Ultrastructure of the Columnar Epithelial Cell Along the Midgut of the *Diatraea saccharalis* (Lepidoptera:Pyralidae) Larvae

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Abstract

The morphological features of the columnar epithelial cell in the midgut of *Diatraea saccharalis* larvae were studied by transmission electron microscopy. There are ultrastructural differences in the columnar cell morphology depending on their localization along the midgut. The apical columnar cell surface exhibit protrusions besides the conspicuous microvilli related with microapocrine vesicles; these cytoplasmic protrusions contain many organelles and they increase in number and size from the anterior to the posterior midgut regions. The mitochondria distribution is also variable. They are few in the apical surface of the anterior region and numerous in the posterior one; the opposite is observed in the basal cytoplasm, where the mitochondria are more abundant in the anterior region. Our results show that the morphological variations in the columnar cell morphology along midgut of the sugarcane borer are similar to the ones described for other Lepidoptera species. The secretion carried out by the midgut columnar cell may be related with both the microapocrine process and cytoplasmic protrusion release.

Keywords: midgut, columnar cell, ultrastructure, Lepidoptera, larvae.

Introduction

In most Lepidoptera the midgut is structurally and functionally differentiated along its length (2,6). Mainly four cell types compose the midgut epithelium: columnar, goblet, regenerative and endocrine cells (6). The predominant columnar cells are responsible for processing the diet, secretion of the digestive enzymes and for the uptake of the final product (6). The columnar epithelial cell in *Manduca sexta* shows gradual structural changes from the anterior to the posterior end of the midgut (1). In *Spodoptera frugiperda* the anterior columnar cells have microvilli with dilated tips and displays large secretory vesicles inside the apical cytoplasm (5). Conspicuous basal membrane infoldings in the columnar cells occur in the posterior midgut in *Erinnyis ello* while they are scarce in the anterior midgut (7). The commonly observed apical cell extrusions in insect midgut cells may be responsible for release of secretory material, although they often do not contain secretory granule (6). More often, microvilli also display extrusions or blebs consistent with the microapocrine secretory process (2,5,6). Columnar cells of the *E. ello* release microvillar extrusions containing vesicles into the midgut lumen, and also buds off small, membrane-bounded spherical bodies (7). The sugarcane borer, *Diatraea saccharalis*, is considered the most import pest for the sugarcane crop, affecting also many other plants (3). There is no study on the ultrastructural aspects of the different epithelial cells along their midgut. This work aims to describe the ultrastructure of the columnar cells in different regions of the midgut, in last instar larvae.

