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THE MIDGUT AS A MODEL SYSTEM FOR INSECT K^+-TRANSPORTING EPITHELIA: IMMUNOCYTOCHEMICAL LOCALIZATION OF A VACUOLAR-TYPE H^+ PUMP

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Summary

In *Manduca sexta* midgut, a vacuolar-type H^+-ATPase and a K^+/nH^+ antiport represent the functional elements of the electrogenic K^+ pump. This vacuolar-type ATPase was localized by immunofluorescence and immunogold staining using monoclonal antibodies to defined subunits of the midgut ATPase. The antibodies labelled the membrane projections of the goblet cell apical plasma membrane, the site of active K^+ transport. Furthermore, an immunologically related epitope was localized in the apical brush border of *Manduca* Malpighian tubules. In addition, cross-reactivity of protein bands corresponding to the major subunits of the midgut vacuolar-type ATPase was found in crude homogenates of Malpighian tubules and antennal sensory epithelium of *Manduca* by immunostaining with a polyclonal anti-holoenzyme serum to the midgut ATPase. This comparative immunological approach suggests that a vacuolar-type ATPase may be a common constituent of insect plasma membranes bearing the insect K^+ pump. These results support the hypothesis that a vacuolar-type H^+-ATPase provides an alternative to the Na^+/K^+-ATPase in energizing animal plasma membranes.

Introduction

Electrogenic K^+ transport in insects serves as the energy source for secretion or absorption of nutrients and ions in gastrointestinal epithelia of midgut (Harvey *et al.* 1983), Malpighian tubules (Maddrell, 1971, 1972) and salivary glands (Berridge, 1977) and for generation of receptor current in sensory sensilla (Thurm and Küppers, 1980). Since the first description of active K^+ transport across the midgut epithelium of *Hyalophora cecropia* larvae (Harvey and Nedergaard, 1964), the midgut of lepidopteran larvae has been used widely as a model system (Harvey *et al.* 1983; Moffett and Koch; 1988; reviewed by Dow, 1986).

In searching for the molecular correlate of the insect K^+ pump, Cioffi and Wolfersberger (1983) succeeded in isolating the goblet cell apical membrane, the putative site of active K^+ transport. An ATPase activity was demonstrated in this

Key words: electrogenic K^+ pump, transepithelial ion transport, vacuolar-type ATPase, midgut, Malpighian tubules, *Manduca sexta*.

membrane (Wieczorek *et al.* 1986) and was subsequently purified and characterized as a vacuolar-type ATPase (Schweikl *et al.* 1989; Wieczorek *et al.* 1991). This finding was unexpected because vacuolar-type ATPases are usually situated in endomembranes of acidic organelles. Along with P-type ATPases of plasma membranes and the sarcoplasmic reticulum and F-type ATPases of mitochondria, chloroplasts and bacteria, vacuolar-type ATPases form the third family of primary ion pumps (Pederson and Carafoli, 1987; Forgac, 1989; Nelson, 1989). Finally, transport studies with goblet cell apical membrane vesicles demonstrated the exclusive H^+-specificity of the vacuolar-type H^+ pump and identified, in addition, an ATP-independent K^+/nH^+ antiport (Wieczorek *et al.* 1989, 1991). Based on these results, a vacuolar-type H^+-ATPase and a K^+/nH^+ antiport represent the functional elements of the electrogenic K^+ pump in *Manduca* midgut (Wieczorek *et al.* 1991).

It has to be verified whether this model holds true for active K^+ transport in other insect epithelia, e.g. Malpighian tubules or sensory sensilla. Indeed, it is likely that all ouabain-insensitive active ion transport in insects may be driven by a vacuolar-type H^+-ATPase. In this case, the primary ion pump would be identical, but it would energize different secondary active transport systems, e.g. active Cl^- transport in locust rectum (Hanrahan and Phillips, 1982) by Cl^-/H^+ symport or active Na^+ transport in Malpighian tubules of the blood-sucking insect *Rhodnius prolixus* (Maddrell, 1972) by Na^+/H^+ antiport. As there is no Na^+/K^+ pump in the midgut epithelium of *Manduca*, the concept might be generalized even further. Consequently, Wieczorek *et al.* (1989, 1991) proposed that the vacuolar-type H^+-ATPase provides an alternative to the classical Na^+/K^+-ATPase in energizing animal plasma membranes.

To test this hypothesis, an immunological approach was used to examine the occurrence of vacuolar-type ATPases in other animal plasma membranes, especially in those cases where biochemical purification of membranes and proteins is crucially hampered by the complexity of tissue organization. Polyclonal and monoclonal antibodies were raised against the purified midgut ATPase (Wieczorek *et al.* 1991; U. Klein, A. Lepier, B. Förg-Brey and H. Wieczorek, in preparation). This paper focuses on the localization of the vacuolar-type ATPase in the midgut tissue itself by immunocytochemistry using monoclonal antibodies with defined subunit specificity. *Manduca* Malpighian tubules were also investigated by immunocytochemical labelling. In parallel experiments, ion-transporting tissues of *Manduca* were probed immunobiochemically for cross-reactivity with a polyclonal anti-holoenzyme serum to the midgut ATPase.

Materials and methods

Insects

Manduca sexta were reared under standard conditions (Schweikl *et al.* 1989). Midguts and Malpighian tubules were taken from fifth-instar larvae, weighing

5–8 g, whereas antennae, flight muscles and brain tissue were taken from adult moths.

