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REVIEW

MITOCHONDRIA-RICH, PROTON-SECRETING EPITHELIAL CELLS

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Summary

Several transporting epithelia in vertebrates and invertebrates contain cells that are specialized for proton or bicarbonate secretion. These characteristic 'mitochondria-rich' (MR) cells have several typical features, the most important of which is an extremely high expression of a vacuolar-type proton-pumping ATPase (H^+V-ATPase) both on intracellular vesicles and on specific domains of their plasma membrane. Physiological modulation of proton secretion is achieved by recycling the H^+V-ATPase between the plasma membrane and the cytoplasm in a novel type of nonclathrin-coated vesicle. In the kidney, these cells are involved in urinary acidification, while in the epididymis and vas deferens they acidify the luminal environment to allow normal sperm development. Osteoclasts are non-epithelial MR cells that use H^+V-ATPase activity for bone remodeling. In some insects, similar cells in the midgut energize K^+ secretion by means of a plasma membrane H^+V-ATPase. This review emphasizes important structural and functional features of proton-secreting cells, describes the tissue distribution of these cells and discusses the known functions of these cells in their respective epithelia.

Key words: intercalated cell, H^+V-ATPase, anion exchanger, carbonic anhydrase, kidney, epididymis, amphibian bladder, amphibian epidermis, turtle bladder.

Introduction

Several vertebrate transporting epithelia contain a subset of epithelial cells that are highly specialized for proton or bicarbonate secretion (Al-Awqati, 1996; Brown, 1989; Madsen and Tisher, 1986; Schuster, 1993; Steinmetz, 1986; Whitear, 1975). The presence of these characteristic cells in the kidney collecting duct (Schachowa, 1876), the amphibian urinary bladder (Schiefferdecker, 1881) and the amphibian epidermis (Schulze, 1876) has been known for over a century, and they are now known to play a vital role in proton translocation and related epithelial ion-transport processes. These cells have several typical features, listed in Table 1, that can be used to group them together as a family of cells that were originally referred to as 'mitochondria-rich' (MR) cells because they have more mitochondria than the adjacent cells from the same epithelium. The most important feature of these cells that is critical for their function is an extremely high expression of a vacuolar-type proton-pumping ATPase (H^+V-ATPase) both on intracellular vesicles and on specific domains of their plasma membrane (Brown *et al.* 1988*a*). This review will focus on some key features of MR cells from a variety of epithelia.

Intercalated cells (kidney collecting duct)

The intercalated cell phenotype

Intercalated cells were first described as a distinct population of cells in the kidney collecting duct by Schachowa (1876). They have several characteristic morphological features revealed by electron microscopy, including many mitochondria, abundant intracellular vesicles and apical microvilli that are often very well-developed (Kaissling, 1982; Madsen and Tisher, 1986) (Fig. 1A). These cells, which comprise about 30–40 % of the total epithelial cells in collecting ducts, contain high levels of carbonic anhydrase II (Brown *et al.* 1983; Dobyan *et al.* 1982; Holthofer *et al.* 1987; Lonnerholm *et al.* 1985; Rosen, 1972*a*; Spicer *et al.* 1979), a feature that first indicated a potential role in distal urinary acidification. An interesting peculiarity of intercalated cells in rat kidney is that the Na^+/K^+-ATPase is virtually undetectable by immunocytochemistry on the basolateral plasma membrane, in contrast to most other renal tubular cell types (Kashgarian *et al.* 1985). However, these cells do contain an H^+/K^+-ATPase that may be involved in K^+ reabsorption and proton secretion (Wingo and Smolka, 1995).

At least two subtypes of intercalated cells are present in the kidney cortex, so-called A (or alpha)-cells and B (or beta)-

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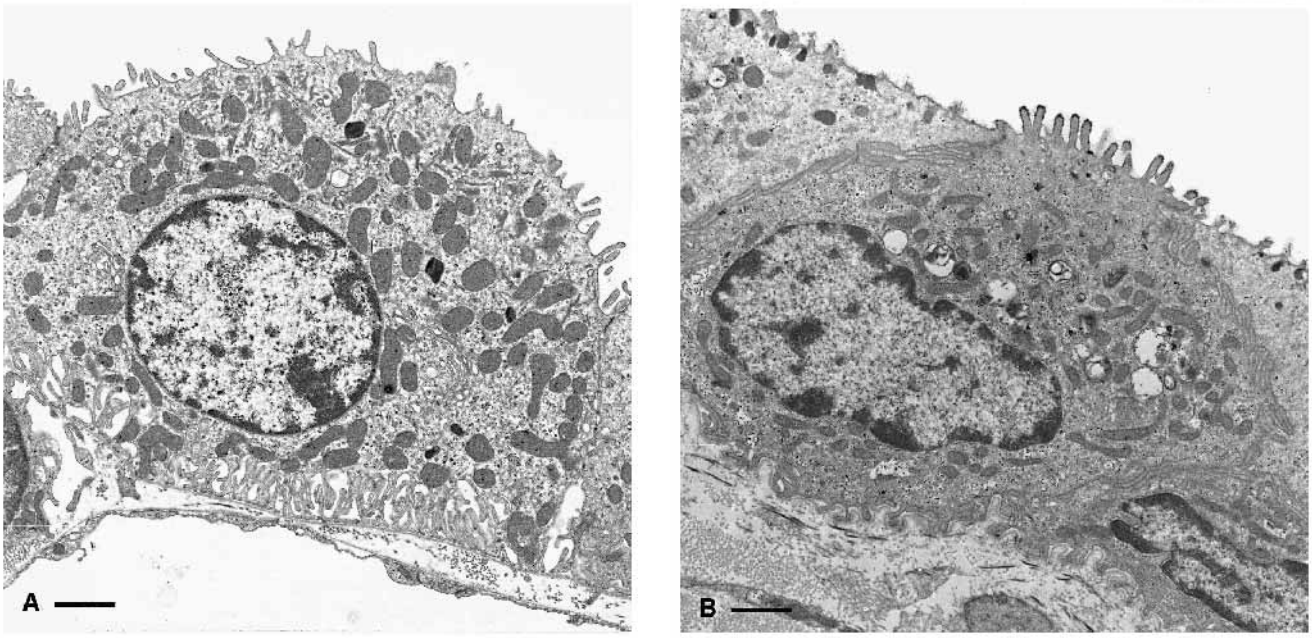


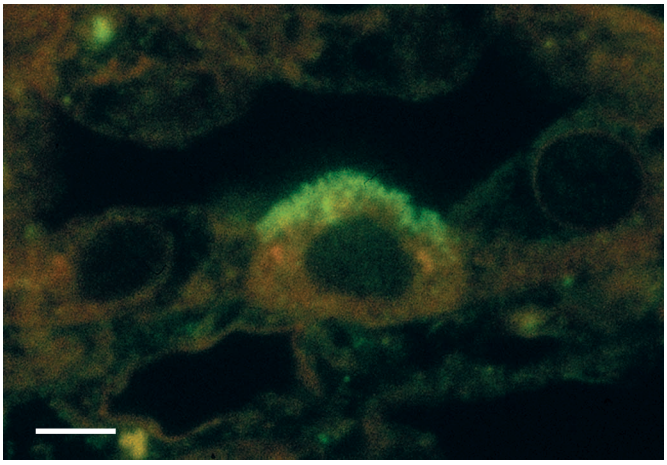
Fig. 1. Conventional thin-section electron micrograph of a rat kidney collecting duct intercalated cell (A) and a toad urinary bladder mitochondria-rich (MR) cell (B). These cells have numerous mitochondria, a large number of intracellular vesicles and well-developed microvilli. Scale bars, 1 μm.

