



## **Deposition of amorphous calcium carbonate within the terrestrial crustacean *Porcellio scaber* by epithelial calcium and proton transport.**

A. Ziegler and S. Hild

Central Facility for Electron Microscopy; University of Ulm, Albert-Einstein-Allee 11, 89069 Ulm

andreas.ziegler@uni-ulm.de / xx49731 5022670

Crustaceans have a mineralized exoskeleton that is replaced in regular time intervals in a process called moulting. Terrestrial isopods moult first the posterior and then the anterior half of the body. Before the moult they store cuticular  $\text{CaCO}_3$  within the ecdysial gap between the old and new cuticle of the first four anterior sternites. These deposits consist of amorphous  $\text{CaCO}_3$  (ACC) originating from the posterior cuticle and an organic matrix forming mainly microspherules. After the moult of the posterior and before the moult of the anterior half of the body the ACC deposits are completely resorbed and used for the quick mineralization of the new cuticle.

The cells of the anterior sternal epithelium (ASE) that are differentiated for ion transport mediate deposition and resorption of ACC. Electron microprobe analysis shows that at least part of the calcium is transported via a transcellular route raising the issue of toxic effects of high intracellular calcium concentrations. By analysing transport molecules within the ASE cells during non transporting and calcium transporting stages it is possible to derive a model for the molecular mechanisms for  $\text{CaCO}_3$  deposition and resorption. Posterior sternal epithelial (PSE) cells can be used as a control tissue. Semi quantitative RT-PCR and *in situ* hybridisation revealed that a plasma membrane  $\text{Ca}^{2+}$  transport ATPase and a  $\text{Na}^+/\text{Ca}^{2+}$  exchange mechanism contribute to epithelial  $\text{Ca}^{2+}$ -transport. *In situ* measurements of the smooth endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA) activity suggest a contribution of the SER to

intracellular  $\text{Ca}^{2+}$ -transport. Deposition and degradation of ACC requires also the transport of protons from and to the ecdysial gap, respectively. Expression analysis suggests a role of a V-type  $\text{H}^{+}$ -ATPase. Immunocytochemistry and ultrastructural localisation of the  $\text{H}^{+}$ -ATPase in high pressure frozen and freeze substituted epithelia indicate a transition of the V-type  $\text{H}^{+}$ -ATPase from the basolateral to the apical plasma membrane compartment between  $\text{CaCO}_3$  deposition and resorption, respectively. The results provide new insights in the mechanisms of epithelial calcium transport and  $\text{H}^{+}$ -transport reversal.

Supported by the DFG within the research program “Principles of Biomineralisation” (SPP 1117) and Zi 368/3-3