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Origin of the Brushborder in the Differentiating Midgut of *Melasoma saliceti* (Chrysomelidae, Coleoptera) Embryos

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The embryonic development of *Melasoma saliceti* takes eight days at room temperature. At the beginning of the 5th day the endoderm cells have already formed a unilayered epithelium of the midgut primordium. The midgut epithelium is formed by flat cells that are not connected by specialized intercellular junctions. Large vesicles can be seen in dilated intercellular spaces of the epithelium. Cytoplasmic projections, similar to microvilli, appear in the vesicles. During the 5th day of development, the vesicles grow and become enclosed by the intercellular junctions of a *zonula adherens* type. During the 6th day of development the cell junctions surrounding the vesicles become transformed into a septate type. On the 8th day of development the vesicles come close to the apical sides of the midgut cells and open towards the yolk. At the same time the microvilli spread over the apical surface of the midgut primordium to form the regular brushborder of the larval midgut. In the species studied the vesicles appear to "prefabricate" the apical surfaces of the future midgut epithelium.

Key-words: Insect embryo, development, microvilli formation, ultrastructure.

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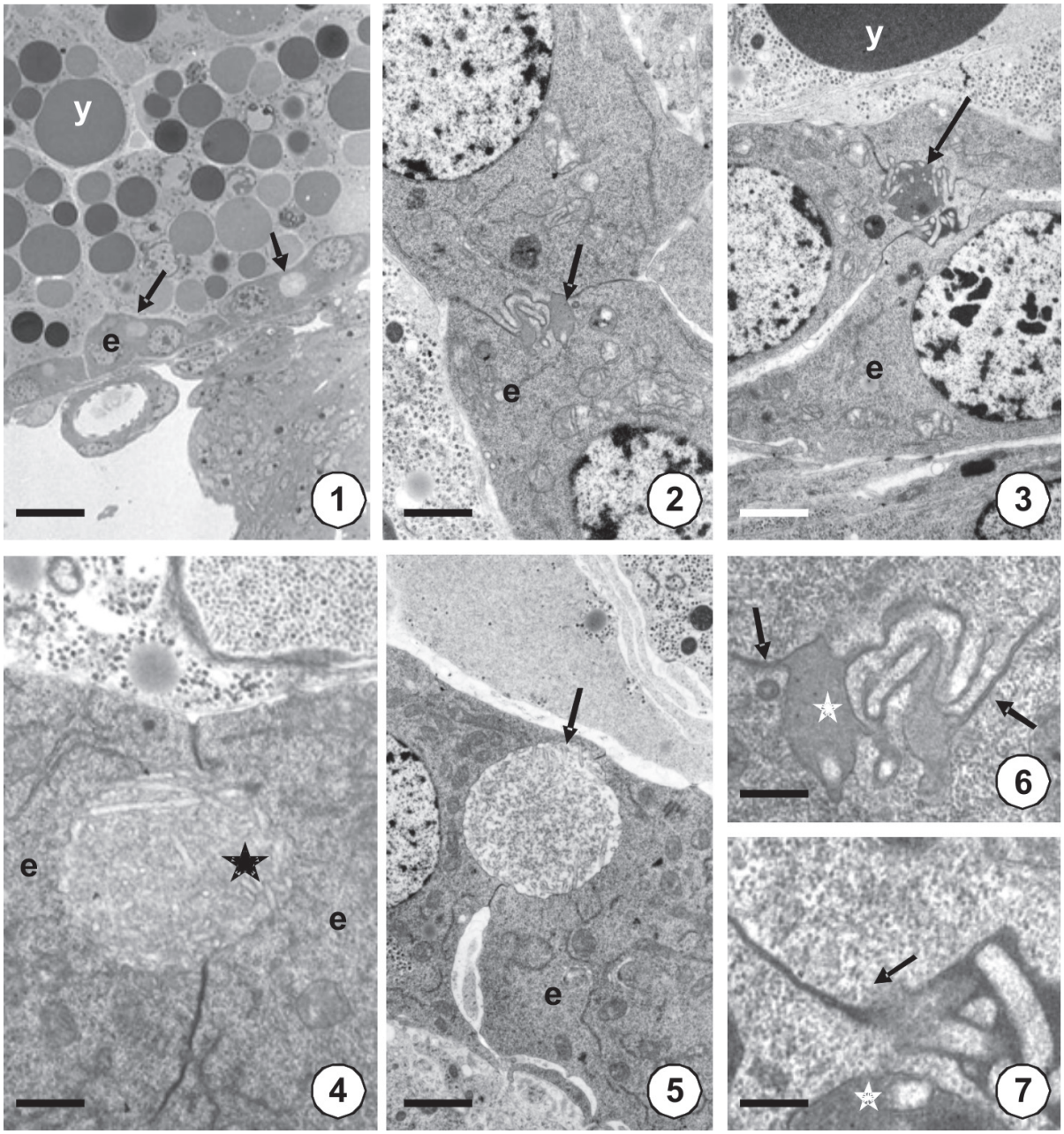
In all insect embryos the foregut (stomodaeum) and the hindgut (proctodaeum) develop by the invagination of ectoderm. The origin of the midgut is, however, different in different insect groups. In some insects e.g. Collembola (JURA 1958, 1966; KRZYSZTOFOWICZ *et al.* 1973; JURA & KRZYSZTOFOWICZ 1977; JURA *et al.* 1987) the midgut is formed from vitellophages, while in others it develops from cells proliferating from the tips of the stomo- and proctodaeum. In still other insects, e.g. *Drosophila*, the midgut develops from a distinct germ layer, namely from the endoderm (TECHNAU & CAMPOS-ORTEGA 1986; BALDWIN *et al.* 1996). There are also some insect species in which many sources of cells that differentiate into the midgut occur (see SCHWALM 1988 for review). Moreover, in some insect species the midgut is formed during embryonic development (BALDWIN *et al.* 1996), but in *Thermobia domestica* and *Lepisma saccharina* it differentiates only during the first and second larval stages (ROST *et al.* 2005; ROST-ROSZKOWSKA *et al.* 2007a).

