Genetics and Molecular Research

Nucleolar-persistence phenomenon during spermatogenesis in genus *Meccus* (Hemiptera, Triatominae)

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Genet. Mol. Res. 15 (1): gmr.15017427
Received August 11, 2015
Accepted November 13, 2015
Published March 24, 2016
DOI http://dx.doi.org/10.4238/gmr.15017427

ABSTRACT. The Triatominae subfamily consists of 150 species in 18 genera, grouped into six tribes. In cytogenetics, triatomines are important biological models because they have holocentric chromosomes and nucleolar persistence in meiosis. The phenomenon of nucleolar persistence has been described for 23 species of triatomine in three genera: *Triatoma*, *Rhodnius*, and *Panstrongylus*. However, new species and genera should be analyzed to assess whether nucleolar persistence is a peculiarity of Triatominae. Thus, this study aimed to analyze nucleolar behavior during spermatogenesis of *Meccus pallidipennis* and *M. longipennis*, focusing on the nucleolar-persistence phenomenon. Through the analysis of spermatogenesis, more specifically of meiotic metaphase, we observed the phenomenon of nucleolar persistence in *M. pallidipennis* and *M. longipennis*, represented by remnants of nucleolar material in metaphase.

Thus, although nucleologenesis of new species, and, especially, new genera, should be analyzed, this study confirms for the first time the phenomenon of nucleolar persistence in the genus *Meccus*. Therefore, we emphasize the importance of new studies in this area in order to assess whether this phenomenon is truly a synapomorphy of these hematophagous insects.

Key words: Cytogenetics; Nucleolus; Meiosis

INTRODUCTION

The triatomines (Hemiptera: Reduviidae) are included in the subfamily Triatominae (Lent and Wygodzinsky, 1979). To date, 150 species in 18 genera have been described (Alevi et al., 2015a). The importance of the triatomines in parasitology is due to these insects being transmitters of the etiologic agent of Chagas disease, *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae) (Tavares and Azeredo-Oliveira, 1996), a flagellate parasite that infects many mammals, including humans (Dias, 2002).

In cytogenetics, triatomines are important biological models because they have holocentric chromosomes, which have diffuse kinetochores. Furthermore, these insects also undergo an unusual form of meiosis in which the segregation of sex chromosomes is post-reductional (Barth, 1956; Ueshima, 1966). In addition, these insects have nucleolar persistence during spermatogenesis (Tartarotti and Azeredo-Oliveira, 1999). Chromosome data are being applied in the taxonomy of these vectors (Alevi et al., 2013a,b,c, 2014a,b, 2015b,c).

Nucleolar persistence is defined by the presence of the nucleolus or nucleolar corpuscles during meiotic metaphase. This phenomenon has been described for 23 species of triatomine within the genera *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., 2013d; Borgueti et al., 2015; Pereira et al., 2015), *Rhodnius* (Morielle and Azeredo-Oliveira, 2004; Morielle-Souza and Azeredo-Oliveira, 2007; Alevi et al., 2014c), and *Panstrongylus* (Tartarotti and Azeredo-Oliveira, 1999). However, new species and genera should be analyzed to assess whether nucleolar persistence is really a peculiarity of the Triatominae subfamily.

The genus *Meccus* was proposed for *Conorhinus phyllosoma*. By means of the cladodios, the rostrum, and the insertion of antennae, *Meccus* was considered synonymous with *Triatoma* (Pinto, 1927). After conducting laboratory studies, Mazzotti and Osório (1942) proposed that three species of *Triatoma* should be considered as subspecies of *T. phyllosoma*. On the basis of unequal size in respect to *Triatoma* species, Carcavallo et al. (2000) proposed revalidation of the genus *Meccus*. The proposed revalidation of the genus was confirmed by Hypša et al. (2002) based on molecular systematics. Currently, the genus consists of six species, *M. phyllosomus*, *M. pallidipennis*, *M. picturatus*, *M. longipennis*, *M. mazzotti*, and *M. bassolsae* (Galvão et al., 2003).