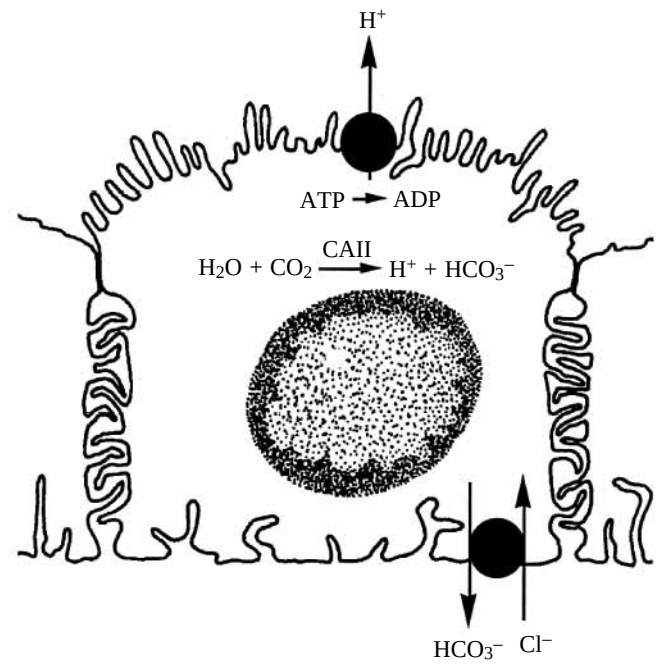
Table 1. *Phenotypic characteristics of mitochondria-rich cells*

Characteristic	Notes
Abundant mitochondria Fig. 1	MR cells with abundant mitochondria form a mosaic in transporting epithelia with cells having fewer mitochondria
Apical microvilli and/or microplicae Fig. 1	The apical plasma membrane can be organized into a complex series of microvilli or broader plate-like extensions of the membrane known as microplicae; this apical amplification is increased when proton secretion is stimulated by vesicle exocytosis
Active endocytotic pathway Figs 4E, 5	Apical fluid-phase markers such as FITC–dextran or horseradish peroxidase are actively internalized by MR cells, but endocytosis is heterogeneous among MR cells in the same epithelium
H^+V-ATPase-coated cytoplasmic vesicles Fig. 4E	The vesicles involved in endo- and exocytosis have a distinct cytoplasmic coat of studs that contain the cytoplasmic domains of H^+-ATPase molecules
Rod-shaped intramembranous particles Fig. 4D	By freeze–fracture electron microscopy, rod-shaped IMPs are present on plasma membranes and on intracellular vesicles
Plasma membrane and vesicular H^+V-ATPase Figs 2 (top), 3, 4B	The plasma membrane and cytoplasmic vesicles contain high levels of a H^+V-ATPase
Cytosolic carbonic anhydrase type II	MR cells have high levels of cytosolic CAII compared with the surrounding epithelial cells

MR, mitochondria-rich; IMPs, intramembranous particles; CAII, cytosolic carbonic anhydrase type II.

cells. These subtypes are involved in proton secretion and bicarbonate secretion, respectively, and their functions have been characterized in a number of studies (Al-Awqati, 1996; Brown, 1989; Kaissling, 1982; Madsen *et al.* 1991; Schuster, 1993; Steinmetz, 1986). A-cells have an H^+V-ATPase on their apical plasma membrane and on intracellular vesicles (Alper *et al.* 1989; Brown *et al.* 1988*a*), and they express the alternatively spliced kidney form of the band 3 Cl^-/HCO_3^- exchanger (AE1) on their basolateral plasma membrane (Alper *et al.* 1989; Drenckhahn *et al.* 1985; Holthofer *et al.* 1987; Schuster *et al.* 1991) (Figs 2, 3). B-cells contain no detectable AE1, but show a remarkable variability in the cellular location of the H^+V-ATPase (Alper *et al.* 1989; Bastani *et al.* 1991; Brown *et al.* 1988*a*; Brown and Sabolic, 1993). Cells that are traditionally identified as B-cells by the absence of AE1 staining can have apical, basolateral, bipolar or diffuse intracellular vesicular staining with anti-H^+V-ATPase antibodies in rat kidney (Fig. 3). However, in mouse and





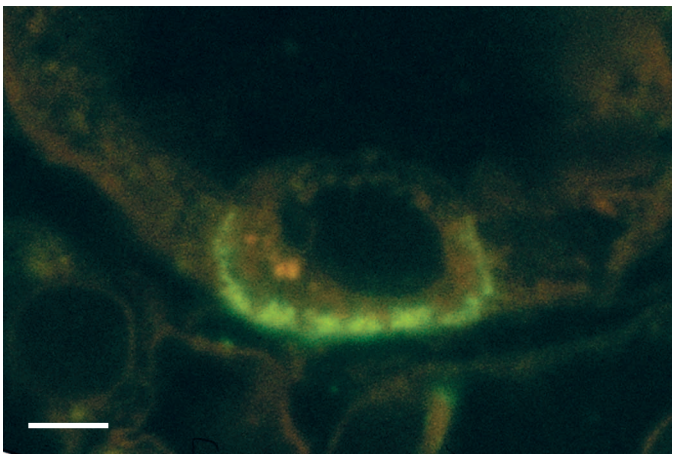


Fig. 2. Typical A-type intercalated cells from the rat kidney collecting duct showing apical H^+V-ATPase (top) and basolateral band 3 (AE1) anion exchanger (bottom) visualized by indirect immunofluorescence. These cells are also rich in carbonic anhydrase II (CAII), which catalyzes the hydration of CO_2 to produce H^+ and HCO_3^- (middle). Scale bars, 3 μm.

rabbit, cells with unique and strong basolateral staining for H^+V-ATPase are rarely found – most cells appear diffusely stained by immunofluorescence in these species (Breton *et al.* 1995; Schuster *et al.* 1991). Basolateral membrane staining can, however, be detected by immunogold staining in some rabbit B-cells (Verlander *et al.* 1994). Intercalated cells express the so-called 'kidney' isoform of the H^+V-ATPase 56 kDa subunit. A distinct 'brain' isoform of the 56 kDa subunit is expressed in the proximal tubule (Nelson *et al.* 1992). Interestingly, other MR cells that have been examined by immunocytochemistry also express the 'kidney' isoform of this 56 kDa subunit (Brown *et al.* 1992; D. Brown, unpublished data), with the exception of the osteoclast, which expresses the 'brain' isoform (see below).

Anion (Cl^-/HCO_3^-) exchangers in intercalated cells

The localization, function and regulation of Cl^-/HCO_3^- anion exchange proteins in intercalated cells are especially complex (Emmons and Kurtz, 1993). New data have challenged the simple picture in which A-cells have basolateral AE1 and B-cells have a functionally detectable apical anion exchanger that has not yet been identified at the protein level (Schuster and Stokes, 1987; Weiner *et al.* 1994). A third type of intercalated cell, the gamma-cell, can have both apical and basolateral anion exchangers, at least in rabbit cortical collecting ducts (Emmons and Kurtz, 1994). Studies in cultured cells have suggested that the apical exchanger in B-cells is also AE1 (van Adelsberg *et al.* 1993), but other work has shown only very low levels of AE1 expression in B-cells (Fejes-Toth *et al.* 1994). Furthermore, no reports have yet shown reversal of polarity of AE1 by immunocytochemistry (Hayashi *et al.* 1988), although it is possible that apically located AE1 might not be recognized by available antibodies, perhaps because of some conformational change in the protein (van Adelsberg *et al.* 1993).

As mentioned above, the immunocytochemical detection of apical anion exchangers has remained elusive until recently. It has now been shown that AE3 isoforms of the Cl^-/HCO_3^- exchanger can be expressed both apically and basolaterally in intercalated cells (Alper *et al.* 1995), thus adding an additional degree of complexity to an already complicated picture.

