

Fine Structure of the Midgut Epithelium of *Atelura formicaria* (Hexapoda: Zygentoma: Ateluridae), with Special Reference to Its Regeneration and Degeneration

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Magdalena M. Rost-Roszkowska, Jitka Vilimova, and Łukasz Chajec (2010) Fine structure of the midgut epithelium of *Atelura formicaria* (Hexapoda: Zygentoma: Ateluridae), with special reference to its regeneration and degeneration. *Zoological Studies* 49(1): 10-18. *Atelura formicaria* belongs to a basal hexapod group, the Zygentoma. Its midgut epithelium is composed of epithelial cells, which are responsible for digestion, secretion, and absorption, and regenerative cells, which form regenerative nests. The midgut epithelium ultrastructure was compared to that described for other zygentoman groups, the Lepismatidae, and the Archaeognatha, a group closely related to the Zygentoma. Among regenerative cells, we distinguished midgut stem cells (resting regenerative cells), which are able to proliferate and differentiate, and differentiating regenerative cells. Just before mitotic division in the cytoplasm of stem cells, many cisterns of endoplasmic reticulum and electron-dense granules appear. During mitosis, the electron-dense granules are still present, but are not visible in the resting regenerative cells. A morphological sign of midgut stem cell differentiation is the accumulation of mitochondria just above the nuclei. They gradually assume characteristic features of epithelial cells during elongation toward the midgut lumen. Proliferation and differentiation of regenerative cells are caused by processes of degeneration (apoptosis and necrosis), which intensively occur in the midgut epithelium of *A. formicaria*. <http://zoolstud.sinica.edu.tw/Journals/49.1/10.pdf>

Key words: Midgut epithelium, Differentiation, Regeneration, Degeneration.

In insects, digestion, absorption, and secretion (and excretion in taxa lacking Malpighian tubules) occur in the midgut (Krzysztofowicz et al. 1973, Szklarzewicz and Tylek 1987, Okuda et al. 2007, Rost-Roszkowska et al. 2008). Not surprising, therefore, the midgut epithelium is comprised of several kinds of cells (i.e., digestive, goblet, endocrine, and regenerative cells). These cells experience intensive degeneration but are replaced by regenerative cells that act as stem cells. Recently, stem cells and their participation in tissue re-modeling and regeneration after degeneration (apoptosis and/or necrosis) have been major topics in the literature (Hakim et al.

2001, Li and Xie 2005, Park and Takeda 2008, Parthasarathy and Palli 2008, Park et al. 2009).

The ultrastructure of the midgut epithelium and degeneration and regeneration of various cells have not received much attention in basal hexapod groups such as the Protura, Collembola, Diplura, Archaeognatha, and Zygentoma (Dallai 1966 1977, Krzysztofowicz et al. 1973, Biliński and Klag 1979, Humbert 1979, Lauga-Reyrel 1980, Klag et al. 1981, Szklarzewicz and Tylek 1987, Xué and Dallai 1992, Rost et al. 2005, Rost 2006a b, Rost-Roszkowska 2008a, Rost-Roszkowska and Undrul 2008); only digestive (epithelial) and regenerative cells were reported to be cellular constituents of

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the midgut. However, the Zygentoma, which is comprised of the Lepidotrichidae, Nicoletiidae, Ateluridae, Protrinemuridae, Maindroniidae, and Lepismatidae, is considered closely related to winged insects (Kjer 2004, Regier et al. 2004, Grimaldi and Engel 2005). Therefore, it is likely that other types of cells reported as common in pterygote midguts are also present in the Zygentoma.

We examined the ultrastructure of the midgut of 4 species, 2 belonging to the Lepismatidae (Zygentoma), i.e., *Thermobia domestica* and *Lepisma saccharina* (Rost et al. 2005, Rost 2006a, Rost-Roszkowska et al. 2007) and 2 to the Machilidae (Archaeognatha), i.e., *Machilis hrabei* and *Lepismachilis notata* (data not published). Their midgut epithelium is composed of columnar cells and regenerative nests. However, there is no information about the ultrastructure of the midgut epithelium in the Ateluridae (Zygentoma). Herein, we present the results of our study on the midgut of *Atelura formicaria*. It is hoped that this information will facilitate a comparison of the midgut epithelium of 2 representatives of 2 groups of the Zygentoma, the Ateluridae and Lepismatidae, and the Archaeognatha, and hopefully provide new insights into the organization of the midgut epithelium in basal hexapod groups.

