The Journal of Experimental Biology 203, 137–145 (2000)
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JEB2340

H^+V-ATPase-DEPENDENT LUMINAL ACIDIFICATION IN THE KIDNEY COLLECTING DUCT AND THE EPIDIDYMIS/VAS DEFERENS: VESICLE RECYCLING AND TRANSCYTOTIC PATHWAYS

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Accepted 21 October; published on WWW 13 December 1999

Summary

Many vertebrate transporting epithelia contain characteristic 'mitochondria-rich' cells that express high levels of a vacuolar proton-pumping ATPase (H^+V-ATPase) on their plasma membrane and on intracellular vesicles. In the kidney cortex, A-cells and B-cells are involved in proton secretion and bicarbonate secretion, respectively, in the distal nephron and collecting duct. A-cells have an H^+V-ATPase on their apical plasma membrane and on intracellular vesicles, whereas the cellular location of the H^+V-ATPase can be apical, basolateral, bipolar or diffuse in B-cells. The rat epididymis and vas deferens also contain a distinct population of H^+V-ATPase-rich epithelial cells. These cells are involved in generating a low luminal pH, which is involved in sperm maturation and in maintaining sperm in an immotile state during their passage through the epididymis and vas deferens. In both kidney and reproductive tract, H^+V-ATPase-rich cells have a high rate of apical membrane recycling. H^+V-ATPase molecules are transported between the cell surface and the cytoplasm in vesicles that have a well-defined 'coat' structure formed of the peripheral V_1 subunits of the H^+V-ATPase. In addition, we propose that B-type intercalated cells have a transcytotic pathway that enables them to shuttle H^+V-ATPase molecules from apical to basolateral plasma membrane domains. This hypothesis is supported by data showing that A-cells and B-cells have different intracellular trafficking pathways for LGP120, a lysosomal glycoprotein. LGP120 was found both on the basolateral plasma membrane and in lysosomes in B-cells, whereas no LGP120 was detectable in the plasma membrane of A-cells. We propose that the 'polarity reversal' of the H^+V-ATPase in B-intercalated cells is mediated by a physiologically regulated transcytotic pathway that may be similar to that existing in some other cell types.

Key words: intercalated cell, H^+V-ATPase, proton pump, anion exchanger, carbonic anhydrase, LGP120, kidney, epididymis.

Introduction

Several vertebrate transporting epithelia contain a population of specialized cells that transport protons or bicarbonate across the epithelial barrier (Al-Awqati, 1996; Brown and Breton, 1996; Madsen and Tisher, 1986; Schuster, 1993). These cells are often referred to as 'mitochondria-rich' (MR) cells (Brown and Breton, 1996), because they contain large numbers of mitochondria. They also share several characteristic features, including high levels of cytosolic carbonic anhydrase and membrane-associated H^+V-ATPase. In the kidney, these cells are located in the collecting duct and connecting segment of the urinary tubule and are known as intercalated cells. The epididymis and vas deferens also contain similar, but not identical, epithelial cells that express high levels of H^+V-ATPase on their luminal plasma membranes as well as in intracellular vesicles (Brown et al., 1997). This review will examine selected aspects of the cell biology of proton-transporting cell types that are related to the regulation of transepithelial acid–base transport in these two distinct organ systems.

Kidney intercalated cells

At least two subtypes of intercalated cell are present in the kidney cortex, so-called A (or alpha)-cells and B (or beta)-cells. These subtypes are involved in proton secretion and bicarbonate secretion, respectively, in the distal nephron and collecting duct (Al-Awqati, 1996; Brown and Breton, 1996; Madsen and Tisher, 1986; Schuster, 1993). A-cells have an H^+V-ATPase on their apical plasma membrane and on intracellular vesicles, and they express the kidney homolog of the band 3 Cl^-/HCO_3^- exchanger (AE1) on their basolateral plasma membrane (Alper et al., 1989; Drenckhahn et al., 1985; Schuster et al., 1991) (Fig. 1). B-cells contain no detectable AE1, but the cellular location of the H^+V-ATPase can be