Regulation of proton pumping in intercalated cells

The capacity of A-type intercalated cells to secrete protons is regulated under physiological conditions by shuttling H^+V-ATPase molecules to and from the plasma membrane *via* a specialized intracellular acidic vesicular compartment (Bastani *et al.* 1991, 1994; Brown, 1989; Madsen *et al.* 1991; Schuster, 1993; Schwartz *et al.* 1985). Under conditions of systemic acidosis or basolateral perfusion of isolated collecting tubules with high-[CO_2] solutions, vesicle exocytosis results in the delivery of more pumps to the apical plasma membrane and an

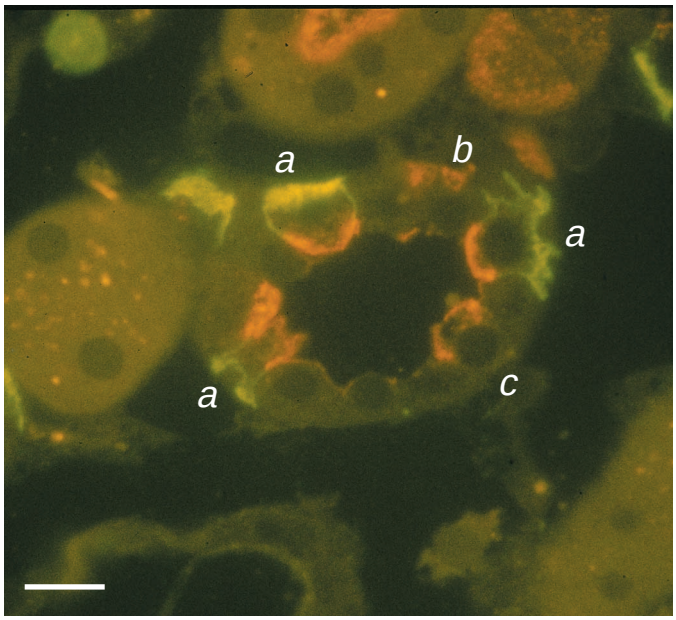


Fig. 3. 5 µm cryostat section of a rat kidney cortical collecting tubule showing H^+V-ATPase (56 kDa subunit) staining in orange and band 3 (AE1) staining in yellow/green. In this figure, three patterns of intercalated cell staining are seen, but four patterns can sometimes be detected in other images. Typical A-cells have apical proton pumps and basolateral AE1 (*a*). Intercalated cells that have no detectable AE1 can have proton pumps concentrated at the basolateral pole (not shown), at both the basolateral and apical poles (*b*) or at the apical pole (*c*). Thus, H^+V-ATPase staining alone cannot be used to distinguish A- and B-type intercalated cells in the renal cortex. Scale bar, 10 µm.

increase in H^+ secretion. Upon reversal of the stimulus, pumps are removed from the membrane by endocytosis. The vesicles that are involved in this shuttling process are highly characteristic and have a well-defined 'coat' structure formed of the peripheral, cytoplasmic V_1 subunit complexes of H^+V-ATPases (Brown *et al.* 1987*a*) (Fig. 4A,E). Protein-A–gold immunocytochemistry using antibodies against the H^+V-ATPase revealed a dense labeling of the apical plasma membrane (Fig. 4B) and of the cytoplasmic side of the coated vesicles (Brown *et al.* 1987*a*). Large regions of the cytoplasmic surface of the apical membrane of toad bladder MR cells are decorated by stud-like projections that are seen as regular, hexagonal arrays by the rapid freezing and freeze-drying technique (Fig. 4C) (Brown *et al.* 1987*a*). Identical arrays of studs are also seen on phospholipid liposomes in which purified H^+V-ATPase molecules have been reconstituted, providing strong evidence that this coating material is related to the H^+V-ATPase (Brown *et al.* 1987*a*). Rod-shaped intramembranous particles (IMPs) are found on the plasma membrane of MR cells (Fig. 4D) and on intracellular vesicles by freeze–fracture electron microscopy (Humbert *et al.* 1975; Orci *et al.* 1981; Stetson *et al.* 1980). The endocytotic process involved in H^+V-ATPase recycling does not involve clathrin (Brown and Orci, 1986; Brown *et al.* 1987*b*), and the mechanism underlying this internalization process is unknown. Recent work has also failed to detect caveolin, a protein associated with another endocytotic pathway involving caveolae (Rothberg *et al.* 1992), in these endocytotic vesicles (S. Breton and D. Brown, unpublished results). In collecting duct principal cells, the 'kidney' isoform of the 56 kDa peripheral V_1 subunit has been detected in association with water-channel-containing endosomes, which do not contain other subunits of the proton pump and which do not acidify their lumen (Sabolic *et al.* 1992). It is possible, therefore, that the 56 kDa subunit has a function that can be independent of the H^+V-ATPase and that is, in some as yet undefined way, involved in a novel endocytotic mechanism in intercalated cells, as well as in other cell types.

In parallel with the cellular redistribution of H^+V-ATPases during acidosis and alkalosis, it has been shown that the expression of AE1 protein and/or mRNA in A-type intercalated cells is increased during acidosis and decreased during alkalosis (Alper *et al.* 1991; Da Silva *et al.* 1991; Fejes-Toth *et al.* 1994). These changes would modulate proton and bicarbonate secretion in parallel with the observed rearrangement of H^+V-ATPases under these conditions.

Interconversion of intercalated cell subtypes?

It is still controversial whether these intercalated cell phenotypes represent distinct cellular subtypes or whether polarized AE1 expression and H^+V-ATPase localization can both be reversed within the same cell, under different physiological conditions (Al-Awqati, 1996; Koichi *et al.* 1992; Madsen *et al.* 1991; Schuster, 1993). Results from a number of studies are inconclusive, because of different possible interpretations of the data, and a complete discussion of this complex issue is not the aim of this review. However, cells with basolateral AE1 always have apical or subapical H^+V-ATPases, but it is clear that AE1-negative cells, classified therefore as B-cells, can also have apical H^+V-ATPases (Fig. 3C) (Alper *et al.* 1989). H^+V-ATPase staining of intercalated cells cannot, therefore, be used alone to distinguish A- and B-cells in the renal cortex. Thus, transcytosis of H^+V-ATPases from apical to basolateral membranes, or *vice versa*, appears to be a feature of AE1-negative cells, but may not occur in 'typical' A-cells. For example, basolaterally located H^+V-ATPases have never been found in the inner stripe or inner medulla of the collecting duct, where only 'typical' A-cells are found. However, it should be borne in mind that, as mentioned above, the intensity of basolateral AE1 staining of A-intercalated cells in the cortex is dependent on acid–base status and further confounds the precise identification of A- and B-cells in this region of the kidney.

Recent data from our laboratory have also shown that A-cells and B-cells have different intracellular trafficking pathways for the lysosomal glycoprotein LGP120 that may parallel the ability of B-cells (but not that of A-cells) to insert H^+V-ATPases into apical and basolateral membrane domains (Breton and Brown, 1995). B-cells may insert newly synthesized LGP120 first into their basolateral plasma