MATERIALS AND METHODS

Adult specimens of *A. formicaria* were collected in Bohemia centralis, Prague (Vinicna Street) in the Czech Republic (May to Sept. 2008). After decapitation, the material was fixed in 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) with saccharose (at 4°C for 2 h). After dehydration in a graded series of alcohol (50%, 70%, 80%, 90%, 96%, and 100%, each for 15 min) and acetone (15 min), the material was embedded in Epon 812. Semi- and ultrathin sections were cut on a Leica UCT25 ultramicrotome (Poznan, Poland). Semithin sections stained with 1% methylene blue in 0.5% borax were observed with an Olympus BX60 light microscope (Warsaw, Poland). After staining with uranyl acetate and lead citrate, ultrathin sections were analyzed with a Hitachi H500 transmission electron microscope (Tokyo, Japan).

RESULTS

Fine structure of the midgut epithelium of *A. formicaria*

The midgut of *A. formicaria* was sack-like in shape and was devoid of midgut caeca characteristic for insects. Its epithelium rested on a non-cellular basal lamina and was composed of epithelial and regenerative cells. The latter formed characteristic regenerative nests (Fig. 1). Approximately 26-30 regenerative nests with several dozen regenerative cells occurred in each transverse section through the midgut. Among regenerative cells, 2 types of cells could be distinguished: resting and differentiating cells (Fig. 2).

The basal regions of epithelial cells, which were rich in mitochondria, were strongly constricted and protruded deeply between adjacent regenerative nests (Fig. 3). The nuclei of epithelial cells were situated at 2/3 of the cell height. Near the nuclei and just beneath the apical membranes, which formed microvilli, were many cisterns of rough (RER) and smooth (SER) endoplasmic reticulum, and Golgi complexes (Fig. 4). Cisterns of endoplasmic reticulum frequently formed circular structures. Many spherites also appeared near the nuclei. Among the midgut epithelial cells, smooth septate junctions, gap junctions and pleated septate junctions were observed.

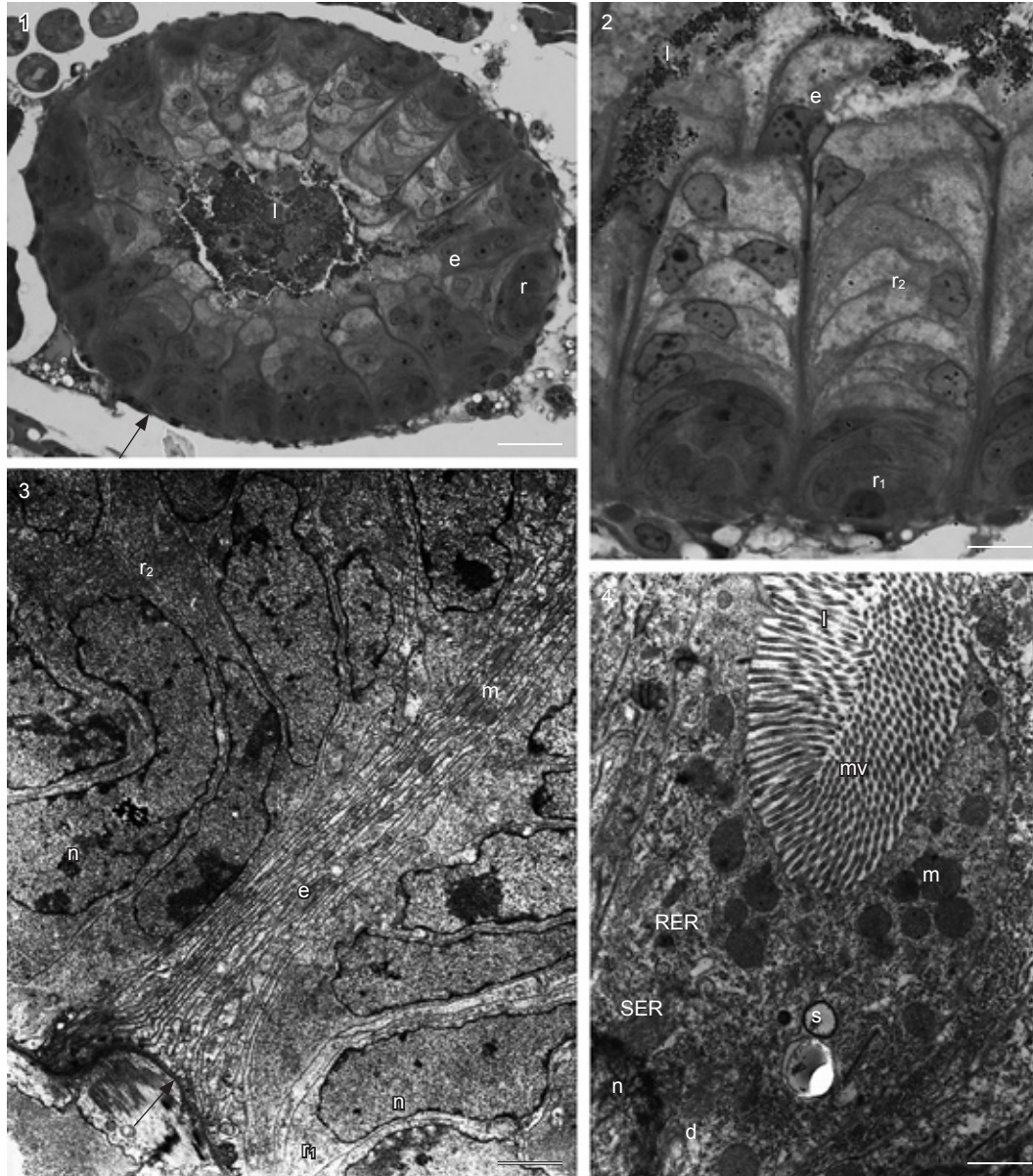
Differentiation and degeneration of midgut epithelial cells in *A. formicaria*

