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Source: Zoological Science, 15(1) : 93-96

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.93

ZOOLOGICAL SCIENCE **15**: 93–96 (1998)

Multilaminar/Vesicular Bodies Accumulating Glycoconjugates in Primary Spermatocytes of Cricket, *Gryllus bimaculatus*

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ABSTRACT—Lectin cytochemistry was carried out on thin sections of 6th-instar cricket testis using two GalNAc-specific lectins, *Dolichos biflorus* agglutinin (DBA) and *Helix pomatia* agglutinin (HPA), and the binding sites in primary spermatocytes were surveyed. Gold particles showing DBA-binding are observed specifically in dense-body clusters. These bodies are about 100-300 nm in diameter and exhibit multilaminar or multivesicular structure. HPA can bind to the dense-body clusters and another kind of larger multivesicular structures. These bodies seem to contain some heterogeneous substances, and sometimes show an autophagosome-like structure. The ultrastructures of these organelles surveyed in conventional Epon sections confirmed the structures of these multilaminar/vesicular bodies in cricket spermatocytes, which may play certain roles in intracellular circulation and degradation of glycoconjugates.

INTRODUCTION

Spermatogenesis is an excellent example of cell differentiation involving dramatic changes in morphological and biochemical aspects. Various stage-specific proteins are known in this process (Myles, 1993), and evidence for their glycosylation in some developmental changes has been noted (Maylie-Pfenninger, 1994; Millette and Scott, 1984). We are interested in how sugar chains are expressed in the entire process of the spermatogenesis. Our previous study on the male germ cells of crickets by mean of lectin cytochemistry at light microscopical level showed the developmental changes of several kinds of glycoconjugates (Suzuki and Nishimura, 1995, 1997). Among them, we took notice of the early-spermatocyte-specific glycoconjugates recognized by GalNAc-specific lectins, especially *Dolichos biflorus* agglutinin (DBA) (Suzuki and Nishimura, 1997). The expression of these molecules was temporal and disappeared in later stages. Preliminary cytochemical analysis at the electron microscopical level using a GalNAc-specific lectin, soybean agglutinin (SBA), revealed a cluster of dense bodies with SBA-binding activity (Suzuki and Nishimura, 1995). In the present study we attempted further to locate the lectin-binding sites in the primary spermatocytes using two GalNAc-specific lectins, DBA and *Helix pomatia* agglutinin (HPA). We ascertained that the dense-body cluster accumulates glycoconjugates which are recognized by DBA and HPA. Very large bodies as well accumulate HPA-binding activity. These organelles are considered to play a crucial role in the intracellular circulation and degradation process of glycoconjugates.

MATERIALS AND METHODS

Crickets, *Gryllus bimaculatus*, were reared on an artificial mouse diet (MF; Oriental Yeast Co. Ltd., Tokyo). Testes of 6th-instar animals were fixed in picric acid-paraformaldehyde solution (Stefanini *et al.*, 1967) overnight at 4°C. After fixation tissues were washed with 50 mM NH_4Cl in PBS for 30 min. The specimens were dehydrated in ethanol and embedded in Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, Germany) at 4°C. Ultrathin sections were mounted on collodion-coated nickel grids and treated by the following procedure for lectin cytochemistry.

Two *N*-acetylgalactosamine-specific lectins conjugated with biotin were used in this study: *Dolichos biflorus* agglutinin (DBA, purchased from Vector Laboratories, Inc., Burlingame, CA, USA) and *Helix pomatia* agglutinin (HPA, purchased from E-Y laboratories, Inc., San Mateo, CA, USA). Ultrathin sections on nickel grids were floated on a drop of 50 mM Tris-HCl buffered saline, pH 7.4 (TBS) for 5 min, then transferred on a drop of TBS containing 1% bovine serum albumin (BSA-TBS) for 15 min to block the non-specific sites. Grids were then incubated with biotinylated lectin diluted in BSA-TBS (10 μg/ml) for 1 hr, washed with TBS, and then incubated with anti-biotin antibody conjugated with 15 nm-gold particle (BioCell Research Laboratories, Cardiff, UK) diluted in TBS (1:20) for 30 min. After gold-labelling, they were washed successively with TBS and distilled water and dried. All incubations were carried out at room temperature. Thin sections were counterstained with uranyl acetate (5 min) and lead citrate (30-45 sec), and observed in a JEOL 100S electron microscope operated at 80 kV. In control experiments, biotinylated lectins were preincubated with 0.1 M of *N*-acetylgalactosamine for 30 min before incubation with the sections. Reduced binding of lectins were observed in these sections.

