Degeneration of the midgut epithelium in *Epilachna* cf. *nylanderi* (Insecta, Coccinellidae): apoptosis, autophagy, and necrosis

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Abstract: This study investigates mechanisms of adaptation to metal toxicity peculiar to the midgut epithelium of *Epilachna* cf. *nylanderi* (Mulsant, 1850) (Coccinellidae). This species of beetle has currently been identified in only one locality in South Africa and is known to feed on the nickel hyperaccumulator *Berkheya coddii* Roessl. (Asteraceae), an endemic plant species of the South African ultramafic ecosystem. Our focus involves an analysis of the morphological features of cells forming the midgut epithelium, which is the first organ exposed to toxic levels of metals ingested by the insect. Through the three key processes of apoptosis, necrosis, and autophagy, excess metals are eliminated from the organism and homeostatic conditions are maintained. Apoptosis and necrosis are both known to be involved in the degradation of midgut epithelial cells, while the role of autophagy is mainly implicated in the disintegration of the organelles of cells. This study reports on the participation of these three key degenerative processes in the removal of excess metals based on targeted observations of the insect midgut epithelium by light and electron microscopies. Additionally, the TUNEL reaction was specifically used to detect apoptosis.

Résumé : Notre étude explore les mécanismes d'adaptation à la toxicité des métaux spécifiques à l'épithélium du tube digestif moyen d'*Epilachna* cf. *nylanderi* (Mulsant, 1850) (Coccinellidae). Cette espèce de coléoptère a été actuellement retrouvée dans une seule localité d'Afrique du Sud et est connue pour s'alimenter de *Berkheya coddii* Roessl. (Asteraceae), une plante hyperaccumulatrice de nickel et endémique à l'écosystème ultramafique de l'Afrique du Sud. Notre étude se concentre sur l'analyse des caractéristiques morphologiques des cellules qui forment l'épithélium du tube digestif moyen, le premier organe à être exposé aux niveaux toxiques des métaux ingérés par l'insecte. À travers les trois processus essentiels d'apoptose, de nécrose et d'autophagie, les métaux excédentaires sont éliminés de l'organisme et les conditions homéostatiques maintenues. L'apoptose et la nécrose sont toutes deux connues comme étant impliquées dans la dégradation des cellules épithéliales du tube digestif moyen, alors que l'autophagie est surtout impliquée dans la désintégration des organites cellulaires. Notre étude décrit la participation de ces trois processus de dégénérescence essentiels au retrait des métaux excédentaires à partir d'observations ciblées de l'épithélium du tube digestif moyen par microscopies photonique et électronique. De plus, nous avons utilisé la réaction TUNEL spécifiquement pour détecter l'apoptose.

[Traduit par la Rédaction]

Introduction

Plants that accumulate metals may use them in protective mechanisms against herbivory and fungal attacks (Boyd 2007). However, some herbivorous insect species have adapted themselves to using these plants as good dietary resources. Given that an excess of consumed metals might become toxic, these insects have had to develop strategies to prevent, decrease, or repair the effects caused by excess metals that have entered their bodies. One such strategy is to intensify the rate of metal excretion either through binding these excess metals to metallothioneins or by sequestering them in intracellular granular structures (Hopkin 1989; Migula 1996; Migula et al. 2007).

Berkheya coddii Roessl. (Asteraceae), an endemic plant species from South African ultramafic ecosystems, is 1 of 318 nickel hyperaccumulators reported worldwide (Reeves and Baker 2000), and 1 of 5 reported from South Africa (Morrey et al. 1989; Smith et al. 2001; Migula et al. 2007). This species can store up to 76 100 mg $Ni \cdot kg^{-1}$ dry mass in leaves (Mesjasz-Przybyłowicz et al. 2004*a*). Several herbivorous insect species have been found associated with this plant, feeding on it with no significant effects on their population dynamics (Migula et al. 2005). Mechanisms allowing

