Presence of chromatoid bodies in the *Rhodnius* genus detected by cytochemical analysis

K.C.C. Alevi[1], N.F.C. Castro[1], J. Oliveira[2], J.A. Rosa[2] and M.T.V. Azeredo-Oliveira[1]

[1]Laboratório de Biologia Celular, Departamento de Ciências Biológicas, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto, SP, Brasil
[2]Laboratório de Parasitologia, Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Júlio de Mesquita Filho", Araraquara, SP, Brasil

Corresponding author: K.C.C. Alevi
E-mail: kaiochaboli@hotmail.com

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ABSTRACT. In addition to the epidemiological importance regarding the transmission of Chagas disease, triatomines are also important biological models for cellular studies, because they have holocentric chromosomes, post-reductional meiosis for sex chromosomes, and nucleolar persistence. Although the nucleolus is present during spermiogenesis, it was suggested that it is inactivated and all transcriptional activity necessary for cell differentiation is supported by the chromatoid body (CB). Thus, considering the importance of CB to triatomine spermatogenesis, this paper aims to analyze the presence of this organelle in the *Rhodnius* genus. The testicles of five adult *R. neglectus* and *R. prolixus* males were analyzed after semi-fine sections were performed, and the material was subjected to silver ion impregnation. The CB organelle was found in the spermatid of both species. Most CB studies focused on the *Triatoma* genus. This study described the existence of CB in *Rhodnius*, confirming thus the importance of this cytoplasmic organelle to spermiogenesis of these insect vectors of Chagas disease.

Key words: Chromatoid body; Nucleolus; Spermiogenesis

INTRODUCTION

In addition to the epidemiological importance regarding the transmission of Chagas disease, triatomines are also important biological models for cellular studies, because they have holocentric chromosomes with diffuse kinetochores. Moreover, these insects also exhibit unusual meiosis in which the segregation of sex chromosomes is post-reductional (Barth, 1956; Ueshima, 1966). In addition, these insects have a peculiar behavior regarding the nucleolus during spermatogenesis, which is termed nucleolar persistence (Tartarotti and Azeredo-Oliveira, 1999).

Nucleolar persistence is defined by the presence of the nucleolus or nucleolar corpuscles during meiotic metaphase, and it has currently been described in 25 triatomine species belonging to the *Triatoma* (Severi-Aguiar and Azeredo-Oliveira, 2005; Severi-Aguiar et al., 2006; Morielle-Souza and Azeredo-Oliveira, 2007; Bardella et al., 2008; Costa et al., 2008; Alevi et al., 2013; Borgueti et al., 2015; Pereira et al., 2015), *Rhodnius* (Morielle and Azeredo-Oliveira, 2004; Morielle-Souza and Azeredo-Oliveira, 2007; Alevi et al., 2014a), *Panstrongylus* (Tartarotti and Azeredo-Oliveira, 1999), and *Meccus* genera (Madeira et al., 2016).

Although the nucleolus is present during triatomine spermiogenesis, transcriptional activity does not occur, because it is inactivated by epigenetic factors (Severi-Aguiar and Azeredo-Oliveira, 2005; Alevi et al., 2014b; Borgueti et al., 2015). Therefore, all transcriptional activity necessary for cell differentiation during spermiogenesis is supported by the chromatoid body (CB) (Alevi et al., 2014b, Borgueti et al., 2015).

The CB was discovered by von Brunn (1876) in the cytoplasm of white rat spermatids, and its functions include the "reserving" or "stocking" of RNA and proteins for various cell differentiation events that occur during spermiogenesis. Borguetti et al. (2015) suggested that this cytoplasmic organelle is formed during the triatomine nucleolar persistence phenomenon, and that it stores all RNA transcribed by persistent nucleolar material.

Most CB studies focused on the *Triatoma* genus (Silistino-Souza et al., 2012; Borgueti et al., 2015). Therefore, taking the importance of CB for triatomine spermatogenesis, this study aimed to analyze the presence of this organelle in *Rhodnius*.

