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Chromatoid body and haploid gene activity: Actinomycin D induced morphological alterations

LEENA-MAIJA PARVINEN, PENTTI T. JOKELAINEN and MARTTI PARVINEN

Institute of Biomedicine, Department of Anatomy, University of Turku, Finland
Department of Anatomy, University of Michigan, Ann Arbor, Mich., U.S.A.

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New evidence about the dependence of the chromatoid body on the haploid gene activity during early spermiogenesis has been obtained in rats by morphological studies after intratesticular injection of Actinomycin D. Normally, this organelle shows an irregularly lobulated structure of dense filamentous material but following AMD administration, it appears as a ring in the electron microscopic sections, in the phase contrast microscopic and light microscopic preparations of living and sectioned material. The alterations appeared 12 h after the injection, which corresponds to the time required for the incorporation of the radioactivity to this organelle after a pulse with tritiated uridine.

Martti Parvinen, Institute of Biomedicine, Department of Anatomy, University of Turku, SF-20520 Turku 52, Finland

The chromatoid body is a reticulum of electron dense fibrils located in the cytoplasm of spermatogenic cells. Although a number of histological (BENDA 1891; NIESSING 1897; SUD 1961a), cytochemical (DAOUST and CLERMONT 1955; SUD 1961b; EDDY 1970) and ultrastructural studies (COMINGS and OKADA 1972; FAWCETT et al. 1970; SUSI and CLERMONT 1970) have been performed on the chromatoid body since its discovery (VON BRUNN 1876) controversy still surrounds the origin, composition, function and ultimate fate of this organelle.

The chromatoid body is thought to arise as a multiloculated, irregularly shaped mass in association with mitochondria in the cytoplasm of the spermatocytes during the pachytene stage of the meiotic prophase (FAWCETT et al. 1970; SUSI and CLERMONT 1970; COMINGS and OKADA 1972) and apparently remains as a distinct cytoplasmic entity throughout the two meiotic divisions (JOKELAINEN et al. 1976). The chromatoid body is seen as the most prominent organelle during the early spermiogenesis (SUSI and CLERMONT 1970), where it is first seen to condensate shortly after the 2nd meiotic division (PARVINEN and JOKELAINEN 1974; JOKELAINEN et al. 1976) and thereafter to be located on the nuclear surface in association with an increased number of nuclear pores (FAWCETT et al. 1970). During this period, the chromatoid body shows rapid movements on the nuclear surface and transient contacts with intranuclear and cytoplasmic organelles (PARVINEN and JOKELAINEN 1974). Time-lapse cinemicrographic and subsequent electron microscopic analyses suggest a transport of material between the chromatoid body, the haploid nucleus and the Golgi complex, the latter organelle being active in building up the acrosomic system (SÖDERSTRÖM and PARVINEN 1976a).

The function and chemical composition of the chromatoid body has been a matter of debate until recent years. Although earlier histochemical studies at the light microscopic level suggested the precence of RNA in the chromatoid body (DAOUST and CLERMONT 1955; SUD 1961a and b), electron microscopic histochemical study has not supported the presence of RNA in this organelle (EDDY 1970). Recently, the chromatoid body of the round nucleated spermatids has been shown to incorporate radioactivity after incubation with

tritiated uridine (SÖDERSTRÖM and PARVINEN 1976b). The incorporation is slow, lasting at least 14 hours. This suggests a dependence of the chromatoid body on the haploid gene activity during early spermiogenesis in the young round-nucleated spermatids (MONESI 1965, 1971; KIERSZENBAUM and TRES 1975; SÖDERSTRÖM and PARVINEN 1976c).

In order to further characterize the dependence of the chromatoid body on the haploid gene activity, we have studied the effects of actinomycin D on this organelle in rats, using morphological methods. Actinomycin D specifically binds to loci in DNA molecules active in RNA synthesis and thus inhibits the formation of RNA (MÜLLER and CROTHERS 1968; SOBELL et al. 1971). The actinomycin D has been shown to bind specifically to spermatogenic cells in certain stages of development (BRACHET and HULIN 1969; DARZYNKIEWICZ et al. 1969) and to induce morphological changes, especially in the nucleoli of the primary spermatocytes (BARCELLONA and BRINKLEY 1973).

