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Study of the nucleolar cycle and ribosomal RNA distribution during meiosis in triatomines (Triatominae, Heteroptera)

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Abstract

Aspects of nucleolar activity during spermatogenesis were assessed in three triatomine species (*Panstrongylus megistus*, *Rhodnius pallescens* and *Triatoma infestans*) using cytochemical and fluorescent staining techniques. Toluidine blue and a variant of critical electrolytic concentration (CEC) allowed the discrimination of rRNA providing structural details of the nucleolus and RNA distribution during meiotic cell division. Acridine orange fluorochrome staining permitted the differentiation of nucleic acids, and silver-ion impregnation made possible the observation of pre-nucleolar bodies (PNBs). Our results support the phenomenon known as ''persistence of the nucleolar material'', and the hypothesis of post-meiotic reactivation of rRNA genes. Nucleolar organizer regions (NORs) were observed in some metaphasic spermatogonial chromosomes in *P. megistus* and *T. infestans*. In *P. megistus* at diplotene–diakinesis, NORs were also detected in one of the sex chromosomes and in an autosome. Therefore, it may be inferred that, in triatomines, the nucleolus does not completely disappear, but persists in the form of small bodies that get together to form the next nucleolar cycle which, in the case of meiosis, will be completed if fertilization occurs and a new zygote is formed.

Keywords: Nucleolus; NORs; PNBs; Spermatogenesis; *Panstrongylus megistus*; *Rhodnius pallescens*; *Triatoma infestans*

1. Introduction

The nucleolus is a nuclear structure common to all eukaryotic cells, responsible for ribosome biogenesis, which is characterized by a series of events that involve the transcription of rRNA genes, the processing of pre-ribosomal RNAs, and the assembly of pre-ribosomal particles (Scheer et al., 1997).

The formation of the nucleolus is a dynamic process in cell development and is known as nucleologenesis. Under transmission electron microscopy, the nucleolus usually exhibits three major nucleolar domains: central fibrillar component (FC), dense fibrillar component (DFC), and granular component (GC) (*pars granulosa*). However, in plants and animal cell cycles, these domains are disassembled and reassembled during nucleologenesis (Ochs et al., 1985; Fakan and Hernandez-Verdun, 1986; Schwarzacher and Wachtler, 1993; Wachtler and Stahl, 1993; Medina et al., 1995; Dundr et al., 1997; Zatsepina et al., 1997; Olson et al., 2000).

The presence of the nucleolus during cell division, a phenomenon known as ''persistence of the nucleolar material'', has been observed in dynoflagellates and in malignant mammalian cells in the form of pre-nucleolar bodies (PNBs). The persistence of PNBs during mitosis and meiosis supports the idea that the nucleolar material is not completely disrupted during metaphase and anaphase. However, the functional significance of this finding remains unclear. It may be hypothesized that these bodies carry ''primary'' or ''new'' material or even represent a source of nucleolar RNA for the daughter cells while the new nucleolus is being organized (Wachtler and Stahl, 1993; Mello, 1995).

Several studies have attempted to characterize pre-nucleolar bodies, which have been described as dense fibrogranular structures of variable size and shape containing RNA and acidic proteins (B23, C23, No38) detectable by silver impregnation (Ochs et al., 1985; Flechon and Kopecny, 1998). According to Medina et al. (1995), resumption of the pre-ribosomal RNA synthesis in daughter cells during telophase is necessary for the aggregation of previously formed PNBs in the nucleolar organizer region (NOR), so that the nucleolus is reorganized.

