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Contribution of the golgi Complex—Endoplasmic reticulum system during spermiogenesis in three species of phytophagous bugs (Hemiptera : Pentatomidae)

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Abstract

The participation of the Golgi complex—endoplasmic reticulum system during spermiogenesis in 3 species of phytophagous bugs (Acrosternum aseadum, Euchistus heros and Nezara viridula) were analysed at the ultrastructural level, after postfixation with osmium tetroxide\potassium iodide. Intense staining was found in the nuclear envelope, endoplasmic reticulum cisternae, Golgi complex, and in a meshwork of tubules scattered throughout the cytoplasm. It is shown that these compartments present a reducing environment. The presence of this kind of staining on the spermatid suggests an important participation of these structures on the differentiation process of this cell.

Keywords: Cell differentiation; potassium iodide; trasmission electron microscopy; osmium impregnation; Acrosternum aseadum; Euchistus heros; Nezaraviridula

1. Introduction

The spermatozoa are very specialized and highly differentiated cells. They have lost various organelles essential to cell metabolism, while the remaining organelles are modified in a manner unparalleled in other processes of cell differentiation. The main compartment of a typical insect spermatozoon are the head, containing the nucleus and acrosome, and the tail, which contains the axoneme and mitochondrial derivatives (for reviews, see Phillips, 1970; Baccetti, 1972).

The sperm nucleus development is characterized by 2 phenomena : the change from a spherical to a highly asymmetric configuration, and the chromatin conversion from a dispersed to a very condensed state (Fawcett et al., 1971; Tokuyasu, 1974). The acrosome, rich in hydrolases, is covered by the acrosomal and plasma membranes (Baccetti, 1972; Báo et al., 1989).

The sperm tail is characterized by a 9+9+2 microtubular pattern in the axoneme, flanked by 2 mitochondrial derivatives containing crystalline material (Phillips, 1970). Heteropteran spermatozoa have certain characteristics in common that are not found in other insects : 2–3 crystalline bodies within the mitochondrial derivatives and bridges between the mitochondrial derivatives and 2 of the axonemal microtubular doublets (Afzelius et al., 1976; Dallai and Afzelius, 1980; Dolder, 1988; Báo and de Souza, 1994a).

Scanty information is available on the Golgi complex—endoplasmic reticulum system of insects germ-cells. Cytochemical data in spermatids of mosquitoes showed the presence of acid phosphatase activity, demonstrating the participation of these compartments in the differentiation process of spermatids (Ndiaye and Mattei, 1992; Báo and de Souza, 1994b).

In the present study, we analyse at the ultrastructural level the morphology of the Golgi complex—endoplasmic reticulum system during spermiogenesis of three species of phytophagous bugs after postfixation of the cells with OsO4\KI.

2. Materials and methods

The insects utilized were male adults of the phytophagous bugs Acrosternum aseadum, Euchistus heros, and Nezara viridula (Hemiptera : Pentatomidae), obtained from a colony maintained in the National Center of Genetic Resource (CENARGEN), Brasília—Brazil.

2.1. Transmission electron microscopy

The testes were dissected and fixed for 2 h in a solution containing 2.5% glutaraldehyde, 4% paraformaldehyde, 3% sucrose and 5 mM CaCl2 in 0.1M cacodylate buffer, pH 7.2. After fixation, the specimens were rinsed in buffer, and postfixed in 1% osmium tetroxide, 0.8% potassium ferricyanide, and 5 mM CaCl2 in the same buffer. The material was dehydrated in acetone and embedded in Spurr resin.

2.2. Osmium tetroxide\potassium iodide impregnation

The testes were fixed for 2 h with 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2, washed once in the same buffer, twice in a solution of 1% potassium iodide (KI) in distilled water and then left for 48 h at room temp. in the dark in a 1% OsO4–1% KI solution (Locke and Huie, 1983). Thereafter, the specimens were washed with 1% KI in distilled water, dehydrated in acetone and embedded in Spurr resin.

Ultrathin sections, unstained or stained with uranyl acetate and lead citrate, were observed by transmission electron microscope.

3. Results

Spermiogenesis in insects involves nuclear elongation, chromatin condensation, acrosomal formation, and flagellar development, along with formation of the axoneme as well as mitochondrial derivatives. This process involved the Golgi complex—endoplasmic reticulum system.