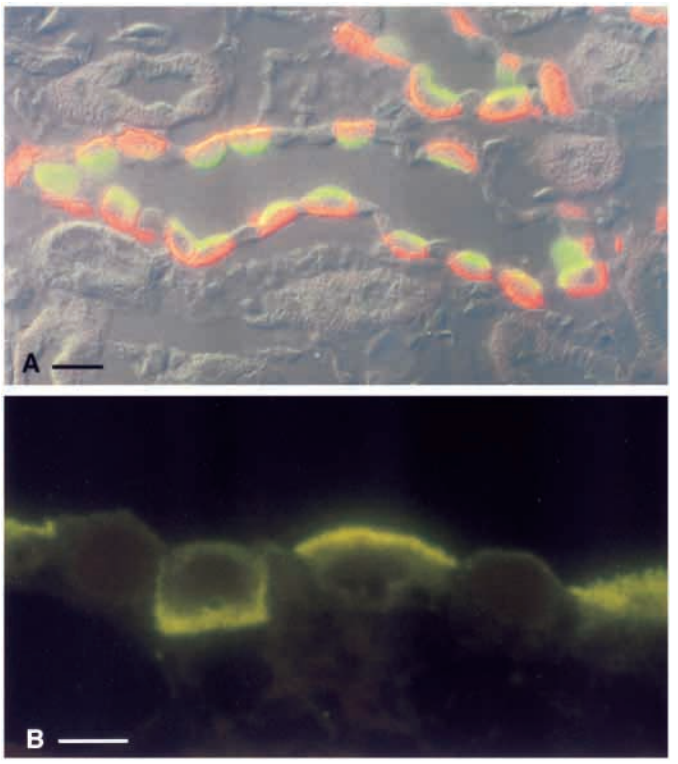

Fig. 1. Immunofluorescence localization of H^+V-ATPase and the AE1 anion exchanger in rat kidney intercalated cells. (A) A combined immunofluorescence/differential interference contrast (DIC) image of an inner stripe collecting duct from rat kidney. Only A-cells are present in this region: they have an apical H^+V-ATPase (green) and basolateral AE1 (red). (B) A 1 μm thick section from a cortical collecting duct stained with anti-H^+V-ATPase antibodies. In the central area of the image, a typical A-cell with intense apical proton pump staining is adjacent to a B-cell, which has well-defined basolateral staining. Scale bars, 15 μm (A) and 5 μm (B).

apical, basolateral, bipolar or diffuse (Alper et al., 1989; Brown et al., 1988a,b; Drenckhahn et al., 1985; Schuster et al., 1991). This complex array of phenotypes is probably at least partially responsible for the different functional categories of intercalated cells (including so-called gamma-cells) that have been identified using intracellular fluorescence pH measurements and ion-substitution experiments (Emmons and Kurtz, 1994; Weiner and Hamm, 1990). In the kidney medulla, only A-type intercalated cells are found, and they account for approximately 40 % of the epithelial cell population in the outer medulla (Brown et al., 1988b). This number falls to approximately 10 % in the initial portion of the inner medulla, and intercalated cells are completely absent from the terminal two-thirds of the papillary collecting duct. A- and B-cells together also make up approximately 40 % of the total number of cells in the cortical collecting duct, with approximately equal numbers of each subtype being present in rat kidney. This differentiated cellular profile in various regions of the kidney corresponds to functional observations; cortical collecting ducts are capable of either net proton or bicarbonate secretion (McKinney and Burg, 1977), whereas medullary collecting ducts can secrete only net acid (Lombard et al., 1983). All intercalated cells contain a large amount of cytoplasmic carbonic anhydrase (CAII) (Brown et al., 1983; Kim et al., 1990), which is also a key protein in supporting the proton-transporting capacity of these cells by providing membrane-associated pumps and transporters with a supply of protons and bicarbonate (Fig. 2).

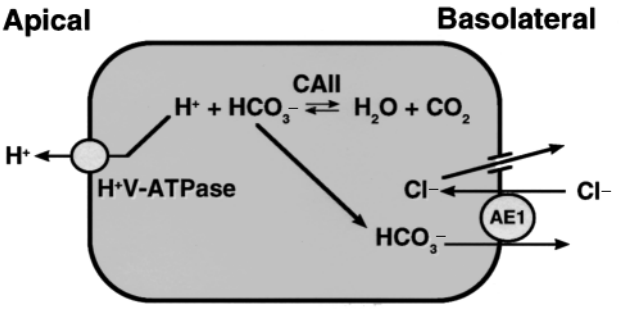

Fig. 2. Diagram illustrating the interaction among proteins that contribute to transepithelial proton translocation in renal intercalated cells. Cytoplasmic carbonic anhydrase (CAII) generates protons and bicarbonate that are subsequently transported by the membrane-associated H^+V-ATPase and the AE1 Cl^-/HCO_3^- exchanger, respectively. Cl^- is recycled across the basolateral membrane *via* a Cl^- channel.

Proton-secreting cells in the epididymis and vas deferens

The luminal fluid along much of the male reproductive tract is maintained at an acidic pH (Carr et al., 1985; Levine and Marsh, 1971; Rodriguez et al., 1990). This low pH is involved in sperm maturation and in maintaining sperm in an immotile state during their passage through the epididymis and vas deferens, probably in conjunction with factors including specific proteins, weak acids and other ions (Hinton and Palladino, 1995). The rat epididymis and vas deferens contain a distinct population of epithelial cells that express high levels of H^+V-ATPase on their luminal plasma membranes as well as on intracellular vesicles (Brown et al., 1992). We have shown that H^+V-ATPases and the cytoplasmic enzyme CAII are co-localized in the same cell type (Breton et al., 1996, 1999). These cells are the so-called ‘apical’ cells or ‘narrow’ cells in the head (caput) portion of the epididymis and the ‘light’ cells or ‘clear’ cells in the body (corpus) and tail (cauda) (Reid and Cleland, 1957; Sun and Flickinger, 1979).