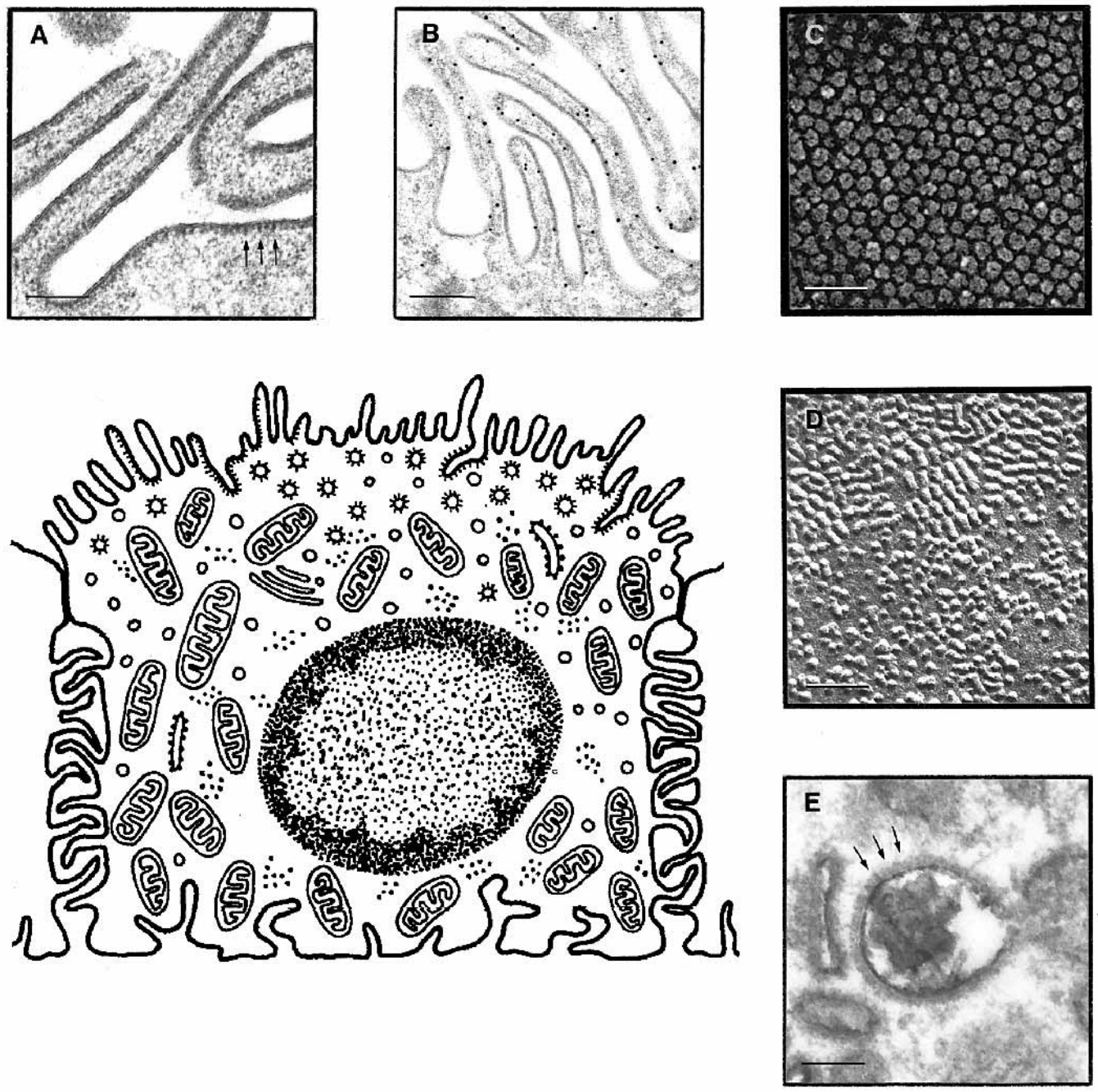


Fig. 4. Morphological features of mitochondria-rich (MR) cell membranes as seen by conventional electron microscopy (A,E), by immunogold staining of the vacuolar-type proton-pumping ATPase (H^+V-ATPase) (B), by rapid-freeze, deep-etch microscopy (C) and by freeze–fracture electron microscopy (D). Horseradish peroxidase uptake by a coated endocytic vesicle is shown in E. In thin sections, parts of the plasma membrane (A) and the membrane of endocytotic vesicles (E) are coated with many studs (arrows); these studs contain the cytoplasmic domain of the H^+V-ATPase. By freeze–fracture electron microscopy, the plasma membranes of MR cells contain numerous rod-shaped intramembranous particles (IMPs) about 16 nm in length (D). By rapid-freeze, deep-etch microscopy, the studs are seen to be arranged in tightly packed hexagonal arrays on the cytoplasmic surface of the plasma membrane (C). Scale bars, 0.1 μm (A), 0.15 μm (B), 30 nm (C), 40 nm (D), 0.15 μm (E). The drawing shows a typical MR cell.

membrane, before re-targeting the protein to lysosomes. In contrast, LGP120 appears to be directly targeted to lysosomes in A-cells, with no detectable accumulation at the cell surface.

Finally, using FITC–dextran as a marker of fluid-phase endocytosis in the kidney, we have found that not only typical A-cells with basolateral AE1 have a high rate of apical endocytotic activity (Fig. 5), but that AE1-negative cells (B-cells) in the collecting duct and connecting tubule often also show an extremely high rate of apical endocytosis (S. Breton, P. King and D. Brown, unpublished results). Many previous

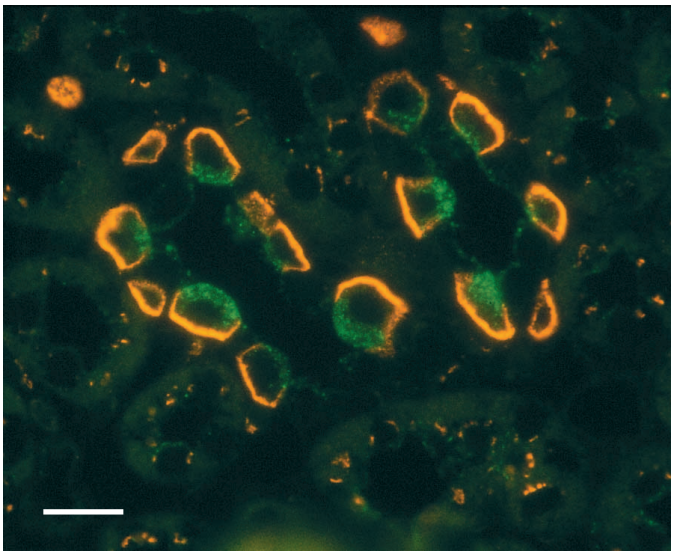


Fig. 5. 5 µm cryostat section of the inner stripe of the outer medulla of a rat kidney injected intravenously for 15 min with FITC–dextran (green). This section was double-labeled to reveal the band 3 (AE1) anion exchanger by immunofluorescence (orange). Typical A-cells with basolateral band 3 have a very high rate of endocytosis, as demonstrated by their numerous apical vesicles labeled with FITC. In cells of the thick ascending limb of Henle, Golgi staining for AE1 is detectable. Scale bar, 10 µm.

studies, particularly in isolated perfused tubules and cell culture (Koichi *et al.* 1992; Schwartz *et al.* 1985; van Adelsberg *et al.* 1994), have utilized apical endocytosis as a means of classifying A-cells (endocytosis-positive) and B-cells (endocytosis-negative). Clearly, this is an oversimplification, because both cell types can display active apical endocytosis *in vivo*. In addition, remodeling of bicarbonate-secreting B-cells, probably by endocytosis of apical membrane components including an anion exchanger and a protein binding peanut lectin, has been shown to occur after an acid load in isolated perfused rabbit collecting tubules (Satlin and Schwartz, 1989). Recently, an extracellular matrix protein has been proposed as a regulator of intercalated cell phenotype on the basis of its ability to reverse the functional polarity of intercalated cells in culture (van Adelsberg *et al.* 1994).

Intercalated cell development

Intercalated cells are found in collecting ducts and connecting segments prior to birth. In the rat kidney, they are most common in the developing papilla and rarer in the outer medulla and cortex (Kim *et al.* 1994; Matsumoto *et al.* 1993). Cells with apical H^+V-ATPases and cells with basolateral H^+V-ATPases appear in collecting ducts and connecting segments simultaneously during development. In the 3 weeks after birth, the situation reverses and the intercalated cell population increases in the cortex and outer medulla, but decreases considerably in the inner medulla (Brown *et al.* 1983; Kim *et al.* 1994; Madsen *et al.* 1988). It is not known to what extent selective cell death is involved in the disappearance of these cells in the inner medulla, although apoptotic figures can be found in some B-intercalated cells in this region after birth, while A-cells appear to be deleted by extrusion from the epithelium and shedding into the lumen (Kim *et al.* 1996). Alternatively, it has been repeatedly suggested that the different cell types in the collecting duct are developmentally related, and that principal and intercalated cells may interconvert (Evan *et al.* 1980; Hancox and Komender, 1963; Ordonez and Spargo, 1976; Richet and Hagege, 1975). Although there is no direct evidence *in vivo* showing such a relationship, studies *in vitro* using immuno-isolated A- and B-intercalated cells and principal cells indicate that antigens characteristic of one cell type can appear in cultures that originally did not contain this cell type. Thus, purified cultures of B-intercalated cells eventually give rise to cells that stain positively with monoclonal antibodies directed against A-cell and principal cell antigens (Fejes-Toth and Naray-Fejes-Toth, 1992, 1993).

Our recent studies on mice that are deficient in carbonic anhydrase type II show that, at birth, both intercalated and principal cells are present in medullary collecting ducts, but that in the adult mouse, intercalated cells are virtually absent from all regions of the medulla (Breton *et al.* 1995). These results suggest either that intercalated cells are selectively depleted from these collecting ducts or that they convert during postnatal differentiation into principal cells. The total number of cells in the collecting ducts of these mice is unchanged compared with normal littermates, indicating either a conversion of intercalated cells into principal cells or a concomitant increase in principal cell proliferation to replace intercalated cells lost by an as yet undetermined mechanism. Our finding of some 'hybrid' cells that contain both intercalated (AE1) and principal (aquaporin 2) cell markers (S. Breton and D. Brown, unpublished data) suggests that earlier work, which provided circumstantial evidence for a potential interconversion of these two functionally quite distinct cell types, merits further investigation.

