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Fine Structure and Differentiation of the Midgut Epithelium of *Allacma fusca* (Insecta: Collembola: Symphypleona)

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Magdalena Maria Rost-Roszkowska and Agnieszka Undrul (2008) Fine structure and differentiation of the midgut epithelium of *Allacma fusca* (Insecta: Collembola: Symphypleona). *Zoological Studies* **47**(2): 200-206. *Allacma fusca* belongs to a less well known, primitive, wingless insect group. The aim of our study was to describe all changes which accompany midgut epithelium differentiation of adult specimens of *A. fusca*. The midgut epithelium of *A. fusca* is composed of columnar cells with an epithelial character. No regenerative cells, which are commonly responsible for midgut epithelium regeneration, were observed. Therefore the growth of the entire epithelium depends on an increase in the dimensions of epithelial cells. Epithelial cells are not able to proliferate, as was earlier suggested, in the 1st larval stage of this species. The characteristic regionalization in the organelle arrangement was observed like in all epithelia responsible for secretion, transport, and excretion. However during the insect's lifespan, distinct differences appear between all epithelial cells. *Allacma fusca* does not have Malpighian tubules; thus the midgut epithelium is also responsible for excretion. This process is connected with urospherites which accumulate in epithelial cells. Their structure suggests that they might be identified with type A granules described for many Pterygota insects.
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Key words: Midgut epithelium, Differentiation, Stem cells, Urospherites

The midgut epithelium of insects is responsible for enzyme production, digestion, secretion, and in insects devoid of Malpighian tubules, excretion (Paclt 1956, Anderson and Harvey 1966, Humbert 1979, Chapman 1998). These functions are possibly due to the characteristic ultrastructure of epithelial cells which form the midgut epithelium. In the majority of insects, their cytoplasm has distinct regionalization in organelle arrangements, and as a consequence, basal, perinuclear, and apical regions appear (Klag et al. 2002, Rost 2006a b, Rost-Roszkowska et al. 2007a). The midgut epithelium is formed during embryogenesis or less commonly, during larval stages (Dallai 1966, Krzysztofowicz et al. 1973, Rost et al. 2005, Rost-Roszkowska et al. 2007a b). In the middle of larval development, together with the growth of insect bodies, the midgut epithelium is formed by an increasing number of cells. Depending on the kind of food, it will differentiate during larval stages or in adult specimens. In the majority of insect species, the midgut epithelium is composed of columnar cells with an epithelial character and regenerative cells. Endocrine and goblet cells have been described in some insect groups. Regenerative cells form groups called regenerative nests or crypts. They may also be located singly among epithelial cells (Billingsley 1990, Sadrud-Din et al. 1996, Diaz et al. 2000 2003, Garcia et al. 2001, Silva-Olivares et al. 2003, Rost et al. 2005, Rost-Roszkowska et al. 2007a). They are regarded as stem cells. However, in some cases, the lack of regenerative cells among epithelial cells has been described (Krzysztofowicz et al. 1973, Lauga-Reyrel 1980). In those cases, the regeneration process seems to be problematic.

There are only a few studies concerning the ultrastructure, development, and differentiation

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of the midgut epithelium in primitive, wingless insects and they mainly refer to species belonging to Zygentoma, Arthropleona (Collembola), or Protura (Dallai 1966 1975 1977, Krzysztofowicz et al. 1973, Biliński and Klag 1979, Klag et al. 1981, Rost 2006a). We know even less about these processes in insects of the collembolans suborder Symphypleona (Rost-Roszkowska et al. 2007b). Additionally it is worth mentioning that due to a recent study, collembolans are thought to be a separate animal order, which developed independently of insects (Nardi et al. 2003).

Our studies have revealed the process of midgut epithelium differentiation in adult specimens of *Allacma fusca*, a species which belongs to the Symphypleona (Collembola). Because of this process, the midgut epithelium in adult specimens might be responsible for digestion and secretion, as well as excretion, because *A. fusca* does not posses Malpighian tubules. Additionally, this species seems to be very interesting considering the lack of regenerative cells in its midgut epithelium.

