Micron 43 (2012) 954–960

Contents lists available at SciVerse ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Chromatoid body: Remnants of nucleolar proteins during spermatogenesis in triatomine (Heteroptera, Triatominae)

Rosana Silistino-Souza[a], Rita Luiza Peruquetti[b], Sebastião Roberto Taboga[a], Maria Tercília Vilela de Azeredo-Oliveira[a,*]

[a] Department of Biology, São Paulo State University – UNESP/IBILCE, Rua: Cristóvão Colombo, 2265, Jardim Nazareth, 15054-000 São José do Rio Preto, SP, Brazil
[b] Center of Health Sciences, Sacred Heart University – USC, Bauru, SP, Brazil

ARTICLE INFO

Article history:
Received 28 September 2011
Received in revised form 2 February 2012
Accepted 23 March 2012

Keywords:
Chromatoid body
Triatoma sordida
Triatoma infestans
Fibrillarin nucleolar protein
Spermatogenesis

ABSTRACT

In this study, we analyzed fibrillarin nucleolar protein expression in CBs of spermatogenic cells from testicular follicles of *Triatoma sordida* and *Triatoma infestans*. In the structural and ultrastructural analysis, it was used impregnation by silver ions, immunocytochemistry, immunofluorescence and transmission electron microscopy using antibodies against fibrillarin. Regarding the results, the fibrillarin nucleolar protein marked the nucleus and some cytoplasmic spots of germ cells during spermatogenesis in triatomines. These data suggest that fibrillarin could be a constituent of the CB that was most likely derived from nucleolar fragmentation. This is the first time that fibrillarin protein expression has been shown in the CB during spermatogenesis progression in triatomines. The knowledge regarding CB constituents may help to expand the understanding of the physiological role of this structure and the role that it plays in the reproductive biology of triatomines, which are vectors of Chagas Disease.

1. Introduction

Chagas disease constitutes one of the greatest health problems in the last decades. Worldwide, it is estimated that there are about 10–12 million infected persons. Multinational control initiatives have drastically reduced prevalence and incidence, mainly through insecticide-based elimination of domestic vector population (blood sucking bugs of the subfamily Triatomine) (Abad-Franch et al., 2011). This evidence demonstrates the importance of new strategies for the control of the triatomines as well as of the Chagas disease. In this sense, studies focusing on the natural history and basic biology of this animal group are still required.

The Chromatoid body (CB), which are structure-specific of reproductive cells, were discovered 130 years ago by Von Brunn in 1876 (Yokota, 2008).

CB is transferred to the residual body during the spermiogenesis process of mammals (Breucker et al., 1985), and it has been seen in association with the late annulus at the end of spermatozoon development (Fawcett et al., 1970; Setchell, 1978). Moreover, they were frequently observed in association with the cisternae of the Golgi complex during spermiogenesis in rats and mice, which indicates that this structure might participate in the formation of the acrosome. Additionally, the CB has been found to be attached to the axoneme, which suggests that this organelle plays a role in the development of the sperm tail (Peruquetti et al., 2008, 2010a). Some mutations observed in the TDR1/MTR-1 protein and OX3 histocompatibility antigen, which are present in the CB, cause sterility in mice, and show an important role of the CB in the process of spermatogenesis (Head and Kresge, 1985; Chuma et al., 2006).

Some authors have shown that the CB is located in a strategic position near the nuclear envelope, which makes it possible for this structure to participate in the regulatory network that controls the spermatogenic process through post-transcriptional mechanisms (Kotaja and Sassone-Corsi, 2007; Nagamori and Sassone-Corsi, 2008). Many studies in the literature describe the relationship between nucleolar material fragmentation during the spermatogenesis process and CB formation (Comings and Okada, 1972; Andonov, 1990; Peruquetti et al., 2008, 2010b, 2011).