Primary antibodies and secondary labels

Rabbit polyclonal anti-holoenzyme serum was raised against the purified vacuolar-type ATPase (Wieczorek *et al.* 1991). Mouse monoclonal antibodies were raised against the purified ATPase, screened with the detergent-solubilized ATPase obtained from the membrane fraction 'band B2' (Cioffi and Wolfersberger, 1983) and purified as described in detail elsewhere (U. Klein, A. Lepier, B. Förg-Brey and H. Wieczorek, in preparation). They were used either as cell-culture supernatant or as a protein-G affinity-purified immunoglobulin (IgG) fraction eluted in 0.1 mol l^{-1} glycine–HCl, pH 2.7, and immediately neutralized by addition of 1 mol l^{-1} Tris–HCl, pH 8. Purified monoclonal antibody stock solutions had protein concentrations of about 0.5–1.0 mg ml^{-1}, determined by $OD_{280\,nm} \times 0.75$ = mg IgG per ml, and contained up to 50 % of bovine IgG from the 10 % foetal calf serum in the cell culture medium.

Goat anti-rabbit antibodies conjugated with alkaline phosphatase, sheep anti-mouse antibodies conjugated with FITC and mouse IgG were purchased from Sigma (Deisenhofen, FRG); goat anti-mouse antibodies conjugated with 1 nm colloidal gold were kindly provided by Bruno Humbel (University of Utrecht, The Netherlands).

Immunobiochemistry

For immunoblot analysis, vacuolar-type ATPase was purified from the larval midgut (Schweikl *et al.* 1989; Wieczorek *et al.* 1990). Membranes from crude homogenates of larval Malpighian tubules, adult antennae, flight muscle or brain were prepared by washing the dissected tissues in a buffer consisting of 16 mmol l^{-1} Tris–HCl, pH 8.1, 0.32 mmol l^{-1} EDTA and 200 mmol l^{-1} mannose, followed by homogenizing in the same buffer without mannose in an Ultra-Turrax (T25, Jahnke and Kunkel, FRG), and by centrifuging the homogenates for 1 h at 100 000 ***g***. The antennal homogenate was filtered through several layers of antiseptic gauze before centrifugation. All steps were performed at 0–4 °C. Membrane pellets were treated for 5 min with 2 % sodium dodecylsulphate (SDS) and 2 % 2-mercaptoethanol at 95 °C. Protein determination, SDS polyacrylamide gel electrophoresis (SDS–PAGE), gel blotting and immunostaining were performed as described earlier (Schweikl *et al.* 1989; Wieczorek *et al.* 1990, 1991).

Immunofluorescence microscopy

Pieces of posterior midgut tissue and of Malpighian tubules were dissected under cold PLP fixative (2 % paraformaldehyde, 0.075 % lysine, 0.01 mol l^{-1} sodium *meta*-periodate in 0.1 mol l^{-1} sodium phosphate buffer, pH 7.3, McLean and Nakane, 1974), left in the fixative for 2 h, washed in phosphate buffer and transferred *via* 10 % (w/v) sucrose in phosphate buffer for four times 15 min into 30 % (w/v) sucrose in phosphate buffer as a cryoprotectant for at least 12 h; all

steps were performed at 0–4 °C. For embedding, the tissue was surrounded by cryo-embedding media (Tissue-Tek II, Miles Laboratories Inc., Naperville, Illinois, USA) and frozen in melting iso-pentane (about −160 °C). 9–12 μm thick sections were cut in a cryostat (Microm HM500, Heidelberg, FRG) at −20 °C and collected on coverslips coated with 0.05 % aqueous poly-L-lysine (Sigma).

For immunofluorescence staining, the cryosections were pre-incubated successively in (i) 0.1 mol l^{-1} sodium phosphate buffer, pH 7.5, 0.5 mol l^{-1} NaCl and 0.02 % NaN_3 (PBSN) with 0.01 % Tween 20 (Sigma), (ii) PBSN with 50 mmol l^{-1} NH_4Cl to block free aldehyde groups of the fixative, (iii) PBSN and (iv) PBSN with 0.5 % gelatine (Serva, Heidelberg, FRG; blocking solution) to block unspecific protein binding sites; for 5 min each. The sections were then incubated in the primary antibody solution consisting of the protein-G affinity-purified IgG fraction diluted in blocking solution to about 0.05 mg protein per ml overnight at 4 °C in a moist chamber. For controls, the primary antibody solution was replaced by mouse IgG or by PBSN. After removal of unbound antibodies by three washes in PBSN, the sections were placed in FITC-conjugated sheep anti-mouse antibody diluted in PBSN 1:50 for 1 h at room temperature in the dark. Finally, the sections were washed three times in PBSN and mounted in Eukitt (Kindler, Freiburg, FRG). The stained sections were examined and photographed with a Leitz EP 20 microscope equipped for epifluorescence illumination (excitation/emission filter set BP 450–490, RKB 510, LP 515). For photographic documentation a Kodak TMax 100 film exposed as 400 ASA was used.

Immunogold electron microscopy

Larval midgut was dissected under cold PLP fixative, fixed for 2 h at room temperature and washed in 0.1 mol l^{-1} sodium phosphate buffer, pH 7.3. The fixed specimens were dehydrated over a graded series of dimethyl formamide (DMF), subsequently infiltrated with mixtures of Lowicryl K4M (Lowi, Waldkraiburg, FRG) and DMF 1:2, 1:1 and 2:1, for 2 h each, and finally in two changes of 100 % Lowicryl, one overnight at room temperature and the other for 3 h at 4 °C. Polymerization was performed for 24 h at 4 °C under ultraviolet illumination (Altman *et al.* 1984). Thin sections were cut on an ultramicrotome (Microm MT6000 XL, Heidelberg, FRG) and collected on uncoated nickel grids.