Usually in insect embryos or early larvae, individual cells which are precursors of the midgut gather around the yolk mass. If the sources of the midgut cells are vitellophages, they crawl through the yolk and come close to the interior surface of the yolk plasmalemma. If the midgut precursors come from tips of the stomo- and the proctodaeum or the endoderm cells (as is the case in *Melasoma saliceti*), they place themselves on the outer surface of the yolk plasmalemma. Initially the cells are flattened and are not connected to each other. As the number of cells grows they change shape. They become cubic and eventually columnar in shape (ROST-ROSZKOWSKA *et al.* 2007b). When the cells assume a cubic shape, intercellular junctions start to appear between them. Initially the cells are joined only by *zonulae adherentes*. During the further differentiation of the epithelium, septate or continuous junctions develop (MÜLLER & BOSSINGER 2003). In almost every species studied to date epithelium microvilli appear on the apical surface of the midgut only after the midgut has differentiated into columnar epithelium. At first the microvilli are short and irregularly spaced, but eventually they become longer and form a regular brushborder. In only one case, in *Clitumnus ex-*



Figs 1-7. Fig. 1. 5th day of *Melasoma saliceti* development. Midgut rudiment (e) surrounding the yolk (y). Numerous vesicles (arrows) in the midgut epithelium. Light microscope. Bar = 7.4 μm. Fig. 2. 5th day of embryogenesis. Between the midgut epithelial cells (e) the intercellular spaces widen (arrow). TEM. Bar = 1.11 μm. Fig. 3. 5th day of embryogenesis. Initially, the enlarged spaces are irregular in shape (arrow). Midgut epithelium (e), yolk (y). TEM. Bar = 1.6 μm. Fig. 4. 5th day of embryogenesis. The intercellular spaces (asterisk) between epithelial cells (e) become regularly spherical. TEM. Bar = 0.75 μm. Fig. 5. 5th day of embryogenesis. Within the "vesicles" (arrow) microvilli develop. Midgut epithelium (e). TEM, bar = 1.6 μm. Fig. 6. At the beginning of the 5th day, around the enlarged spaces (asterisk), the neighboring cells are connected only with the junctions of the *zonula adherens* type (arrows). TEM. Bar = 0.4 μm. Fig. 7. 6th day of development. Around the "vesicles", beside the *zonulae adherentes* septate junctions (arrow) develop and within the "vesicles" lumen (asterisk) microvilli appear. TEM. Bar = 0.3 μm.

tradentatus, do the microvilli develop before the differentiating midgut cells assume a columnar shape (KADIRI & LOUVET 1982). In this species, microvilli develop within dilatations of the intercellular spaces between growing epithelial cells in the differentiating midgut cells.