The regenerative nests were composed of several dozen regenerative cells among which were resting and differentiating cells (Figs. 2, 5). Each regenerative nest included approximately 10-20 resting regenerative cells. The electron-dense cytoplasm had numerous mitochondria and free ribosomes. Their nuclei were oval (Fig. 6). In cells just before mitotic division, many cisterns of endoplasmic reticulum and electron dense granules were visible (Fig. 6). Pleated septate junctions were observed between adjacent regenerative cells (Fig. 6a). During mitotic division, the electron-dense granules were still evident (Fig. 7) but were no longer visible in resting regenerative cells. Mitotic division occurred continuously in all regenerative nests. Differentiating cells, which showed various stages of differentiation, were clustered above resting and dividing regenerative

cells (Figs. 2, 8).

The first morphological sign of the differentiation of regenerative cells into epithelial cells was the accumulation of mitochondria just above the nuclei, in the cytoplasm which would soon elongate toward the midgut lumen (Fig. 9). Cisterns of RER and SER and Golgi complexes gradually appeared. Regenerative cells elongated,

assuming features of epithelial cells. Cisterns of RER and SER, and Golgi complexes together with the nucleus shifted toward the apical cytoplasm. The more distal was the cell in the regenerative nest, the more epithelial features it possessed. Initially, differentiating cells did not contact the midgut lumen (Fig. 2), so their apical cytoplasm lacked microvilli. The apical membrane



Figs. 1-4. Midgut epithelium of *Atelura formicaria*. **1.** The epithelium is composed of epithelial cells (e) and regenerative nests (r). Arrow, basal lamina; l, midgut lumen. Light microscopy, scale bar = 62.5 μm . **2.** Regenerative nests formed by resting (r_1) and differentiating (r_2) regenerative cells. e, epithelial cells; l, midgut lumen. Transmission electron microscopy, scale bar = 20.8 μm . **3.** Basal regions of epithelial cells (e) rich in mitochondria (m) protruding between regenerative nests. r_1 , resting and r_2 , differentiating regenerative cells; n, nuclei of regenerative cells, arrow, basal lamina. Transmission electron microscopy, scale bar = 1.67 μm . **4.** Apical cytoplasm with many cisterns of rough (RER) and smooth endoplasmic reticulum (SER), and mitochondria (m). d, Golgi complexes; mv, microvilli; l, midgut lumen; n, nucleus; s, spherites. Transmission electron microscopy, scale bar = 1.33 μm