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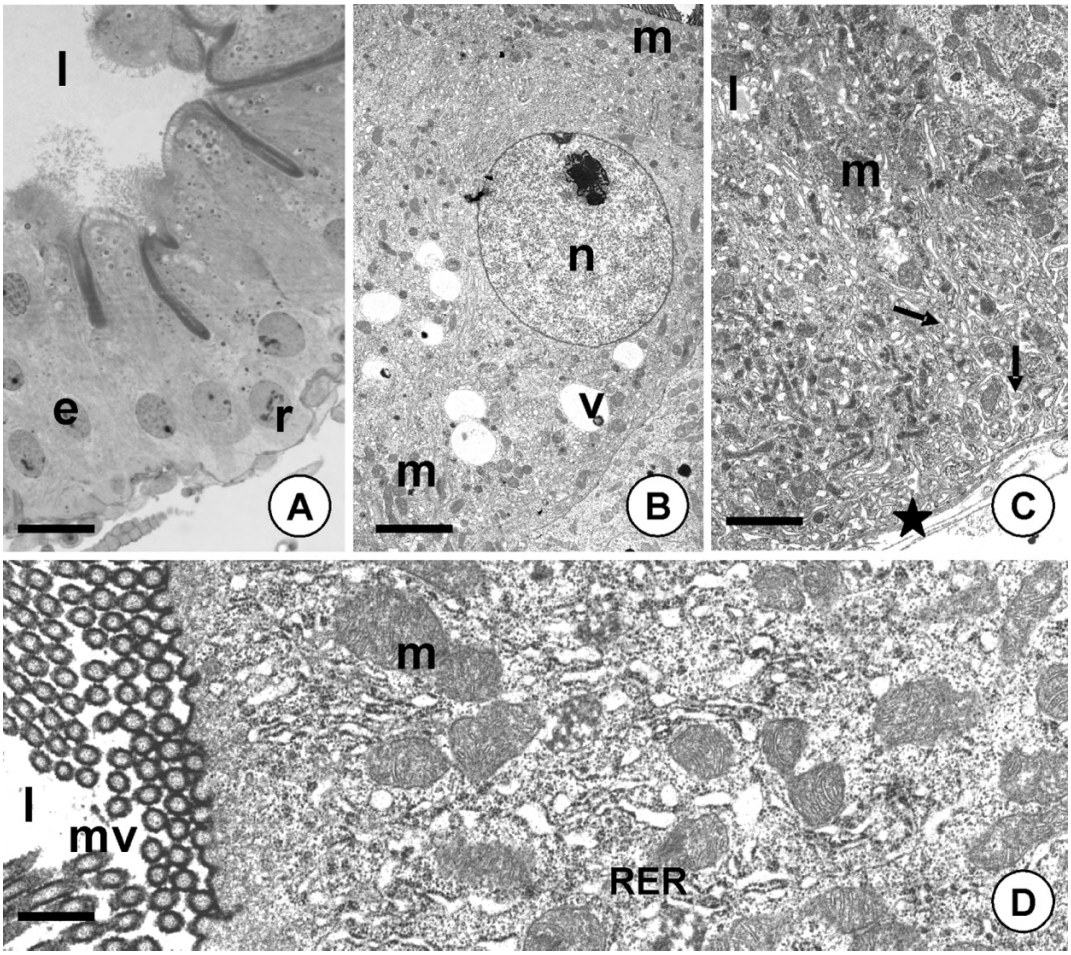
Fig. 1. Midgut epithelial cells in *Epilachna* cf. *nylanderi*. (A) Midgut epithelium of *E. nylanderi* is composed of epithelial cells (*e*) and singly distributed regenerative cells (*r*). Midgut lumen (*l*). Light micrograph; scale bar = 19.2 μm. (B) Cytoplasm of epithelial cells shows the regionalization in organelle distribution. Nucleus (*n*), mitochondria (*m*), and vacuoles (*v*), which are responsible for accumulation of metals. Transmission electron micrograph (TEM); scale bar = 4 μm. (C) Basal region of epithelial cells. Basal lamina (star), basal membrane forming numerous folds (arrows), mitochondria (*m*), and lipid droplets (*l*). TEM; scale bar = 1.42 μm. (D) The cytoplasm of the apical region in epithelial cells. Midgut lumen (*l*), microvilli (mv), mitochondria (*m*), and cisterns of rough endoplasmic reticulum (RER). TEM; scale bar = 0.63 μm.



these insects to cope with excess nickel were intensively studied recently for a chrysomelid beetle, *Chrysolina pardalina* (Fabricius, 1781), which efficiently eliminates nickel (Klag et al. 2002; Przybyłowicz et al. 2004).

The present study seeks to identify mechanisms of adaptation to nickel toxicity developed by another beetle, the ladybird beetle *Epilachna* cf. *nylanderi* (Mulsant, 1850) (Coleoptera: Coccinellidae, Epilachninae), known from only one locality in South Africa. Both, the larva and adults feed on *B. coddii* leaves. Laboratory tests confirmed their ability to live on leaves of three other nickel hyperaccumulators from these areas — *Berkheya zeyheri* (Sond. and Harv.) Oliv. & Hiern subsp. *rehmannii* (Thell.) Roessl. var. *rogersiana* (Thell.) Roessl., *Senecio anomalochrous* Hilliard, and *Senecio coronatus* (Thunb.) Harv., which grow on ultramafic soils containing high concentrations of nickel, chromium, and iron (Augustyniak et al. 2002; Mesjasz-Przybyłowicz et al. 2004*b*).

The midgut cells are the first line of defence against excessive levels of metals in insects. For this reason, this study is focused on searching for morphological features of the gut that might be important for the proper distribution of metals or their elimination, thereby protecting the insects against their toxicity. Food in the insect midgut is often enveloped by the peritrophic matrix, which among many functions protects the epithelium and allows the circulation of digestive enzymes in the endoectoperitrophic space (Terra 1996). The midgut epithelium is mainly composed of the epithelial and the regenerative cells. The first group of cells is responsible for digestibility of food through the production and excretion of enzymes and the absorption of the digests. Regenerative cells located between basal regions of epithelial cells replace them when they are lost as a result of abrasion or aging processes (Cavalcante and Cruz-Landim 1999). The insect midgut epithelium degenerates, according to all functions combined with digestion, and new cells differentiate from cells that are able to proliferate, known as regenerative cells.