For conventional electron microscopic analysis, cricket testes were fixed in 2.5% glutalaldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 hr and post-fixed in 1% OsO_4 for 1 hr at room temperature. They were dehydrated through a graded series of ethanol and embedded in Epon. Ultrathin sections were mounted on copper grids and stained with uranyl acetate (10 min) and lead citrate (5 min).

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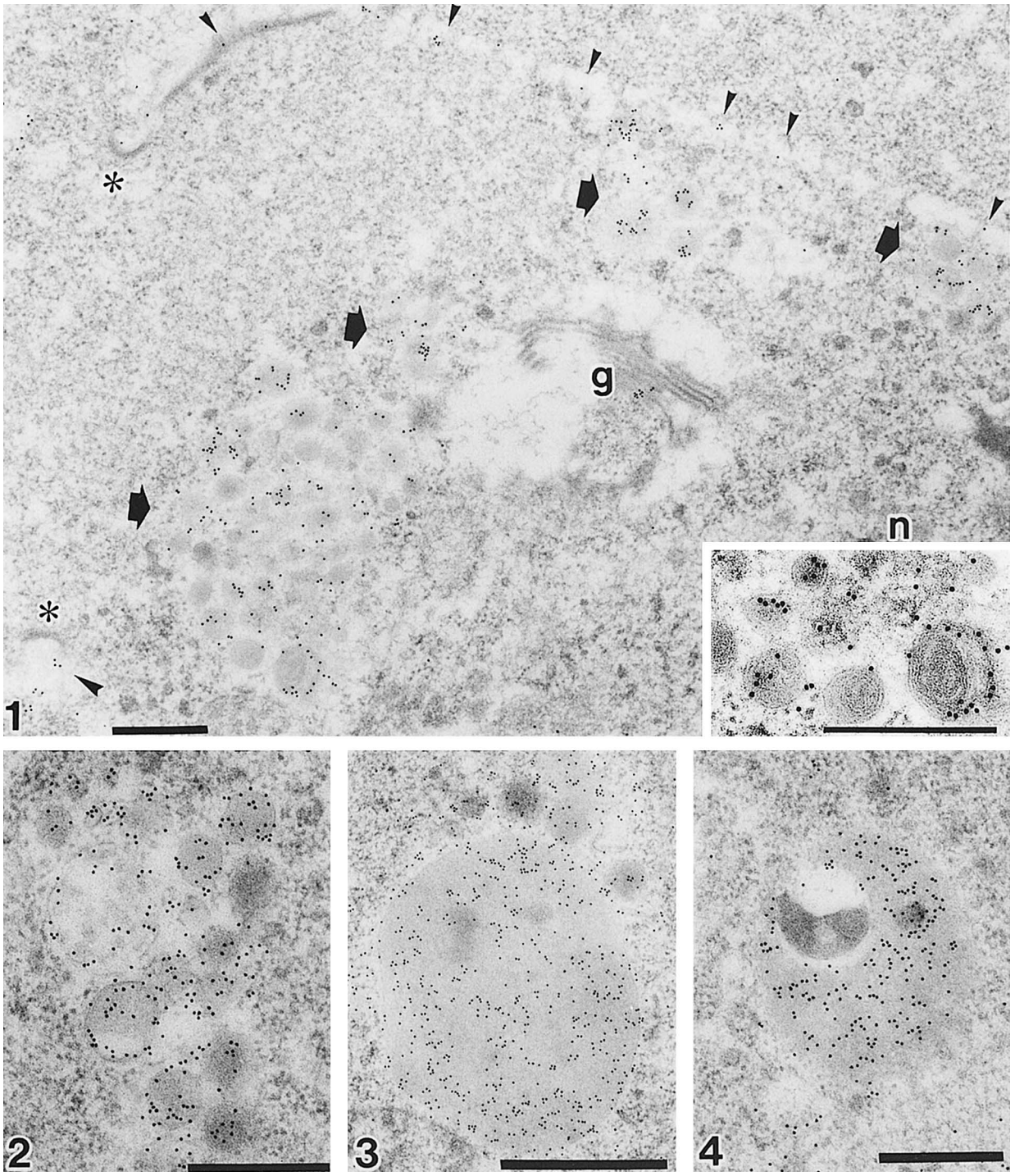