reached the midgut lumen because of gradual degeneration of epithelial cells. Degenerated cells were discharged into the midgut lumen forming a space for new epithelial cells. Then the apical and perinuclear cytoplasm of fully differentiated epithelial cells underwent vacuolization and became electron lucent (Fig. 10). This was the first morphological sign that necrosis was beginning. Organelles decreased in number. Apical membranes, forming lobular evaginations (Fig. 10), ruptured, and their cytoplasm, together with organelles, was discharged into the midgut lumen (Fig. 11). Necrosis proceeded continuously during the entire life of adult specimens, but it occurred more intensively when the midgut lumen was filled with nourishment. At such times, differentiation of regenerative cells also proceeded more quickly.

In the midgut epithelium of *A. formicaria*, cell death proceeded in an apoptotic manner. The nucleus in an apoptotic cell assumed a lobular shape, and its condensed chromatin accumulated both near the nuclear envelope and in the central nuclear region (Fig. 12). Eventually, it was fragmented. Concurrently, the cytoplasm of an apoptotic cell became electron dense (Fig. 13), and its gradual shrinkage led to its separation from the basal lamina and adjacent epithelial cells. Finally, the apoptotic cell was discharged into the midgut lumen (Fig. 14), where it assumed a lobular shape with numerous blebs. Its cytoplasm was still electron dense with abundant free ribosomes, cisterns of endoplasmic reticulum, mitochondria, single Golgi complexes, and fragments of the nucleus. Neither the formation of apoptotic bodies nor phagocytosis was observed. In the midgut lumen, apoptotic cells disintegrated.