Little is known about cell death in insect tissues and organs in response to environmental stressors. Apoptosis and necrosis are processes that enable the persistence of homeostasis in multicelullar organisms. Because of the regulation

Fig. 2. Apoptosis in the midgut epithelial cells of *Epilachna* cf. *nylanderi*. (A) Nucleus (*n*) of epithelial cell changes its shape into a lobular form, signalling the beginning of apoptosis. Nucleolus (nu). TEM; scale bar = 1.52 μm. (B) Nucleus (*n*) of apoptotic cell undergoes fragmentation (arrows). Mitochondria (*m*) and microvilli (mv). TEM; scale bar = 1.25 μm. (C) Cytoplasm of apoptotic cell (*a*) becomes electron dense. Nucleus (*n*) of epithelial cell (*e*) and regenerative cell (*r*). TEM; scale bar = 2.17 μm. (D) Detection of apoptotic cells in the midgut epithelium (*e*) by TUNEL (cross section). TUNEL-positive nuclei of the epithelial layer are stained in red (arrows). Midgut lumen (*l*). Fluorescence micrograph; scale bar = 122 μm. (E) Apoptotic cell (*a*) is completely removed into the midgut lumen (*l*), where it undergoes digestion. Mitochondria (*m*), microvilli (mv), and peritrophic matrix (star). TEM; scale bar = 1.58 μm.

of cell number, these processes cause the balance of cell proliferation. Thus both apoptosis and necrosis are responsible for cell elimination; however, they definitely differ in their source, course, and changes that they cause. Necrosis is defined as an incidental and passive cell death caused by disruptive external factors (chemical, physical, and biological) (Kõműves et al. 1985; Guimarães and Linden 2004). Apoptosis is recognized as an actively regulated physiological process that enables removal of useless or unexploited cells (Jacobson et al. 1997; Proskuryakov et al. 2002, 2003; Schöck and Perrimon 2002; Guimarães and Linden 2004). Autophagy also plays an important role in maintaining homeostasis. It is responsible for disintegration of organelles utilized by vacuoles and (or) lysosomes (Klionsky and Emr 2000; Lee et al. 2002; Levine and Klionsky 2004; Lockshin and Zakeri 2004; Tettamanti et al. 2007).

The main anatomical features of the alimentary canal of *Epilachna* sp. were described using light microscopy more than 70 years ago in the Mexican bean beetle, *Epilachna corrupta* Mulsant, 1851 (Burgess 1932). The aim of our study was to analyze all processes of degeneration (apoptosis, necrosis, and autophagy) in the midgut epithelium of *E. nylanderi*, which in the natural environment is the obligatory monophage of *B. coddii*, with the use of transmission electron microscopy (TEM).

Materials and methods

The insects were collected from one site in Mpumalanga Province, South Africa. They have not been encountered thus far at other ultramafic sites overgrown with dense populations of *B. coddii* (Asteraceae) during 7 consecutive years of field studies.

According to the description of the South African Museum of Natural History, the ladybird beetle *Epilachna* cf. *nylanderi* is probably a new species because of its long-term geographical isolation and host-plant specificity. It has morphological features similar in description to that of *E. nylanderi* collected in Namibia.

Light microscopy and TEM

Isolated from about 40 adult specimens, midguts of *E. nylanderi* were fixed in 2% OsO4 in 0.1 mol·L^{-1} phosphate buffer with saccharose (1.5 h at 4 °C), dehydrated in the graded series of ethanol (50%, 70%, 90%, 95%, and 100%, each for 15 min), then acetone (15 min), and embedded in Epon 812. Semi- and ultra-thin sections were cut on a Leica Ultracut UCT25 ultramicrotome. Semi-thin sections were stained with 1% methylene blue in 0.5% borax and observed with an Olympus BX60 light microscope. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H500 transmission electron microscope at 75 kV.

TUNEL assay

Isolated midguts (from 10 adult specimens) were fixed in 4% paraformaldehyde in Tris-buffered saline (TBS) for 20 min at room temperature, placed in TBS containing 0.1% Triton X-100, and embedded in tissue-freezing medium (Tissue-Tek®; Sakura Finetek USA, Inc., Torrance, California). Cryostat sections were cut (5 μm of thickness) and mounted on slides covered with 1% gelatin. Slides were incubated in permeabilization solution (0.1% sodium citrate) (2 min on ice at 4 °C), washed in TBS (3 × 5 min), and stained with TUNEL reaction mixture (In situ Cell Death Detection Kit, TMR red; Roche, Penzberg, Germany) (60 min at 37 °C in the dark). Negative controls were prepared according to labeling protocol (In situ Cell Death Detection Kit, TMR red; Roche, Penzberg, Germany). Slides were analyzed with an Olympus BX60 fluorescence microscope.

Results

The midgut epithelium of *E. nylanderi* contains epithelial cells and regenerative cells singly distributed among epithelial ones (Fig. 1A). Many epithelial cells degenerate during the life of the beetle and regenerative cells are responsible for the restoration of midgut epithelium after degeneration. Because of the complexity of processes combined with regenerative cells, their structure and differentiating are described in a separate article (M.M. Rost-Roszkowska, I. Poprawa, J. Klag, P. Migula, J. Mesjasz-Przybyłowicz, and W. Przybyłowicz, unpublished data). The cytoplasm of epithelial cells shows distinct regionalization in organelle distribution (Fig. 1B), and consequently basal (Fig. 1C), perinuclear, and apical regions are distinguished (Fig. 1D).