For immunogold staining, selected grids were pre-incubated according to steps i–iv of the protocol given for the fluorescence microscopy, but instead of PBSN, 20 mmol l^{-1} Tris–HCl, pH 7.3, 0.5 mol l^{-1} NaCl and 0.02 % NaN_3 (TBSN) was used, the NH_4Cl step was omitted, the blocking solution was 1.5 % gelatine in TBSN and incubation steps lasted 10 min each. Incubation in the primary antibody lasted overnight at 4 °C in a moist chamber. As primary antibody, cell culture supernatant was used undiluted. For controls, the primary antibody solution was replaced by blocking solution. Since labelling with other mouse monoclonal antibodies failed under these conditions, no further controls were run for unspecific binding of mouse IgG. After removal of unbound antibodies by three washes in TBSN, the sections were placed in 1 nm gold-conjugated goat anti-

mouse antibody diluted 1:100 in blocking solution for 1 h at room temperature. After washing in TBSN and water, the sections were post-fixed for 10 min at room temperature using half-strength Karnovsky (2 % paraformaldehyde, 2.5 % glutaraldehyde in 0.1 mol l^{-1} sodium phosphate buffer, pH 7.4; Karnovsky, 1965). The gold label was silver-enhanced according to Danscher (1981) with developing times of about 30 min at room temperature. The sections were finally stained with 2 % aqueous uranyl acetate for 30 min at 40 °C and lead citrate for 10 min at room temperature (Venable and Coggeshall, 1965).

For better structural preservation in conventional electron microscopy, the midgut tissue was dissected under cold half-strength Karnovsky solution and fixed in the same solution for 2 h at 4 °C. Following an incubation in 1 % OsO_4 in 0.1 mol l^{-1} sodium phosphate buffer, pH 7.3, at room temperature for 1 h and three washes in 0.1 mol l^{-1} sodium maleate buffer, pH 6.0, for 5–10 min each, the specimens were treated with 2 % uranyl acetate in maleate buffer for 1.5 h at 40 °C (Karnovsky, 1967) and finally dehydrated through a graded ethanol series. Embedding was performed over propylene oxide in Epon 812. Malpighian tubules were also fixed with cold half-strength Karnovsky solution overnight at 4 °C and in 1 % OsO_4 in 0.1 mol l^{-1} sodium phosphate buffer, pH 7.4, for 2 h at room temperature. Embedding was performed after dehydration in a graded ethanol series *via* propylene oxide in Durcupan.

Semi-thin sections of 0.6 μm were stained with 1 % Azur II in water and 0.1 % Methylene Blue in 1 % sodium tetraborate, mixed 1:1 according to Richardson *et al.* (1960). Ultrathin sections were stained automatically for 1 h at 40 °C in uranyl acetate and 30 min at 27 °C in lead citrate (Ultrastainer, LKB 2168, Bromma, Sweden). All sections were examined and photographed in a Philips CM 10 transmission electron microscope.

Results

Specificity of monoclonal antibodies

The monoclonal antibodies were directed against the purified native vacuolar-type ATPase from *Manduca* midgut. When tested in immunoblots of the SDS-denatured ATPase, the monoclonal antibodies recognized defined subunits of the ATPase (Fig. 1). The antibody from the hybridoma clone 206-2 recognized the 67×10^3 M_r band, which represents the presumed catalytical subunit (Forgac, 1989), the antibody from 47-5, despite being of monoclonal origin, bound to the 28×10^3 and 16×10^3 M_r bands, the latter presumably forming the proton channel (Forgac, 1989), and the antibody from 37-4 recognized the 40×10^3 M_r band, a glycosylated subunit, detected first by immunostaining with the anti-holoenzyme serum (see Fig. 5B, lane 1; Wieczorek *et al.* 1991; U. Klein, A. Lepier, B. Förg-Brey and H. Wieczorek, in preparation).

Immunofluorescence labelling of midgut

The midgut is a monolayer epithelium composed of columnar and goblet cells

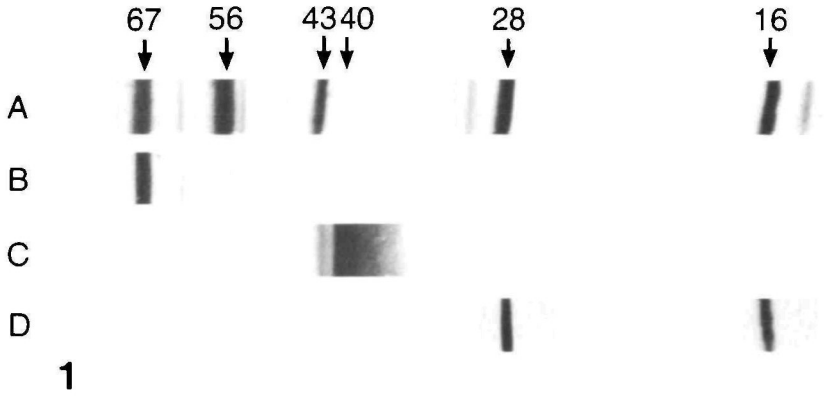

Fig. 1. Subunit-specificity of the monoclonal antibodies to the vacuolar-type ATPase from *Manduca sexta* midgut. Blots of SDS–PAGE. (A) Purified vacuolar-type ATPase (about 3.5 μg protein per lane) from goblet cell apical membrane, amido black staining of total protein; (B–D) immunostaining of vacuolar-type ATPase by protein-G affinity-purified monoclonal antibodies, about 0.1 μg IgG per ml, visualized by secondary antibodies conjugated with alkaline phosphatase, using 206-2 (B), 37-4 (C) and 47-5 (D). Numbers give the relative molecular masses ($\times 10^{-3}$) of vacuolar-type ATPase subunits.