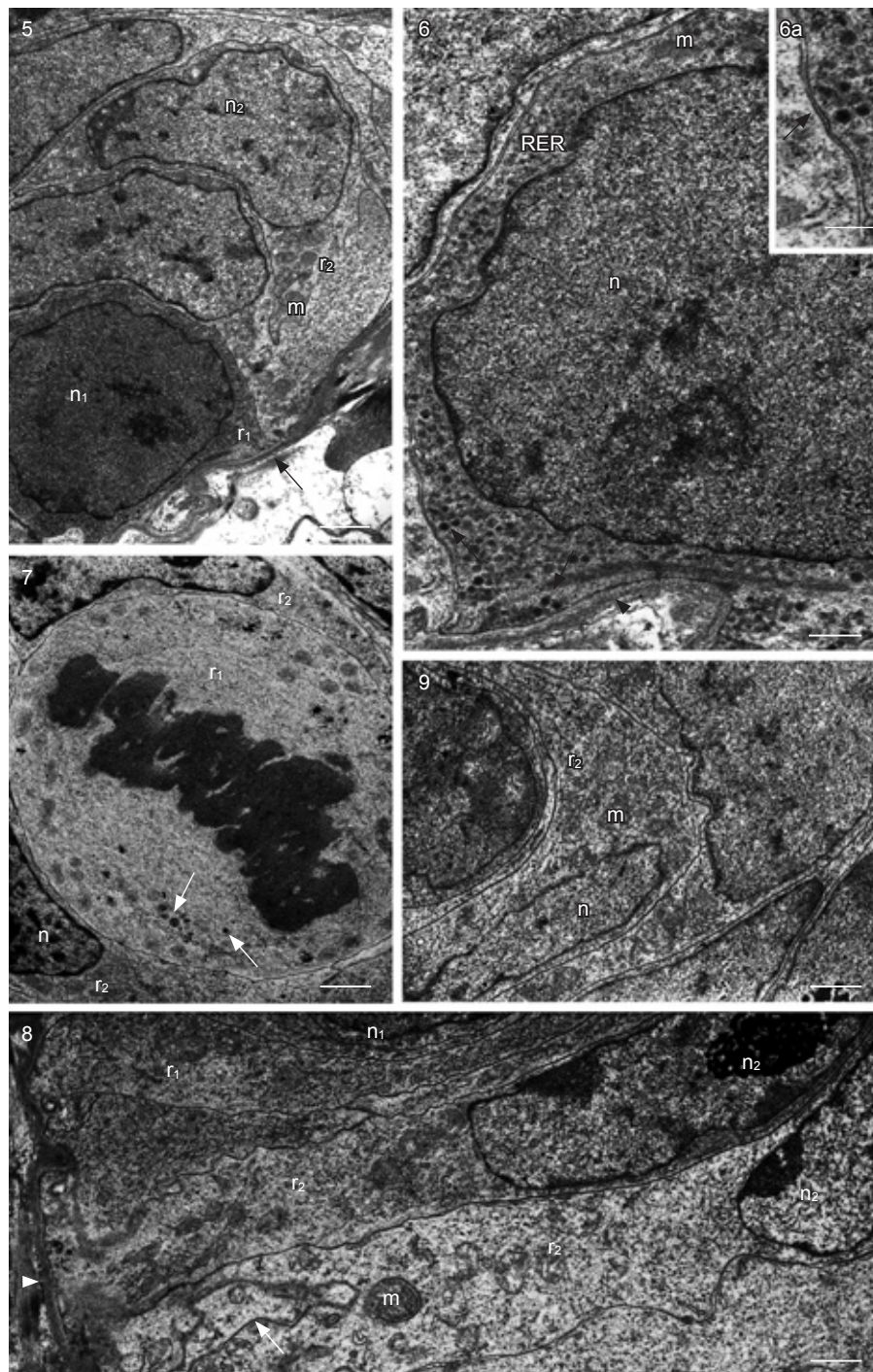
DISCUSSION

In many basal hexapod groups (Protura, Collembola, Diplura, Archaeognatha, and Zygentoma), the structure of the midgut epithelium at the ultrastructural level was described, but those descriptions mainly concentrated on its organization (Dallai 1966 1975 1977, Krzysztofowicz et al. 1973, Biliński and Klag 1979, Humbert 1979, Lauga-Reyrel 1980, Klag et al. 1981, Xué and Dallai 1992, Pigino et al. 2005, Rost 2006a b, Rost-Roszkowska 2008a, Rost-Roszkowska and Undrul 2008). The midgut epithelium in those groups is composed of epithelial and regenerative cells, but information on other epithelial cells, common

in pterygotes (e.g., goblet or endocrine cells) is lacking (Chayka and Farafonova 1980, Endo and Nishiitsutsuji-Uwo 1981, Montuenga et al. 1989, Levy et al. 2004, Rost-Roszkowska et al. 2008). Regenerative cells are individually formed between basal regions of epithelial cells, e.g., in *Podura aquatica* (Collembola, Arthropleona) (Rost 2006b) or *Filientomon takanawanum* (Protura) (Rost-Roszkowska et al. 2009), but they form regenerative groups called regenerative nests as in species belonging to the Archaeognatha (*Lepismachilis notata* and *Machilis hrabei*) (our studies, unpublished data), or the Lepismatidae of the Zygentoma (*Thermobia domestica* and *Lepisma saccharina*) (Rost et al. 2005, Rost 2006a, Rost-Roszkowska et al. 2007). Regenerative cells have not been described in some species of Collembola (Jura 1958, Krzysztofowicz et al. 1973, Lauga-Reyrel 1980, Rost-Roszkowska and Undrul 2008); in those hexapods, epithelial cells may regenerate by epithelial cells themselves. *Atelura formicaria* belongs to the Ateluridae, which together with the Nicoletiidae, Lepidotrichidae, Protrinemuridae, Maindroniidae, and Lepismatidae comprise the order Zygentoma (Kjer 2004, Regier et al. 2004, Gimaldi and Engel 2005). The ultrastructure of the midgut epithelium, in association with degeneration and regeneration, was analyzed only in the Lepismatidae (Rost et al. 2005, Rost 2006a, Rost-Roszkowska et al. 2007). Similar analyses in the remaining groups of Zygentoma may help elucidate interfamily relationships of these taxa.

The midgut epithelium in *A. formicaria*, as with other wingless ectognathans we have analyzed, is composed of epithelial cells and regenerative nests. In transverse sections through the midgut of *A. formicaria*, we observed about 26-30 regenerative nests, whereas in *T. domestica* and *L. saccharina* (Lepismatidae), we observed only about 4-6 and 8-12 nests, respectively (Rost 2006a). The high number of regenerative nests in *A. formicaria* causes a strong constriction of the basal regions of epithelial cells placed between regenerative nests. Therefore, many mitochondria are packed into narrow parts of the basal cytoplasm. Similar constrictions of basal regions in epithelial cells were observed in the midgut epithelium of the Orthoptera (Pterygota); however, in that group, regenerative cells form regenerative crypts, which protrude into the hemocoel (Srivastava 1997, Illa-Bochaca and Montuenga 2006, Rost-Roszkowska 2008b).