MATERIALS AND METHODS

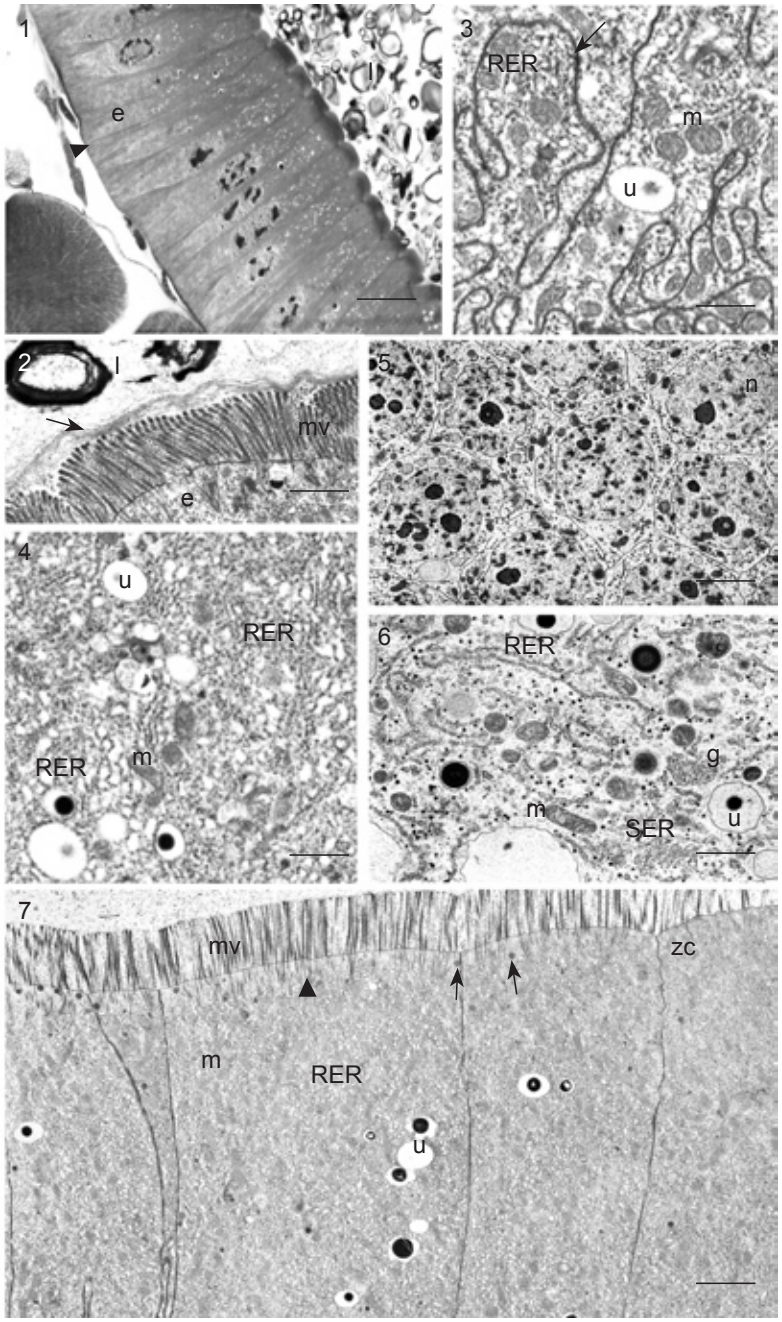
Animals for investigation were obtained from the laboratory culture of *A. fusca* (Insecta: Collembola: Symphypleona). Adults specimens were collected in Bieszczady mountains (Poland) from June to Aug. Adult specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 (for 24 h at 4°C) and postfixed in 2% OsO_4 (for 1.5 h at room temperature). After dehydration in a graded series of alcohol (30%, 50%, 70%, 90%, 95%, and 100% for 15 min each) and acetone (15 min) the material was embedded in Epon 812. Semi- and ultrathin sections were cut on a Leica Ultracut UCT25 ultramicrotome (Leica, Wetzlar, Germany). Semi-thin sections were stained with 1% methylene blue in 0.5% borax and analyzed with an Olympus BX60 light microscope (Olympus, Tokyo, Japan). Ultrathin sections were stained with uranyl acetate and lead citrate. They were examined with a Hitachi H500 transmission electron microscope (Hitachi, Tokyo, Japan).

