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CELL TYPE-SPECIFIC ANTIGENS IDENTIFIED WITH MONOCLONAL ANTIBODIES IN THE ACCESSORY GLANDS OF MALE MEALWORM BEETLES

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INTRODUCTION

The accessory gland complex of Tenebrio molitor consists of two sets of paired glands: the bean-shaped (BAG) and the tubular glands (TAG) (Fig. 1). These glands undergo a change in competence correlated with the mid-pupal ecdysterone peak. After eclosion, the BAG develops a complex pattern of eight cell types (Dailey et al., 1980), and produces at least 40 new proteins. Many of these proteins are secreted and formed into the spermatophore, a multilayered structure used to transmit sperm to the female during mating (Happ, 1984). We are interested in identifying cell type-specific antigens, and following their production, secretion and eventual role in spermatophore formation.

Monoclonal antibodies were generated against BAG secretory masses (plugs) and screened against Towbin electroblots of accessory gland proteins. Immunoelectron microscopy was used to determine the sub-cellular localization of antigen by the avidin-biotin peroxidase complex (ABC) technique. Methods used for these studies are reported in Black and Happ (1985) and Bricker et al., (1985).

RESULTS

Two clones were recovered which recognized proteins specific to the BAGs. One antibody (PL 3.4) recognized a single protein with two allelic forms (29Kd and 27.5Kd MW), which was secreted into the plug with an apparent loss of 4Kd MW (Fig. 2a). A second antibody (PL 6.3) recognized a 9.5Kd protein and a 5Kd breakdown product (Fig. 2b). Both

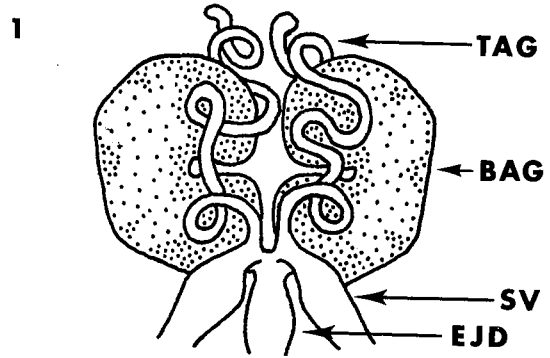

Figure 1. Accessory glands of Tenebrio molitor. EJD = ejaculatory duct, SV = seminal vesicle

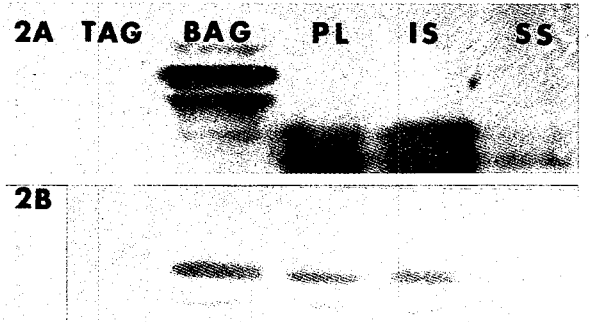

Figure 2. Immunoblot of accessory tissues. A = PL 3.4, B = PL 6.3, PL = plug, IS = insoluble spermatophore, SS = soluble spermatopore.

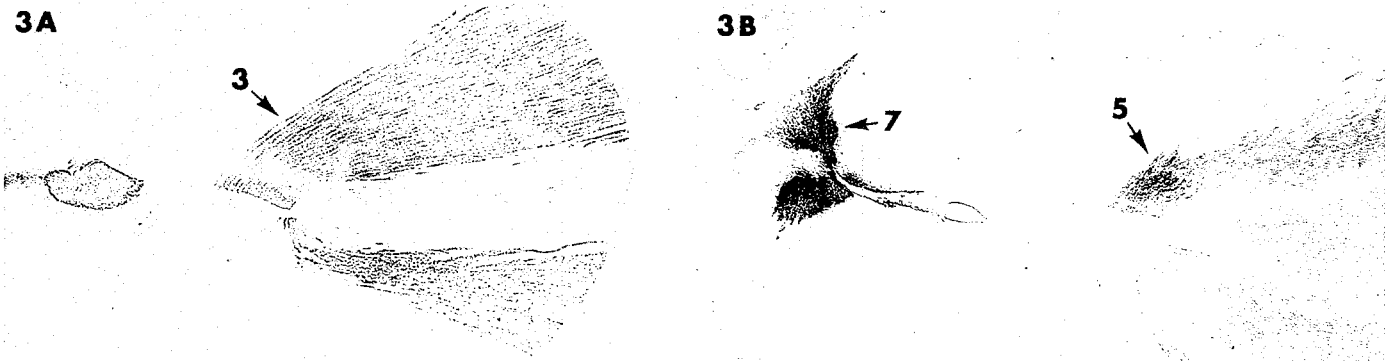

Figure 3. Immunohistochemical localization of (A) PL 3.4 antigen to cell type 3 (400X) and (B) PL 6.3 antigen to cell types 5 and 7 of the BAG (300X).