In most insects, regenerative cells in the



Figs. 5-9. Regenerative nests in the midgut epithelium of *Atelura formicaria*. **5.** Electron-dense cytoplasm and circular nucleus (n_1) in a resting regenerative cell (r_1). Arrow, basal lamina; r_2 , differentiating regenerative cell; n_2 , nucleus of a differentiating cell; m, mitochondria. Transmission electron microscopy, scale bar = 1.48 μm . **6.** A regenerative cell just before mitotic division. Arrowhead, basal lamina; RER, cisterns of rough endoplasmic reticulum; arrows, electron-dense granules; m, mitochondria; n, nucleus. Transmission electron microscopy, scale bar = 0.57 μm . **6a.** Septate junction (arrow) between adjacent regenerative cells. Transmission electron microscopy, scale bar = 0.24 μm . **7.** Mitotic division of a regenerative cell (r_1). Arrows, electron-dense granule in the cytoplasm of a regenerative cell; r_2 , differentiating regenerative cell with nucleus (n). Transmission electron microscopy, scale bar = 1.25 μm . **8.** Differentiating regenerative cells (r_2) near resting ones (r_1). n_1 , n_2 , nuclei of regenerative cells. External regenerative cells assumed epithelial features. Arrowhead, basal lamina; arrow, slight folds in the basal membrane; m, mitochondria. Transmission electron microscopy, scale bar = 1.19 μm . **9.** Accumulation of mitochondria (m) at the beginning of the differentiation process of regenerative cells (r_2). n, nucleus. Transmission electron microscopy, scale bar = 0.95 μm .

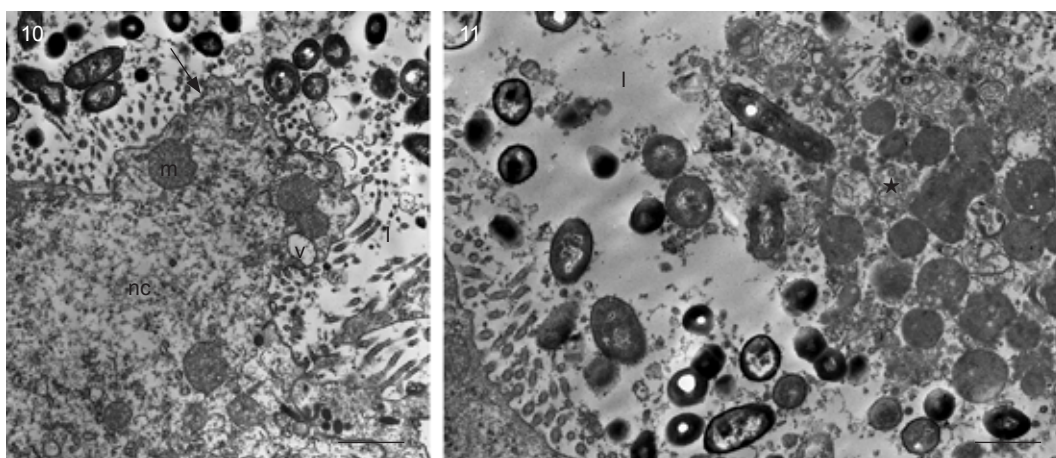
midgut epithelium are treated as stem cells, and they are able to intensively proliferate to produce epithelial cells (Srivastava 1997, Cruz-Landim 1999, Hakim et al. 2001, Evangelista and Leite 2003, Neves et al. 2003, Martins et al. 2006, Rost 2006a b, Baton and Ranford-Cartwright 2007, Rost-Roszkowska 2008b, Park and Takeda 2008, Parthasarathy and Palli 2008, Park et al. 2009). Therefore, damaged midgut epithelium or single cells may be replaced by those that are newly differentiated. In *A. formicaria*, distinct functional differentiation among regenerative cells in each regenerative nest was observed. These cells, which are capable of mitotic division (resting cells), often occur with differentiating cells. Among the latter, mitotic division was not observed, implying that only resting regenerative cells can function as stem cells. Initially, stem cells proliferate; then some begin to differentiate, whereas others remain as a pool of cells capable of self-renewal. Among differentiating regenerative cells, those that rest externally in the regenerative nests possess many features of epithelial cells. They do not have microvilli and do not contact the midgut lumen, but they can participate in synthesis (by the presence of cisterns of RER and SER) and transport (by the folds in the basal membrane with numerous mitochondria).