The nucleolus represents a highly dynamic nuclear compartment involved in multiple functions and able to promptly respond to variations of cell metabolic needs (Malatesta et al., 2011). In this connection, it has been also suggested that nucleolar proteins, fundamental for the structural and functional organization of the organelle, may be active component of other nucleoplasmic structures. The fibrillarin is a key small nucleolar protein in eukaryotes, which has an important role in pre-rRNA processing during ribosomal biogenesis (Amin et al., 2007). This protein is associates with the U3, U8, and U13 small nuclear RNAs and is located in the dense fibrillar component (Nicol et al., 2000). Moreover, fibrillarin also is essential for early embryonic development (Newton et al., 2003).

* Corresponding author. Tel.: +55 17 32212378; fax: +55 17 32212390.
E-mail address: tercilia@ibilce.unesp.br (M.T. Vilela de Azeredo-Oliveira).

0968-4328/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.micron.2012.03.017

The observation that the CBs are involved in key steps for the relocation of nucleolar proteins have suggested that this nuclear component could have also a role in the nucleoplasmic transport of ribosomal proteins (Cisterna et al., 2006; Cisterna and Biggiogera, 2010). Additionally, its location has been reported to be in several successive stages of spermatogenesis, and it has also been associated with the nuclear pore complex during mammalian spermatogenesis (Ventelä et al., 2003).

Studies using transmission electron microscopy have revealed the presence of a CB in the germ cells of annelids (Dhainaut, 1970), insects (Mahowald, 1962), ascidians (Kessel, 1966), fish (Peruquetti et al., 2010b), mammals (Peruquetti et al., 2008, 2010a) and amphibians (Al-Mukhtar and Webb, 1971; Peruquetti et al., 2011).

Research has been directed mainly toward identifying the chemical and molecular composition of CBs. Cytochemical analysis showed that the CB possesses RNA, polysaccharides, and basic proteins (Werner and Werner, 1995; Haraguchi et al., 2005). Recent studies using molecular biology techniques have shown that CBs are in a region where RNA is stored and processed in reproductive cells (Ventelä et al., 2003; Kotaja et al., 2006; Kress et al., 2007). Arginine-rich basic proteins and polysaccharides (Krimer and Esponda, 1980), ribonucleoprotein snRNP, and hnRNP are found in the CB by means of specific polyclonal and monoclonal antibodies, especially during early stages of spermiogenesis. Monoclonal antibodies directed against the proteins PI/P2, which are components of the largest ribosomal subunit, detect these antigens in the CB and in the dense nucleolar fibrillar center. This suggests that the CB can act as a source of mRNA and/or as an RNA processing center during late stages of spermiogenesis, when the nucleus of the spermatid becomes progressively more inactive (Biggiogera et al., 1990; Moussa et al., 1994).

The investigations show that the CB is composed of fine fibrillar material, and in many cases, it is surrounded by numerous small vesicles (Andonov, 1990). Primary spermatocytes have materials that are spread around the nucleus and that will originate from the CB. These materials are still not aggregate, and early spermatids reveal the CB condensed to its final shape. The CB also may be present in association with mitochondria (Russell and Frank, 1978; Peruquetti et al., 2008). Others authors describe the CB as a lobulated and finely filamentous perinuclear granule (Nagamori and Sassone-Corsi, 2008) and also as an accumulation of dense fibrous material, which occurs in the cytoplasm of germ cells in the animal kingdom (Eddy, 1975; Saffman and Lasko, 1999).

Despite the growing number of studies related to the CB, its origin, composition and function are not yet fully understood. As for the triatomine, there are also no studies that show the CBs in germ cells. Because of the importance of the triatomines as the main vectors of Chagas disease, the research related to its reproductive aspects has been of great importance. Thus, the purpose of this study is to investigate the expression of the fibrillarin protein, one of the main nucleolar acidic proteins, as a possible component of the CB in spermatogenic cells of triatomines.

