Nucleolar Persistence: Peculiar Characteristic of Spermatogenesis of the Vectors of Chagas Disease (Hemiptera, Triatominae)

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Abstract. All species of triatomines are considered potential vectors of Chagas disease and the reproductive biology of these bugs has been studied by different approaches. In 1999, nucleolar persistence during meiosis was observed in the subfamily for the first time. Recently, it has been observed that all species within the genus *Rhodnius* exhibit the same phenomenon, suggesting that it may be a synapomorphy of the triatomines. Thus, this article aims to analyze the nucleolar behavior during spermatogenesis of 59 triatomine species. All analyzed species exhibited nucleolar persistence during meiosis. Recently, it has been suggested that nucleolar persistence may be fundamental for the spermatogenesis of these vectors, since it is related to the formation of the chromatoid body. Therefore, we emphasize that this phenomenon is a peculiarity of the Triatominae subfamily and that further studies are required to analyze whether the nucleolar material that persists is active.

The Triatominae subfamily is composed of 150 species (148 living species and two fossils), grouped into 18 genera and five or six tribes. All triatomine species are considered potential vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease.¹

Chagas disease is a neglected disease that has no cure. The main way to minimize the incidence of this disease in human populations is through vector control.¹ It is estimated that more than 5 million people are infected by *T. cruzi*, the parasite that causes Chagas disease, and that 70 million still live at risk, which places this illness among the most serious parasitic diseases in the world.¹ Thus, improving the knowledge on several fields on triatomine vector potentiality (such as ecology² and biology³) may provide important information for control measures.

The reproductive biology of these bugs has been studied by different approaches, such as cytogenetic,⁴ structural,⁵ and ultrastructural⁶ analysis. Furthermore, the spermatogenesis of the triatomines is characterized as cystic,^{5,6} and it has been suggested that, during imaginal molt (transition from the fifth instar nymph to adult), the cell division is disrupted, aiming to reduce energy costs, and the differentiation into sperm is stimulated to ensure the paternity of the adult male.⁷

In 1999, Tartarotti and Azeredo-Oliveira,⁸ while studying the spermatogenesis of *Panstrongylus megistus* and *Panstrongylus herreri* (= *Panstrongylus lignarius*), noted that these triatomines exhibited a different nucleolar behavior than the one described for other eukaryotes: the nucleolus persisted during all stages of meiosis. The authors characterized this phenomenon as nucleolar persistence.

Recently, Alevi and others⁹ have observed that all species within the genus *Rhodnius* feature nucleolar persistence during meiosis as well, and they suggested that analyses of the nucleolar behavior should be carried out in a large range of

species of triatomines to examine whether that characteristic is a synapomorphy of the Triatominae subfamily.

Thus, this article aims to analyze the nucleolar behavior during spermatogenesis of 59 triatomine species to determine whether nucleolar persistence occurs in all bugs of the Triatominae subfamily.

We analyzed at least two adult male specimens of each species (*Cavernicola pilosa*, *Psammolestes tertius*, *Rhodnius brethesi*, *Rhodnius colombiensis*, *Rhodnius domesticus*, *Rhodnius ecuadoriensis*, *Rhodnius milesi*, *Rhodnius montenegrensis*, *Rhodnius nasutus*, *Rhodnius neglectus*, *Rhodnius neivai*, *Rhodnius pallenscens*, *Rhodnius pictipes*, *Rhodnius prolixus*, *Rhodnius robustus*, *Rhodnius stali*, *Dipetalogaster maxima*, *Eratyrus cuspidatus*, *Meccus pallidipennis*, *Meccus longipennis*, *Mepraia spinolai*, *Nesotriatoma bruneri* (= *Nesotriatoma flavida*), *Panstrongylus lignarius*, *Panstrongylus megistus*, *Triatoma arthurneivai*, *Triatoma baratai*, *Triatoma brasiliensis*, *Triatoma brasiliensis macromelasoma*, *Triatoma carcavalloii*, *Triatoma circummaculata*, *Triatoma costalimai*, *Triatoma delpontei*, *Triatoma dimidiata*, *Triatoma garciabesi*, *Triatoma guasayana*, *Triatoma guazu*, *Triatoma infestans*, *Triatoma juazeirensis*, *Triatoma jurbergi*, *Triatoma klugi*, *Triatoma lectularia*, *Triatoma lenti*, *Triatoma maculata*, *Triatoma matogrossensis*, *Triatoma melanica*, *Triatoma melanocephala*, *Triatoma petrochiae*, *Triatoma platensis*, *Triatoma protracta*, *Triatoma pseudomaculata*, *Triatoma pintodiasi*, *Triatoma rubrovaria*, *Triatoma sherlocki*, *Triatoma sordida*, *Triatoma tibiamaculata*, *Triatoma vandeae*, *Triatoma vitticeps*, *Triatoma williamsi*, *Triatoma wygodzinskyi*) that were provided by the “Insetário de Triatominae,” from Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Araraquara, São Paulo, Brazil, and by the “Insetário do Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos,” from Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. The bugs were dissected, the testicles were removed, the slides were mounted through cell squashing, and stained through silver impregnation.¹⁰

The analysis of the nucleolar behavior during the meiosis of these bugs has highlighted that all species feature the nucleolar persistence phenomenon. Therefore, as the observed results were the same for all triatomines, we represent the

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FIGURE 1. Nucleologenesis of *Dipetalogaster maxima* (A–D). Note the nucleolar persistence phenomenon (C, D, arrow). Bar = 10 μ m.

nucleologenesis (Figure 1A–D) and the nucleolar persistence phenomenon (Figure 1C and D, arrow) with *D. maxima*. Our results are in agreement with the initial works carried out for the *Triatoma*,^{11–17} *Meccus*,¹⁸ *Rhodnius*,^{9,19} and *Panstrongylus*⁹ genera, which likewise have exhibited this phenomenon during spermatogenesis of these vectors.

Recently, it has been suggested that the nucleolar persistence can be essential for the spermiogenesis of these vectors, since during this differentiation stage, the nucleolus—although present—is inactive by epigenetic factors, and the chromatoid body (CB) is an essential organelle to supply all the transcriptional activity required for spermiogenesis.²⁰ Thus, it has been suggested that the nucleolus that persists during meiosis can be a structure responsible for forming the CB, because if it is active, all the transcribed RNA is directed to the formation of the CB.¹⁷

Therefore, we describe the nucleolar persistence as a synapomorphy of the triatomines and suggest that further analysis should be conducted to assess whether the persistent nucleolus is really active, thus clarifying the importance of the persistence of the nucleolus for spermatogenesis of these vectors of Chagas disease.

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