Figs. 1–4. Sections of early primary spermatocytes labeled with biotinylated lecins, DBA (Fig. 1) and HPA (Figs. 2–4), and 15 nm-gold conjugated anti-biotin antibody.

Fig. 1. Gold particles showing DBA-binding are conspicuous in the dense bodies (arrows). Many vesicular bodies gather to form clusters. Multilaminar or multivesicular structures are found in these bodies. DBA-gold also bind to the stacks of Golgi apparatus (g) and cell membrane (arrow heads). Insignificant signals are found in the nucleus (n) nor on its membrane. The intercellular bridge are found between asterisks, at the sides of which the thickened structures are observed. The inset shows a part of dense-body cluster at higher magnification. Scale bars, 500 nm.

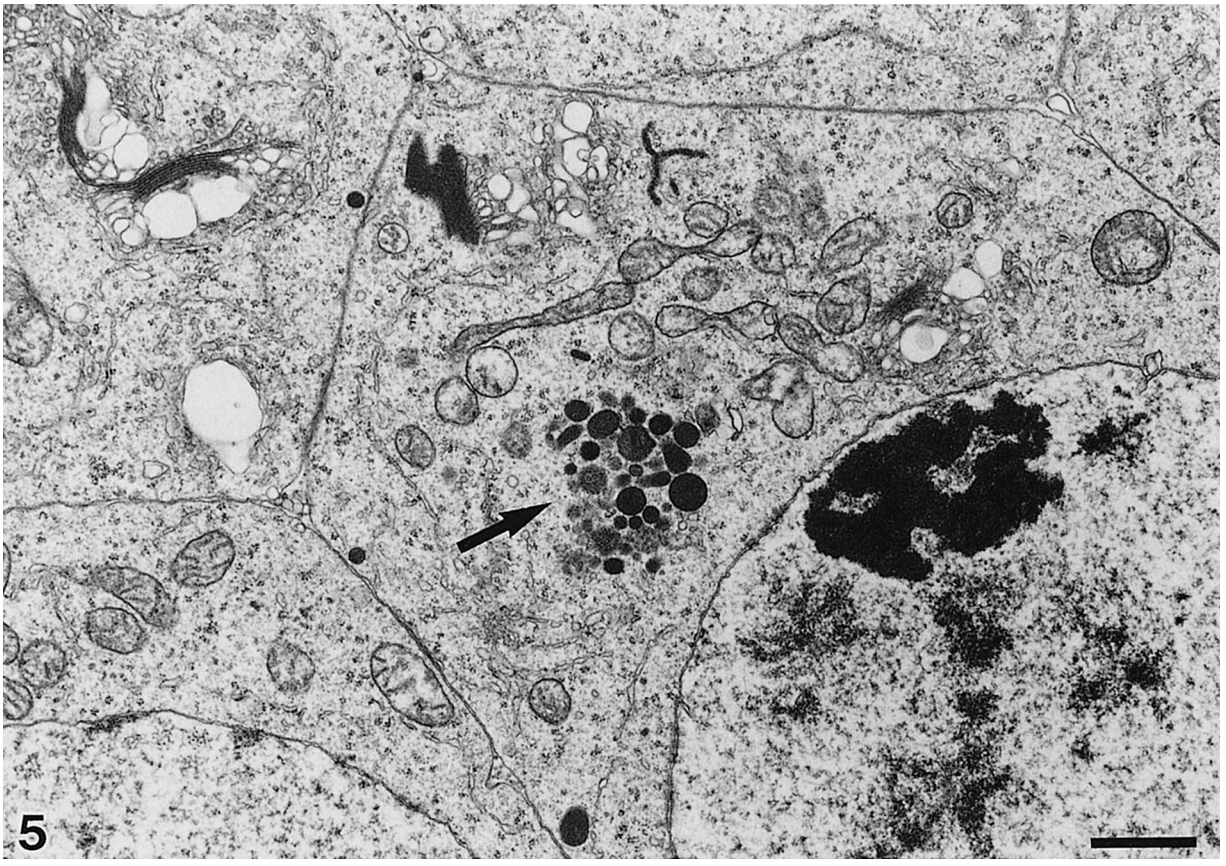
Fig. 2. An HPA-labeled dense-body cluster. Multilaminar structures are found in small dense bodies, while the largest body among this cluster exhibits obvious multivesicular structure. Scale bar, 500 nm.