In collaboration with Dr Peter Smith at the BioCurrents Research Center, Marine Biology Laboratory, Woods Hole, MA, USA, a proton-selective, non-invasive microelectrode was used to detect apical proton secretion in the isolated vas deferens. We showed that the proton flux is inhibited rapidly by the H^+V-ATPase inhibitor bafilomycin (Breton et al., 1996) and more slowly by the carbonic anhydrase inhibitor acetazolamide (Breton et al., 1998a) (Fig. 3). These results indicate that H^+V-ATPase-driven proton transport is a major source of luminal acidification in the vas deferens, although other acid–base transporters, including an Na^+/H^+ exchanger

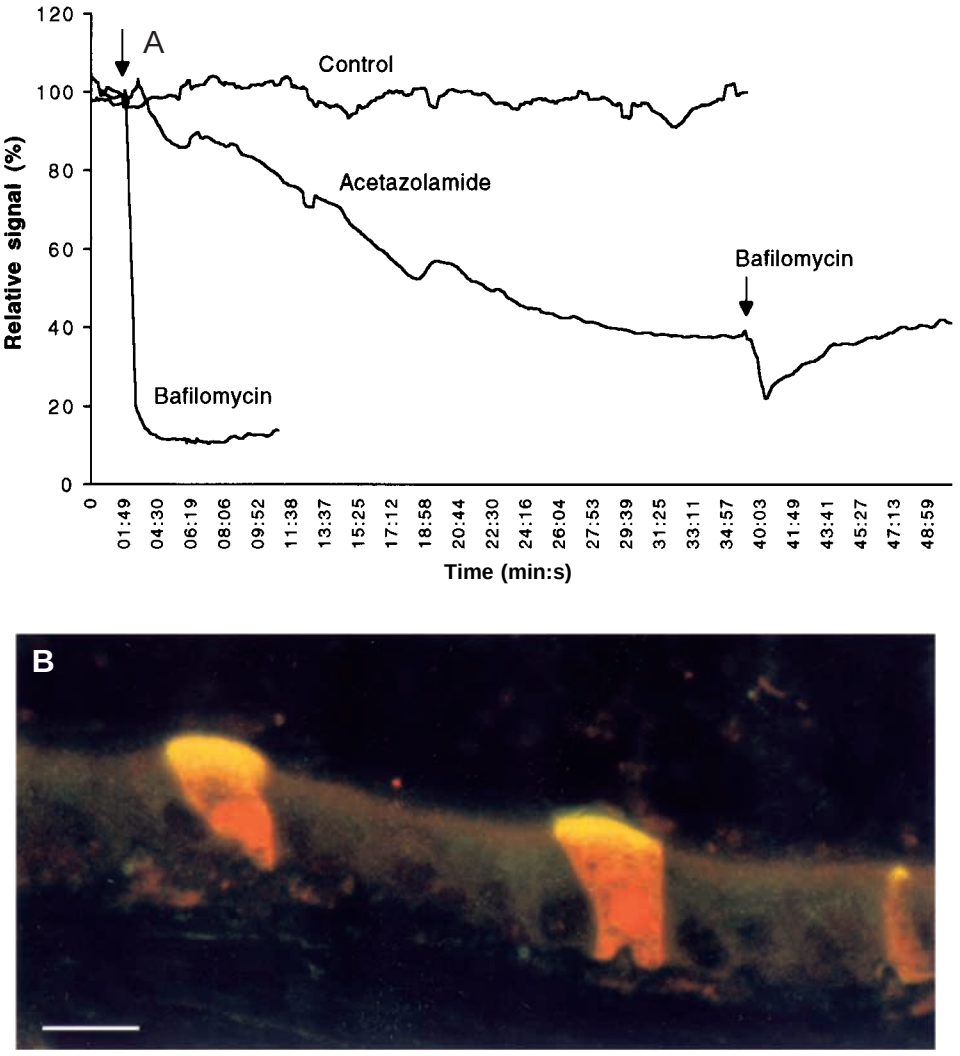

Fig. 3. (A) Real-time measurement of proton secretion in the split-open vas deferens, using a proton-selective, self-referencing microelectrode. Proton secretion by the control vas deferens remains stable for at least 1 h. The H^+V-ATPase inhibitor bafilomycin (1 μmol l^{-1}) causes a rapid reduction in the rate of proton secretion. The carbonic anhydrase (CAII) inhibitor acetazolamide (100 μmol l^{-1}) also causes a reduction in the rate of proton secretion that is of a similar final magnitude to that seen with bafilomycin, but it occurs over a longer period. No further inhibition is seen when bafilomycin is added after acetazolamide. (B) Immunofluorescence localization of CAII and H^+V-ATPase in proton-secreting cells of the rat vas deferens. This double-stained image shows CAII in red throughout the cytosol. The H^+V-ATPase appears as a yellow fluorescence and is located at the apical pole of these specialized cells. Scale bar, 10 μm.