The degeneration of midgut cells in the analyzed species proceeds either in an apoptotic or in a necrotic way. In young specimens, just after pupation, only apoptosis is observed, while necrosis is involved together with aging. Distinct apoptosis involves individual cells of the entire epithelium. Necrosis proceeds much more intensively on the ventral side of the midgut. At the end of the insects' life, when necrosis is observed in all insects tissues, apoptosis is detained and the entire epithelium undergoes necrosis.

Alterations within the nucleus are the first signs of the beginning of apoptosis. The nucleus achieves a lobular shape (Fig. 2A), forming numerous folds and blebs, and finally undergoes fragmentation (Fig. 2B). The cytoplasm of an apoptotic cell becomes electron dense with numerous free ribosomes and cistern of rough (RER) and smooth (SER) endoplasmic reticulum. Some SER and RER cisterns degenerate forming abundant membranous structures. Apoptotic cells still maintain contact with neighboring epithelial cells (Fig. 2C). Shrinkage of apoptotic cells and the beginning of differentiation of new epithelial ones cause the gradual separation of the apoptotic cells from the basal lamina.

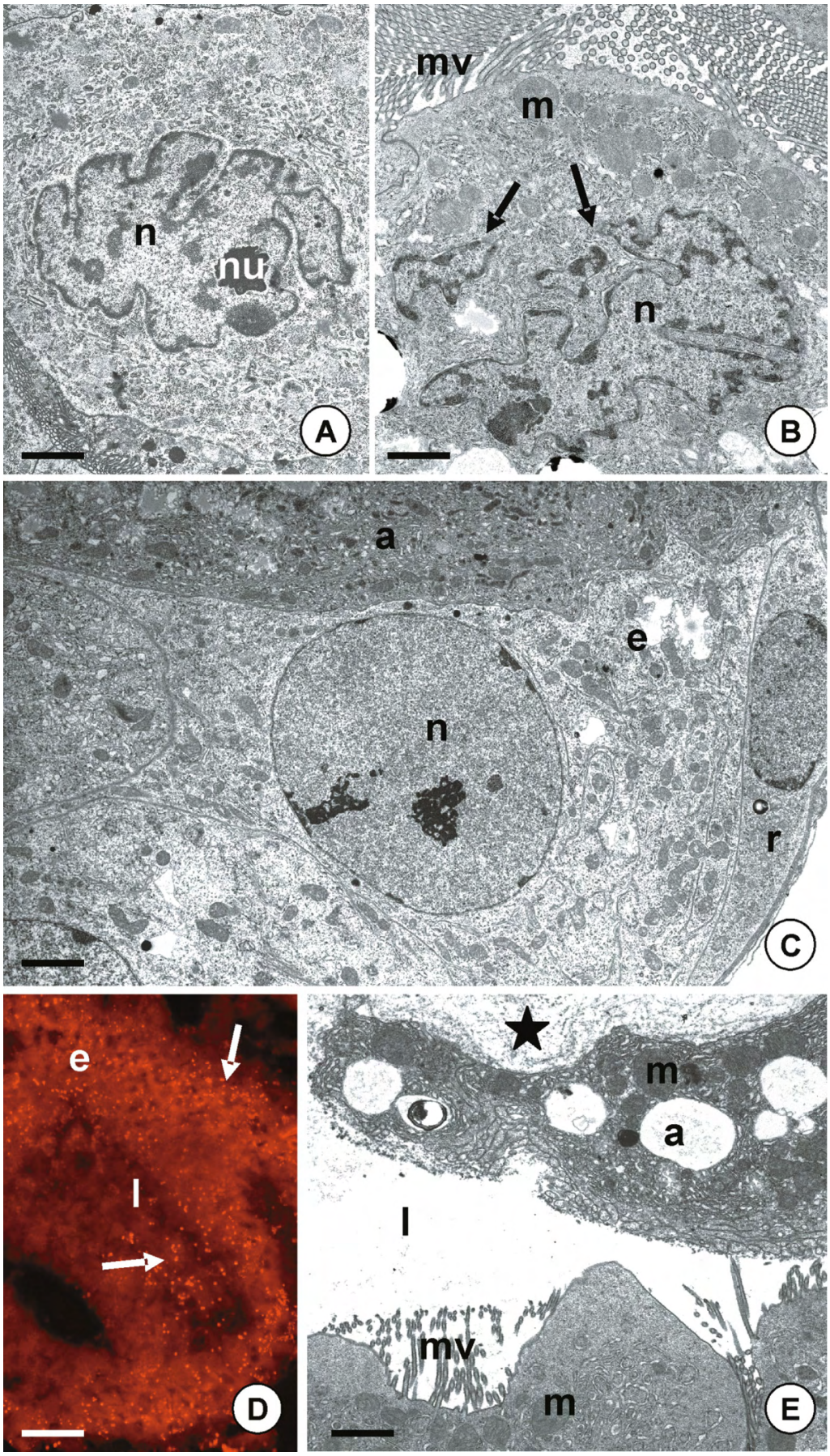
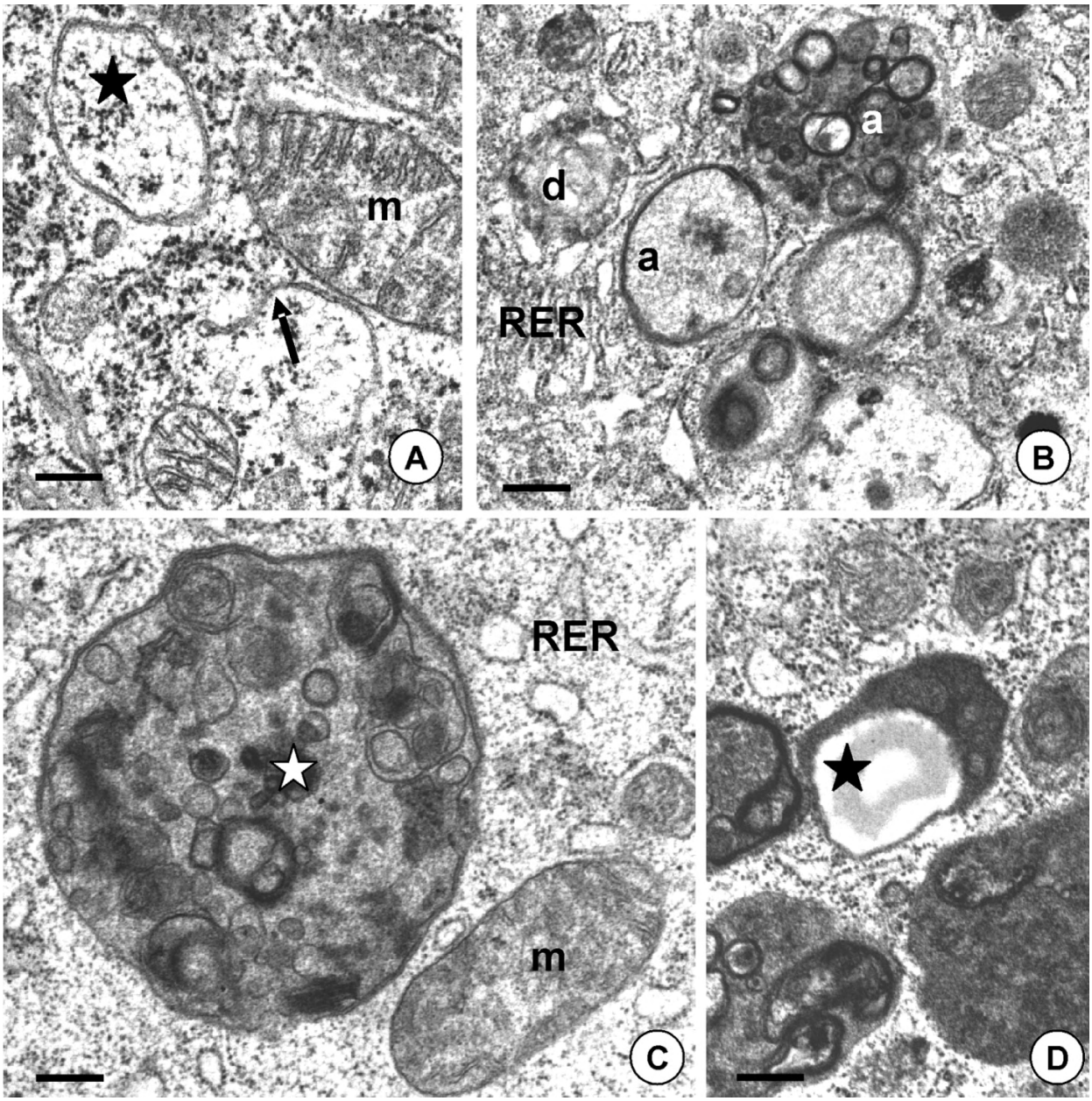


Fig. 3. Autophagy in the midgut epithelial cells of *Epilachna* cf. *nylanderi*. (A) Cisterns of endoplasmic reticulum extending (arrow) towards the neighbor organelles. Mitochondrium (*m*) and newly formed autophagosome (star). TEM; scale bar = 0.32 μm. (B) Autophagosomes (*a*) with disintegrating organelles. Dictyosomes (*d*) and rough endoplasmic reticulum (RER). TEM; scale bar = 0.4 μm. (C) Autophagosome with remains of digested organelles (star). Mitochondria (*m*) and rough endoplasmic reticulum (RER). TEM; scale bar = 0.45 μm. (D) Degenerating organelles in the autophagosome interior (star). TEM; scale bar = 0.3 μm.



Apoptotic cells are shifted into the midgut lumen just beneath the peritrophic matrix by the newly differentiated cells (Figs. 2D, 2E). In the midgut lumen, they undergo fragmentation and digestion.