A structure called a “stem niche” includes differentiating regenerative cells, neighboring epithelial cells, and extracellular spaces between cells and the basal lamina. This stem niche represents a setting that sends signals to resting regenerative cells. The signals stimulate resting regenerative cells to either divide or differentiate

(Fuchs et al. 2004, Li and Xie 2005, Illa-Bochaca and Montuenga 2006, Moore and Lemischka 2006).

Intercellular septate junctions between regenerative cells in all regenerative nests were important in our studies of *A. formicaria*. Until now, only intercellular junctions (e.g., smooth septate junctions, spot desmosomes, gap junctions, and pleated septate junctions) were described between midgut epithelial cells; intercellular junctions between regenerative cells in regenerative cell groups were not described (Xué and Dallai 1992, Rost 2006a b, Rost-Roszkowska and Undrul 2008, Fialho et al. 2009). In *A. formicaria*, pleated septate junctions develop, which are responsible for cell adhesion and the tightness of extracellular spaces. It is likely that these junctions enable the maintenance of such high numbers of regenerative cells in regenerative nests. It suggests these same junctions may serve as a component of a stem niche. The junctions are the 1st intercellular junctions between newly formed epithelial cells, whereas smooth septate and gap junctions are formed between differentiated cells.

In *A. formicaria*, midgut epithelial cells degenerate from necrosis, and the process intensifies when the midgut lumen is filled with nourishment. Degeneration of the cells may stimulate resting stem cells to proliferate. Necrosis is a common process observed in insect midgut epithelium and is required for proper functioning of the epithelium. It occurs in a cyclic (connected to molting cycles) or continuous manner (during the entire life) (Garcia et al. 2001, Hakim et al. 2001, Takeda et al. 2001, Evangelista and Leite 2003,