RESULTS

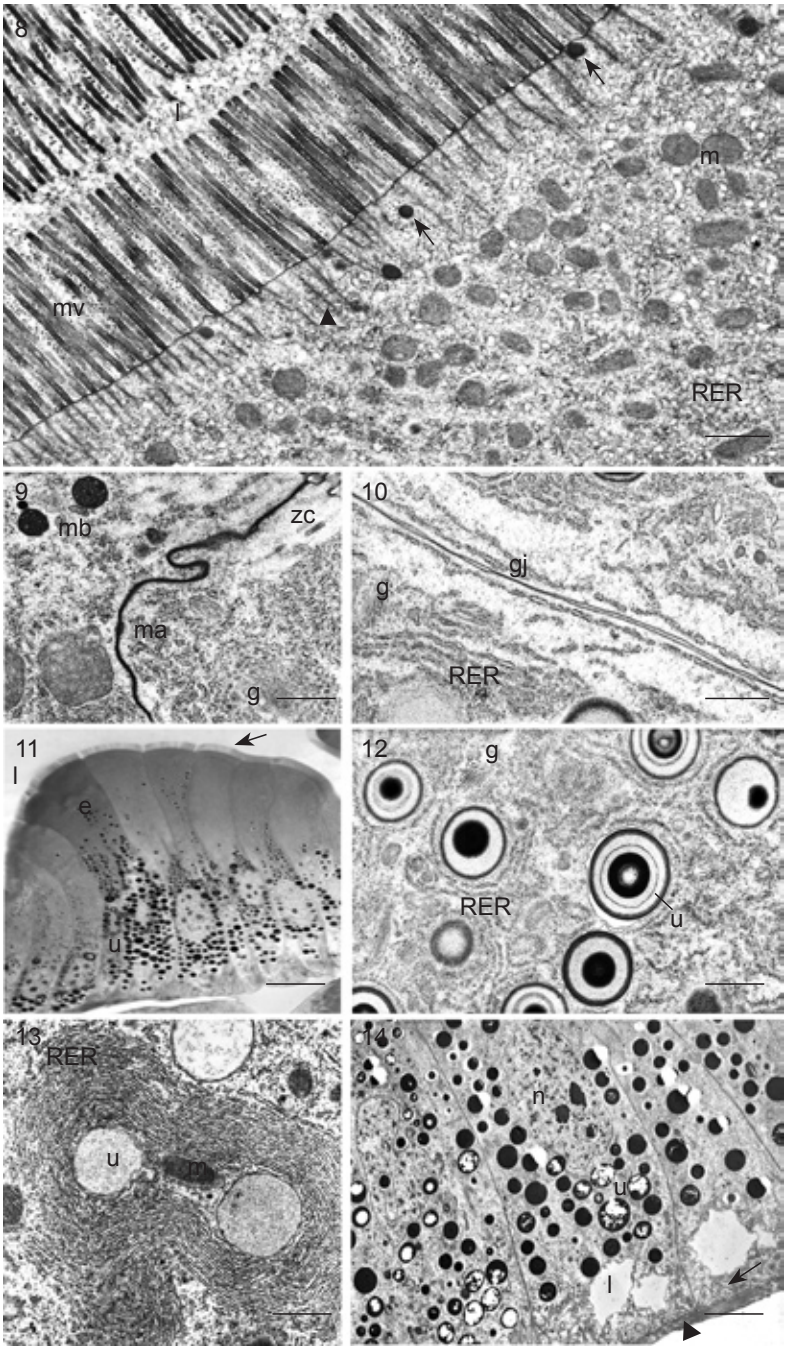
The midgut epithelium of adult specimens of *A. fusca* is composed of columnar epithelial cells, which lie on a non-cellular basal lamina (Fig. 1). Regenerative cells were not observed. The epithelium is separated from the midgut lumen by a peritrophic membrane (Fig. 2). Distinct regionalization in organelle distribution in the cytoplasm of epithelial cells was observed.

