Subcellular Processes in Plants and Animal Cells

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Cuticle formation during marsupial development of the crustacean Porcellio scaber: Imaging and analysis

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Introduction: Crustacean exoskeleton is a chitin-protein matrix, formed apically by a single-layered epidermis. Organic cuticle constituents are hierarchially ordered in horizontal layers and incorporate minerals, mostly calcium carbonate and phosphate. The data on *de novo* formation of crustacean cuticle during embryogenesis provide knowledge of cell biology of chitin-based mineralized matrices, biomineralization processes and the role of hard biological materials. In this study several intramarsupial developmental stages of terrestrial isopod *Porcellio scaber* are examined, including analysis of cuticle ultrastructure, localization of N-acetylglucosamine (chitin monomer) and presence of mineral component. The embryonic development of terrestrial isopods takes place in the fluidic environment of the female marsupium. Embryos hatch into mancae and continue their development in marsupium for another week. Our results are compared with two model arthropod species, insect *Drosophila melanogaster* with non-mineralized exoskeleton and different reproductive strategy [1, 2] and aquatic amphipod crustacean *Parhyale hawaiensis* [3].

Methods: A detailed staging system through twenty progressive stages [4] was used to characterize the stages of *P. scaber* embryos and mancas, isolated from the marsupium. Conventional ultrastructural study and labelling of N-acetylglucosamine with WGA lectin were performed on ultrathin sections. The elemental composition of the cuticle was examined in methanol-fixed specimens by energy-dispersive x-ray spectroscopy (EDXS) in scanning electron microscope.

Results and discussion: Ultrastructural and compositional study of *Porcellio* epidermal extracellular matrices was performed in two sequential stages of late embryos and in two sequential stages of marsupial mancae. A substantial layer of apical matrix, synthesized by embryonic epidermis, is observed in late embryo in stage 16. It is elaborated underneath the vitelline membrane and consists of fibrous material without any prominent pattern, lined distally by a thin dense layer (Figure 1). Morphologically it resembles the *Drosophila* and *Parhyale* embryonic cuticle [1, 3]. Our results indicate that this matrix differs from adult chitinous cuticle also in composition as WGA labelling is not conspicuous. The matrix persists during stages of embryo bending, indicating its flexibility. In the further development of late embryo (stage 18) the formation of the electron dense epicuticle and homogenous procuticle begins underneath the matrix (Figure 2). Prominent positive WGA labelling with similar pattern as in adults is shown in cuticles of advanced stages 19 and 20 that already display characteristic sublayers in procuticle, indicating their similarity in chitin-protein arrangement. EDXS analysis reveals that cuticle in early mancae just after hatching is not strongly calcified in comparison to adult. Cuticle calcification appears much more prominent in advanced marsupial mancae where cuticle becomes ultrastructurally more similar to adult cuticle (Figures 3, 4). These results suggest the importance of exoskeleton formation and calcification and its involvement in animal mobility which was observed already within marsupium. It is noteworthy to expose the role of the exoskeleton during the release of mancae from the marsupium followed by absence of maternal care in the external environment.

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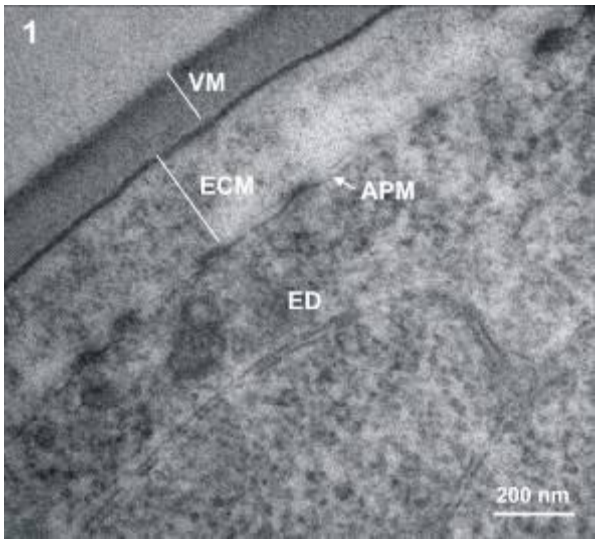

Figure 1. Late embryo of *P. scaber*, stage 16: Ultrastructure of apical extracellular matrix (ECM), lying between the vitelline membrane (VM) and apical plasma membrane (APM) of epidermal cell (ED).

