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# PRELIMINARY LOCALIZATION OF A CELL TYPE-SPECIFIC ANTIGEN IN THE ACCESSORY GLANDS OF THE MALE MEALWORM BEETLE BY IMMUNOELECTRON MICROSCOPY

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The reproductive accessory gland complex of male Tenebrio molitor consists of two sets of paired glands: the bean-shaped (BAGs) and the tubular-shaped accessory glands (TAGs).[1] Unlike the TAG, which contains a single cell type, the BAG contains at least seven cell types differentiated by the ultrastructure of their secretory granules. The BAG also synthesizes a wider variety of proteins.[2] As the gland becomes mature, granules are secreted into the lumen of the BAG, forming the plug, and are molded into the spermatophore, a complex structure used to transmit sperm to the female during mating. Up to this point it has been difficult to correlate individual protein species with specific granule types. Therefore, we have turned to monoclonal antibody technology and immunoelectron microscopy to investigate the production, localization and fate of BAG and spermatophore proteins.

Monoclonal antibodies were made against plug antigens.[3] Spleen cells from mice immunized with secretory plug material were fused with SP2 myeloma cells. The resulting hybridomas were selected in HAT media and were screened and characterized by enzyme-linked immunosorbant assay (ELISA) and immuno-electroblotting. Positive cell lines were cloned and injected into mice for antibody-containing ascites fluid. One recovered clone (PL 6.3) secretes antibody against a pair of antigens (MW=100Kd). The adult-specific antigens were identified in BAG, in secretory plug, and in spermatophore homogenates. Examination of immunochemically stained paraffin-embedded sections revealed that the antigens were restricted to cell type 7.

We used immunoelectron microscopy to determine the sub-cellular localization of PL 6.3 antigen. BAGs from mature males were fixed in 4% paraformaldehyde in phosphate buffer pH 7.2 for 1 hr, dehydrated through alcohol and embedded in Epon. Thin sections (silver) were cut on a Reichert OM-U2 microtome and collected on nickel grids. Sections were etched in 5% $H_2O_2$ for 10 min. After incubation in blocking sera, sections were incubated in primary antibody (diluted 1/20) for 30 min in anti-mouse IgG avidin-biotin peroxidase conjugate for 60 min (Vectastain, Vector Labs), and were stained with DAB for 10 min.[4] Controls for immunohistochemical study consisted of treatment with pre-immune sera, and omission of primary or secondary antibody (Fig. 2). Sections were examined on a Philips 201 at 40 kV.

The antigens recognized by antibody PL 6.3 were located primarily within the secretory granules of cell type 7 and rarely within the cytoplasm of the BAG cell (Fig. 3 and 4). Although perfect morphological integrity was not maintained by the fixation protocol necessary to preserve antigenicity, examination of adjacent thin sections stained with uranyl acetate and lead citrate confirmed granule type identification (Fig. 1). This antibody, and others currently under development will provide tools for mapping granule movement and modification in BAG development and in spermatophore formation.



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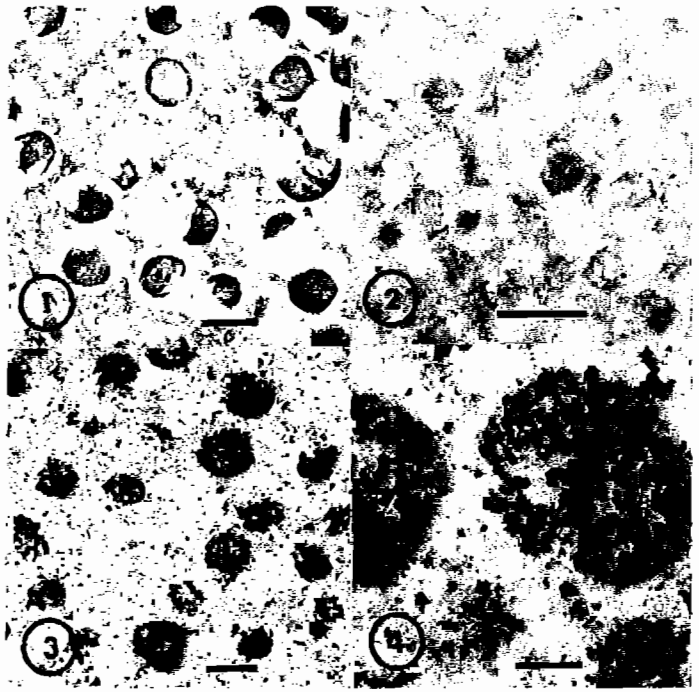


Fig 1-4 Section of BAG containing cell type 7 granules.
1. Stained with uranyl acetate and lead citrate. Bar = 0.5 μm.
2. Stained with avidin-biotin peroxidase (ABP) complex without primary antibody. Bar = 1.0 μm.
3. Stained with monoclonal antibody PL 6.3 and ABP complex. Bar = 0.5 μm.
4. Highly magnified view of type 7 granules showing localization of ABP complexes. Bar = 0.25 μm.