Materials and Methods

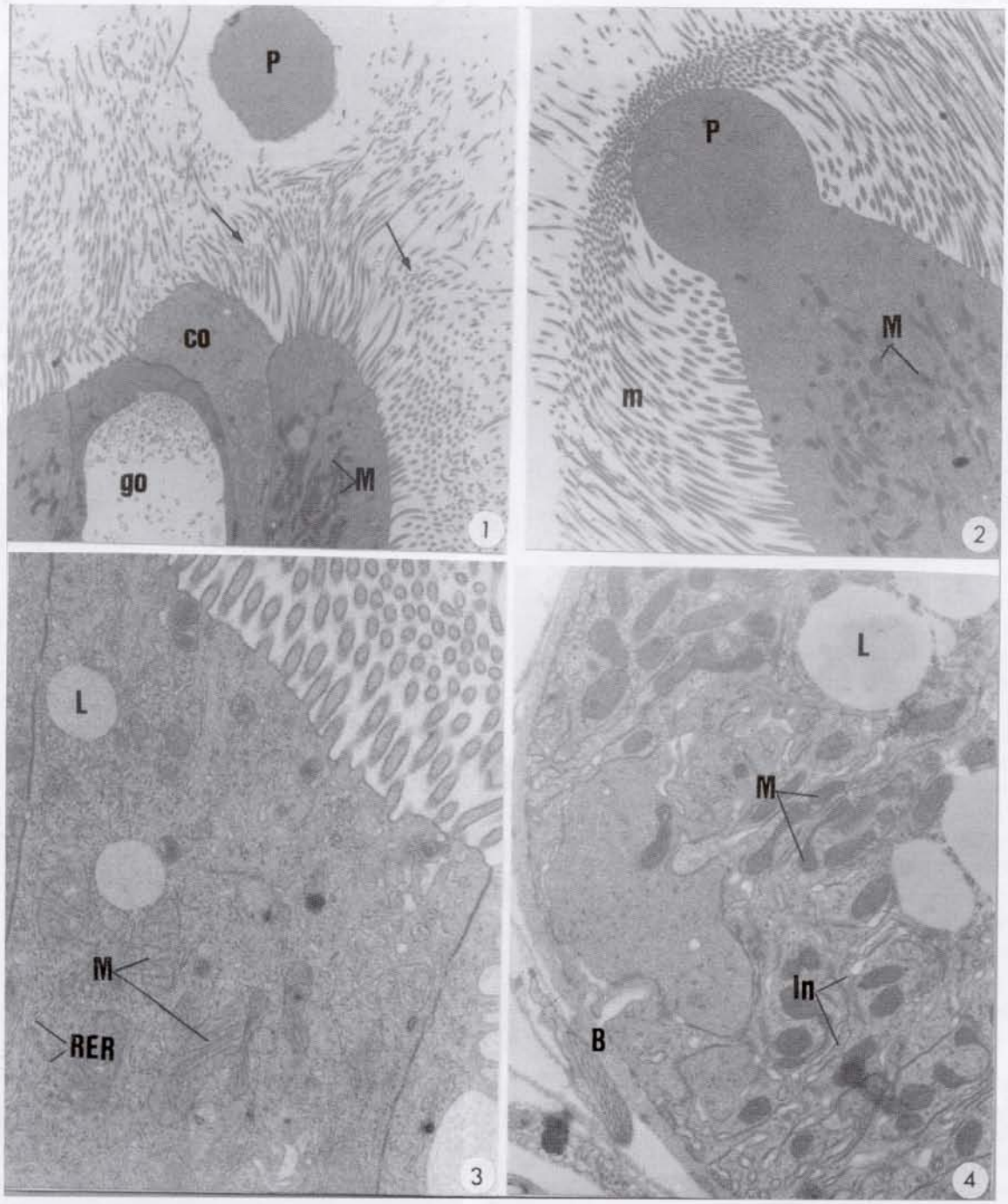
. The larvae of *D. saccharalis* were reared on artificial diet (4) and maintained under controlled temperature (25-27°C) and humidity (70%). Fragments of midgut (anterior, middle and posterior regions) were removed from last instar larvae and conventionally prepared for transmission electron microscopic observations. The midgut fragments were fixed in 2% glutaraldehyde – 4% paraformaldehyde solution in 0.1M phosphate buffer (pH 7.3) for 24h, post-fixed in 1% osmium tetroxide in the same buffer for 2h, dehydrated through a graded series of acetone and embedded in Araldite resin. Ultrathin sections were double stained with uranyl acetate and lead citrate and

examined under a Philips CM100 transmission electron microscope.