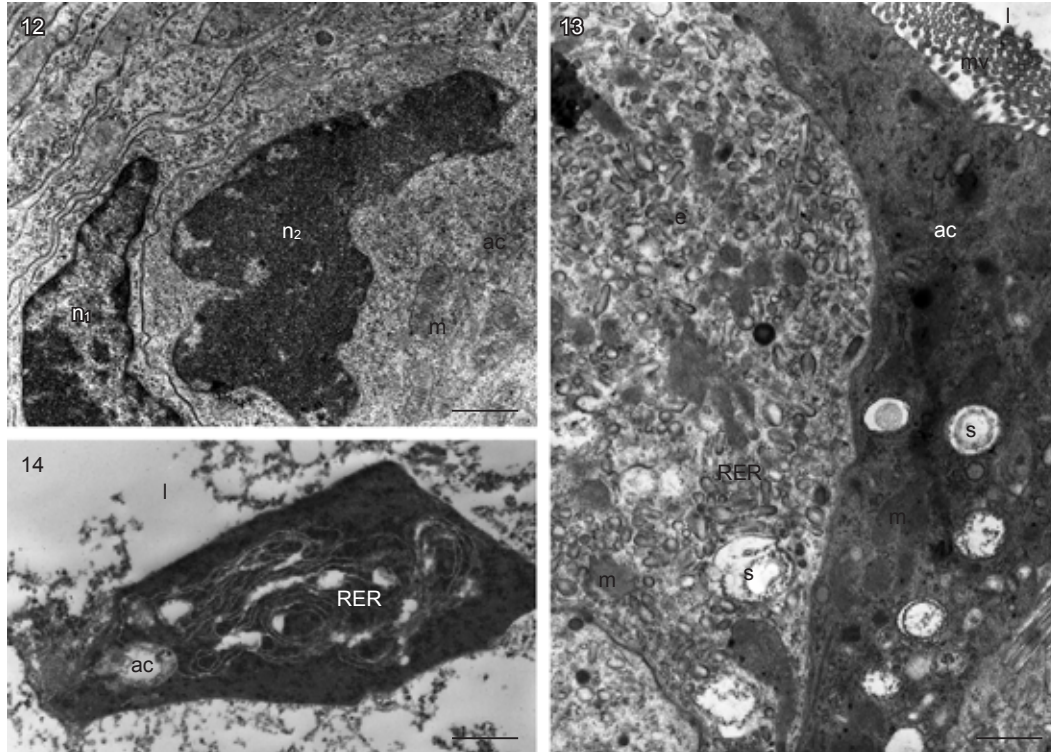


Figs. 10 -11. Necrosis in the midgut epithelium in *Atelura formicaria*. **10.** Electron-lucent cytoplasm of a necrotic cell (nc) with vacuoles (v). Arrow, apical membrane evagination; l, midgut lumen; m, mitochondria. Transmission electron microscopy, scale bar = 1.04 μm . **11.** Organelles (asterisk) discharged into the midgut lumen (l). Transmission electron microscopy, scale bar = 0.87 μm .

Rost 2006a b, Rost-Roszkowska 2008b). In *A. formicaria*, necrosis is intensively activated when the midgut lumen is filled with nourishment, but it continuously proceeds during the entire lifespan of adults. Necrosis is caused by mechanical damage and external factors and is sometimes associated with holocrine excretion (Kömüves et al. 1985, Jimenez and Gilliam 1990, Guimarães and Linden 2004). In *A. formicaria*, apoptosis, which is treated as programmed cell death, occurs, as does necrosis. Apoptosis was described in the midgut epithelium of some insect species (Pipan and Rakovec 1980, Gregorc and Bowen 1997, Uwo et al. 2002, Vaidyanathan and Scott 2006, Parthasarathy and Palli 2007, Tettamanti et al. 2007, Vilaplana et al. 2007, Rost-Roszkowska 2008a, Rost-Roszkowska et al. 2008 2009, Parthasarathy and Palli 2008, Park et al. 2009). During apoptosis, a cell shrinks because of water elimination, and intercellular junctions between apoptotic and adjacent cells disappear. The nucleus of a cell undergoing apoptosis becomes lobular, and its chromatin becomes fragmented and

electron dense. Then the entire cell fragments. But there are some items that should be explained, i.e., the formation of apoptotic bodies (fragments of apoptotic cells) and their phagocytosis. According to Pipan and Rakovec (1980) and Vaidyanathan and Scott (2006), apoptotic bodies are phagocytized by neighboring cells or an entire apoptotic cell is absorbed by them. However, in some insects, the phagocytosis of apoptotic cells was not reported; it is likely that midgut epithelial cells cannot phagocytize them. As in some other insect species, apoptotic cells of *A. formicaria* were discharged into the midgut lumen, where they were further disintegrated and digested.

Our studies show that in *A. formicaria*, the midgut epithelium is regenerated and degenerated in a manner similar to that described for the Lepismatidae and Archaeognatha. It is also composed of epithelial cells and regenerative nests. An analysis of the midgut epithelial structure in the remaining groups of the Zygentoma (Nicoletiidae, Lepidotrichidae, Protrinemuridae, and Maindroniidae) will hopefully contribute to an



Figs. 12-14. Apoptosis of midgut epithelial cells in *Atelura formicaria*. **12.** Lobular-shaped nucleus (n_2) in an apoptotic cell (ac) with condensed chromatin. m, mitochondria; n_1 , nucleus of an adjacent epithelial cell. Transmission electron microscopy, scale bar = 1.05 μm . **13.** Electron-dense cytoplasm of an apoptotic cell (ac). e, adjacent epithelial cell; RER, cisterns of rough endoplasmic reticulum; mv, microvilli; l, midgut lumen; m, mitochondria; s, spherite. Transmission electron microscopy, scale bar = 0.63 μm . **14.** Apoptotic cell (ac) with electron-dense cytoplasm in the midgut lumen (l). Cisterns of RER. Transmission electron microscopy, scale bar = 0.74 μm .

understanding of interfamilial relationships of the Zygentoma and to a reconstruction of the ground plan of the midgut epithelium of the Ectognatha.

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