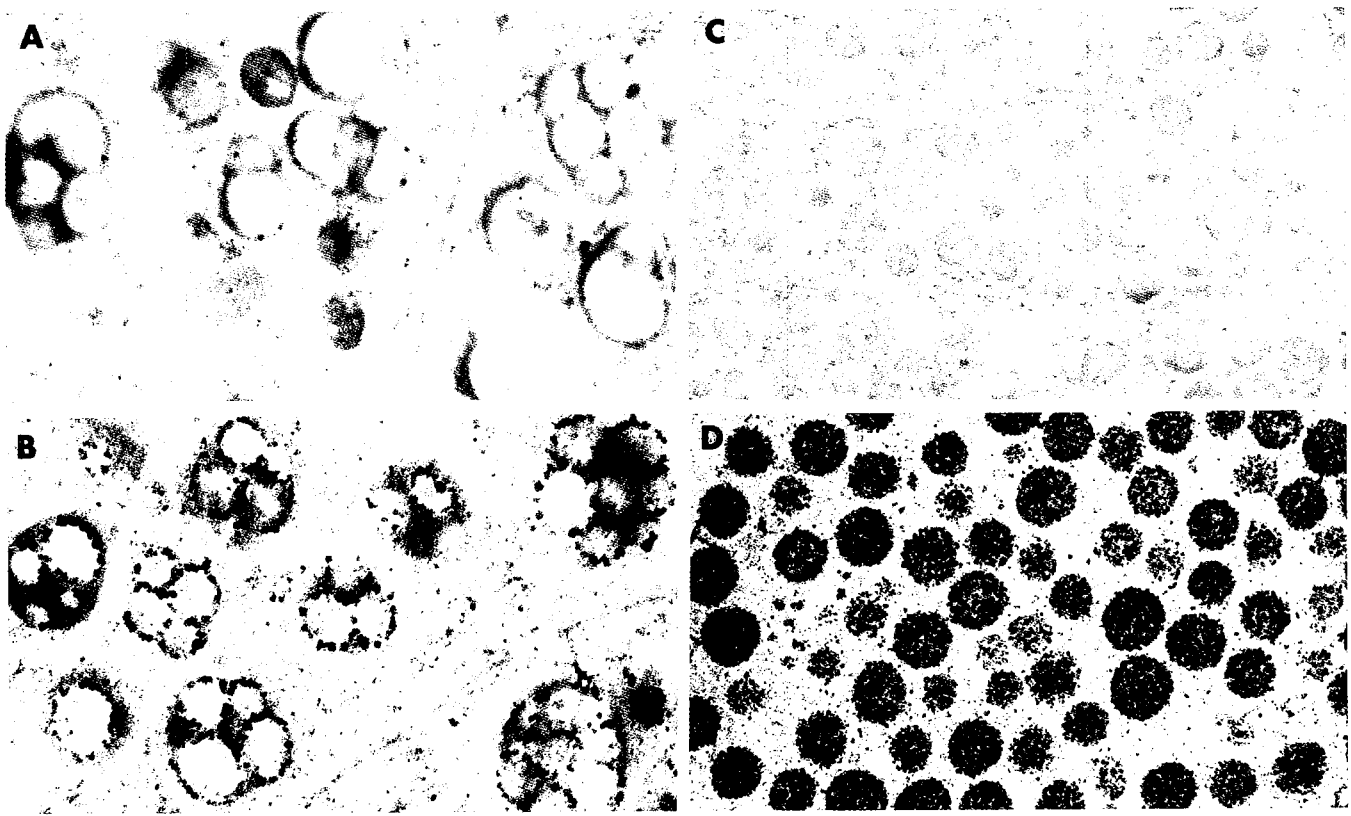

Figure 4. Immunoelectron microscopic localization of antigen by the ABC method. (A) type 3 granules, control and (B) type 3 granules, PL 3.4 antibody (30,000X); (C) type 7 granules, control and (D) type 7 granules, PL 6.3 antibody (14,000X).

antigens (PL 3.4 and PL 6.3) are incorporated into the water-insoluble spermatophore, and are differentiation-specific, reaching detectable levels by day 2 of adult life. Each antigen was cell type specific, with PL 3.4 restricted to type 3, and PL 6.3 found in cell types 5 and 7 (Fig. 3). Immunoelectron microscopic localization studies showed antigen was present within the characteristic granules of each cell type, with only trace amounts detected in the corresponding cytoplasm (Fig. 4). Antigen PL 6.3 was uniformly distributed through type 5 and 7 granules (type 5 not shown), however PL 3.4 antigen was concentrated in the cortex of type 3 granules.

These monoclonal probes will facilitate our studies of differentiation, pattern formation, and steroid-influenced development.

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