(Fig. 2A, posterior region; Cioffi, 1979; Hakim *et al.* 1988). Columnar cells bear an apical brush border of microvilli and have a highly folded basal membrane. Goblet cells are characterized by a large cavity, formed during embryogenesis by fusion of an intracellular vacuole with the apical membrane (Hakim *et al.* 1988). The apical membrane is thrown into projections extending into the goblet cell cavity and bears membrane particles on its cytoplasmic side (Cioffi, 1979; Fig. 2B,C) that are thought to be part of the K^+ pump (Harvey *et al.* 1981, 1983).

When the posterior midgut was inspected for binding of monoclonal antibodies 206-2, 47-5 and 37-4, a clear labelling of the area of the folded goblet cell apical membrane was found (Fig. 3A–C). Labelling was fainter in the apical portion of the goblets, where the membrane projections were less dense. No obvious labelling of the cell interior, of the basal membrane or of the densely packed microvilli of the columnar cells was detected. The same type of labelling was found with other monoclonal antibodies binding to defined subunits of the vacuolar-type ATPase (anti-67$\times 10^3$ M_r: 221-9, 184-3, 86-3, anti-20$\times 10^3$ M_r: 224-3; not shown). Control incubations with mouse IgG for unspecific binding of mouse antibodies or with blocking solution for unspecific binding of secondary goat anti-mouse antibody used instead of primary antibody (Fig. 3D) showed almost no background labelling; the autofluorescence of the tissue was negligible.

Immunogold labelling of the midgut

Although mitochondria are not present within the projections of the goblet cell apical membrane in the posterior region of the midgut, they are abundant close to the membrane (Cioffi, 1979; Fig. 2B). Both ATPase families, the

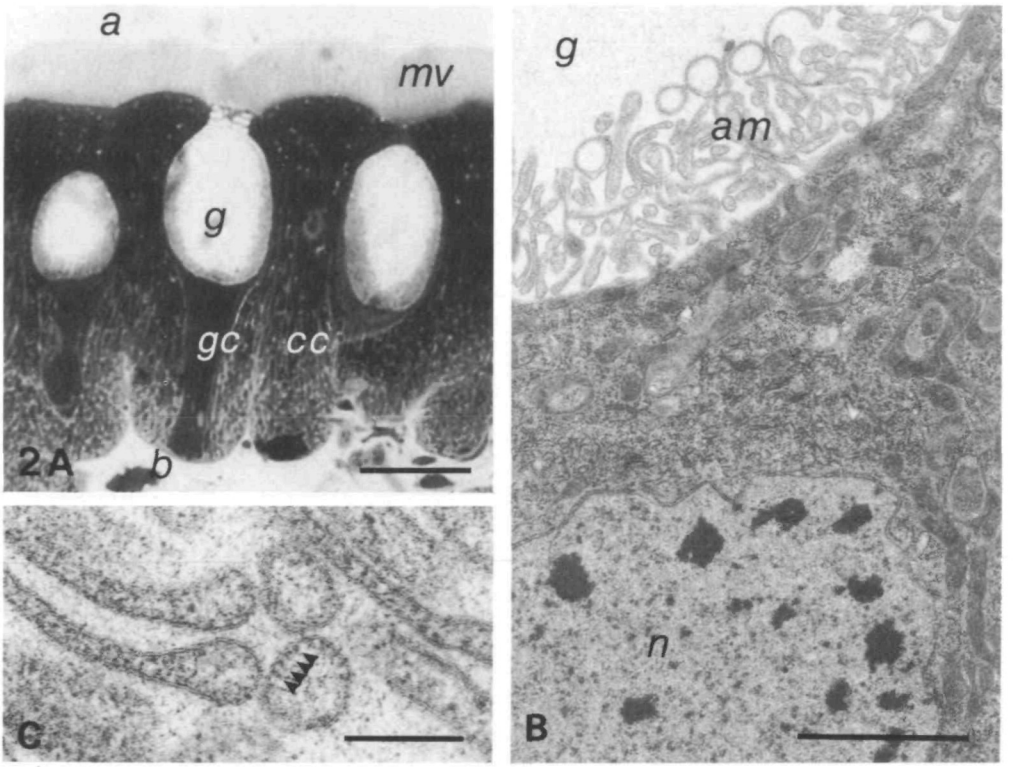

Fig. 2. Morphology of the posterior midgut epithelium of *Manduca sexta*. Conventional light and electron microscopy. (A) Cellular organization of the epithelium, semithin section, Richardson staining, scale bar 10 μm; (B) apical part of goblet cell, ultrathin section, scale bar 2 μm; (C) detail of goblet cell apical membrane foldings with membrane particles ('portasomes', arrowheads), scale bar 0.2 μm. *cc*, columnar cell; *gc*, goblet cell; *a*, apical; *am*, apical membrane; *b*, basal; *g*, goblet cell cavity; *mv*, microvilli; *n*, nucleus.