Finally, cells with rod-shaped IMPs, which are present in all MR cell membranes and may represent the 'V_2' membrane region of H^+V-ATPases (see below for discussion of rod-shaped IMPs), are present even in the rabbit mesonephric kidney (Schiller and Tiedemann, 1981), and more recent data have confirmed the presence of A-type intercalated cells in mesonephric rat and rabbit kidney (Matsumoto *et al.* 1993, 1994), indicating a complex developmental relationship among the different collecting duct cell types.

Mitochondria-rich cells in amphibians and reptiles

Special epithelial cell subtypes were first described in amphibian epidermis by Rudneff (1865) and in amphibian urinary bladder by Schiefferdecker (1881). While the nature of these cells was unknown, the *camera lucida* illustrations prepared by these authors provided a highly accurate reflection of the morphology and distribution of MR cells in these tissues. Similar cells are also found in the turtle urinary bladder, and

this system proved to be a powerful model in which to examine transepithelial proton secretion in many pioneering studies (Steinmetz, 1986).

The turtle urinary bladder

The turtle bladder is an epithelium with a high capacity to translocate protons. It has been widely used as a model system to examine different aspects of proton secretion, including the cellular biology of the specialized cell type that is primarily responsible for acid–base transport, the MR cell or the CAII-rich cell, as it is commonly referred to in this tissue (Schwartz *et al.* 1972, 1982; Steinmetz, 1985). A series of functional studies on the turtle bladder provided critical insights into mechanisms of acid–base handling by the renal collecting duct, and these are discussed in reviews by Steinmetz (1985, 1986). The MR cells in the turtle bladder have many of the morphological features of renal intercalated cells, but so far immunocytochemical localization of H^+V-ATPases in reptilian bladder has not been performed. However, using the characteristic membrane-associated studs (originally named portasomes) as a morphological marker of the V_1 peripheral region of H^+V-ATPases (Harvey, 1980), it was shown that α- and β-type MR cells having an opposite polarity of the H^+V-ATPase could be identified (Stetson and Steinmetz, 1985). On the basis of this observation, a model for proton and bicarbonate transport across transporting epithelia, activated by a proton-pumping V-ATPase, was developed (Stetson *et al.* 1985) and the bicarbonate-secreting cells were identified as MR cells (Fritsche and Schwartz, 1985). Bicarbonate secretion is linked to the activity of an H^+V-ATPase, presumably located in the basolateral membrane of β-type MR cells (Fritsche *et al.* 1989). In addition, vibrating probe studies provided direct evidence that MR cells were the source of the proton current in turtle bladder (Durham and Nagel, 1986). The turtle bladder was also the first tissue in which regulation of apical proton secretion by exo- and endocytosis of acidic vesicles containing H^+V-ATPases was demonstrated (Cannon *et al.* 1985; Dixon *et al.* 1986; Gluck *et al.* 1982; Stetson and Steinmetz, 1983).

The amphibian urinary bladder

In parallel with observations on the turtle bladder, the amphibian bladder was also found to be an acidifying epithelium (Ludens and Fanestil, 1972). Cells bearing the hallmark features of MR cells are found in variable numbers in the urinary bladder of many amphibian species (Frazier, 1978; Keller, 1963; Orci *et al.* 1975; Peachy and Rasmussen, 1961; Rosen, 1972*b*; Wade, 1976) (Fig. 1B). A key observation that provided the basis for many studies on the cellular origin of the proton flux in this tissue was that toads (*Bufo marinus*) from the Dominican Republic and from Columbia had considerably different capacities to acidify their urine (Rosen *et al.* 1974). Morphological examination revealed that the poor acidification ability of Dominican toads correlated with a paucity of MR cells in the bladder epithelium, whereas Columbian toads, which could acidify vigorously, had considerably greater numbers of MR cells. Thus, the amphibian bladder provided another system in which acidification was related to the presence of specialized MR cells. The morphology of these MR cells was specifically modified during stimulation of acid secretion (Frazier, 1978), and MR cells appear to be specific targets for aldosterone binding in toad bladder (Sapirstein and Scott, 1975). The cytoplasmic ‘studs’ that coat the inner surface of MR cell membranes, as well as many intracellular vesicles, were first identified as cytoplasmic subunits of an H^+V-ATPase by rapid-freeze, deep-etch microscopy studies on toad bladder membranes (Brown *et al.* 1987*a*).

The amphibian skin

The amphibian epidermis is another acid-secreting tissue that contains MR cells (Machen and Erlij, 1975). In the skin, they are often referred to as flask cells, because of their typical elongated shape, with a wide base tapering to a narrow neck. Epidermal MR cells are rich in carbonic anhydrase (Rosen and Friedley, 1973), have elongated IMPs seen by freeze–fracture (Brown *et al.* 1978) and show characteristic morphological features of the MR cell family (Whitear, 1975). Recent work has demonstrated a band-3-like anion exchanger in MR cells of *Bufo marinus* skin (Devuyst *et al.* 1993), but it was located apically. In contrast, we have found that a band-3-like anion exchanger is basolaterally located in MR cells, while proton pumps are apically located (S. Breton and D. Brown, unpublished data; Fig. 6). In addition to their role in transepithelial proton movement, it has also been shown that the H^+V-ATPase in these cells energizes transepithelial Na^+ transport in this tissue (Harvey, 1992), although the MR cells

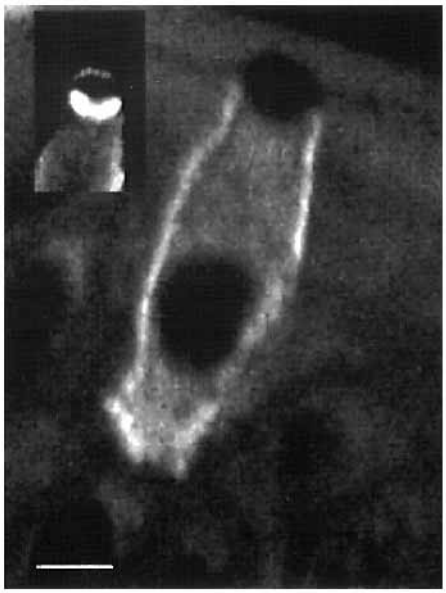


Fig. 6. Flask cell from amphibian (*Bufo marinus*) skin stained with an antibody that recognizes a C-terminal epitope that is found in both AE1 and AE2 Cl^-/HCO_3^- exchangers. The inset shows the same cell after subsequent double staining with an antibody against the 56 kDa subunit of the H^+V-ATPase. Basolateral AE1/AE2 staining is also apparent in the inset. Scale bar, 5 μm.

themselves are not the principal site of amiloride-sensitive transepithelial Na^+ transport (Dorge *et al.* 1990; Ehrenfeld *et al.* 1976). However, several studies have shown that epidermal flask cells are the pathway for the high transepithelial chloride conductance in this tissue (Foskett and Ussing, 1986; Katz and Scheffey, 1986; Larsen *et al.* 1987). This conductance is regulated by β-adrenergic agonists *via* cyclic AMP (Willumsen *et al.* 1992). The functional state of epidermal MR cells is modulated by aldosterone (Devuyst *et al.* 1991*a*) and prostaglandins (Yorio *et al.* 1991).

The number of flask cells varies considerably among different regions of skin in the same animal and also among different species. In several species, the number of epidermal flask cells can be increased by maintaining the animals in distilled water and decreased by maintaining them in saline solutions (Budtz *et al.* 1995; Devuyst *et al.* 1991*b*; Ilic and Brown, 1980; Katz and Gabbay, 1988). Many flask cells are shed during each molting cycle and replaced by differentiation of epithelial cells, although the lineage of the new cells is not clear (Muto *et al.* 1990).