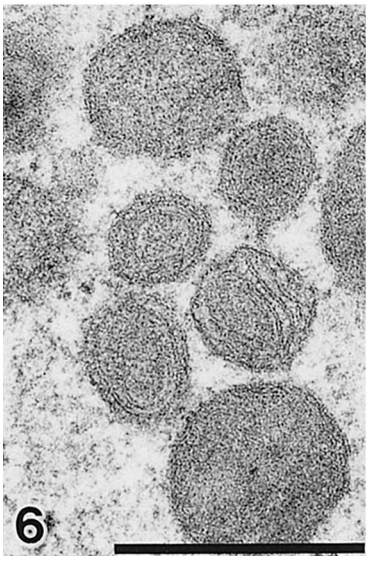
Fig. 3. Large multivesicular structure accompanied with several small bodies. Scale bar, 1 μm.

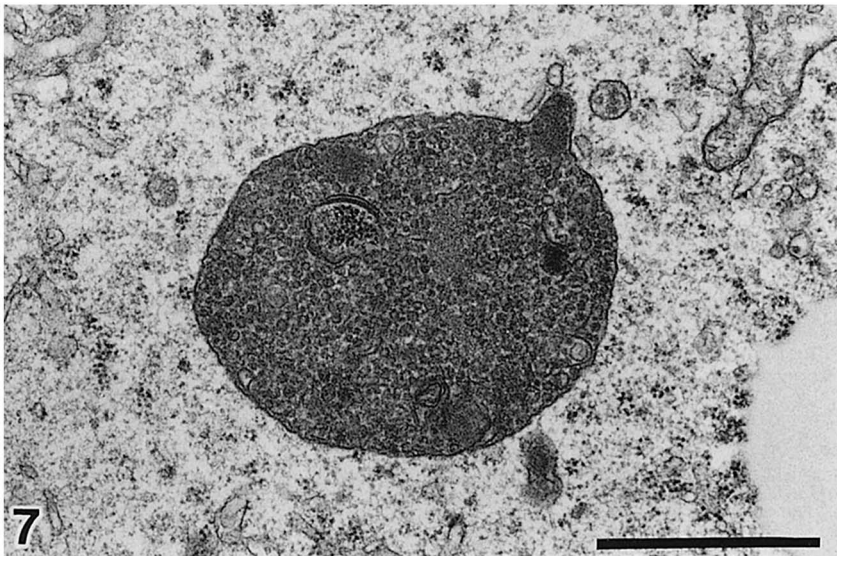
Fig. 4. An autophagosome-like body labeled with HPA-gold. The large inclusion showing a dense crescent are not labeled. Scale bar, 500 nm.