Autophagy is a process that may be observed in any of the epithelial cells in both young and old specimens. It proceeds much faster in some epithelial cells, so autophagosomes of different stages are observed. Expanded cisterns of endoplasmic reticulum (Fig. 3A) surround fragments of cytoplasm with organelles, e.g., mitochondria. Subsequently, the cytoplasm with organelles is completely enclosed inside the newly formed autophagosome (Fig. 3B). Numerous dictyosomes and cisterns of endoplasmic reticulum accumulate in the vicinity of the autophagosome (Figs. 3B, 3C). Organelles are gradually digestedin the formed autophagosomes; eventually residual bodies are formed (Figs. 3D, 4B–4D).

When there are many aytophagosomes in epithelial cells, no distinct morphological signs of their degeneration are seen. At first, numerous small buds were observed on the surface of the nucleus (Fig. 4A). The nuclear envelope has a distinct layer of nuclear lamina. The buds separate from the nucleus retaining their envelope, which is similar to the nuclear envelope. Presumably it is the first morphological sign of necrosis beginning in these cells. The nucleolus be-

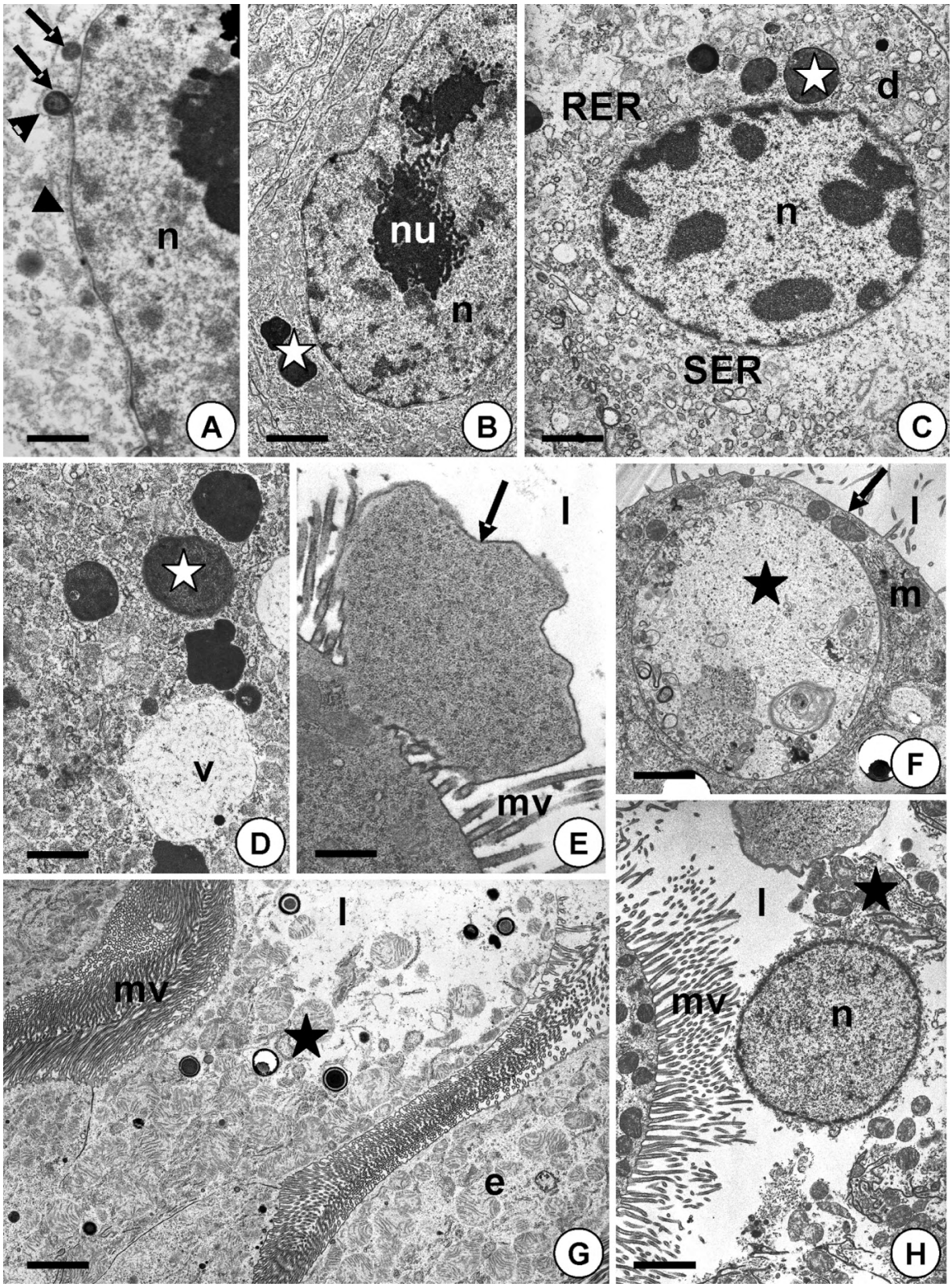
Fig. 4. Necrosis in the midgut epithelial cells of *Epilachna* cf. *nylanderi*. (A) Small buds (arrows) of nucleus (*n*) are formed. Nuclear lamina (arrowhead) of buds is similar to that of the nuclear envelope. TEM; scale bar = 0.8 μm. (B) Nucleolus (nu) in the nucleus (*n*) of necrotic cell diffuses. Residual bodies (star). TEM; scale bar = 1.4 μm. (C) Chromatin in the nucleus (*n*) becomes electron dense. Dictyosomes (*d*) and cisterns of smooth (SER) and rough (RER) endoplasmic reticulum swell. Residual bodies (star). TEM; scale bar = 1.6 μm. (D) Cytoplasm of necrotic cell possesses numerous residual bodies (star) and vacuoles (*v*). TEM; scale bar = 1.2 μm. (E) Apical membrane forms blebs (arrow) into the midgut lumen (*l*). Microvilli (mv). TEM; scale bar = 0.8 μm. (F) Autophagosome, which possesses remains of organelles (star), appear in apical blebs (arrow). Midgut lumen (*l*) and mitochondria (*m*). TEM; scale bar = 2 μm. (G) Apical cell membrane ruptures and all organelles (star) are removed into the midgut lumen (*l*). Microvilli (mv) and epithelial cell (*e*). TEM scale bar = 2.8 μm. (H) Organelles (star) of necrotic cell in the midgut lumen (*l*). Nucleus (*n*) and microvilli of epithelial cell (mv). TEM scale bar = 1.85 μm.