During midgut epithelium differentiation, numerous transitions of the epithelial cytoplasm are set, until the time at which the functions of absorption, secretion, digestion, and excretion are fully developed. However, at the beginning of its differentiation, in the basal cytoplasm where the basal membrane forms numerous folds, many mitochondria and cisterns of rough endoplasmic reticulum (RER) were observed (Fig. 3). The latter forms both closed, circular structures and the typical lamellar arrangement (Fig. 4). Nuclei with heterochromatin clusters situated near the nuclear envelope have many nucleoli (Fig. 5). In the perinuclear region, numerous cisterns of smooth. endoplasmic reticulum (SER), Golgi complexes, single mitochondria, and cisterns of RER were observed (Fig. 6). In the apical cytoplasm, abundant mitochondria, minor and major vesicles with electron-lucent content, and circular structures formed from cisterns of RER were present. During differentiation of the epithelium, mitochondria mainly accumulated in the apical cytoplasm (Fig. 8). Electron-dense granules, which also appeared in this region, moved toward the apical membrane, where they removed their contents into the midgut lumen (Figs. 7, 8). Apical membranes formed microvilli with abundant cytoskeletal elements, which also protruded into the apical cytoplasm forming characteristic roots (Figs. 7, 8). The entire cytoplasm was rich in free ribosomes. Between the apical regions of adjacent epithelial cells, intercellular junctions of zonula continua (smooth septate junctions) and macula adherens were present (Fig. 9). Between the perinuclear and basal regions, pleated septate and gap junctions could be observed (Fig. 10).

At the beginning of midgut epithelium differentiation, urospherites appeared only in the perinuclear cytoplasm (Figs. 3, 6, 7). Their number and volume greatly increased, so that they also began to occupy the basal (Figs. 11, 12) and apical cytoplasm of epithelial cells. Accumulation of RER cisterns could be observed during their formation (Fig. 13). When urospherites appeared near the nuclear envelope, initially small and gradually larger lipid droplets occurred in the basal cytoplasm (Fig. 14). In the perinuclear region, lamellar bodies arose, and nuclei often took on lobular shapes. The numbers of RER cisterns in



Figs. 1-7. 1. Midgut epithelium (e), composed of columnar cells resting on the non-cellular basal lamina (arrowhead). l, midgut lumen. Light microscopy, scale bar = 26 μm. **2.** Peritrophic membrane (arrow) separating the midgut epithelium (e) from its lumen (l). mv, microvilli. Transmission electron microscopy, scale bar = 2.6 μm. **3.** Basal membrane of epithelial cells forming numerous folds (arrow) between which mitochondria (m) and cisterns of RER (RER) can be observed. The beginning of urospherite (u) formation is evident. Transmission electron microscopy, scale bar = 2.5 μm. **4.** Cisterns of rough endoplasmic reticulum (RER) forming numerous circular structures and the typical lamellar arrangement. m, mitochondria; u, urospherites. Transmission electron microscopy, scale bar = 2.63 μm. **5.** Nuclei (n) possessing many clusters of heterochromatin situated near the nuclear envelope and a few nucleoli. Transmission electron microscopy, scale bar = 2.9 μm. **6.** Longitudinal section through the perinuclear cytoplasm of epithelial cells. m, mitochondria; RER and SER, cisterns of rough endoplasmic reticulum and smooth. endoplasmic reticulum; g, Golgi complexes; u, urospherites. Transmission electron microscopy, scale bar = 1.3 μm. **7.** Beginning of epithelial differentiation. The apical region of the epithelial cytoplasm exhibits electron-dense granules (arrows), mitochondria (m), cisterns of rough endoplasmic reticulum (RER), and single urospherites (u). Microvilli (mv) of the apical membrane possess filaments which form characteristic roots (arrowhead). The zonula continua junction (zc) is visible between apical regions of adjacent cells. Transmission electron microscopy, scale bar = 2.9 μm.