mitochondrial and the vacuolar-type ATPases, are thought to be monophyletic and bear sequence homologies in related subunits (Nelson, 1989). To exclude the possibility that the large amount of labelling seen in the immunofluorescence experiments was due to cross-reactivity with epitopes of the mitochondrial ATPase, the localization of antibody label was further defined on the ultrastructural level by immunogold staining. The area of the projections of the goblet cell apical membrane was densely labelled when probed with the monoclonal antibody 206-2 (Fig. 4). No specific labelling of mitochondria or other membranes, such as microvilli, was detected. Background staining of goblet cell cavity, midgut lumen, haemolymph space and cytoplasm of the columnar cell was equally low. There was, however, a slightly, but significantly, enhanced staining above background in the cytoplasm of the goblet cell (Fig. 4). This staining might be specific and due to cross-reaction with an immunologically related epitope of endomembrane vacuolar-type ATPases. As this staining was restricted, however, to the goblet cell

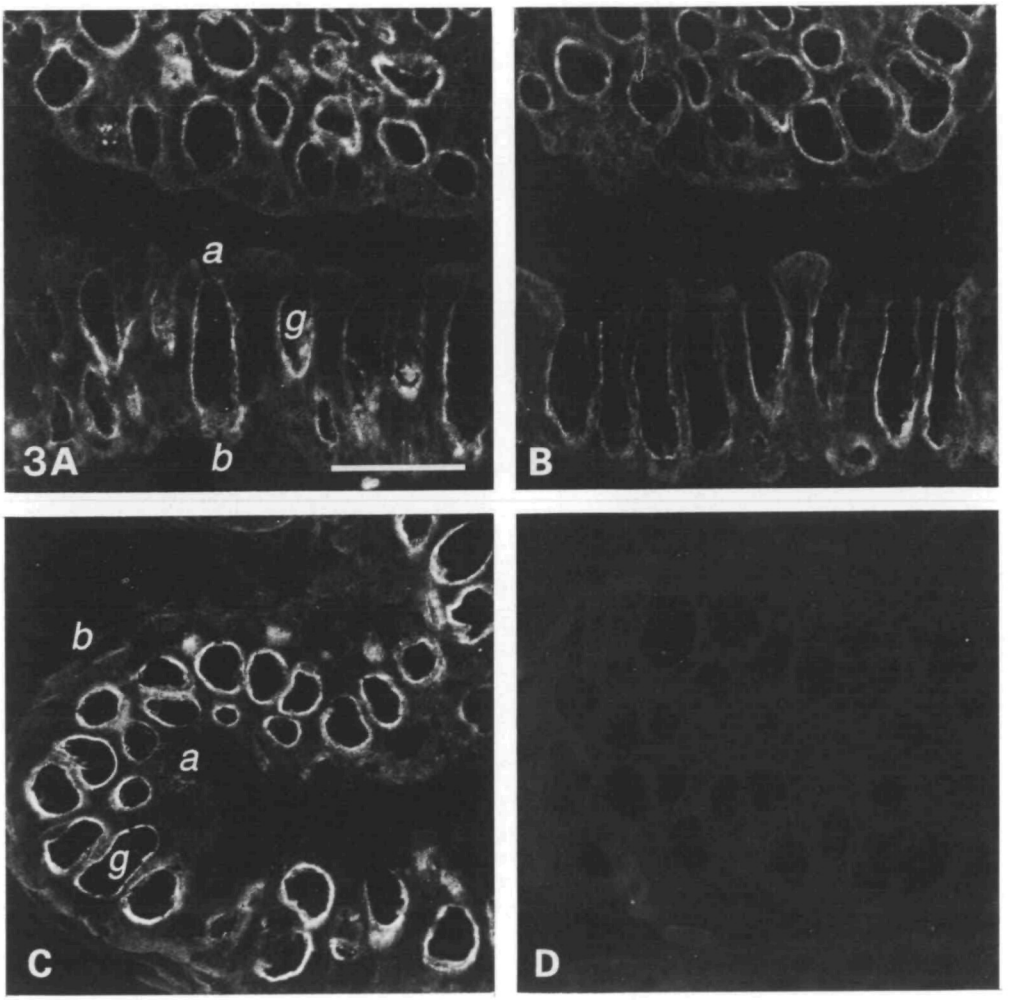

Fig. 3. Immunofluorescence labelling of posterior midgut using monoclonal antibodies. Cryosections of midgut labelled with protein-G affinity-purified monoclonal antibodies (about 0.05 mg IgG per ml), visualized by FITC-conjugated anti-mouse antibodies; scale bar 20 μm. (A) Labelling with antibody 47-5; (B) with 206-2; (C) with 37-4; (D) control section labelled with secondary antibody only. *a*, apical; *b*, basal; *g*, goblet cell cavity.

cytoplasm, it is more likely that the antibody recognized the plasma membrane vacuolar-type ATPase on its biogenetic pathway.

Immunobiochemical probing of insect K^+-transporting epithelia

In a first attempt to search for vacuolar-type ATPases in other insect ion-transporting epithelia, several tissues of *Manduca* were inspected for cross-reactivity with polyclonal anti-holoenzyme antibodies to the midgut ATPase. The membrane extracts of crude homogenates of all tissues exhibited a complex