Proton-pump-rich cells in the epididymis and vas deferens

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 in intracellular vesicles (Brown *et al.* 1992) (Fig. 7). These cells have many morphological features of MR cells, including the presence of rod-shaped IMPs on their apical plasma membrane (Brown and Montesano, 1980). An earlier histochemical study showed a distinct population of epithelial cells in the epididymis that contained large amounts of carbonic anhydrase (Cohen *et al.* 1976), an indication that these cells might be involved in proton secretion. It was then shown that carbonic-anhydrase-rich cells appear gradually in the epithelium after birth and are present at adult levels after 4–5 weeks (Membre *et al.* 1985). We have shown that H^+V-ATPases and CAII are co-localized in the same cell type (Breton *et al.* 1996). 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). The morphology of apical and clear cells is quite distinct, although both share an extremely high level of apical endocytotic activity, similar to that of renal intercalated cells (Al-Awqati, 1996; Brown *et al.* 1987*b*; Moore and Bedford, 1979). Furthermore, aldosterone binds avidly to epididymal clear cells (Hinton and Keefer, 1985), and clear cells are selectively ablated from parts of the epididymis by ethane dimethane sulfonate (Klinefelter *et al.* 1994; Liu *et al.* 1993) in parallel with a decrease in fertility. The presence of CAII in the epididymis prompted studies to determine whether the CA inhibitor acetazolamide would inhibit luminal acidification. Conflicting data were obtained. In one study, acetazolamide did partially inhibit acidification (Au and Wong, 1980), whereas subsequent work showed no effect on epididymal pH (Caflisch, 1992), possibly due to a failure to deliver an appropriate dose of the inhibitor to the CAII-rich cells.

In collaboration with Dr Peter Smith at the Woods Hole Marine Biology Laboratory, MA, the proton-selective, non-invasive vibrating probe has been used to detect a bafilomycin-sensitive proton secretion in the lumen of the vas deferens (Breton *et al.* 1996). Bafilomycin is a specific inhibitor of the H^+V-ATPase. Up to 80 % of the transepithelial proton flux in the vas deferens was inhibited by bafilomycin, implicating H^+V-ATPase-driven proton transport as the major source of luminal acidification. Because the 'apical' and 'clear' cells contain large numbers of H^+V-ATPase molecules on their apical plasma membranes, it is likely that they are analogous in function to renal intercalated cells and that they are primarily responsible for luminal proton secretion into the excurrent duct system. However, at least in rat epididymis, no basolateral anion exchanger has yet been identified, and the cells do not stain with anti-AE antibodies that strongly label the basolateral plasma membrane of renal intercalated cells (Breton *et al.* 1996; Brown *et al.* 1992).

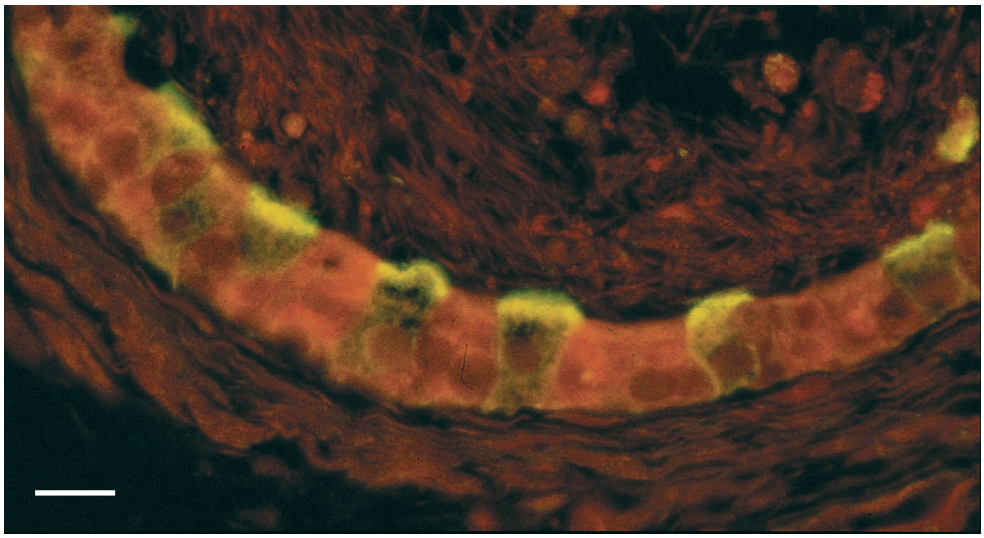


Fig. 7. Section of rat epididymis (tail region) stained to show the mosaic of H^+V-ATPase-rich mitochondria-rich (MR) cells (yellow) that are involved in acidifying the luminal fluid in this region of the male reproductive tract. In some parts of the epididymis, these cells comprise up to 40 % of the total epithelial cells. The antibody used to stain these cells was prepared against the 56 kDa subunit of the H^+V-ATPase. The red background is Evan's Blue counterstain. Abundant sperm in the tubule lumen and the smooth muscle layer beneath the epithelium are stained with Evan's Blue. Scale bar, 10 μm.

The luminal fluid along much of the male reproductive tract is maintained at an acidic pH in many species (Carr *et al.* 1985; Levine and Marsh, 1971; Rodriguez *et al.* 1990). It has been proposed that low pH is required for sperm maturation and that it is involved 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). Previous work on the perfused epididymis implicated an Na^+/H^+ exchanger in the process of acidification (Au and Wong, 1980), but on the basis of our observations, it is likely that the H^+V-ATPase-rich MR cells described above play a key role in the maintenance and modulation of luminal pH in this tissue.

The osteoclast – a non-epithelial MR cell

Osteoclasts reabsorb bone by creating a specialized extracellular acidic space between the bone-facing domain of their plasma membrane and the bone surface. The osteoclast membrane in this region is highly ruffled, and its cytoplasmic surface is decorated with typical H^+V-ATPase studs. Immunocytochemistry and functional assays have shown that this membrane contains a high level of H^+V-ATPase (Blair *et al.* 1989; Vaananen *et al.* 1990), while the non-absorptive surface of the osteoclast contains a band-3-like anion exchanger (Baron, 1989; Blair *et al.* 1993). In addition, like other MR cells, osteoclasts express abundant cytosolic carbonic anhydrase (Baron, 1989). The H^+V-ATPase is, however, distinct from the intercalated cell enzyme in that the 56 kDa subunit expressed in osteoclasts is the 'brain' isoform (Bartkiewicz *et al.* 1995; van Hille *et al.* 1994) that is mainly expressed in the kidney in proximal tubules and not in intercalated cells (Nelson *et al.* 1992).

The functional polarity necessary for vectorial proton secretion is generated in this non-epithelial cell by its adherence to the surface of bone (Baron, 1989). Interestingly, the bone-facing membrane surface also contains lysosomal membrane markers (reminiscent of the cell surface staining for LGP120 that is characteristic of B-type intercalated cells in the kidney), and soluble lysosomal enzymes are secreted from this surface (Baron *et al.* 1990). The lacuna formed between the osteoclast membrane and the bone surface can be considered in several respects to be an extracellular lysosome that is specialized for bone reabsorption. Intracellular vesicles also contain H^+V-ATPase molecules in the osteoclast, and modulation of the surface density of proton pumps probably occurs by vesicle recycling in a fashion similar to that in other MR cells, including the kidney intercalated cell (Baron *et al.* 1990). Thus, as described in a review (Gluck, 1992), the osteoclast can be considered as a 'unicellular proton-transporting epithelium'. Indeed, osteoclasts are derived from phagocytes, which also have H^+V-ATPases on their plasma membranes (Grinstein *et al.* 1992).