(Au and Wong, 1980) and a Na^+/HCO_3^- cotransporter, may play an important role in more proximal segments of the epididymis (Jensen et al., 1999a). In rat epididymis, the proton-secreting cells do not stain with anti-AE1 antibodies, in contrast to kidney A-type intercalated cells. However, all epithelial cells in the epididymis contain AE2, a different isoform of the anion exchanger, on their basolateral plasma membranes (Jensen et al., 1999b). Its contribution to transepithelial proton secretion remains to be determined.

Membrane recycling in proton-translocating epithelial cells

In the kidney and the reproductive tract, cells rich in proton pumps display a high rate of apical membrane recycling. This recycling can be readily detected by following the internalization of fluid-phase markers, such as fluorescent dextrans, introduced into the tubule lumen (Brown and Breton, 1996; Brown and Sabolic, 1993; Schwartz et al., 1985). The recycling process involves the transport of H^+V-ATPase molecules between the cell surface and the cytoplasm in highly specialized intracellular vesicles that have a well-defined 'coat' structure formed of the peripheral, cytoplasmic V_1 subunits of the H^+V-ATPase (Brown et al., 1987a). In the kidney, systemic acidosis or basolateral perfusion of isolated collecting tubules with high-[CO_2] solutions causes vesicle exocytosis and results in the delivery of more pumps to the apical plasma membrane and an increase in H^+ secretion (Schwartz et al., 1985). Upon reversal of the stimulus, pumps are removed from the membrane by endocytosis. A similar recycling process also occurs in the epididymis (Breton et al., 1998c), but the physiological stimulus that controls this process has not yet been identified. In contrast to the kidney, systemic acidosis does not cause an increase in apical plasma membrane H^+V-ATPase density in the epididymis (S. Breton, L. J. Jensen and D. Brown, unpublished results).

Vesicle coat proteins and endocytosis in proton-secreting cells

Traditionally, endocytosis was thought to be mediated either by clathrin-coated vesicles or by 'smooth' pinocytotic vesicles. Clathrin is involved in the endocytosis of many receptors as well as some other membrane proteins including the facilitated

glucose transporter GLUT4 (Holman and Cushman, 1994) and aquaporin 2 (AQP2) (Brown, 1989; Van Hoek et al., 1998). Endocytosis can also occur *via* a clathrin-independent pathway: these smooth vesicles or caveolae are decorated with a protein called VIP21 or caveolin, which is a substrate for the v-*src* tyrosine kinase in rous-sarcoma-virus-transformed fibroblasts (Glenney and Soppet, 1992). We have shown that endocytosis of the H^+V-ATPase occurs in intercalated cells *via* a clathrin-independent mechanism (Brown and Orci, 1986; Brown et al., 1987b), yet caveolin cannot be detected in these endocytic vesicles (Breton et al., 1998b). Finally, β-COP, another vesicle coat protein associated with vesicle trafficking between the Golgi and the rough endoplasmic reticulum, could not be detected on proton-pump transporting vesicles in the kidney or the epididymis (Breton et al., 1997). It is possible, therefore, that some subunits of the H^+V-ATPase are, in some as yet undefined way, involved in the endocytotic mechanism in these cells.

A- and B-intercalated cell interconversion

Despite considerable effort by many investigators, interconversion of A- and B-type intercalated cells *in vivo* has not been demonstrated directly. The pattern of proton-pump staining clearly changes in the cortical intercalated cell population after acute and chronic acid–base disturbances, with more cells showing tight apical staining in acid conditions, and more showing basolateral staining during alkalosis (Bastani et al., 1991; Sabolic et al., 1997). However, the data can be equally well explained by rearrangements of proton pump polarity in distinct populations of A- and B-cells, without the need to invoke interconversion between these two cell types. This conclusion stands in contrast to data from *in vitro* studies, where an extracellular matrix protein named hensin has been proposed to be a regulator of the intercalated cell phenotype on the basis of its ability to reverse the functional polarity of intercalated cells in culture (Al-Awqati et al., 1998; Vijayakumar et al., 1999).