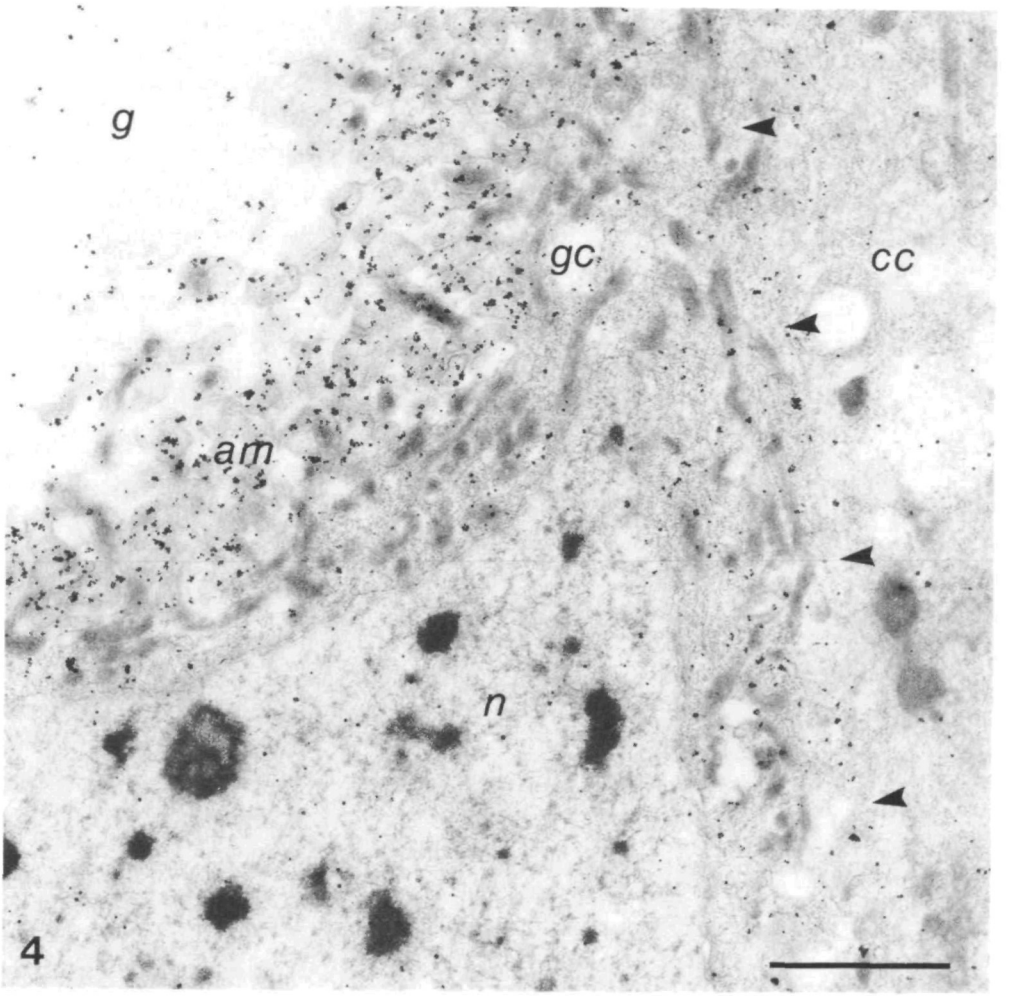

Fig. 4. Ultrastructural localization of monoclonal antibody labelling in posterior midgut. Immunogold-electron microscopy with monoclonal antibody 206-2 in the culture supernatant, visualized by labelling with 1 nm gold-conjugated anti-mouse antibodies, silver-enhanced, scale bar 2.0 μm; note the enhanced background labelling in goblet cell cytoplasm as compared to columnar cell cytoplasm. *cc*, columnar cell; *gc*, goblet cell; *am*, apical membrane; *g*, goblet cell cavity; *n*, nucleus. Arrowheads show cell border line between goblet and columnar cell.

protein pattern (Fig. 5A), but showed specific cross-reactions with only a few bands in immunoblots (Fig. 5B). The positions of these bands in the extracts of Malpighian tubules, antennae and brain clearly corresponded to the major subunits of the vacuolar-type ATPase derived from midgut (Fig. 5B). This evidence reveals a high level of immunologically related vacuolar-type ATPase in these tissues. A similar labelling of muscle extract was not found. The muscle tissue was used as a control, because it is supposed not to contain large amounts of

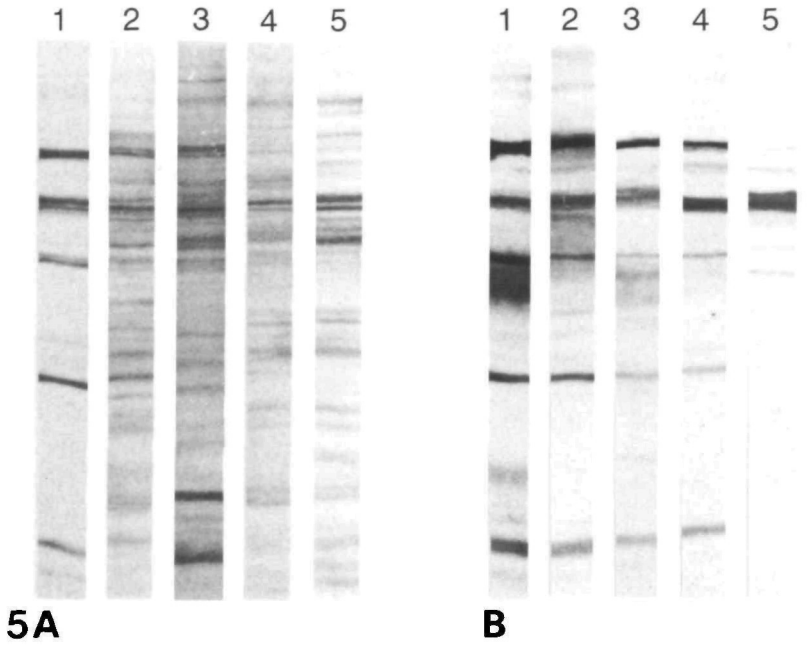

Fig. 5. Cross-reactivity of crude homogenates of ion-transporting tissues of *Manduca sexta* with polyclonal anti-holoenzyme serum to the midgut ATPase. Blots of SDS extracts after SDS–PAGE; about 5 μl applied per lane. 1, purified vacuolar-type ATPase from midgut (0.35 mg protein per ml); 2, Malpighian tubules (1.0 mg protein per ml); 3, antennae (0.8 mg protein per ml); 4, brain (1.0 mg protein per ml); 5, muscles (0.9 mg protein per ml). (A) Amido black staining of total protein; (B) immunostaining with polyclonal anti-holoenzyme serum, diluted $1:10^5$ in 1% gelatine–TBSN, visualized by anti-rabbit antibodies conjugated with alkaline phosphatase.