RESULTS

Lectin cytochemistry was carried out on thin sections of 6th-instar cricket testis and the reactivity was surveyed in its early stages of primary spermatocytes. As shown in Figs. 1–4, several sizes of electron-dense bodies are labeled by GalNAc-binding lectins. Gold particles showing DBA-binding are observed specifically in dense-body clusters (Fig. 1). These bodies are about 100-300 nm in diameter and exhibit multilaminar or multivesicular structure. Weak binding of DBA-gold is observed as well on cell membranes and Golgi stacks. Another GalNAc-specific lectin, HPA, can bind to the dense-







Figs. 5–7. Epon sections of early primary spermatocytes.
Fig. 5. A cluster of many dense bodies (arrow) is conspicuous at the center of this picture. Scale bar, 1 μm.
Fig. 6. Relatively small bodies often exhibit multilaminar structures. Scale bar, 500 nm.
Fig. 7. A large multivesicular body fused with a smaller vesicle. Highly heterologous contents are seen in this large dense body. Scale bar, 1 μm.

body clusters (Fig. 2) and very large multivesicular structures (Figs. 3, 4). The example of them shown in Fig. 3 reaches up to 2 μm in diameter. It is not clear whether DBA also bind these structures, although a few gold particles have been observed in some sections with back-ground like labelling. These bodies seem to contain some heterogeneous substances showing different degrees of electron density. Several small multilaminar bodies with gold particles are often found together with the larger body (Fig. 3). Figure 4 shows an autophagosome-like body with HPA-binding activity. The electron-dense crescent-shaped body in this large body are not labeled with HPA-gold.

The ultrastructures of above mentioned organelles were surveyed more precisely in conventional Epon sections (Figs. 5–7). Clusters of electron-dense bodies found in primary spermatocytes exhibit similar characteristics as those observed in the specimen for lectin cytochemistry (Fig. 5). These bodies exhibit multilaminar or multivesicular structure (Fig. 6). Very large multivesicular bodies are also observed (Fig. 7). Such large bodies contain various vesicular structures and multilaminar bodies. Figure 7 seems to show the fusion of a small body with the larger one.

DISCUSSION

Evidence of the alteration of lectin-binding pattern in the early stages of the primary spermatocytes of cricket (Suzuki and Nishimura, 1997) attracted our interest into the cytochemical localization of such lectin-binding molecules. Although little has been reported in male germ cells about the intracellular circulation of glycoconjugates, some distinctive organelles should participate in such a cellular event.

The present lectin cytochemical analysis uncovered several vesicular bodies accumulating glycoconjugates specific to GalNAc-lectins. These structures were frequently found as a cluster of electron-dense bodies. Although the existence of such clusters have long been known as Y-granules in male germ cells (Gatenby, 1922; Gatenby and Beams, 1935), their function and origin are still unknown. But the evidence of the accumulation of certain glycoproteins, which may have either complex- or hybrid type *N*-linked oligosaccharide with terminal α-*N*-acetylgalactosamine residue (Suzuki and Nishimura, 1997), in the dense-body cluster suggests some function of this organelle in the processing of glycoprotein. This possibility is also confirmed by the morphological observation. These glycoprotein-containing bodies often show multilaminar structures. Similar ultrastructure was reported in recent papers, for example, in the endocytic compartment known as MHC class II compartment (MIIC) (Calafat *et al*., 1994; Peters *et al*., 1991). MIIC has a multilaminar structure with the lysosomal proteins and serves on the way between endosome and lysosome. It has been also known that the multilaminar or multivesicular ultrastructures often found in the autophagic vacuoles participate in the protein degradation mechanism (Seglen and Bohley, 1992). Thus we have an idea that the multilaminar/vesicular bodies in cricket spermatocytes may play a similar role in the protein processing mechanism. The weak binding of DBA on the Golgi stacks and cell surfaces implies one of the possible routes of the vesicular bodies. Although the precise nature of such molecules labeled by DBA-gold has yet to be elucidated, their early primary spermatocyte-specific expression suggests its contribution to some characteristic cellular event at this stage.

We also found larger multivesicular bodies containing glycoconjugates recognized by HPA. These large bodies include various heterologous structures and their morphological characteristics suggest their lysosomal nature. This may be one of the possible structures participating in the protein degradation process of cricket spermatocytes. The morphological resemblance between the structure shown in Fig. 7 and yolk granules also remind us of the description of male-yolk by Gatenby (Gatenby, 1922).

ACKNOWLEDGMENTS

We are grateful to Dr. M. Obika and Dr. Y. Nakajima, Keio University, for their helpful advice and discussions on electron microscopy.

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(Received October 12, 1997 / Accepted November 19, 1997)