comes scattered (Fig. 4B) and nuclear chromatin forms dense masses (Fig. 4C). Cisterns of RER and SER, as well as dictyosomes enlarge and swell (Fig. 4C). Numerous autophagosomes are present in their cytoplasm (Figs. 4B–4D). Cytoplasm of the basal region becomes electron lucent and the number of organelles gradually decreases. Small vacuoles appear and some mitochondria begin to swell. Apical regions of epithelial cells initially do not show any signs of degeneration. However, the apical membrane forms large protrusions and blebs that extend into the midgut lumen (Fig. 4E), with an absence of organelles. The number of microvilli decreases. Organelles from the apical cytoplasm flow gradually into the bleb. In some cases a large autophagosome is shifted into the apical bleb (Fig. 4F). At this stage the process of nucleus budding is now not observed, but abundant double-membraned structures with the nuclear material appear in the cytoplasm of the necrotic cell. Eventually the apical membrane breaks (Fig. 4G) and all organelles together with autophagosomes are moved into the midgut lumen (Fig. 4H), where they undergo disintegration. Even if the apical membrane has been destroyed, the vacuolization of the cytoplasm in necrotic cells is not visible.

Discussion

The insect midgut epithelium degenerates just before each molting, successively during the entire life span (Humbert 1979; Garcia et al. 2001; Rost 2006*a*, 2006*b*). Accordingly, epithelial cells play a role not only in digestion and secretion, but also in the elimination of unnecessary or even harmful substances, which originate from food. In insects devoid of Malpighian tubules (e.g. Collembola or Diplura), redundant products of metabolism accumulate in specialized structures called urospherites (Krzysztofowicz et al. 1973; Humbert 1979; Pigino et al. 2005; Rost 2006*a*; Rost-Roszkowska et al. 2007; Rost-Roszkowska and Undrul 2008). In higher insects, metals and harmful substances are stored mainly in crystalline structures. Because of degeneration, metals accumulated in membranous structures are shifted into the midgut lumen and are consequently eliminated from the organism. It is one of the strategies through which insects feeding on metal hyperaccumulating plants are able to protect themselves against the harmful effects of metals (Hopkin 1989; Migula 1996; Migula et al. 2007). *Epilachna nylanderi* belongs to herbivores feeding on *B. coddii*, a plant species capable of nickel hyperaccumulation (Morrey et al. 1989). Thus numerous structures, which resemble the urospherites of primitive wingless insects, are present in the cytoplasm of its epithelial cells. These structures also resemble A-type granules, which are responsible for the accumulation of metals in the midgut of insects (Hopkin 1989). *Epilachna nylanderi* can therefore eliminate epithelial cells containing excess nickel in a continuous manner and the midgut epithelium may fulfill all of its functions. Adaptive mechanisms of coping with excess nickel were analyzed intensively in a chrysomelid beetle *C. pardalina* (Klag et al. 2002; Przybyłowicz et al. 2004). The gut and Malpighian tubules were identified in this species as the major target organs, important for protecting other body parts from potential nickel toxicity (Migula et al. 2003). Another recently studied grasshopper species *Scenoscepa* sp. (at present undescribed) seems to be less adapted (Augustyniak et al. 2006).