vacuolar-type ATPases. However, the muscle extract exhibited a cross-reactivity in one protein band at about 56×10^3 M_r, the origin of which is unknown.

Immunofluorescence labelling of Malpighian tubules

Cryosections of Malpighian tubules were probed with the monoclonal antibody 37-4 to the midgut ATPase. The cellular organization of the tubular epithelium is very simple, consisting of only one type of cell bearing numerous microvilli at the apical border facing the tubular lumen (Fig. 6A,B). Heavy fluorescence labelling was observed only in the region of the apical brush border (Fig. 6C).

Discussion

Localization of the vacuolar-type ATPase in the midgut plasma membrane

The immunocytochemical investigation of the midgut localized the vacuolar-type H^+-ATPase at the goblet cell apical membrane in agreement with evidence from biochemical studies (Wieczorek *et al.* 1986; Schweikl *et al.* 1989). Earlier

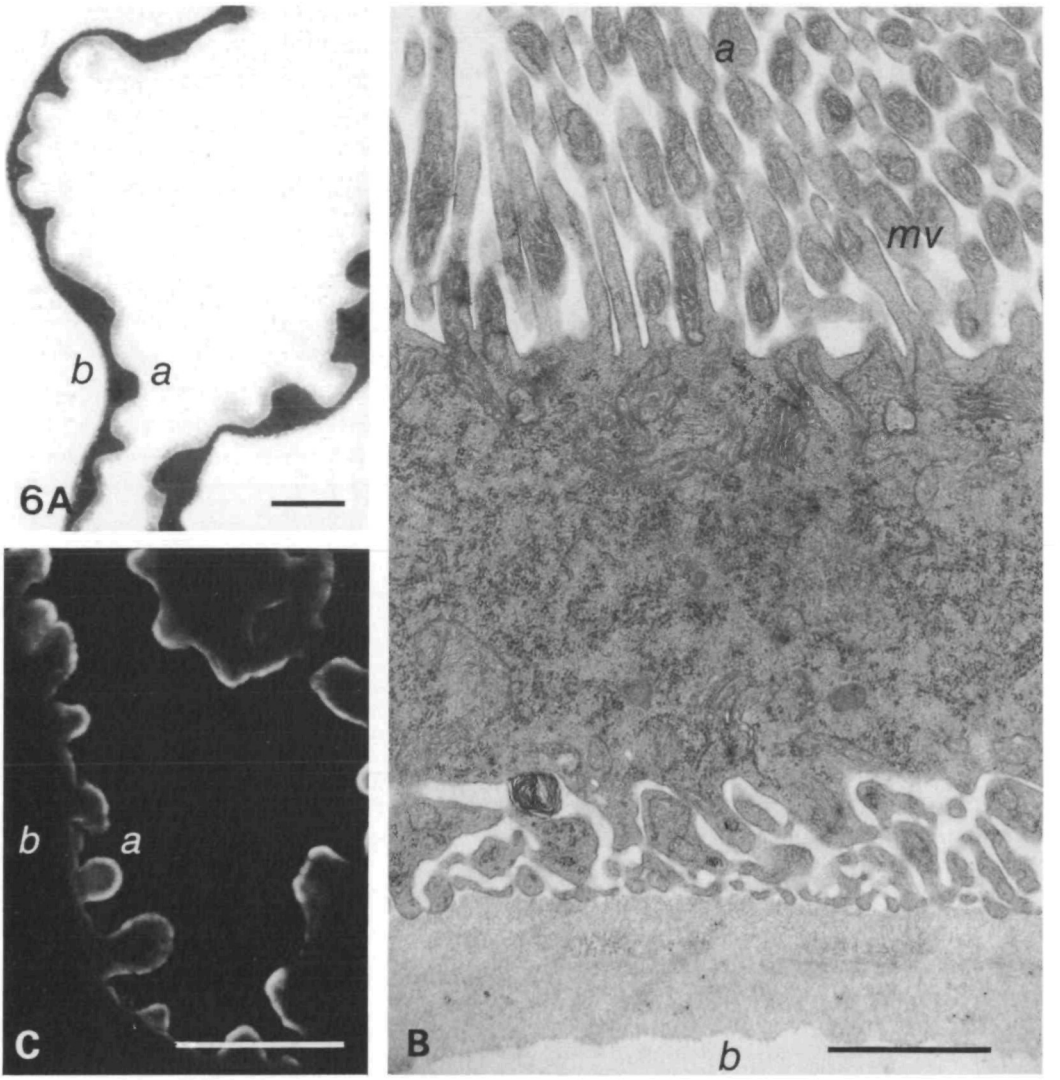

Fig. 6. Immunofluorescence labelling of Malpighian tubules using a monoclonal antibody to the midgut ATPase. (A) Conventional light microscopy of tubule, semithin section, Richardson staining, scale bar 10 μm; (B) conventional electron microscopy of epithelial cells, ultrathin section, scale bar 1 μm; (C) cryosection, labelled with protein-G affinity-purified monoclonal antibody 37-4 (0.1 mg IgG per ml), visualized by FITC-conjugated secondary antibodies, scale bar 10 μm. *a*, apical; *b*, basal; *mv*, microvilli.