Similar pictures of microvilli developing inside the intercellular spaces can be seen on the apical surfaces of growing regenerating cells in the midguts of adults. In this case, however, the microvilli belong to the growing young cell only (CRUZ-LANDIM 1999). In the present paper we de-

scribe intercellular vesicles with well-developed microvilli within the embryonic midgut precursor of a holometabolous insect for the first time. We suppose that the vesicles are filled with "prefabricated" microvilli formed by the cell membranes with molecules characteristic for the apical cell surface.

It is well known that epithelia have diversified surfaces (LECUIT 2003; KNOBLICH 2000). Different specialized molecules are present on the apical surface while others occur on the basolateral surface. These differences are connected with diversified functions of the surfaces. It is also well established that the differences in the distribution of the specialized molecules are maintained by intercellular junctions in the epithelial cells.

Material and Methods

The embryonic development of *Melasoma saliceti* takes 8 days at room temperature. The embryos were fixed in 2.5% glutaraldehyde in a 0.1M phosphate buffer at pH=7.4 (1.5h at room temperature), postfixed in 1% osmium tetroxide in a phosphate buffer at pH=7.4 (2h at room temperature) and dehydrated in a graded series of ethanol (50%, 70%, 90%, 96%, 100%) and acetone (each for 15 min at room temperature). The material was embedded in Epon 812. Semi- and ultrathin sections were cut using a Leica UCT25 ultramicrotome. Semithin sections were stained with 1% methylene blue in 0.5% borax and analyzed using an Olympus BX100 microscope. Ultrathin sections after staining with uranyl acetate and lead citrate were examined with a Hitachi H500 transmission electron microscope.

Results

The embryonic development of *Melasoma saliceti* takes eight days at room temperature. The endoderm cells initially form two cellular bands along the lateral sides of the yolk and soon spread over the yolk mass (for a description of the formation of the midgut in *Melasoma saliceti* see: ROST-ROSZKOWSKA *et al.* 2007b). At the beginning of the 5th day the endoderm cells have already formed a unilayered epithelium of the midgut primordium. The midgut epithelium is formed by flat cells that are not connected by specialized intercellular junctions. During the 5th day of development, numerous large vesicles become visible under the light microscope (Fig. 1). It is possible to observe the sequential steps of the vesicle formation using an electron microscope. The intercellular space widens between neighboring cells (Fig. 2). Initially the enlarged spaces are irregular in shape (Figs 2, 3) but soon grow and change into spherical balloon-like structures (Fig. 4). Cytoplasmic projections similar to microvilli appear in the vesicles (Fig. 5). The microvilli are not very numerous at first. During the 5th day of development, the vesicles grow and become enclosed by intercellular junctions. The cell membranes within the junctions are denser for electrons than the cell membrane covering other cell surfaces. The intercellular junctions formed in these places take on the appearance of *zonulae adherentes* (Fig. 6).

On the 6th day of embryonic development, the endoderm cells change shape and grow into cubic epithelium. At the same time the intercellular vesicles also grow and become filled with microvilli embedded within a very electron-dense substance (Fig. 8). At this time the neighboring cells are not connected by any specialized junctions, but the cell junctions surrounding the vesicles become transformed into the septate type (Fig. 7).