Results

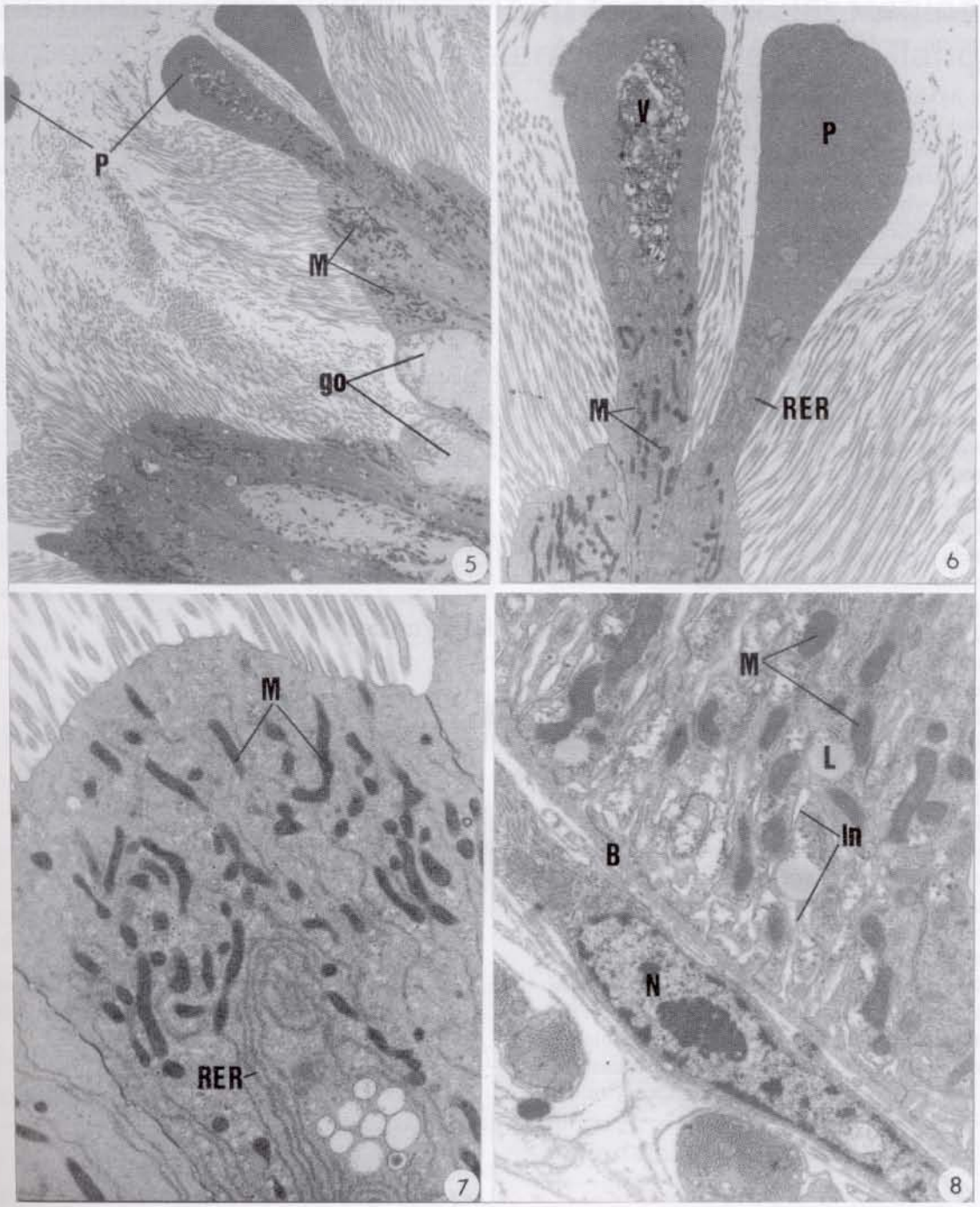
The apical surface of the columnar epithelial cells along the midgut exhibit conspicuous microvilli (Figs 1-3, 5-7). There are cytoplasmic protrusions among the microvilli; they increase in number, size and content from the anterior (Figs 1, 2) to the posterior region (Figs 5,6). The mitochondria are scarce in the apical cytoplasm of the anterior region (Fig. 4) whereas they are numerous and quite electron-dense at the posterior one (Figs 5, 7).