In vivo, as mentioned above, cells with basolateral AE1 always have apical or subapical H^+V-ATPases, but AE1-negative cells, classified as B-cells, can also have apical H^+V-ATPase (Alper et al., 1989). Basolaterally located H^+V-ATPases have never been found in collecting ducts in the inner stripe of the outer medulla, where only A-cells are present. This distribution may reflect the inability of A-cells in this region to insert proton pumps into the basolateral membrane domain, suggesting that they do indeed represent a distinct cell type. However, the tubular and extracellular environment of the medulla might be such that the A-cell phenotype is favored in this kidney region. Future work addressed at modulating this environment to render it more similar to that of the cortex might provide some answers to the issue of whether A-cells can indeed convert to B-cells under appropriate conditions. These issues have been discussed in previous reviews, to which the reader is referred (Al-Awqati, 1996; Al-Awqati et al., 1998; Brown and Breton, 1996; Schuster, 1993).

Transcytotic pathways in intercalated cells

Type B intercalated cells can insert H^+V-ATPases either apically or basolaterally, and some even have a bipolar distribution of H^+V-ATPase (Alper et al., 1989; Bastani et al., 1991; Brown et al., 1988a,b). Our working hypothesis is that B-cells have an active transcytotic pathway analogous to that present in intestinal epithelial cells, e.g. where the polymeric immunoglobulin (Ig) receptor is first inserted into the basolateral plasma membrane and subsequently re-internalized and transported to the apical plasma membrane after ligand binding. A 14 amino acid domain at the C terminus of the receptor determines initial basolateral targeting, and phosphorylation of Ser664 stimulates endocytosis and transcytosis (Casanova et al., 1990). The transcytotic vesicles fuse with an apical recycling endosomal compartment before the Ig receptor is resorted and finally inserted into the apical membrane (Apodaca et al., 1994). Hepatocytes also have an especially active transcytotic pathway (Bartles and Hubbard, 1990). Microtubules (Hunziker et al., 1990) and GTP-binding proteins (Bomsel and Mostov, 1993) have been implicated in the process of transcytosis, and brefeldin A also inhibits transcytosis (Hunziker et al., 1991).

Some data from our laboratory suggest that A-cells and B-cells have different intracellular trafficking pathways for the lysosomal glycoprotein LGP120 that may be relevant to the ability of B-cells (but not A-cells) to shuttle H^+V-ATPases between apical and basolateral membrane domains by transcytosis. Using antibodies against LGP120 (provided by Dr Ira Mellman, Yale University), B-intercalated cells showed variable levels of this lysosomal membrane protein on their basolateral plasma membranes, as well as in lysosomes (Fig. 4). The distribution of LGP120 on the plasma membrane was similar to that of the H^+V-ATPase in each individual B-cell. In A-cells, however, LGP120 was detectable only in lysosomes and not at the cell surface. This observation suggests that B-cells may insert newly synthesized LGP120 first into their basolateral plasma membrane, before re-targeting the protein to lysosomes. This indirect pathway of lysosomal membrane protein trafficking to lysosomes *via* the cell surface has been previously reported in other cell types (Gough and Fambrough, 1997; Nabi et al., 1991). However, other studies have shown that this transient cell surface expression is not necessary for lysosomal targeting (Harter and Mellman, 1992). Indeed, LGP120 may be directly targeted to lysosomes in A-cells, with no detectable accumulation at the cell surface, at least by immunocytochemical analysis.

Effect of microtubule disruption on LGP120 localization in intercalated cells

Because re-internalization and subsequent transcytosis of some proteins from the basolateral membrane of epithelial cells depends on an intact microtubular network, the distribution of LGP120 in cells from colchicine-treated rats was examined. Rats were injected with colchicine for 8–12 h as previously described (Brown et al., 1991; Gutmann et al., 1989), and fixed