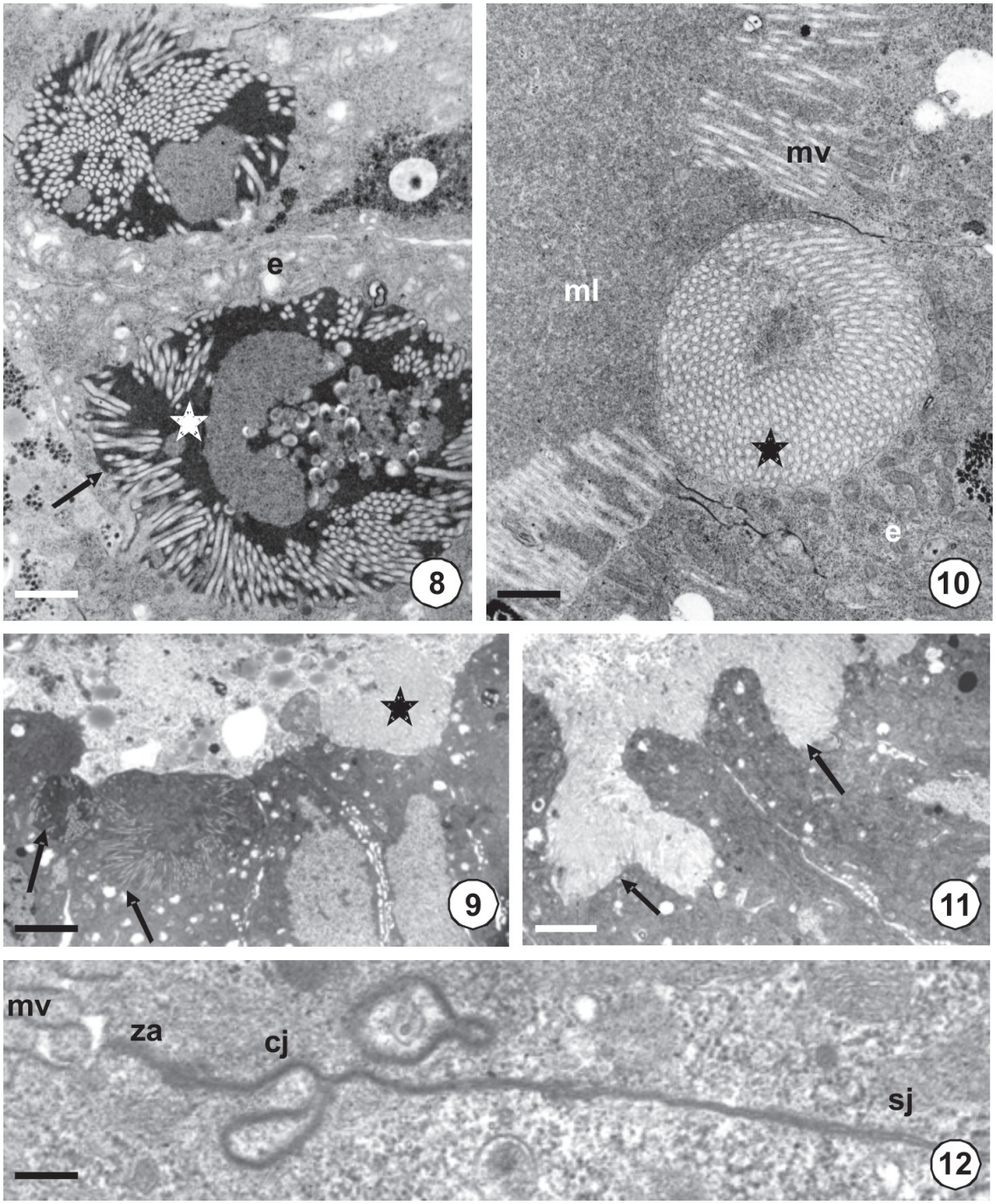
On the 7th day of development, the midgut cells have attained a columnar shape and the vesicles have moved to their apical sides along with the elongating cells (Fig. 9). The microvilli embedded within the vesicles become densely distributed. The apical surfaces of the midgut primordium which face the yolk mass are completely smooth and do not contain microvilli. Specialized intercellular junctions of the *zonula adherens* type appear close to the apical surfaces of the columnar cells.

On the 8th day of development, the vesicles come close to the apical sides of the midgut cells and open towards the yolk (Fig. 10). At the same time the microvilli spread over the apical surface of the midgut primordium forming the regular brushborder of the larval midgut (Fig. 11). Afterwards the first larva comes out of the eggshell and the yolk is quickly digested. At the same time well-developed junctional complexes with the *zonulae adherentes* continuous junctions and septate junctions appear between the midgut cells (Fig. 12).

Discussion

In the embryos of *Melasoma saliceti* the midgut forms from endoderm cells migrating over the yolk surface from anterior and posterior endoderm anlagens. On the 5th day of development the endoderm cells completely cover the yolk surface with a one-layered epithelium.