2. Materials and methods

2.1. Insects

The *Triatoma infestans* and *Triatoma sordida species*, order Heteroptera, family Reduviidae, subfamily Triatominae were used. Ten adult male specimens that had not been infected with the protozoan *Trypanosoma cruzi* were provided by the Triatominae Insectarium, Department of Biological Sciences, College of Pharmaceutical Sciences, at the UNESP/FCF – Araraquara Campus in the state of Sao Paulo, Brazil.

2.2. Transmission electron microscopy

The testicular follicles of adult triatomines ($n = 10$) were placed in 3% glutaraldehyde plus 0.25% tannic acid solution in Milloning's buffer, pH 7.3 for 3 h (Cotta-Pereira et al., 1976). After washing with the same buffer, the follicles were placed in 1% osmium tetroxide. The material was dehydrated in a series of ascending acetones (30–100%) and embedded in Araldite (*Electron Microscopy Sciences*). Ultrathin sections (50 nm) were cut and contrasted with lead citrate and uranyl acetate. Image capture and analysis were performed using an Olympus BX40 microscope with the image analysis system Axiovision LE version 4.8 for Windows. The samples were evaluated using a LEO-ZEISS 906 (Zeiss, Cambridge, UK) transmission electron microscope operated at 80 kV.

2.3. Placement, processing and embedding for immunocytochemistry, immunofluorescence and ultrastructural immunocytochemistry

To carry out the techniques of immunocytochemistry, immunofluorescence and ultrastructural immunocytochemistry, the testicular follicles of the adult triatomines were placed in 4% paraformaldehyde and 0.5% glutaraldehyde diluted in a 0.1-M phosphate buffer, pH 7.4, for 24 h, at 4 °C. They were then washed in a 0.1-M phosphate buffer, pH 7.4, at 4 °C, dehydrated through a graded series of methanol, and embedded in LRGold resin (Sigma–Aldrich, UK). Sections (1 μm thick) for immunocytochemistry and immunofluorescence and (70 nm thick) for ultrastructural immunocytochemistry were cut on an ultramicrotome (Reichert Ultracut; Leica, Austria) and placed on nickel grids for immunogold labeling.

2.4. Immunocytochemistry

For fibrillarin detection, 1-μm sections of testicular follicles were prepared; antigen retrieval was then performed in citrate buffer, pH 6.0, at 96–98 °C, for 60 min. Followed by incubation with Background Sniper solution (Biocare Medical, Concord, CA, USA), for 15 min to block nonspecific protein-linkage. Sections were sequentially incubated overnight at 4 °C with mouse anti-fibrillarin primary antibody (Abcam, Cambridge, MA), which was used as a nucleolar marker and diluted 1:100 in BSA 1%. The blockade of endogenous peroxidases was obtained by covering the slide with 3% H_2O_2 in methanol, for 30 min. The sections were then incubated with the biotinylated anti-rabbit followed by avidin–biotin complex ABC kit (Santa Cruz Biotechnology, Santa Cruz, CA) for 45 min at 37 °C.

The reactions mentioned above were revealed with diaminobenzidine (DAB), and the sections were counterstained with hematoxylin. The negative control was obtained by omission of the primary antibody. The image capture and analysis was performed an Olympus BX40 microscope with an image analysis system Axiovision LE version 4.8 for Windows.

2.5. Immunofluorescence

One-μm-thick sections of the testicular follicles of the triatomines were prepared and incubated with a 5% Image-iT Signal Enhancer solution to block nonspecific links. The samples were incubated with mouse polyclonal anti-fibrillarin antibody (Abcam, Cambridge, MA), which was used as a nucleolar marker, and stored overnight at 4 °C and then treated for 1 h with the secondary antibody Alexa 488 anti-rabbit immunoglobulin G (Molecular Probes, Eugene, OR). Samples were also counterstained with DAPI (4′,6′-diamidino-2-phenylindole). The preparations were observed under a fluorescence microscope, and the data were collected using