H^+V-ATPase-rich cells in insect transporting epithelia

Specialized transporting epithelia from many insects contain cells that closely resemble MR cells. For instance, ion-secreting cells of Malpighian tubules and sensory sensilla contain abundant mitochondria. In the lepidopteran midgut, the 'goblet cell', which also contains numerous mitochondria, has long been a model for active K^+ transport, since K^+ is the principal extracellular and intracellular cation in this tissue (Harvey *et al.* 1983). The apical surface of goblet cells in lepidopteran midgut is involuted to form a deep well surrounded by a highly specialized membrane with many microvilli (Anderson and Harvey, 1966). In this respect, goblet cells are similar to the flask cells of amphibian kidney collecting ducts (Brown, 1978). The microvillar membrane is decorated with cytoplasmic studs that resemble the F_1 region of mitochondrial F_1F_2-ATPase, and these studs were referred to as 'portasomes' because of their proposed involvement in transmembrane transport processes, particularly K^+ excretion (Harvey, 1980; Harvey *et al.* 1983). It is now known that they are part of the peripheral 'V_1' region of an H^+V-ATPase complex that is abundant in this membrane domain (Russell *et al.* 1990; Wieczorek *et al.* 1989, 1990). In the midgut, this high concentration of H^+V-ATPases has a well-defined role in K^+ secretion. The electrical gradient formed by active proton pumping is used to energize the secretion of K^+, which is found at high levels in the diet of leaf-eating insects, *via* an H^+/K^+ antiporter that is located in the same membrane domain (Wieczorek *et al.* 1991; Lepier *et al.* 1994).

Similar portasomes are found in cells of the insect salivary gland, Malpighian tubules and all sensory sensilla (Harvey *et al.* 1983). In cockroach salivary gland, an H^+V-ATPase has been localized in apical microvillar membrane domains in the distal duct cells (Just and Walz, 1994).

Miscellaneous cells with some MR cell characteristics

In addition to the examples discussed above, application of freeze–fracture microscopy or (immuno)cytochemical procedures to a variety of tissues has revealed cells that have some characteristics of MR cells, although the complete battery of diagnostic assays has not yet been performed for these cells. Listed in no particular order, these cells include the following types.

(1) A characteristic mitochondria-rich pneumocyte in turtle lung, distinct from type I and type II pneumocytes, which has rod-shaped IMPs on its plasma membrane (Bartels and Welsch, 1984).

(2) The supporting cells in rodent olfactory mucosa, which have rod-shaped IMPs (Kerjaschki and Horandner, 1976). However, the supporting cells do not have high levels of carbonic anhydrase. Instead, the adjacent olfactory sensory cells in this epithelium are rich in carbonic anhydrase (Brown *et al.* 1984*b*). This is an unusual example of these two features of the MR cell family being present in different cells within the same epithelium. Furthermore, some taste bud cells in rats are also rich in carbonic anhydrase, but other features that would link them to the MR cell family have not yet been studied (Brown *et al.* 1984*a*). The detection of high levels of

carbonic anhydrase in these cells supported an earlier proposal that this enzyme was involved in the sense of taste (Hansson, 1960).

(3) In the intestine of several species, including humans, scattered cells containing abundant carbonic anhydrase have been reported (Lonnerholm, 1977, 1983; Lonnerholm and Wistrand, 1983), and some cells with linear IMP arrays that resemble rod-shaped IMPs have been found (Neutra, 1979). About 1 % of the epithelial cells lining the hen coprodeum also contain large numbers of rod-shaped IMPs on their plasma membrane, but this number can be increased to 20 % by feeding animals a Na^+-deficient diet (Eldrup *et al.* 1980). This observation prompted the authors to speculate that the rod-shaped IMPs were Na^+ channels.

(4) Specialized 'flask' cells in the amphibian kidney collecting duct have rod-shaped IMPs (Brown, 1978) and are rich in carbonic anhydrase (Jonas, 1981). These are likely to be the amphibian homolog of the mammalian kidney intercalated cell.

(5) Rod-shaped IMPs have been detected in endometrial cells lining the uterus of rats after treatment with progesterone (Murphy *et al.* 1979) and after implantation (Murphy *et al.* 1982).

(6) Proton pumps and cytoplasmic studs are present on membranes of cells of the chorioallantoic membrane villus cavity cells in chick embryos (Narbaitz *et al.* 1995). The acidification activity of these cells is required to eliminate protons produced during metabolism of the embryo and to release calcium from the shell for use in embryonic growth.

Dissociation between proton pumps and rod-shaped IMPs

In all cases so far examined, cells that have membrane-associated rod-shaped IMPs by freeze–fracture electron microscopy also have an active plasma-membrane-associated proton-pumping ATPase, and it has been postulated that these IMPs are the membrane-embedded 'V_2' regions of (V_1V_2) H^+V-ATPases. However, the reverse situation is not always true. Some cells that have immunocytochemically detectable H^+V-ATPases on their plasma membrane have no detectable rod-shaped IMPs by freeze–fracture electron microscopy. Two examples are found in the kidney. The apical plasma membrane of the proximal tubule in rat has a high level of functional and antigenically detectable proton pumps, yet has no typical rod-shaped IMPs (Brown *et al.* 1988*b*; Orci *et al.* 1981). The same applies to the initial portion of the thin descending limb of Henle (long loops) in rat kidney (Orci *et al.* 1981). In contrast, the thick ascending limb of Henle contains proton pumps (Brown *et al.* 1988*b*) and has readily detectable rod-shaped IMPs that are located in small clusters throughout the apical plasma membrane (D. Brown and S. Breton, unpublished data). In the epididymis, apical cells in the head have typical rod-shaped IMPs (Brown and Montesano, 1980), yet clear cells in the body and tail do not (Brown and Montesano, 1981), despite expressing high levels of H^+V-ATPase (Brown *et al.* 1992).

In addition, freeze–fracture studies on bovine medullary H^+V-ATPases reconstituted into pure phospholipid vesicles did not reveal the presence of 'convincing' rod-shaped IMPs (Brown *et al.* 1987*a*). Thus, the identity of the rod-shaped IMP remains enigmatic. Although circumstantial evidence correlates the presence and number of these structures with the activity of MR cells (Stetson and Steinmetz, 1983; Stetson *et al.* 1980), it remains to be proved that they are indeed the morphological correlate of the V_2 transmembrane complex of the H^+-translocating V_1V_2-ATPase. Alternatively, they could be a membrane protein that is often located in the same membrane domain as the H^+V-ATPase or they could reflect an organizational state of the enzyme, perhaps in conjunction with specific lipid membrane components, that is variable among different cell types and under different physiological conditions.

Conclusions

In this review, we have brought together a considerable amount of scattered information related to a class of highly specialized cells collectively known as mitochondria-rich (MR) cells. These cells are found in many epithelia in which proton and/or bicarbonate secretion is functionally important. A variety of physiological stimuli modify proton transport and related ion-transport processes in these tissues, and MR cells are intimately involved in the regulatory response at several levels. Because of the highly polarized, but variable, expression of transport proteins in these cells, they make an intriguing model in which to examine the establishment and regulation of functional epithelial cell polarity.

Future studies on these cells will have several goals, including dissecting intracellular pathways and signaling molecules that are responsible for the polarized targeting of proton pumps and anion exchangers in these cells; elucidating factors involved in the developmental expression of MR cells and their characteristic battery of transporting proteins; determining the relationship between MR cells and the surrounding epithelial cells in adult tissues; and last, but not least, providing definitive evidence concerning the potential interconversion of MR cell subtypes, especially in the kidney collecting duct.

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References

AL-AWQATI, Q. (1996). Plasticity in epithelial polarity of renal intercalated cells: targeting of the H^+-ATPase and band 3. *Am. J. Physiol.* **270**, C1571–C1580.

ALPER, S. L., NATALE, J., GLUCK, S., LODISH, H. F. AND BROWN, D. (1989). Subtypes of intercalated cells in rat kidney collecting duct defined by antibodies against erythroid band 3 and renal vacuolar H^+-ATPase. *Proc. natn. Acad. Sci. U.S.A.* **86**, 5429–5433.

ALPER, S. L., SABOLIC, I., TYSZKOWSKI, R. AND BROWN, D. (1991). Metabolic acidosis and alkalosis modulate anion exchanger (AE1) expression in kidney collecting ducts. *J. Am. Soc. Nephrol.* **2**, 693a.

ALPER, S. L., STUART-TILLEY, A. K., YANNOUKKAKOS, D. AND BROWN, D. (1995). AE3 anion exchanger immunolocalization in rodent kidney: evidence for apical and basolateral isoforms. *J. Am. Soc. Nephrol.* **6**, 372a.

ANDERSON, E. AND HARVEY, W. R. (1966). Active transport by the *Cecropia* midgut. II. Fine structure of the midgut epithelium. *J. Cell Biol.* **31**, 107–134.