Degeneration plays an important role not only during embryogenesis, tissue and organ differentiation, but also enables homeostasis maintenance in adult organisms (Nassif et al. 1998; Vaux and Korsmeyer 1999; Schöck and Perrimon 2002; Proskuryakov et al. 2003). Necrosis in the midgut epithelium of insects has been described as a process in which organelles are removed to the midgut lumen and epithelial cells undergo lysis (Rost 2006*a*, 2006*b*; Rost-Roszkowska 2008*a*). It is thought that necrosis might be caused by mechanical damage, external factors, or that it could be combined with holocrine excretion (Kõműves et al. 1985; Jimenez and Gilliam 1990; Guimarães and Linden 2004). During necrosis, the cell swells, all of its organelles become distended, and the entire cytoplasm undergoes strong vacuolization and becomes electron lucent (Proskuryakov et al. 2003). As a result the apical membrane breaks, sometimes forming large blebs into the midgut lumen. All organelles pass through the midgut lumen where they disintegrate. The remains of cell membranes are separated from the basal lamina and are also shifted towards the midgut lumen. A similar mechanism of necrosis was observed in *E. nylanderi*, where distinct blebs of apical membrane are formed just before its rupture. However, cell vacuolization is not so apparent. It might be combined with rapid and dynamic processes of degeneration in all cells that contain numerous metal accumulating structures. A surprising result was the formation of buds with nuclear material inside and distinct nuclear lamina. Necrosis is not only a passive process, because it can also be regulated by programmed events (Proskuryakov et al. 2003). Probably in *E. nylanderi*, this is a morphological sign that necrosis has started in these cells. Since no other typical necrotic changes were present in these cells, it would have been the beginning of a genetically regulated necrosis pathway of programmed cell death. The first signal is sent from the nucleus, which proceeds to small fragmentized structures resembling accessory nuclei. Eventually the cell gets a signal to self-destruct, which pro-



ceeds as a typical necrosis pathway. The mode of nuclear bud formation with distinct nuclear lamina described above resembles the process of accessory nuclei formation, which has been described for arthropodan oocytes (Meyer et al. 1979; Szklarzewicz et al. 1993; Biliński and Kloc 2002; Świątek 2005).

In typical programmed cell death, the cell, owing to water elimination, undergoes shrinkage and intercellular junctions between apoptotic and neighboring cells disappear.

The nucleus initially takes a lobular shape and eventually undergoes fragmentation. Its chromatin becomes electron dense. The entire cell is fragmented. Apoptotic bodies are phagocyted by the neighboring cells or the entire apoptotic cell is absorbed by them (Vaidyanathan and Scott 2006).

This mode of apoptosis has been observed in the midgut epithelia of several 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). Apoptosis of individual cells of *E. nylanderi* proceeds in the manner described above, but apoptotic cells are removed into the midgut lumen where they undergo further disintegration and a final digestion. Midgut epithelial cells do not have phagocyte abilities, so they do not absorb remains of the apoptotic cells. Such cells are digested in the midgut lumen. However, Pipan and Rakovec (1980) observed in *Apis mellifera carnica* Pollmann, 1879 endocytosis of individual apoptotic cells by neighboring epithelial ones. A process similar to that observed in *E. nylanderi* has been documented for *Allacma fusca* (L., 1758) (Collembola) (Rost-Roszkowska 2008*b*). In that species, apoptotic cells are shifted into the midgut lumen where they form a layer of apoptotic cells and eventually undergo disintegration. In *E. nylanderi*, a layer of apoptotic cells beneath the peritrophic matrix is not formed. In many insects species having midgut cells infected by viruses or parasites, apoptosis is the mechanism used to eliminate cells damaged by them (Han et al. 2000; Baton and Ranford-Cartwright 2004; Vaidyanathan and Scott 2006). This mechanism is also important in blood-feeding insects when the entire organism is exposed to toxic haem activity (Okuda et al. 2007). Studies on *E. nylanderi* contribute to our knowledge of the role of apoptosis. We suggest that apoptosis participates in the removal of excessive amounts of metals, which at existing concentrations might be toxic even though small amounts are essential to the organism.

Autophagy is the process that enables disintegration of cytoplasmic components. First, the cell gets rid of organelles and then undergoes degeneration. In the midgut epithelial cells of the analysed species, autophagy proceeds intensively and the autophagosomes in various stages of differentiation are present in epithelial cells. Membranes of endoplasmic reticulum surround organelles, which are eventually completely enclosed inside such formed autophagosomes and undergo disintegration. If too many autophagosomes appear, epithelial cells would not be able to disintegrate them. Therefore, one of the irreversible pathways (apoptosis or necrosis) is initiated. Residual bodies with remains of the organelles are removed into the midgut lumen during one of these pathways. Autophagocytosis has been described in midgut epithelial cells in cabbage white (*Pieris brassicae* (L., 1758)) larvae (Kõműves et al. 1985), fruit flies (*Drosophila melanogaster* Meigen, 1830) (Lee et al. 2002), and tobacco budworms (*Heliothis virescens* (Fabricius, 1777)) (Tettamanti et al. 2007). Autophagy is also taken into consideration as a kind of cell death, which is responsible for cytoplasmic component degradation (Klionsky and Emr 2000; Lee et al. 2002; Levine and Klionsky 2004; Lockshin and Zakeri 2004; Levine and Yuan 2005; Tettamanti et al. 2007). Moreover, the fact that in *E. nylanderi* autophagy has been observed in all cells undergoing necrosis confirms this suggestion as well.

Our studies characterize the degenerative processes of the midgut epithelium of *E. nylanderi*. Probably because of these processes, the midgut cells might serve as the first line of defense against toxic amounts of metals. To further increase our understanding of how these processes are involved in excess metal elimination, it would be helpful to carry out a comparative study with specimens fed on *B. coddii* plants that do not carry such high amounts of nickel.

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