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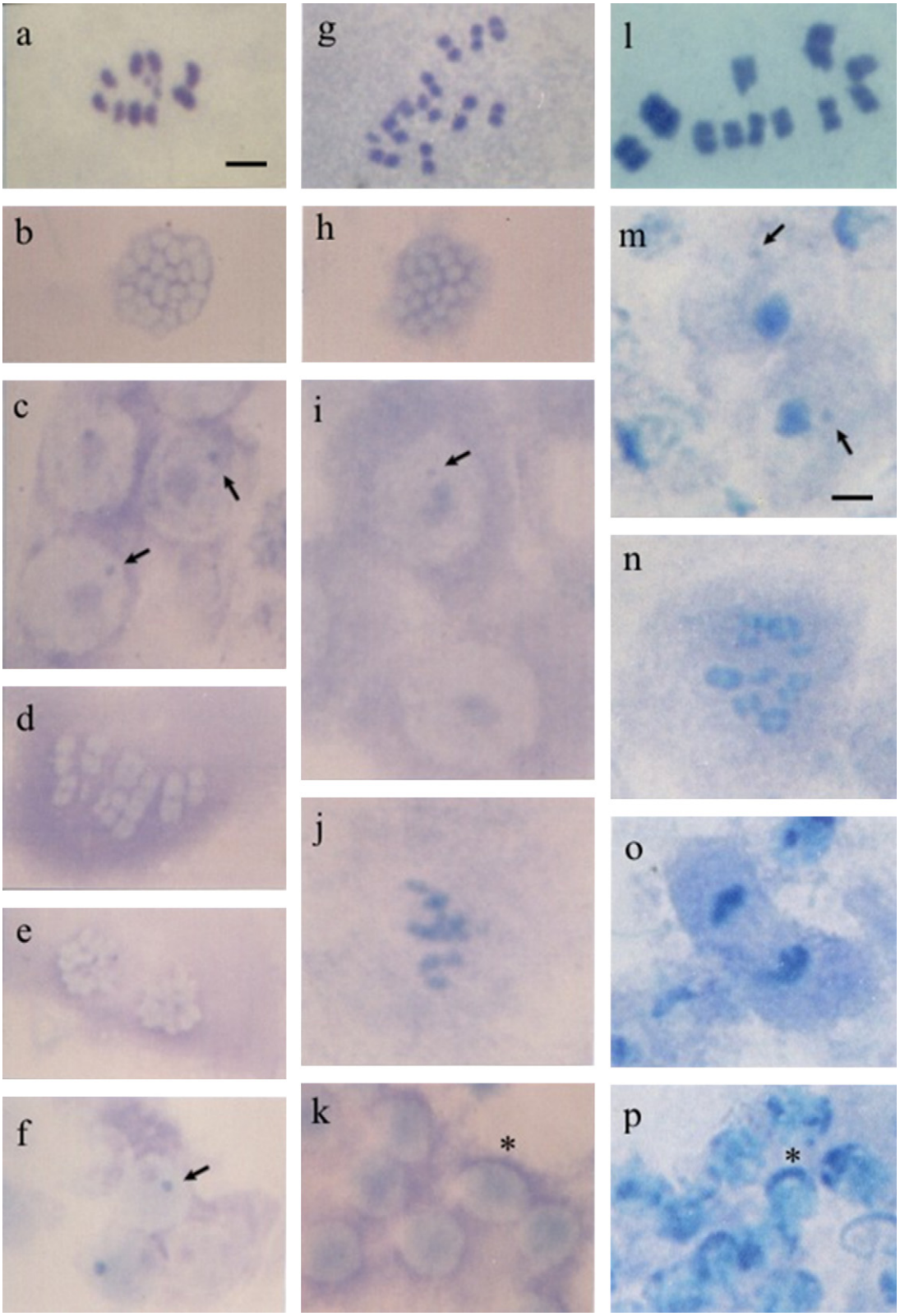

Fig. 1. *Panstrongylus megistus* (a–f), *Rhodnius pallescens* (g–k) and *Triatoma infestans* (l–p) seminiferous tubules submitted to the staining techniques: toluidine blue (TB) (a, g, l) and a variant of CEC (b–f, h–k, m–p). MI (g, l) and MII (a) chromosomes were metachromatic. In cells submitted to the CEC variant, RNA stained either blue or violet and DNA stained green. Spermatogonial metaphases (b, h). Nuclei at meiotic prophase I (c, i, m), arrows indicate nucleolar bodies. First meiotic division metaphases (d, j, n). Meiotic anaphase (o). Meiotic telophase (e). Spermatids at early spermiogenesis (f, k, p) the arrow and asterisks indicate RNAr persistence. Bars = 10 μm (a–l) and 5 μm (m–p).