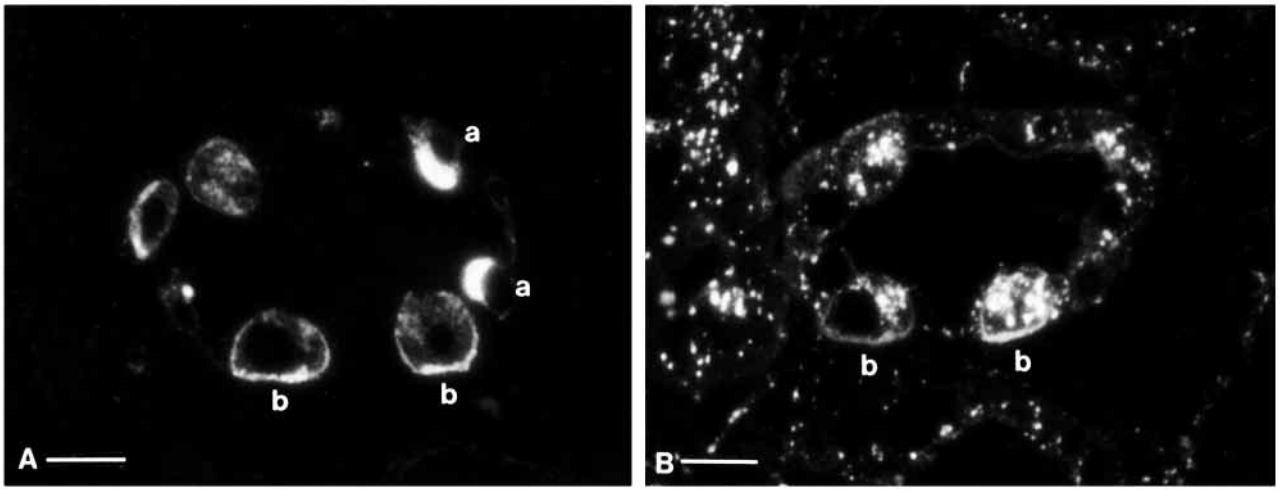

Fig. 4. Indirect immunofluorescence of rat kidney cortical collecting duct showing the localization of H^+V-ATPase (A) and the lysosomal glycoprotein LGP120 (B) in adjacent 1 μm thick cryostat sections. A-type intercalated cells with apical H^+V-ATPase (a) (A) have a punctate intracellular distribution of LGP120, consistent with lysosomal staining. In contrast, B-type intercalated cells with basolateral H^+V-ATPase (b) (A) show variable levels of basolateral LGP120 staining in addition to intracellular lysosomal staining (b) (B). As expected, lysosomes in other tubule segments are also stained with LGP120 in B. Scale bars, 10 μm.

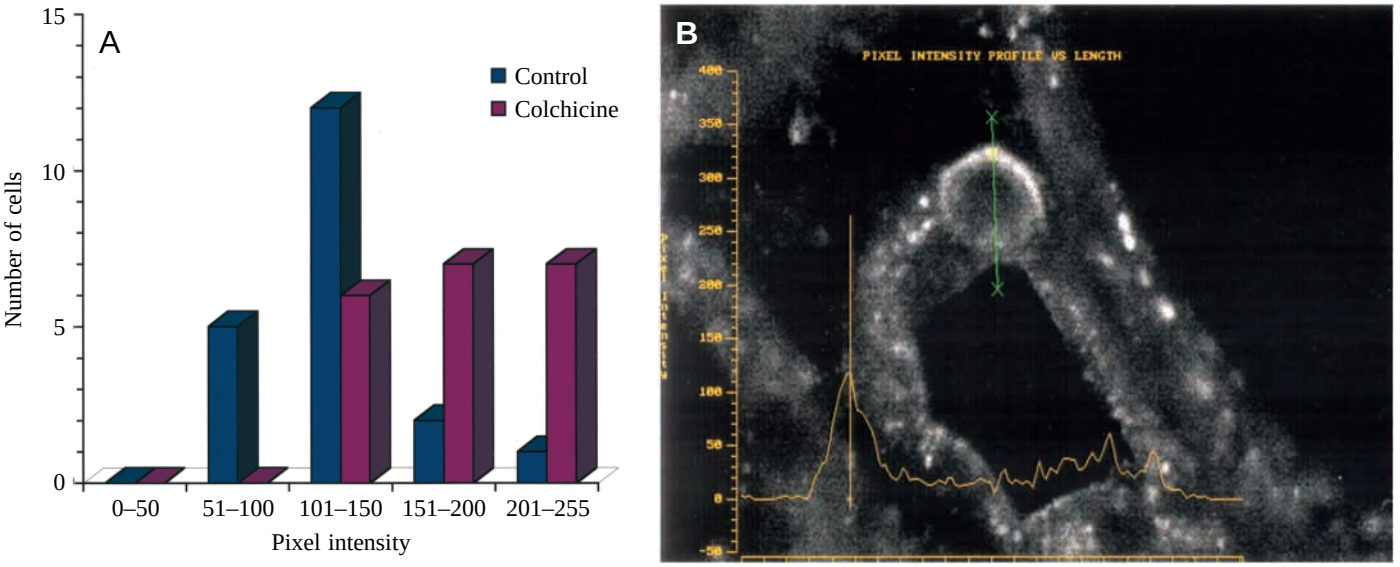

Fig. 5. (A) Quantitative image-analysis of staining for the lysosomal glycoprotein LGP120 at the basolateral membrane of B-type intercalated cells. In control kidneys, a range of expression levels is seen in B-cells, varying from poorly stained to intensely stained. After treatment of rats with the microtubule-disrupting agent colchicine, a clear shift in the staining pattern of B-cells is seen. The relative number of B-cells showing high levels of basolateral LGP120 staining is increased. (B) Confocal microscope image showing LGP120 staining of a cortical collecting duct after colchicine treatment, with an overlay of the image-analysis procedure used to quantify basolateral plasma membrane staining. The linear pixel intensity was measured along the apical-to-basolateral axis of each cell (indicated by the green line) using software bundled with the BioRad confocal operating system. The peak pixel intensity of the cell shown in this image was approximately 120 units, where 0 is black and 255 is pure white. Note that there is very little intracellular LGP120 staining in this cell after microtubule disruption.

kidneys were examined by immunofluorescence microscopy. In B-cells, significantly more LGP120 was located on the basolateral plasma membrane, as assessed by quantitative fluorescence imaging (Fig. 5) indicating that, in the absence of microtubules, LGP120 was delivered to the basolateral cell surface but was not re-internalized and directed towards lysosomes. Consistent with this idea, considerably less intracellular lysosomal staining for LGP120 was seen in B-cells from colchicine-treated rats (Fig. 6).