evidence for the localization of the K^+ pump in the midgut came from microelectrode studies of the voltage profiles (Wood *et al.* 1969; Moffett and Koch, 1988), K^+-flux analysis (Blankemeyer and Harvey, 1978) and X-ray microanalysis of potassium concentrations (Dow *et al.* 1984). Immunocytochemical investigations with antibodies directed to the vacuolar-type ATPase from beet

tonoplasts yielded similar results in labelling the goblet cell apical membrane (V. E. W. Russell, M. Reuveni and W. R. Harvey, personal communication). Localization of all vacuolar-type ATPases studied so far has been based primarily on biochemical analysis of isolated membrane fractions. Confirmatory immunocytochemical evidence is available for only a few tissues: the bovine kidney mitochondria-rich cells (Brown *et al.* 1987), maize root tip cells (Hurley and Taiz, 1989) and chicken osteoclasts (Blair *et al.* 1989).

Localization of the vacuolar-type ATPase in other insect tissues

Immunoblots of different *Manduca* tissues stained with a polyclonal anti-holoenzyme serum to the midgut ATPase exhibited clear cross-reactivity of protein bands corresponding to the major ATPase subunits. In Malpighian tubules and antennal sensilla, the vacuolar-type ATPase is very probably localized in the putative K^+-transporting cells of these epithelia, i.e. on the apical plasma membrane of both tubular cells and auxiliary cells (see below). The cross-reactivity of the brain might be due to enrichment of neurosecretory vesicles and, therefore, may be caused by endomembrane vacuolar-type ATPases.

For Malpighian tubules, the conclusions drawn from the immunoblots were supported immunocytochemically; the monoclonal antibody 37-4 to the midgut ATPase cross-reacted with the apical region of the tubular cells. This localization of the label corresponds with the presumed site of active K^+ transport (Maddrell, 1971, 1972). Evidence for a vacuolar-type ATPase function in Malpighian tubules was derived from physiological studies in *Drosophila hydei*, where an ion-transport mechanism similar to that of the midgut goblet cell was proposed based on inhibition of urine formation by the specific inhibitor of vacuolar-type ATPases, Bafilomycin A_1 (Bertram *et al.* 1991).

For sensory sensilla, immunoelectron microscopy of various sensilla types on the antenna of *Antheraea pernyi* using specific monoclonal antibodies to different subunits of the midgut ATPase localized immunologically related epitopes in the apical membrane of the auxiliary cells (Klein and Zimmermann, 1991). These findings support the hypothesis that a K^+ pump is situated in this membrane, energizes the transepithelial voltage and is involved in driving the receptor current (Thurm and Küppers, 1980).

The vacuolar-type ATPase as a candidate for the portasomes

The analogy between the ultrastructure of the F_1 particles of the mitochondrial ATPase (Fernandéz-Moràn *et al.* 1964; cf. Kagawa *et al.* 1979) and the membrane particles found apically in goblet cells (see Fig. 2C) tempted Anderson and Harvey (1966) to propose that they are part of the K^+-transporting mechanism; Harvey *et al.* (1981) named them 'portasomes'. These membrane particles are known to occur as a common feature in ion-transporting insect epithelia (e.g. rectum: Gupta and Berridge, 1966; sensory sensilla: Smith, 1969; Malpighian tubules: Berridge and Oschman, 1969; labial gland: Hakim and Kafatos, 1974; salivary gland: Berridge and Oschman, 1972; midgut: Cioffi, 1979; reviewed by Harvey *et al.*

1981). Emerging knowledge about the molecular structure of vacuolar-type ATPases corroborates this view. Both the vacuolar-type and the mitochondrial-type ATPases are multi-subunit complexes with molecular masses in a similar range. The vacuolar-type ATPase may be organized in a stalked-sphere arrangement similar to that of the mitochondrial-type ATPase (Forgac, 1989; Nelson, 1989). Besides the insect goblet cell apical membrane, particles with a similar appearance have been reported on the cytoplasmic side of membranes known to bear a vacuolar-type ATPase (bovine chromaffin granules: Schmidt *et al.* 1982; plasma membrane and vesicles in bovine kidney mitochondria-rich cells and in toad and turtle urinary bladder: Brown *et al.* 1987; *Neurospora crassa* vacuoles: Bowman *et al.* 1989) and have been identified as part of the vacuolar-type ATPase by immunocytochemistry in the kidney mitochondria-rich cells (Brown *et al.* 1987).

Perspectives

Evidence is growing that electrogenic K^+ transport in insect epithelia in general may be performed by a vacuolar-type H^+-ATPase and a K^+/nH^+ antiport. To support the new paradigm of a vacuolar-type H^+-ATPase as an energizing principle for animal plasma membranes, the comparative immunological approach using antibodies to the midgut ATPase may now be extended from protists to mammals: e.g. in *Entamoeba histolytica*, a vacuolar-type ATPase may be localized in the plasma membrane, as deduced from Bafilomycin A_1-sensitive production of pinocytotic vesicles (Löhden-Bendinger and Bakker-Grunwald, 1990), and in the mammalian inner ear the endocochlear K^+ potential (Sterkers *et al.* 1988) may be energized by a vacuolar-type ATPase.

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