Meccus longipennis is considered the main species of the *Meccus* complex and is responsible for the transmission of *T. cruzi* in West Mexico (Lozano-Kasten et al., 2008). This species is founded in both domestic and wild ecotopes (WHO, 2002; Martínez-Ibarra et al., 2003) and presents a high capacity to colonize human dwellings (Espinoza-Gómez et al., 2002). *Meccus pallidipennis* is also considered to be an important vector of Chagas disease, since it is responsible for 74% of the vectorial transmission to humans in Mexico (Ibarra-Cerdeña et al., 2009). Its

distribution has been associated with different degrees of invasion in human dwellings (Ramsey et al., 2003; Martínez-Ibarra et al., 2011).

Thus, in order to evaluate whether nucleolar persistence is a peculiarity of the Triatominae subfamily, the aims of present study were to analyze nucleolar behavior during spermatogenesis of *M. pallidipennis* and *M. longipennis*, focusing on the phenomenon of nucleolar persistence.

MATERIAL AND METHODS

Seminiferous tubules of five adult males of *M. pallidipennis* and *M. longipennis* (from the "Triatominae Insectarium" installed in Araraquara city, Sao Paulo, Brazil), after being shredded, squashed, and fixed on a cover slip in liquid nitrogen, were stained by silver ion impregnation (Howell and Black, 1980). The biological material was analyzed with a Jenaval light microscope coupled to a digital camera and an image analyzer Axio Vision LE 4.8 (Carl Zeiss Imaging Solutions Gmb H). The images were magnified by a factor of 1000X.

RESULTS

Through the analysis of spermatogenesis, more specifically, of the meiotic metaphase, we observed nucleolar persistence in *M. pallidipennis* (Figure 1) and *M. longipennis*, which was represented by remnants of nucleolar material in metaphase (Figure 1, arrows).

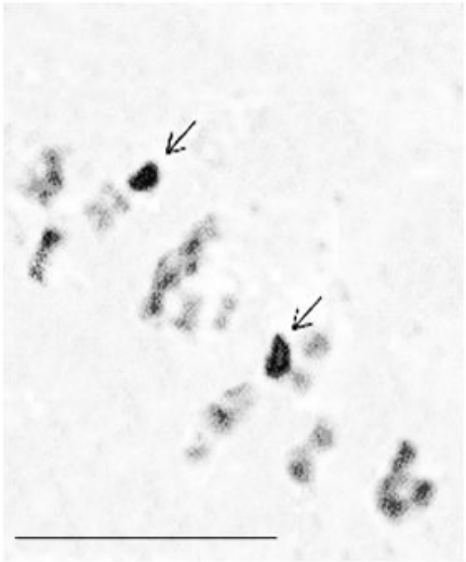

Figure 1. Metaphase I of *Meccus pallidipennis*. Note the nucleolar persistence (arrows). Bar: 10 µm.

DISCUSSION

Spermatogenesis consists of three different phases: spermatocytogenesis, which is a phase of proliferation; meiosis, which is the multiplication phase; and spermiogenesis, which is the

differentiation phase (Johnson et al., 1997). Both species of *Meccus* analyzed (*M. pallidipennis* and *M. longipennis*) presented the phenomenon of nucleolar persistence during meiosis. It is believed that the persistence of the nucleolus or nucleolar remnants during meiosis in triatomines is an important transcription factor that accumulates mRNA in the cytoplasm or in the formation of a chromatoid body (CB) (Borgueti et al., 2015).

Severi-Aguiar and Azeredo-Oliveira (2005) and Alevi et al. (2014d) showed that the nucleolus, although present during spermiogenesis in insects, is inactivated by epigenetic factors. Recently, Silistino-Souza et al. (2012) and Borgueti et al. (2015) described the cytoplasmic organelle CB in the triatomines. Thus, although further studies should be conducted, we believe that the entire mRNA transcript is stored in the CB during nucleolar persistence in meiosis, and that this organelle is responsible for the differentiation of spermatids to sperm cells (Alevi et al., 2014d; Borgueti et al., 2015).

Thus, although nucleologenesis of new species, and, especially, new genera, should be analyzed, this study confirms for the first time the phenomenon of nucleolar persistence in the genus *Meccus*. Therefore, we emphasize the importance of new studies in this area in order to assess whether this phenomenon is truly a synapomorphy of these hematophagous insects.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Processes #2013/19764-0 and #2015/14762-5) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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