Figs. 8-14. 8. Later stage of epithelial differentiation. Electron-dense granules (arrows) are moving toward the apical membrane where they remove their contents into the midgut lumen (l). Mitochondria (m) can be observed to be accumulating in the apical cytoplasm, and the roots of microvilli (mv) are like filaments (arrowhead). Transmission electron microscopy, scale bar = 1.3 μm. **9.** Zonula continua (zc) and macula adherens (ma) junctions are present between the apical regions of adjacent epithelial cells. g, Golgi complexes; mb, multivesicular bodies. Transmission electron microscopy, scale bar = 0.6 μm. **10.** Gap junctions (gj) between perinuclear regions of adjacent cells. RER, cisterns of rough endoplasmic reticulum; g, Golgi complexes. Transmission electron microscopy, scale bar = 0.8 μm. **11.** Longitudinal section through the midgut epithelium. Accumulation of urospherites in epithelial cells (e) is evident. l, midgut lumen; arrow, microvilli. Light microscopy, scale bar = 27 μm. **12.** Urospherites (u) occupying the perinuclear and basal regions of epithelial cells. RER, cisterns of rough endoplasmic reticulum; g, Golgi complexes. Transmission electron microscopy, scale bar = 1.1 μm. **13.** Accumulation of cisterns of rough endoplasmic reticulum (RER) near newly formed urospherites (u). m, mitochondria. Transmission electron microscopy, scale bar = 1.3 μm. **14.** At the end of differentiation process, when urospherites (u) occupy the entire cytoplasm, lipid droplets (l) occur in the basal region. Arrowhead, basal lamina; arrow, folds of the basal membrane; n, nucleus. Transmission electron microscopy, scale bar = 4.8 μm.

the entire cytoplasm and small vesicles situated near the apical membrane increased. However, the quantity of electron-dense granules near the apical membrane decreased. They had completely disappeared by the end of midgut epithelium differentiation. The appearance of single vacuoles in the epithelial cytoplasm was a morphological sign of its degeneration. The entire epithelium was only composed of epithelial cells, and at their anterior and posterior ends, no regenerative cells or regenerative groups were observed. Mitotic divisions of epithelial cells were also not observed. So during each molting, only single epithelial cells were removed, and no new cells were observed to appear.

DISCUSSION

As is known, many insect species belonging to the Collembola and Diplura constitute an important element of the soil mesofauna. The midgut epithelium is the main organ responsible for nutrient and ion absorption. Therefore, the completely formed midgut epithelium is characterized by distinct regionalization in organelle distribution enabling it to fulfill all functions related to digestion, secretion, and transport.

There have only been a few studies examining the ultrastructure of the midgut epithelium of primitive, wingless Collembola, and they mainly focused on collembolans, which belong to the Arthropleona, while *A. fusca* is a member of a less well known group, the Symphypleona (Dallai 1966 1975, Krzysztofowicz et al. 1973, Klag et al. 1981, Rost 2006a, Rost-Roszkowska et al. 2007b).