During the early spermatid phase, the nucleus resembles that of a somatic cell. The surrounding cytoplasm contained a well-developed Golgi complex and mitochondrial complex (Fig. 1A). The spermatid axoneme contained a variety of tubular elements that persisted in the spermatozoon. There were 9 accessory microtubules followed by 9 doublets and a central pair of microtubules (Fig. 1B). Two mitochondrial derivatives, containing 2 or 3 paracrystalline structures, flanked the axoneme (Fig. 1B).

In the early spermatid, a single Golgi complex close to the proacrosomal granule was observed (Fig. 1C). Occasionally, it appeared near the flagellar components (Fig. 1D). Although the Golgi complex typically appeared as a flattened structure, circular and U-shaped patterns were also frequently observed (Fig. 1 C, E). It is made up of 8–10 saccules, with the central saccule showing a uniform thickness and electron-dense contents; it is not fenestrate, which clearly differentiates it from the other saccules.

An elongated cisterna of the endoplasmic reticulum was associated to the cis face of the Golgi complex; several uncoated vesicules were found between the endoplasmic reticulum and the first Golgi cistern, forming a cis Golgi network (Fig. 1E). A trans Golgi network formed by numerous small vesicles and tubules could also be found (Fig. 1E).

Prolonged postfixation with osmium tetroxide for 48 h in the presence of potassium iodide resulted in an effective electron-dense staining of specific cell compartments : the nuclear envelope, the Golgi complex, as well as a meshwork of short and long tubules scattered throughout the cytoplasm (Fig. 2A). Association of such tubular profiles with the nuclear envelope, the Golgi complex, and flagellar structure allowed us to identify them as endoplasmic reticulum cisternae (Fig. 2C). No reaction was observed in proacrosomal granule and the plasma membrane (Fig. 2D).



Fig. 1 (A) *Spermatid of Euchistus heros* in the initial stage of differentiation. The nucleus (N) seems like of a somatic cell; the cytoplasm contains a developed Golgi complex (Gc) and mitochondrial complex (Mc) 13,000x Transverse section of flagellar region of *Nezara viridula* spermatozoon, showing the axoneme (Ax) with 9+9+2 microtubule pattern, 2 mitochondrial derivatives (Md) containing 2 paracrystalline structures (arrowhead). Microtubules (Mt). 70,000x. (c) Early spermatid of N. *viridula showing* a single Golgi complex (Gc) close to the proacrosomal granule 9 (Pa) Nucleus (N)17,000 (D) Transverse section of flagellar region of *E. heros* spermatozoon showing a Golgi complex (Gc) near the flagellar structures. Axoneme (Ax) mitochondrial derivatives (Md).32,500×.(E) Spermatid of *N viridula* showing a cisterna of the endoplasmic reticulum (Er)associated to the cis face (arrowhead) of the Golgi complex (Gc). A trans Golgi network (asterisk) could also be seen[Nucleus (N)33,000x.



Fig. 2. Spermatids after a prolonged postfixation with osmium tetroxide in the presence of potassium iodide. in medial Golgi cisternae (asterisk). Axoneme (Ax); Mitochondrial derivatives (Md). 42,000×. (E) Spermatid of N. viridula showing positive reaction on the cis Golgi cisternae (C), as well as the trans Golgi network (T) and uncoated vesicles (arrowhead) associated to this region. 42,000×.

The Golgi complex compartments present a differentiated impregnation. The cis network and the cis Golgi cisternae, recognized by their close association with endoplasmic tubules, presented a strong positive reaction (Fig. 2 D, E), as well as the trans Golgi network, and uncoated vesicles associated with this region (Fig. 2E). On the other hand, reaction in the medial and trans Golgi cisternae was usually absent (Fig. 2C).

Background staining was observed as finely granular deposits scattered throughout the cytoplasm.

4. Discussion

Heteropteran spermatozoa have common characteristics, which are not found in other insects : 2–3 crystalline bodies within the mitochondrial derivatives and bridges between the mitochondrial derivatives and 2 of the axonemal microtubules (Afzelius et al., 1976; Afzelius et al., 1985; Dallai and Afzelius, 1980; Dolder, 1988; Báo and de Souza, 1994a). But, like other insects, the heteropteran spermatozoa possess a head containing nucleus and acrosome, and the tail, which, contains axoneme and mitochondrial derivatives.