According to the literature microvilli developing within the intercellular spaces can only be seen during the regeneration of the midgut epithelium.



Figs 8-12. Fig. 8. 6th day of development. The "vesicles" become very large and filled with a substance very dense for electrons (asterisk). The cell membranes encircling the "vesicles" are decorated with densely packed microvilli. The apical surfaces of the midgut primordium (e), facing the yolk are completely smooth (arrows). TEM. Bar = 0.7 μm. Fig. 9. 7th day of development. The "vesicles" filled with microvilli come close to the apical surface of the midgut cells (asterisk) while some others are already open (arrows). TEM. Bar = 2.4 μm. Fig. 10. 8th day of embryogenesis. In some regions of the midgut rudiment (e), the "vesicles" (asterisk) do not open at the same time. Microvilli (mv), midgut rudiment lumen (ml). TEM. Bar = 0.7 μm. Fig. 11. 8th day of embryogenesis. Dynamic picture of brushborder (arrows) formation. TEM. Bar = 1.7 μm. Fig. 12. At the moment of larval hatching fully formed junctional complexes can be seen. The individual complex is formed by the *zonula adherens* (za) lying in apical position, the continuous junction (cj) lying just below, and the septate junction (sj). Microvilli (mv). TEM. Bar = 0.3 μm.

However, they only develop on the apical tip of the growing regenerative cell (CRUZ-LANDIN 1999, ROST 2006; ROST-ROSZKOWSKA *et al*. 2010). In the majority of insects the microvilli develop on the apical surfaces of the embryonic midgut cells after they have formed an epithelium (ROST-ROSZ-

KOWSKA *et al.* 2007a, 2007b). Only in *Clitumnus extradentatus* have vesicles developing within the intercellular spaces of the embryonic midgut been described (KADIRI & LOUVET 1982).

At the beginning of the development of *M. saliceti*, the epithelium is flat and the cells forming it are not connected by any specialized intercellular junctions. Large vesicles arise in the intercellular spaces between the epithelial cells during the 5th day of development. Only then the intercellular junctions are formed. The intercellular junctions can be seen because the cell membranes that form the junctions are thicker and denser. Most probably the junctions close the vesicles with a seal and this is why the vesicles acquire a spherical shape (GENOVA & FEHON 2003; SCHULTE *et al.* 2003; WOOD 1990). At the end of the 5th day of development, the junctions develop into septate junctions which are equivalent to the tight junctions of vertebrates (GENOVA & FEHON 2003; SCHULTE *et al.* 2003; WOOD 1990). The appearance of the septate junctions probably enables the vesicles to take a spherical shape. During the 6th and 7th days of development, the junctions around the vesicles transform into typical junctional complexes with the *zonula adherens* junction close to the vesicle lumen and the continuous junction farther on. At the end of embryonic life, the vesicles open to the midgut lumen and the microvilli spread over the apical surfaces of the epithelial cells. Only then does the epithelium acquire the "brushborder". It is very strange that such a method of microvilli "prefabrication" is so rare and it has only been described for one hemimetabolous insect *Clitumnus extradentatus* and for only one holometabolous insect, namely *M. saliceti*.

Another problem arises in connection with this. It is well known that apical-basal polarity seems critical for epithelia to maintain their integrity and undergo the morphogenetic changes that occur during development. The plasma membranes of epithelial cells are subdivided into apical and basolateral compartments which differ in their protein and lipid composition. The process of the establishment of apical-basal cell polarity in a single-layer epithelium is essential for subsequent morphogenetic events. Cell polarity is marked by the concentration of adherens junctions in the apical neck of these epithelial cells, which form a belt-like structure known as the *zonula adherens*. Epithelial polarity is established when distinct apical and basolateral plasma membrane compartments separated by a belt of junctions are formed. Such a case can be seen in developing midgut epithelium of *M. saliceti*. The apical surfaces of the larval midgut epithelium are "prefabricated" during embryonic development within the vesicles formed between the developing epithelial cells. However, the importance of the prefabrication of apical surfaces is unknown. The next step in our studies will be the identification of the distribution of apical and basolateral molecules within the cells of the embryonic midgut.

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