Many adult collembolan specimens are able to molt during their life, when the entire midgut epithelium or only single cells are removed. The midgut epithelium undergoes differentiation, which enables it to take part in digestion, secretion, and absorption. The distinct regionalization in organelle arrangements appears during midgut epithelial differentiation in *A. fusca*. This is related to the functions of the fully developed epithelium (Anderson and Harvey 1966, Berridge and Oshman 1972, Cioffi 1979, Pigino et al. 2005, Rost 2006a b), and it also enables an increase in cell dimensions. In consequence, the entire surface of the midgut epithelium is enlarged. Studies of the midgut epithelium of the 1st larval stage of *A. fusca* revealed that when the midgut lumen is filled with the yolk mass stored during embryogenesis, midgut epithelium cells proceed to differentiate. This enables feeding by the next larval stages (Rost-Roszkowska et al. 2007b). In adult insect specimens, midgut growth continues owing to the growth of already existing cells, total replacement of cells by new populations, or the proliferation and differentiation of regenerative cells, which play the role of stem cells (Reinhard et al. 1972, Wigglesworth 1972, Sehnal 1985, Spies and Spence 1985). More striking is the suggestion that the midgut epithelium might be completely replaced by a new cell population. Newly formed populations of epithelial cells should originate from regenerative or epithelial cells, which possess the ability to proliferate. However, in the literature, it is difficult to find a precise explanation of this problem. Regenerative cells fulfill the role of midgut stem cells and cause the growth and renewal of already degenerated epithelial cells (Reinhard et al. 1972, Spies and Spence 1985, Dow 1986, Billingsley 1990, Sadrud-Din et al. 1996, Hakim et al. 2001, Evangelista and Leite 2003, Rost 2006a b). The midgut epithelium of *A. fusca* is composed solely of epithelial cells. The same phenomenon was observed in some other collembolan species (Jura 1958, Krzysztofowicz et al. 1973, Lauga-Reyrel 1980). The absence of regenerative cells, which play a role as stem cells, might suggest the growth of the midgut epithelium of analyzed species, which proceeds merely by the extension of existing cells. Additionally, it is worth mentioning that the midgut epithelium in *A. fusca* undergoes degeneration through apoptosis (data not shown). Some of the midgut epithelial cells proceed to necrosis, but not before the tissue has been completely differentiated. Because of the complexity of these processes in *A. fusca*, those results are described in a separate article. The gradual increase in the number of midgut epithelial cells correlating with molting cycles has been described for many insect species. Accordingly, larvae are able to grow continuously not only before, but also just after molting (Baldwin and Hakim 1991). It is probable that in the larval stages of *A. fusca*, epithelial cells possess the ability to proliferate, which might be lost in adult specimens. In such a way, the surface of the larval midgut epithelium is extended. It is also possible that epithelial cells possess the ability to divide, but we did not manage to observed that. There is also the probability that epithelial cells proliferate at precise times, as suggested for regenerative cells in other collembolan species, e.g., *Podura aquatica* (Rost 2006a). Epithelial cells might

proliferate in larval stages, but their number does not increase in adult specimens. Taking into consideration that Collembola is a group which developed independent of insects (Nardi et al. 2003), there is a similarity of the midgut epithelium of *A. fusca* with the hepatopancreas of Isopoda. The epithelium of the hepatopancreas is composed of 2 kinds of cells: small (S) and large (B) cells. Regenerative cells or regenerative groups were not observed (Clifford and Witkus 1971, Storch et al. 2002, Žnidaršič et al. 2003). The process of regeneration of the hepatopancreatic epithelium is still problematic. According to some scientists, S cells differentiate into B cells, while others believe that at the anterior and/or posterior ends of the hepatopancreas, S cells play a role as stem cells. There are also studies which state that cells with the ability to proliferate are not present in the epithelium (Prosi et al. 1983, Bettica et al. 1984). The Symphypleona is a less well known collembolan group, and the analysis of the midgut epithelium in some other species might be helpful.

In the primitive, wingless insects of the Collembola and Diplura, which are devoid of Malpighian tubules, the midgut epithelium is also responsible for excretion, because products of metabolism accumulate in specific structures called urospherites (Paclt 1956, Humbert 1979). The presence of these structures suggests their role in accumulating excessive metal ions (Dallai and Burroni 1981, Xue et al. 1990, Pawert et al. 1996, Pigino et al. 2005). van Straalen et al. (1987) and Posthuma et al. (1992) found that in the Collembola, toxic metals first accumulate in the cytoplasm of midgut epithelial cells, and owing to the cyclic renewal of the epithelium just before molting, they are moved outside the body. Urospherites in Diplura have been described as type A storage granules (Pigino et al. 2005). There are many studies which assumed that ions of zinc and manganese accumulate in type A granules (van Straalen et al. 1987, Hopkin 1989, Köhler 2002). These structures have been described in both the cytoplasm of midgut epithelial cells and the epithelium of Malpighian tubules as the granules responsible for metal ion accumulation. They are also regarded as a barrier which inhibits the harmful influence of metal ions from reaching the entire organism. During the gradual differentiation of the midgut epithelium of *A. fusca*, structures described as urospherites or type A granules also gradually form. They were observed in the midgut epithelium of the 1st larval stage of the species analyzed (Rost-Roszkowska et al. 2007b).

As we noted in adult specimens of *Allacma fusca*, together with succeeding molting cycles, only single midgut epithelium cells are removed. This phenomenon is commonly observed in many insect species, in both adults and larvae. The epithelium proceeds through differentiation, and at the end of its life, it completely degenerates.

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