A schematic representation of the proposed trafficking pathways for LGP120 in A- and B-type intercalated cells is shown in Fig. 7. Although this cell-specific difference in

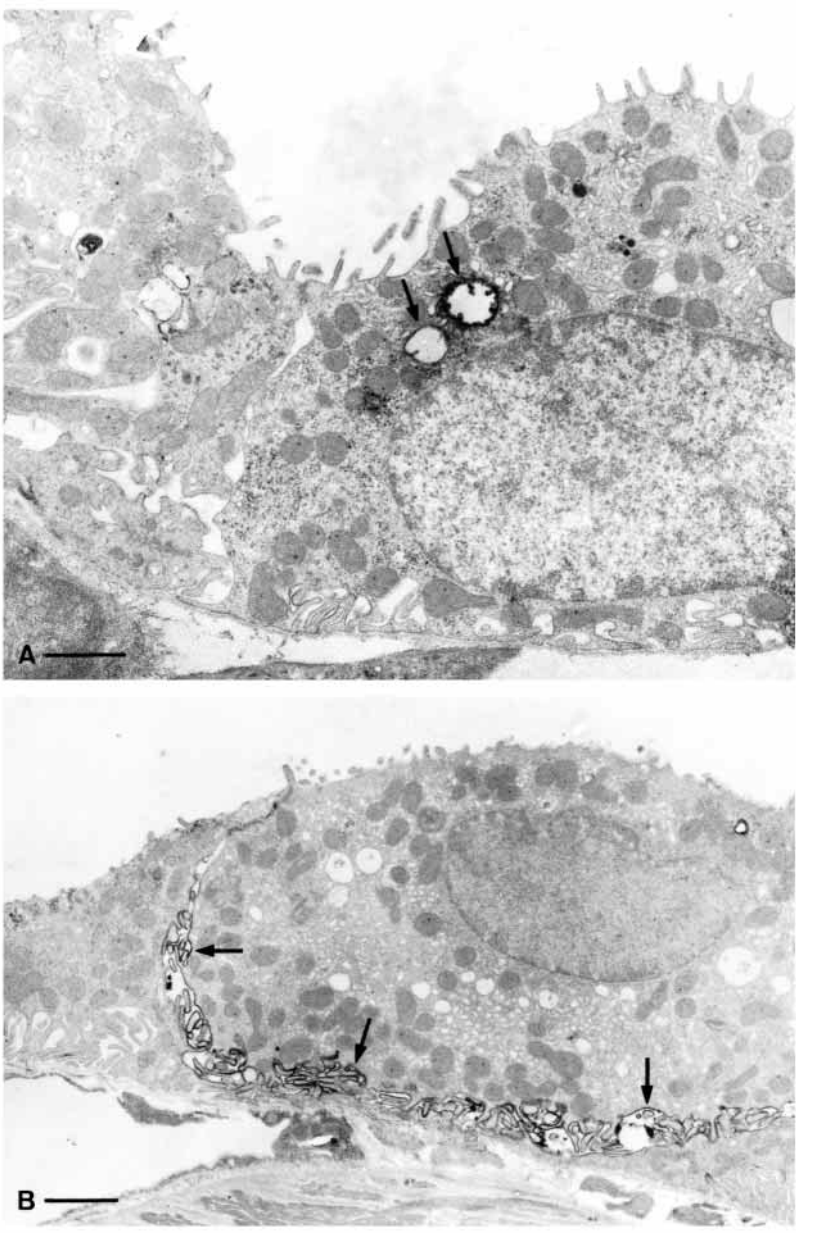

Fig. 6. Electron microscopic immunocytochemistry using horseradish peroxidase to localize the lysosomal glycoprotein LGP120 in intercalated cells from a colchicine-treated rat kidney collecting duct. Antigenic sites are revealed by the electron-dense diaminobenzidine (DAB) reaction product. In A-type cells (A), reaction product is located in some intracellular vesicles (arrows indicate lysosomes). No DAB is detectable at the cell surface. In contrast, B-type intercalated cells (B) show marked basolateral staining for LGP120 (arrows), but very few intracellular structures are labeled. Scale bars, 1 μm.

protein trafficking in these two cell types remains to be confirmed for acid–base transporters, it is supported by observations from Gandhi et al. (1990), showing that another 'apical' marker, ecto 5′ nucleotidase, is also detectable on the basolateral plasma membrane of B-cells. Thus, we propose that the polarity reversal of the H^+V-ATPase, at least in B-type intercalated cells, is mediated by a physiologically regulated transcytotic pathway that may be analogous to that existing in other cell types.

