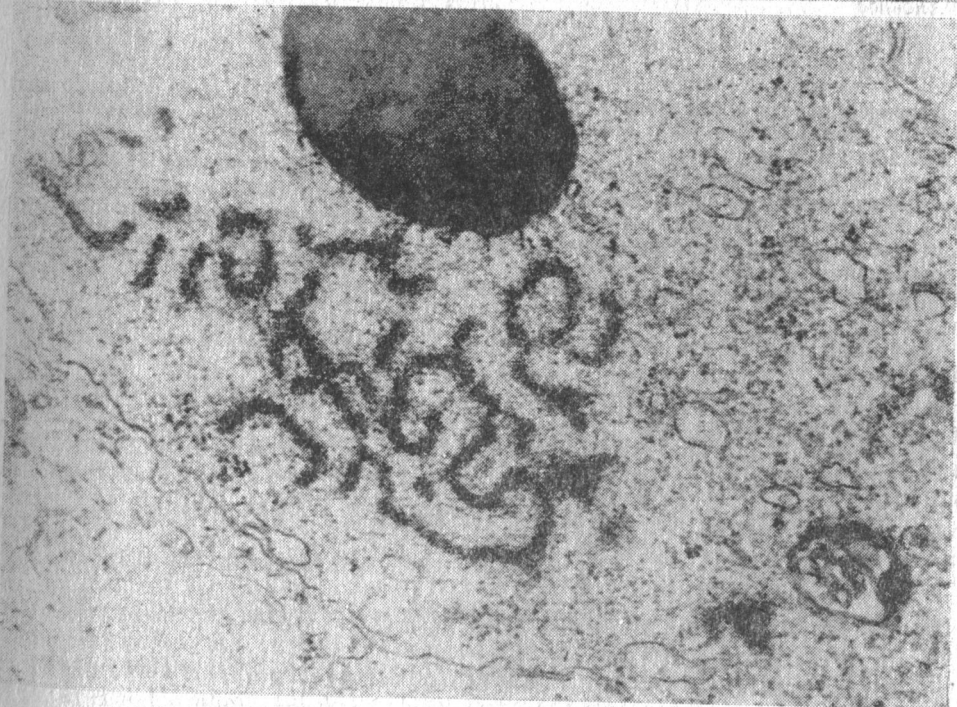
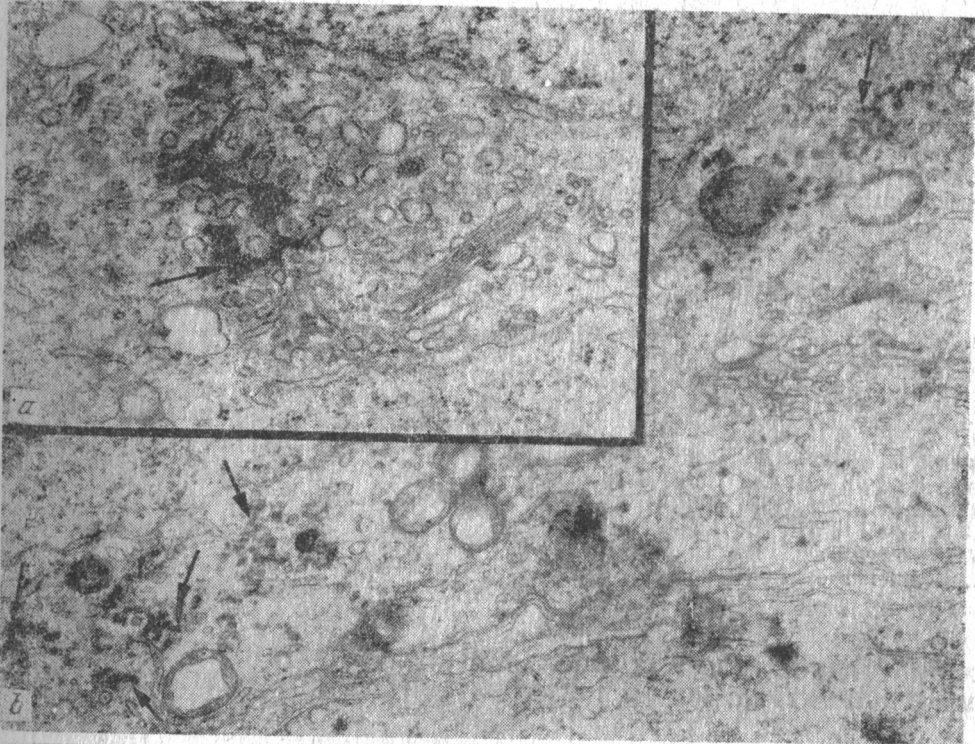
In short, our presumption of principle concerning CB migration processes takes in consideration the possible role of cytoskeletal and cytomatrix elements which are associated with it and described in the present work. It is known that



Fig. 2. Spermatid. Aggregation (enlarged) of dense material of a mature CB. Arrows indicate fine filamentous structures (cytomatrix?) emerging from the surface of the dense material. Double arrow indicates a vesicular evagination of the outer nuclear membrane. In the circle there is an electron-dense material probable «passage» of nuclear substance into the cytoplasm. Magn. x40000.

Fig. 3. Spermatocyte. Small CBs (miniature, immature, forming) are indicated by arrows. Magn. (a) x17000. Magn. (b) x11000.

Fig. 4. Spermatid. Electron-dense material, spiral-like, band-like, located in the caudal region of the cell (disintegrated, involuting CB form?). There are no vesicles and cisternes near the dense material. They are, however, characteristic of perinuclear CB.



MT participate in different intracellular processes related with cell organelles translocation (2). We suppose that these filamentous «evaginations» irradiating from the surface of the dense substance and sometimes configurated as a lattice (fig. 2) can be a structural analogy to the microtrabecular lattice (11). If it is so, the role of CB-associated smooth membranes (endoplasmic reticulum) as Ca^{2+} -concentration regulators can operate both assembly and disassembly of MT and microtrabecular lattice (8), and, intracellular CB motion, respectively.

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ХРОМАТОИДНОЕ ТЕЛО В ГЕРМИНАТИВНЫХ КЛЕТКАХ ЯИЧКА У КРЫСЫ: УЛЬТРАСТРУКТУРА, МОРФОГЕНЕЗ И ВНУТРИКЛЕТОЧНОЕ ДВИЖЕНИЕ

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РЕЗЮМЕ

В настоящей работе приводятся результаты исследований новых ультраструктурных характеристик хроматоидного тела: ассоциированные с хроматоидным телом микротубулы, тонкие филаментозные структуры, irradiирующие от поверхности плотной материи хроматоидного тела, ассоциированные с хроматоидным телом цистерны гладкого эндоплазматического ретикулума. Делается предположение, что эти структуры имеют непосредственное отношение к внутриклеточному движению хроматоидного тела (двигательный аппарат хроматоидного тела?). Гладкие мембраны могут контролировать локальную Ca^{2+} концентрацию в хроматоидном теле и около него и, таким образом, регулировать ассамблирование — дезассамблирование цитоскелетных структур, ассоциирующих с хроматоидным телом.