The basal membrane infoldings of the columnar cell occur in a parallel array extending up to half the height of



Figures 1- 4. Anterior region of the midgut of the sugarcane borer. Fig. 1: Cytoplasmic protrusion (P) and microapocrine secretory vesicles (arrow) among the microvilli of the columnar cell (co); mitochondria (M); goblet cell (go). X 4.600. Fig. 2: A brush border of microvilli (m) lines the lumen; mitochondria (M) and cytoplasmic protrusion (P) with endoplasmic reticulum. X 5.800. Fig. 3: Apical surface with microvilli; cytoplasm with mitochondria (M), lipid droplet (L) and rough endoplasmic reticulum (RER). X 16.800. Fig. 4: Basal cytoplasm with plasma membrane infoldings (In) with associated mitochondria (M); lipid droplets (L); basal lamina (B). X 16.800.

the cell, associated with mitochondria (Figs 4, 8). These cell region is also different along the midgut: there are few openings of the basal membrane infoldings in the underlying extracellular space at the anterior region (Fig. 4) comparing with the ones observed in posterior one (Fig. 8). Beside, the associated mitochondria are numerous at the anterior region (Fig. 4) while they are few at the posterior midgut (Fig. 8).



Figures 5-8. Posterior region of the sugarcane borer midgut. Fig. 5: Cytoplasmic protrusions (P) among the microvilli; dense mitochondria (M); goblet cell (go). X 2.800. Fig. 6: Detail of the apical surface with cytoplasmic protrusions (P) among the microvilli, containing rough endoplasmic reticulum (RER), mitochondria (M) and large vacuole (V) with heterogeneous content. X 6.400. Fig. 7: Apical cytoplasm with many dense mitochondria (M) and rough endoplasmic reticulum (RER). X 13.200. Fig. 8: Basal cytoplasm with plasma membrane infoldings (In), associated with mitochondria (M); lipid droplets (L); basal lamina (B); nucleus (N) of muscle cell. X 16.800.

Discussion

The general ultrastructural morphology of *D. saccharalis* larvae midgut is similar to that described for many Lepidoptera as *M. sexta* (1), *E. ello* (7) and *S. frugiperda* (5).

There are modifications along the midgut concerning the number and morphology of the cytoplasm projection going out of the columnar cells. The protrusions increase in number and size from the anterior to the posterior midgut regions, where they exhibit great amount of cytoplasmic organelles, suggesting that these cells are involved in apocrine secretory process. At the same time, the columnar cell may release material by microapocrine secretory process, as described for *E. ello* (7), based on our observation of microvesicles with electron lucent content among the microvilli, mainly in the anterior midgut region. Microapocrine secretion consists the elimination of small pinched-off single- or double-membrane vesicles from the cell microvilli (2). The absence of secretory granules in the columnar cells let us to believe that they might be secreting digestive enzymes in a different way, related either with the apocrine or the microapocrine process, as observed in *E. ello* (7), *Tenebrio molitor* (2) and *S. frugiperda* (5). Apocrine secretion is thought to be a cell adaptation to enhance the dispersion of secretory vesicle contents release from a water-absorbing epithelium, whereas exocytosis is an efficient secretory mechanism in a water-secreting epithelium (2).

The basal plasma membrane infoldings in the posterior midgut columnar cells of *D. saccharalis*, in contrast to those of the anterior, display regular openings into the underlying extracellular space similar to those found in *E. ello* (7). It is possible that such an arrangement facilitates water secretion, associated with potassium extrusion, mainly by the posterior region of the midgut. The existence of a well-developed brush-border, a large number of mitochondria present in the apical cytoplasm, and basal plasma membrane infoldings with associated mitochondria, should provide the columnar cells with the capacity to transport ions and water (7). The fact that the basal plasma membrane infoldings constitute an extracellular compartment, with restricted access to the hemolymph (because of the restricted openings into the underlying extracellular space), should enable the columnar cells from the anterior midgut region to concentrate solutes in that compartment and in the lumen, which might assist the absorption of water (7).

There are modifications along the midgut of *D. saccharalis* larvae concerning the secreting process number and morphology of the cytoplasm projection going out of the columnar cells. The anterior midgut region is mainly involved in the microapocrine secretory process whereas in the posterior region the releases of cytoplasmic protrusions are predominant. Another difference is related with the amount of mitochondria in the apical and basal cytoplasm: at the anterior midgut they are numerous in the basal cytoplasm while in the posterior region there are many mitochondria concentrated in the apical cytoplasm.

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