Previous studies indicate that the acrosome of insects, as in higher animals, is formed by the Golgi complex (Phillips, 1970; Baccetti, 1972; Yasuzumi, 1974). In the majority of insects, where this process has been studied, the formation of a spherical body, the proacrosomal granule, occurs on the concave side of the Golgi complex. This vesicle, found in early spermatids, between the Golgi complex and nucleus, is gradually modified taking on a characteristic shape in the last stages of spermiogenesis (Báo et al., 1989; Ndiaye and Mattei, 1992). The association of an endoplasmic reticulum cisterna with the cis-face of the Golgi apparatus, as in secretory cells, is well known, and is also common during acrosome formation (Baccetti, 1975; Werner, 1989) that occurs simultaneously with nuclear transformation and flagellar formation in a typical insect spermiogenesis.

In this study, we used osmium tetroxide–potassium iodide (OsO4–KI) to show the Golgi complex—endoplasmic reticulum system of spermatids during differentiation process. The impregnation with OsO4–KI technique consists of the reduction of osmium tetroxide to osmium black after several hours of incubation, by combining osmium with labile S-S bridges present in newly made proteins, before they reach tertiary structure (Locke and Huie, 1983).The essential requirements for consistent staining of the vacuolar system, are osmium tetroxide in the presence of iodide. OsO4–KI acts slowly to break dissulfide bridges via the formation of osmium blacks, than the reaction required several hours at room temperature to produce the kind of staining shown.

This staining in Golgi complex suggests that these compartments have a reducing environment able to reduce osmium in the presence of potassium iodide; this result is also consistent with the fact that the spermatids are in a phase of differentiation, thus needing a continuous supply of proteins for this process. Indeed, it allows to see the participation of Golgi complex—endoplasmic reticulum system on the formation of acrosome and other structures like membranes surrounding the nucleus, the flagellar components and the axoneme. The staining of other structures on spermatic cell confirms the idea that all structures, except nucleus, mitochondria and axoneme, are derived from the Golgi complex—endoplasmic reticulum system (Baccetti, 1975).

Since Bowen, 1924classical studies, the role of the Golgi complex in the formation of the acrosome is well established. A great number of electron microscopical investigations, beginning with Burgos and Fawcett, 1955, have extended our knowledge at the ultrastructural level (Werner, 1989). The Golgi complex is present during almost the entire period of spermatogenesis. Its prominent role, although restricted to early spermatid stages, is the formation of the acrosome (Werner and Werner, 1991). Previous studies have shown that the Golgi complex is fundamental to acrosome formation in Diptera (Perotti, 1969; Warner, 1971; Baccetti, 1972; Báo et al., 1989; Ndiaye and Mattei, 1992), as well as in Chrysomelidae (Báo, 1996) and Homoptera (Báo et al., 1997).

The acrosome may be considered with a secretory granule and a lysosome (Birns and Masek, 1961; Allison and Hartree, 1970); its formation serves as the first example of the role of the Golgi complex in the production of a 'primary lysosome (Goldfischer, 1982). The mammalian sperm acrosome, a membrane-bounded organelle in the sperm head, is derived from vesicles formed in the Golgi apparatus, as are primary lysosomes and secretory granules of somatic cells (Randall and Meizel, 1981).

The most important functions of the Golgi complex are the transport and terminal glycosylation of glycoproteins coming from the endoplasmic reticulum and destined to secretion granule, lysosomes and plasma membrane (Palade, 1975; Mellman and Simons, 1992). Recent studies on the Golgi complex of various cell types have shown the importance of the elements on the trans face of the stacks of saccules in the terminal glycosylation of proteins and in sorting and directing glycoproteins toward their destinations (Roth et al., 1985; Rothman, 1985; Griffiths and Simons, 1986; Taatjes and Roth, 1986; Thorne-Tjomsland et al., 1988). Golgi complex transition vesicules and saccules maintain an environment that is independent of the secretory material passing through them, it is detectable by OsKI staining (Locke and Huie, 1983).

When a routine fixation was used, only few profiles of endoplasmic reticulum could be observed, dispersed throughout the cytoplasm. Nevertheless, using osmium–KI impregnation we observed several profiles throughout the cytoplasm. This fact leads us to consider this technique as a powerful tool to study the distribution and participation of Golgi complex endoplasmic reticulum system on spermiogenesis of hemipteran insects. Acknowledgements

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