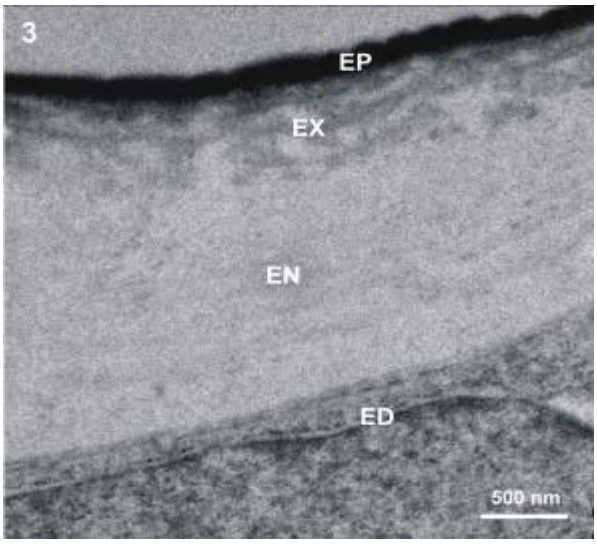

Figure 3. Marsupial manca of *P. scaber*: Ultrastructure of exoskeletal cuticle, overlying the epidermis (ED) and differentiated in three main layers: epicuticle (EP), exocuticle (EX) and endocuticle (EN).

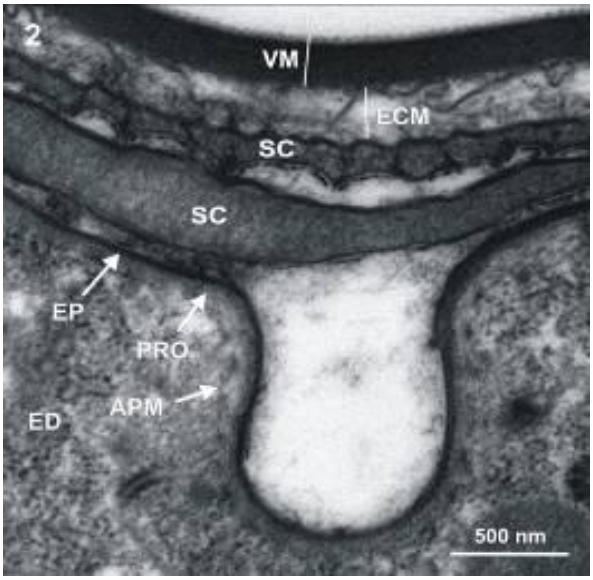

Figure 2. Late embryo of *P. scaber*, stage 18: Ultrastructure of epicuticle (EP) and procuticle (PRO), lying above apical plasma membrane (APM) of epidermal cell (ED) and underneath the matrix (ECM) and the vitelline membrane (VM). Epicuticular scales (SC), one covering the other, are fully developed.

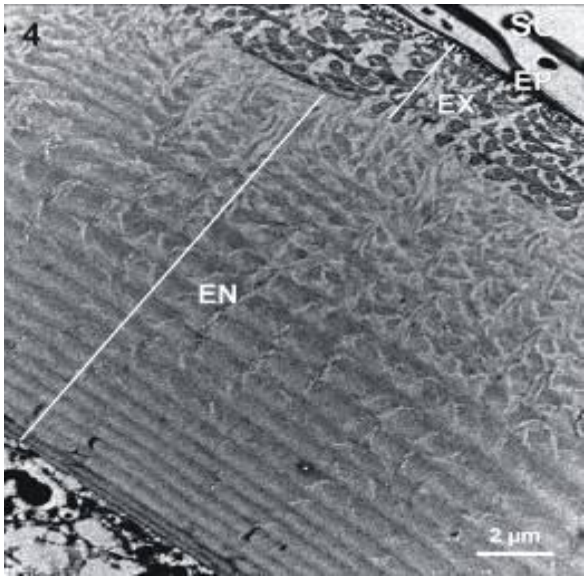

Figure 4. Ultrastructure of *P. scaber* adult exoskeletal cuticle, consisting of distinct horizontal layers: endocuticle (EN) with lamellar chitin-protein sublayers, exocuticle (EX) with chitin–protein fibers arranged in characteristic pattern and thin electron dense epicuticle (EP). Scales (SC) are epicuticular structures.