MATERIAL AND METHODS

Five adult *R. neglectus* and *R. prolixus* males were provided by the "Insectarium of Triatominae" that was installed at the Faculty of Pharmaceutical Sciences, Campus de Araraquara, State of São Paulo, Brazil. The insects were anesthetized according to Alevi et al. (2015), and the testicles were dissected and dipped into a physiological solution of Demerec. The testicles were then exposed, and the seminiferous tubules were separated and fixed in

Karnovsky solution, pH 7.4. The specimens were subsequently post-fixed with 1% osmium tetroxide before being dehydrated in acetone and embedded in Araldite resin. Semi-fine sections were made (0.5 mm thick), and the material was then subjected to silver ion impregnation (Howell and Black, 1980). Additional analysis and image capture were performed via light microscopy using a Zeiss Jenaval with an Axio Vision Rel 4.8 image analyzer.

RESULTS AND DISCUSSION

The CB organelle was found in the spermatids of both species (Figure 1, arrow). The CB is considered a macromolecular complex, which appears to have an important role in the coordination of post-transcriptional control of gene products in male haploid germ cells, and it also aids in determining messenger RNA destinations (Kotaja and Sassone-Corsi, 2007). Because of these characteristics, this structure has important functions associated with spermiogenesis, such as cellular communication between spermatids (Ventelä et al., 2003), mitochondrial targeting to the posterior region of spermatid nuclei (which assists in the formation of the mitochondrial sheath and the spermatozoid flagellum (Fawcett et al., 1970)), and the formation of the spermatozoid acrosome (Söderström and Parvinen, 1976; Tang et al., 1982).

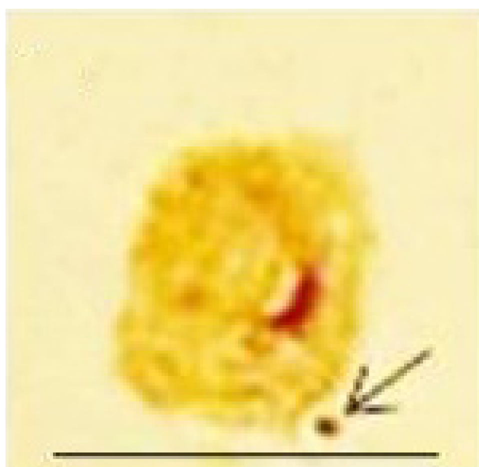

Figure 1. Semi-fine cuts of *Rhodnius neglectus* spermatids that were stained with silver ions. Note the presence of chromatoid body (arrow). Bar: 10 µm.

The origin of this cytoplasmic structure remains uncertain. Some authors support the idea that CB originates from a nuclear product that runs through the pore complex into the cytoplasm of cells (Parvinen and Parvinen, 1979; Parvinen et al., 1997). Other authors claim that CB originates from the accumulation of material from the inter-mitochondrial cytoplasm of spermatids (Fawcett et al., 1970) or from mitochondrial products that were released into the cytoplasm (Reunov et al., 2000). However, other authors believe that CB is a structure derived from fragmentation and migration of nucleolar material from the nucleus to the cytoplasm (Comings and Okada, 1972; Peruquetti and Azeredo-Oliveira, 2009; Peruquetti et al., 2008, 2010).

Silistino-Souza et al. (2012) examined the presence and formation of CB in *T. infestans* and *T. sordida*, and they confirmed the hypothesis that CB originated from nucleolar fragments. This conclusion was based on the results of immunocytochemical analyses where the presence of nucleolar fibrillarin protein was observed in the cytoplasmic organelle. On the other hand, Borgueti et al. (2015) suggested that the formation of CB in triatomines begins during spermatocytogenesis. Thus, the authors related the transcriptional activity during nucleolar persistence to the formation of CB and not to nucleolar fragments as suggested by Silistino-Souza et al. (2012).

Therefore, this study described the existence of CB in *Rhodnius*, confirming thus the importance of this cytoplasmic organelle to spermiogenesis of these insect vectors of Chagas disease.

Conflicts of interest

The authors declare no conflict of interest.

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