Available online at www.sciencedirect.com
ScienceDirect
Micron 39 (2008) 419–425
micron
www.elsevier.com/locate/micron

Meiotic nucleolar cycle and chromatoid body formation during the rat (*Rattus novergicus*) and mouse (*Mus musculus*) spermiogenesis

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Received 12 December 2006; received in revised form 21 February 2007; accepted 22 February 2007

Abstract

The aims of the present study were to follow the nucleolar cycle in spermiogenesis of the laboratory rodents *Rattus novergicus* and *Mus musculus*, to verify the relationship between the nucleolar component and chromatoid body (CB) formation and to investigate the function of this cytoplasmic supramolecular structure in spermatogenic haploid cells. Histological sections of adult seminiferous tubules were analyzed cytochemically by light microscopy and ultrastructural procedures by transmission electron microscopy. The results reveal that in early spermatids, the CB was visualized in association with the Golgi *cisterns* indicating that this structure may participate in the acrosome formation process. In late spermatids, the CB was observed near the axonema, a fact suggesting that this structure may support the formation of the spermatozoon tail. In conclusion, our data showed that there is disintegration of spermatid nucleoli at the beginning of spermatogenesis and a fraction of this nucleolar material migrates to the cytoplasm, where a specific structure is formed, known as the ''chromatoid body'', which, apparently, participates in some parts of the rodent spermiogenesis process.

Keywords: Nucleolus; Nucleologenesis; Chromatoid body; Spermiogenesis; Cytochemistry; Ultrastructure

1. Introduction

The nucleolus is a particular nuclear territory related to the compartmentalization of nuclear functions (Hernandez-Verdun, 1991). It is a large, highly organized, non-membrane-bound subcompartment of the nucleus and is the site of biogenesis of ribosomal subunits (Gerbi et al., 2003).

Nucleolar formation is a dynamic process in the eukaryotic cell cycle denominated nucleologenesis. The interphasic nucleoli generally show three major nucleolar domains in transmission electron microscopy: fibrillar center (FC), dense fibrillar component (DFC) and granular component (GC). During nucleologenesis there is a disintegration and reorganization of these nucleolar domains (Zatsepina et al., 1997).

The nucleolus structure disintegrates and the majority of its proteins migrate to the cytoplasm when the cells start mitotic or meiotic cell division and stop the DNAr transcription during prophase. These proteins remain associated in the nucleolar organizer regions (NORs) of the condensed chromosomes, dissolve in the cytoplasm or connect in the peripheral chromosomic region (perichromosomal sheath). The nucleolus gradually becomes reorganized during the later anaphase when DNAr transcription is reactivated. The nucleolus reorganization initiates when the nucleolar material condenses or collects in discrete structures denominated pre-nucleolar bodies (PNBs) that, subsequently, fuse in the chromosomic RONs of the telophase and earlier interphase cells (Ochs et al., 1985; Wachtler and Sthal, 1993; Mello, 1995; Dundr et al., 2000).

Few studies of the nucleolar cycle were carried out during meiotic cell division (*e.g.* Comings and Okada, 1972; Andonov, 1990; Tartarotti and Azeredo-Oliveira, 1999; Morielle and Azeredo-Oliveira, 2004). Takeuchi and Takeuchi (1990) demonstrate nucleolar fragmentation during rodent spermiogenesis and other studies reported migration of fragmented

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doi:10.1016/j.micron.2007.02.009

nucleolar material to cytoplasm with the formation of a structure denominated chromatoid body (CB) (Comings and Okada, 1972; Andersen, 1978; Paniagua et al., 1986; Andonov, 1990). This structure, a typical cytoplasmic organelle of spermatogenic haploid cells, has a function related to RNA and protein accumulation and/or reserves for later differentiation of the sperm cell (*e.g.* Söderström and Parvinen, 1976a; Moussa et al., 1994; Oko et al., 1996; Figueroa and Burzio, 1998). Recent review by Kotaja and Sassone-Corsi (2007) indicates that the CB is a highly specialized structure might function as an intracellular focal domain that organizes and controls RNA processing in male germ cells. This report suggests that CB as a model in which their functions as a subcellular coordinator of different RNA-processing pathways, centralizing the post-transcriptional mRNA control in the cytoplasm of haploid male germ cells. The majority of the authors, however, have not reached a consensus about what happens to the nucleolus during meiotic cell division. The aims of the present work are to study the nucleolar supramolecular material distribution in several spermatid phases in the seminiferous epithelium of rodents and to compare the nucleolar material distribution with CB formation.

