Cell Tissue Res (1995) 282:343–350

Cell & Tissue Research

Immunocytochemical localization of a vacuolar-type ATPase in Malpighian tubules of the ant *Formica polyctena*

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Received: 26 October 1994 / Accepted: 2 June 1995

Abstract. The presence of a vacuolar-type ATPase in Malpighian tubules of the ant *Formica polyctena* was investigated immunocytochemically, using antibodies to vacuolar ATPases of *Manduca sexta* midgut and bovine kidney. Specific labelling was observed at the brush border of the epithelium, extending along the entire length of the tubules. These findings agree with the current view that a vacuolar ATPase is situated at the apical membrane of Malpighian tubule cells and other insect epithelial cells, being the energizing element of an electrogenic potassium pump. When antibodies were tested on tubules in different secretion conditions prior to fixation, no differences were observed in the distribution of the vacuolar ATPase.

Key words: Vacuolar ATPase – Proton pump – Electrogenic potassium transport – Malpighian tubules – Immunocytochemistry – *Formica polyctena* (Insecta)

Introduction

Vacuolar ATPases (V-ATPases) constitute a third family of ion-motive ATPases, displaying structural and inhibitory properties that distinguish them from F-type and P-type ATPases. They are large enzymes comprised of multiple subunits which, as far as it is known, translocate protons (Pedersen and Carafoli 1987; Forgac 1989). In eukaryotic cells, these pumps have been associated mainly with vacuolar membranes, being responsible for acidification of a variety of subcellular compartments (Mellman et al. 1986; Forgac 1989). However, evidence has been accumulating over recent years for the presence of V-ATPases in the plasma membranes of diverse animal cells capable of proton secretion, such as those in mammalian kidney, amphibian and reptilian urinary bladders, frog skin, avian osteoclasts, and mammalian phagocytic cells (reviewed by Gluck 1992; Harvey 1992). Evidence for a V-ATPase in the plasma membranes of insect gastrointestinal and sensory epithelia (Schweikl et al. 1989; Klein and Zimmermann 1991; Klein et al. 1991) has also been reported. The physiological importance and ubiquity of V-ATPases have led to them being described as among the most fundamental ion pumps in nature (Nelson 1992).

For a long time, several models of insect epithelia (e.g., lepidopteran larval midgut, Malpighian tubules, sensory sensilla, and salivary and labial glands) suggested that a "K^+ pump" or more general "cation pump" was responsible for the transport of K^+ and/or Na^+ across apical membranes (Harvey et al. 1983a,b). The identification of the molecular components of this pump was first achieved in the K^+-secreting goblet cells of lepidopteran (*Manduca sexta*) larval midgut; the presumed "electrogenic K^+ pump" was shown to correspond to a H^+-transporting V-ATPase in parallel with a secondary electrogenic $K^+/2H^+$ antiporter (Schweikl et al. 1989; Wieczorek et al. 1989, 1991; Wieczorek 1992; Lepier et al. 1994). The hypothesis of a similar transport mechanism was then put forward for the above-mentioned transporting epithelia of insects, the common constituent being an apical vacuolar-type H^+-ATPase that would energize different secondary active-transport systems (Wieczorek et al. 1989; Klein et al. 1991; Klein 1992).

In Malpighian tubules, active transport of K^+ and/or Na^+ (depending on the feeding behaviour of the insect species) is coupled to water movements in the formation of an isosmotic primary urine (Maddrell 1977; Phillips 1981). As indicated above, the transport system responsible for the electrochemical gradient of these cations across apical membranes of the Malpighian tubule cells was also thought to be a "cation pump". A first hint of proton-energized secretion in Malpighian tubules arose when the presence of an amiloride-sensitive antiporter, exchanging intracellular K^+ for luminal H^+, was suggested in *Drosophila hydei* (Bertram 1989). Subsequent

This work was supported by grants from the European Community (SCI-CT90–0480), from the Ministerio de Educación y Ciencia DGICYT, Spain (CE 91–0002), and from the Deutsche Forschungsgemeinschaft (Wi 698–3).

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