Material and methods

Young adult rats, derived from the Sprague-Dawley strain, were used. Actinomycin D (Lyovac Cosmegen, Merch Sharp & Dohme, Rahway, New Jersey, USA) was dissolved in Hanks' salt solution and injected intratesticulary in the approximate center of the testis in a dose of 20 μg in a volume of 0.1 ml of the medium. The testes of the control animals received 0.1 ml of the salt solution. A total of 10 animals with survival times of 6, 9, 12, 16 and 24 h were studied. After killing, the testes were removed and the proximal end of each was fixed in Bouin's fluid, embedded in paraffin, sectioned at 5 μm and stained with PAS-hematoxylin technique. The remaining part of the testis was dissected in a fluid having an ionic composition of rat rete testis fluid (TUCK et al. 1970) slightly buffered with phosphate buffer to pH 7.4. Gross damages of the seminiferous tubules were analyzed by a transillumination technique of freshly isolated tubules (PARVINEN and VANHA-PERTTULA 1972). Further analysis was made by phase contrast microscopy of freshly isolated living cells (PARVINEN 1973). Isolated seminiferous tubules containing the whole spermatogenic wave (PEREY et al. 1961) were prepared for light and electron microscopy by taking subsequent segments for phase contrast microscopy and fixation. For light microscopy, the fixed tubular segments were treated as mentioned above. For electron microscopy, the segments were fixed in 3 per cent glutaraldehyde buffered with cacodylate buffer at room temperature, pH 7.2. The samples were postfixed with osmium tetroxide, embedded in Epon and sectioned at 70 nm. The sections were stained with uranyl acetate and lead citrate. Observations were made with Siemens El-Miscope I A.

Results

The transillumination of freshly isolated unstained seminiferous tubules showed a dull segment covering the stages IX–X–XI (LEBLOND and CLERMONT 1952), and occasionally reaching also to stage VIII (Fig. 1) and XII, 12 h after the administration of the drug. The preleptotene and pachytene spermatocytes were specifically killed. Phase contrast microscopy revealed a ring-like appearance of the chromatoid body throughout the steps 1–6 of spermiogenesis (Fig. 2). The ring shape was first visible 12 h after drug administration and the phenomenon was more pronounced at 16 and 24 h. Occasionally, the hole in the middle of the chromatoid body was very large and the dense chromatoid material was seen only as a thin layer. The ring- or hollow sphere-like chromatoid body was also visible in paraffin sectioned preparations (Fig. 3, arrow).

The electron microscopy confirmed the light microscopic findings. At step 1 of spermiogenesis the chromatoid body of untreated cells typically has a multilobular appearance as seen in Fig. 6. Following AMD treatment, the lobulated structure disappears and the chromatoid material condenses at the periphery of the organelle as a relatively thin layer. The fine filamentous structure of the chromatoid material is similar as in normal conditions, and it has the same density. The normal relationships of the chromatoid body to the nucleus (Fig. 4) and to the vesicles and dark particles (Fig. 5) do not seem to be affected. Inside the ring-shaped chromatoid material small particles with equal size, shape and electron density with free ribosomes are seen, as well as occasional vesicles. The earliest signs of the ring-like structure in electron micrographs were seen at 9 h, and the alteration remained unchanged during the survival time of 24 h. No alterations were seen 6 h after AMD injection.