2. Material and methods

2.1. Animals

Investigations were carried out on rats (*Rattus novergicus*) and mice (*Mus musculus*) ranging from 4 to 8 weeks of age, since younger animals have seminiferous tubules with more spermatogonial cells and primary spermatocytes whereas older ones have seminiferous tubules with more spermatids, mature sperm cells and residual bodies. The animals were obtained from the Biology Department Biotherium (IBILCE/UNESP, São José do Rio Preto, SP) and treated according to the recommendations of the Committee on Care and Use of Laboratory Animals from the Institute of Laboratory Animal Resources, National Research Council, ''Guide for the Care and Use of Laboratory Animals'' (Committee on Care and Use of Laboratory Animals, 1980). The animals were anesthetized and killed by carbon dioxide gas (CO_2) inhalation, according to Resolution 714, Brazilian Veterinary Medicine Federal Council. All animals were housed under standard conventional conditions (25 °C, 40–70% relative humidity, 12-h light:12-h dark cycle) and allowed access to chow and water *ad libitum*.

2.2. Light microscopy

The testes of each animal were removed and fixed by immersion in Bouin's fixative solution and Karnovisky fixative solution for 24 h. The material was embedded in paraffin and glycol-methacrylate historesin. Thick sections (1–3 μm) were obtained in *Leica RM* 2155 microtome. Tissue sections were submitted to some ordinary cytological and cytochemical procedures such as Periodic Acid and Schiff (PAS) and Hematoxylin–eosin (HE) according to Ribeiro and Lima (2000), Toluidine Blue (TB) according to Mello and Vidal (1980), modified critical eletrolyte concentration for detecting RNA (CEC) (Mello, 1997), silver ion impregnation (AgNOR) (Howell and Black, 1980), Gömöri's Reticulin (Taboga and Vidal, 2003), Feulgen reaction (Mello and Vidal, 1980), acid and alkaline fast green (Mello and Vidal, 1980). The sections were evaluated in an Olympus BX 60 photomicroscope and documented by an Image Pro-Plus, Media Cybernetics, Version 4.5 for Windows computer software for image analysis.

2.3. Transmission electron microscopy

After dissection, the testes of each animal were removed, sliced into small pieces and samples of the seminiferous tubules were minced and fixed by immersion with 3% glutaraldehyde plus 0.25% tannic acid solution in Millonig's buffer (pH 7.3) containing 0.54% glucose for 24 h (Cotta-Pereira et al., 1976). After washing with the same buffer, samples were post-fixed with 1% osmium tetroxide for 1 h, washed in Millonig's buffer, dehydrated in a graded acetone series, and embedded in Araldite resin. Ultrathin silver sections were cut using a diamond knife and stained with 2% alcoholic uranyl acetate for 30 min (Watson, 1958) followed by 2% lead citrate in sodium hydroxide for 10 min (Venable and Coggeshall, 1965). Samples were evaluated using a Leo-Zeiss 906 (Cambridge, UK) transmission electron microscope.

3. Results

3.1. Light microscopy

PAS reaction was used to identify seminiferous tubule stadia. Seminiferous tubules from stadia I to VII (Leblond and Clermont, 1952) were analyzed because they show A and B spermatogonial cells, primary spermatocytes, spermatids in some development phases and mature spermatozoa, thus facilitating the observation of nucleolar cycle during the spermatogenesis process. Pre-acrosomal granules on nuclear surface and in basophilic regions near the nucleus were observed in cap phase spermatids (Orsi and Ferreira, 1978) (Table 1).

HE stain was used herein for general analysis of seminiferous tubules and showed a basophilic reaction in earlier spermatid cytoplasm, probably in the pre-acrosomal granule formation region (Table 1).

Utilization of the TB reaction verified which nuclear structures (euchromatin, heterochromatin, nucleoli and chromosomes) of seminiferous epithelial cells presented intense metacromasy (Figs. 1 and 4). The TB method was employed as control for the CEC variant method for RNA, in which some regions demonstrated different degrees of metachromasy. By CEC, the nucleolar areas of the spermatogonial cells and primary spermatocytes were shown in blue, while nucleolar areas of the spermatids were not shown (Figs. 2 and 5). Chromatin of all seminiferous epithelial cells showed greenish stain, varying according to their compactation degree and the cytoplasm showed intense basophilia (Figs. 2 and 5). ''Residual corpuscles'' or ''chromatophilic spheres'' originated by