Future directions

Physiologically modulated recycling of proton pumps in acid-transporting cells is important for the normal function of many tissues and organ systems in mammals and many other vertebrate and non-vertebrate species (Brown and Breton, 1996; Wieczorek et al., 1999). It will be important to determine the protein composition of the specialized transporting vesicles that are involved in this process to understand the mechanisms involved in exo- and endocytosis of these novel 'coated' vesicles, which contain none of the other so-called vesicle 'coat' proteins, clathrin, caveolin or β-COP (Breton et al., 1997, 1998b). In contrast, cellubrevin (McMahon et al., 1993) and related vesicle fusion proteins that form a protein complex necessary for vesicle fusion to target membranes seem to be involved in proton pump exocytosis in the epididymis (Breton et al., 1998c) and in cultured renal collecting duct epithelial cells (Alexander et al., 1997). Thus, the potential role of synaptic-vesicle-like fusion proteins in exocytosis of proton pumps should be explored in more detail.

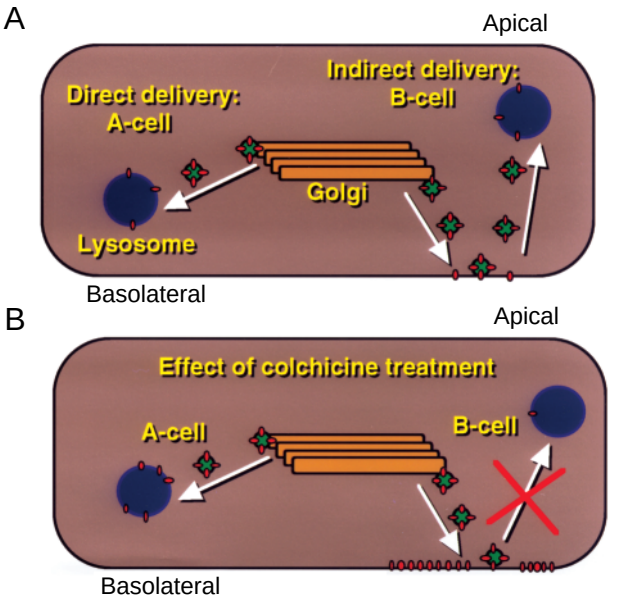

Fig. 7. Diagrams of proposed trafficking pathways for the lysosomal glycoprotein LGP120 (represented by red ovals) in type A and type B intercalated cells. In control cells (A), LGP120 is delivered directly to lysosomes after exit from the Golgi. In B-cells, LGP120 is first inserted into the basolateral plasma membrane, then internalized by endocytosis and subsequently delivered to lysosomes. After microtubule disruption by colchicine (B), LGP120 targeting to lysosomes in A-cells is relatively unaffected, but a large accumulation of LGP120 on the basolateral plasma membrane is seen in B-cells. This may reflect inhibition of re-internalization of LGP120 after microtubule depolymerization, resulting in reduced delivery to lysosomes *via* the indirect pathway.

The opposite polarity of membrane insertion of proton pumps in subpopulations of intercalated cells remains a fascinating biological observation that has not so far been explained in terms of traditional protein sorting paradigms. However, the 56 kDa, or B1, subunit of the H^+V-ATPase that is preferentially expressed in renal intercalated cells and in epididymal proton-secreting cells has a C-terminal DTAL motif (Nelson et al., 1992), which suggests that it is a PDZ domain binding protein. PDZ domains are approximately 90-residue repeats found in a number of proteins implicated in the clustering of ion channels, receptors and other proteins in the plasma membrane. They are named after the first letters of the three proteins that were initially found to contain this amino acid domain – a postsynaptic density protein PSD-95, the *Drosophila* tumor-suppressor protein discs large, and the tight-junction-associated protein ZO-1 (Hall et al., 1998; Wang et al., 1998). Interaction between PDZ-binding proteins and PDZ proteins occurs *via* specific amino acid sequences on their C terminus including, but not restricted to, the consensus sequence, DS/TXL, as for the B1 H^+V-ATPase subunit. The possibility that one of the V-H^+ATPase subunits might associate with these anchoring proteins raises the exciting possibility that proton pumps may be selectively concentrated in some membrane domains *via* this newly discovered intracellular scaffolding system.

This work was supported by NIH grants DK 42956 (D.B.) and DK 38452 (S.B. and D.B.).

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