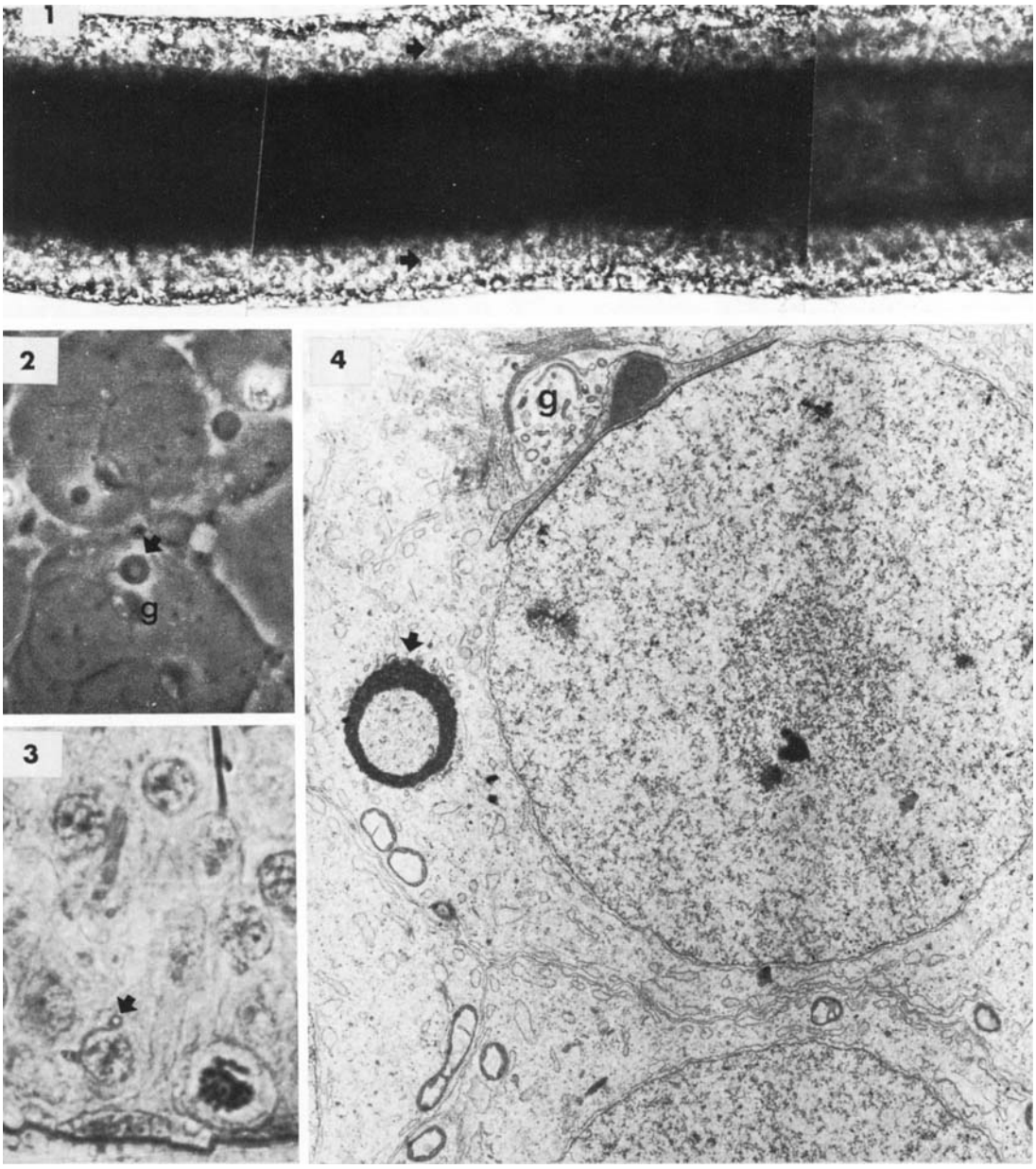
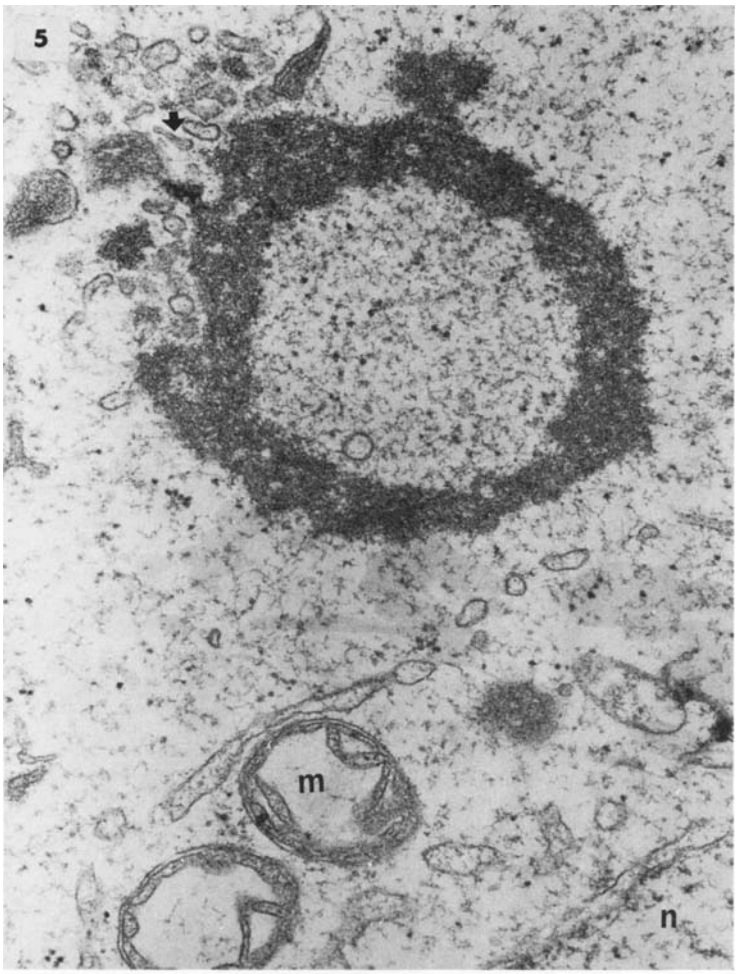


Fig. 1–4. – Fig. 1. Transillumination of a freshly isolated rat seminiferous tubulus 12 h after injection of actinomycin D. A dull segment at stage VIII of spermatogenesis (beginning at the arrows) indicates an injury of pachytene spermatocytes and permits a rapid screening of the effect of actinomycin D. × 140. – Fig. 2. Phase contrast photomicrograph of freshly isolated step 5 spermatids 16 h after actinomycin D administration. A less dense area is seen inside the chromatoid body (arrow). × 1500. – Fig. 3. PAS-hematoxylin stained section of stage V of spermatogenesis 16 h after actinomycin D injection. The ring-shaped chromatoid body is evident (arrow). × 1400. – Fig. 4. Electron micrograph of a step 5 spermatid 16 h after actinomycin D injection, from adjacent segment to the freshly isolated cells seen in Fig. 2. The chromatoid body appears as a relatively regular ring (arrow), the less dense area inside containing particles with the same size, shape and electron density as that of the free ribosomes. g: Golgi complex at the acrosome. × 16,000.



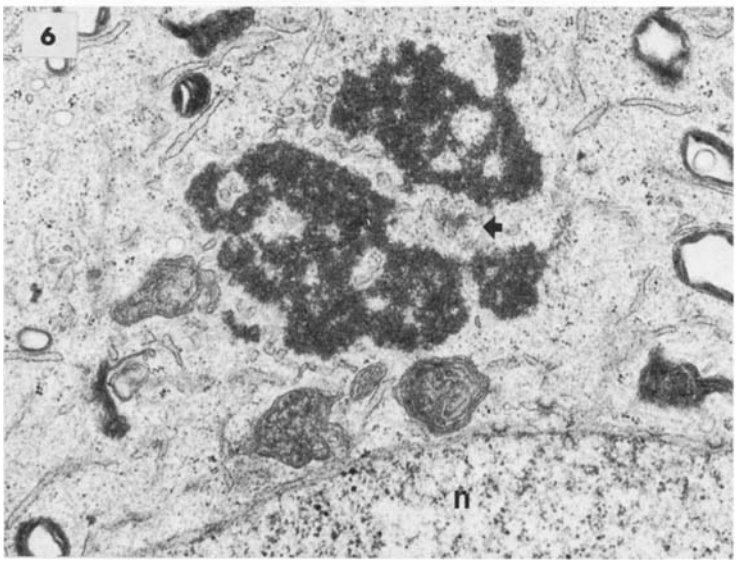


Fig. 5 and 6. – Fig. 5. The ultrastructure of a ring-like chromatoid body in a step 1 spermatid 16 h after actinomycin D injection. Note the normal association with the vesicles (arrow) and the unaffected finely fibrillar structure of the chromatoid material. m: mitochondrion, n: nucleus. × 71,200. – Fig. 6. The ultrastructure of a normal chromatoid body of a step 1 rat spermatid. Note the multilobular finely filamentous chromatoid material and its association with the vesicles (arrow). n: nucleus. × 67,000.

Discussion

The morphological alterations of the chromatoid body caused by the inhibition of RNA synthesis by actinomycin D give support to the theory of the dependence of this organelle on the postmeiotic RNA synthesis, and about the participation of this organelle in the RNA metabolism during the spermiogenesis in general.

It is very difficult to compare the behaviour of the chromatoid body to any other structure of the normal cells. The nucleolar origin of the chromatoid body has been suggested by COMINGS and OKADA (1972). Under certain conditions, associated with reduced RNA synthesis, the nucleolus has been demonstrated to obtain a ring shape, as in some highly differentiated cells (SMETANA et al. 1968, 1970). Ring-shaped nucleoli have also been obtained by treatment with 5-fluorouracil (ROSE et al. 1972) and actinomycin D (SMETANA and POTMĚŠIL 1968). In this respect, the morphological behaviour of the chromatoid body after administration of actinomycin D resembles that of the nucleolus. Furthermore, the similarity of the morphological reaction under the influence of actinomycin D both in the nucleolus and the chromatoid body suggests that the chromatoid body may contain DNA. The chromatoid body reaches the ring shape also physiologically when the RNA synthesis of the spermatids ceases. The chromatoid body moves to the caudal pole of the spermatid and is found as a ring-like structure near the insertion of the flagellum at the time when the chromatin of the spermatid nucleus begins to condense (FAWCETT et al. 1970). This happens in step 8 of spermiogenesis (SUSI and CLERMONT 1970), which coincides with the cessation of the haploid gene activity (MONESI 1965; SÖDERSTRÖM and PARVINEN 1976c).

Recently it has been shown that the chromatoid body of the round-nucleated spermatids becomes slowly labelled after a pulse with tritiated uridine. This occurs ca. 14 h after the labelling of the spermatid nuclei (SÖDERSTRÖM and PARVINEN 1976b). The labelling is inhibited by actinomycin D (SÖDERSTRÖM, pers. comm.). These observations together with cinemicrographic and ultrastructural data about the transport of material between the haploid nucleus and the chromatoid body (SÖDERSTRÖM and PARVINEN 1976a) strengthen the concept of the present work about the dependence of the chromatoid body on the activity of the haploid genome.

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