AU, C. L. AND WONG, P. Y. (1980). Luminal acidification by the perfused rat cauda epididymidis. *J. Physiol., Lond.* **309**, 419–427.

BARON, R. (1989). Polarity and membrane transport in osteoclasts. *Conn. Tissue Res.* **20**, 109–120.

BARON, R., NEFF, L., BROWN, W., LOUVARD, D. AND COURTOY, P. J. (1990). Selective internalization of the apical plasma membrane and rapid redistribution of lysosomal enzymes and mannose 6-phosphate receptors during osteoclast inactivation by calcitonin. *J. Cell Sci.* **97**, 439–447.

BARTELS, H. AND WELSCH, U. (1984). Freeze–fracture study of the turtle lung. II. Rod-shaped particles in the plasma membrane of a mitochondria-rich pneumocyte in *Pseudemys* (*Chrysemys*) *scripta*. *Cell Tissue Res.* **236**, 453–457.

BARTKIEWICZ, M., HERNANDO, N., REDDY, S. V., ROODMAN, G. D. AND BARON, R. (1995). Characterization of the osteoclast vacuolar H(+)-ATPase B-subunit. *Gene* **160**, 157–164.

BASTANI, B., MCENANEY, S., YANG, L. AND GLUCK, S. (1994). Adaptation of inner medullary collecting duct vacuolar H-adenosine triphosphatase to chronic acid or alkali loads in the rat. *Exp. Nephrol.* **2**, 171–175.

BASTANI, B., PURCELL, H., HEMKEN, P., TRIGG, D. AND GLUCK, S. (1991). Expression and distribution of renal vacuolar proton-translocating adenosine triphosphatase in response to chronic acid and alkali loads in the rat. *J. clin. Invest.* **88**, 126–136.

BLAIR, H. C., SCHLESINGER, P. H., ROSS, F. P. AND TEITELBAUM, S. L. (1993). Recent advances toward understanding osteoclast physiology. *Clin. Orthop.* **294**, 7–22.

BLAIR, H. C., TEITELBAUM, S. L., GHISELLI, R. AND GLUCK, S. (1989). Osteoclastic bone resorption by a polarized vacuolar proton pump. *Science* **245**, 855–857.

BRETON, S., ALPER, S. L., GLUCK, S. L., SLY, W. S., BARKER, J. E. AND BROWN, D. (1995). Depletion of intercalated cells from collecting ducts of carbonic anhydrase II deficient (CAR2 null) mice. *Am. J. Physiol.* **269**, F761–F774.

BRETON, S. AND BROWN, D. (1995). The lysosomal glycoprotein LGP120 is selectively inserted into the basolateral membrane of kidney collecting duct (CD) type B-intercalated cells (IC). *J. Am. Soc. Nephrol.* **6**, 305a.

BRETON, S., SMITH, P., LUI, B. AND BROWN, D. (1996). Acidification of the male reproductive tract by a proton pumping H^+ATPase. *Nature Medicine* **2**, 470–473.

BROWN, D. (1978). Freeze–fracture of *Xenopus laevis* kidney: rod-shaped particles in the canalicular membrane of collecting tubule flask cells. *J. Ultrastruct. Res.* **63**, 35–40.

BROWN, D. (1989). Membrane recycling and epithelial cell function. *Am. J. Physiol.* **256**, F1–F12.

BROWN, D., GARCIA-SEGURA, M. AND ORCI, L. (1984*a*). Carbonic anhydrase is associated with taste buds in rat tongue. *Brain Res.* **324**, 346–348.

BROWN, D., GARCIA-SEGURA, M. AND ORCI, L. (1984*b*). Carbonic anhydrase is present in olfactory receptor cells. *Histochemistry* **80**, 307–309.

BROWN, D., GLUCK, S. AND HARTWIG, J. (1987*a*). Structure of the novel membrane-coating material in proton-secreting epithelial cells and identification as an H^+ATPase. *J. Cell Biol.* **105**, 1637–1648.

BROWN, D., HIRSCH, S. AND GLUCK, S. (1988*a*). An H^+ATPase is present in opposite plasma membrane domains in subpopulations of kidney epithelial cells. *Nature* **331**, 622–624.

BROWN, D., HIRSCH, S. AND GLUCK, S. (1988*b*). Localization of a proton-pumping ATPase in rat kidney. *J. clin. Invest.* **82**, 2114–2126.

BROWN, D., ILIC, V. AND ORCI, L. (1978). Rod-shaped particles in the plasma membrane of the mitochondria-rich cell of amphibian epidermis. *Anat. Rec.* **192**, 269–276.

BROWN, D., LUI, B., GLUCK, S. AND SABOLIC, I. (1992). A plasma membrane proton ATPase in specialized cells of rat epididymis. *Am. J. Physiol.* **263**, C913–C916.

BROWN, D. AND MONTESANO, R. (1980). Membrane specialization in the rat epididymis. I. Rod-shaped intramembrane particles in the apical (mitochondria-rich) cell. *J. Cell Sci.* **45**, 187–198.

BROWN, D. AND MONTESANO, R. (1981). Membrane specialization in the rat epididymis. II. The clear cell. *Anat. Rec.* **201**, 477–483.

BROWN, D. AND ORCI, L. (1986). The 'coat' of kidney intercalated cell tubulovesicles does not contain clathrin. *Am. J. Physiol.* **250**, C605–C608.

BROWN, D., ROTH, J., KUMPULAINEN, T. AND ORCI, L. (1983). Immunohistochemical localization of carbonic anhydrase in postnatal and adult rat kidney. *Am. J. Physiol.* **245**, F110–F118.

BROWN, D. AND SABOLIC, I. (1993). Endosomal pathways for water channel and proton pump recycling in kidney epithelial cells. *J. Cell Sci.* (Suppl.) **17**, 49–59.

BROWN, D., WEYER, P. AND ORCI, L. (1987*b*). Non-clathrin coated vesicles are involved in endocytosis in kidney collecting duct intercalated cells. *Anat. Rec.* **218**, 237–242.

BUDTZ, P. E., CHRISTOFFERSEN, B. C., JOHANSEN, J. S., SPIES, I. AND WILLUMSEN, N. J. (1995). Tissue kinetics, ion transport and recruitment of mitochondria-rich cells in the skin of the toad (*Bufo bufo*) in response to exposure to distilled water. *Cell Tissue Res.* **280**, 65–75.

CAFLISCH, C. R. (1992). Acidification of testicular and epididymal fluids in the rat after surgically-induced varicocele. *Int. J. Androl.* **15**, 238–245.

CANNON, C., VAN ADELSBERG, J., KELLY, S. AND AL-AWQATI, Q. (1985). Carbon dioxide-induced exocytotic insertion of H^+ pumps in turtle bladder luminal membrane: role of cell pH and calcium. *Nature* **314**, 443–446.

CARR, D. W., USSELMAN, M. C. AND ACOTT, T. S. (1985). Effects of pH, lactate and viscoelastic drag on sperm motility: a species comparison. *Biol. Reprod.* **33**, 588–595.

COHEN, J. P., HOFFER, A. P. AND ROSEN, S. (1976). Carbonic anhydrase localization in the epididymis and testis of the rat: histochemical and biochemical analysis. *Biol. Reprod.* **14**, 339–346.

DA SILVA, J., JR, PERRONE, R. D., JOHNS, C. A. AND MADIAS, N. E. (1991). Rat kidney band 3 mRNA modulation in chronic respiratory acidosis. *Am. J. Physiol.* **260**, F204–F209.

DEVUYST, O., BEAUJEAN, V. AND CRABBÉ, J. (1991*a*). Aldosterone and

chloride conductance of amphibian skin. *Biochim. biophys. Acta* **1066**, 268–270.

DEVUYST, O., BEAUJEAN, V. AND CRABBÉ, J. (1991*b*). Effects of environmental conditions on mitochondria-rich cell density and chloride transport in toad skin. *Pflügers Arch.* **417**, 577–581.

DEVUYST, O., ROTT, R., DENEF, J. F., CRABBÉ, J. AND KATZ, U. (1993). Localization of a Band 3-related protein in the mitochondria-rich cells of amphibian skin epithelium. *Biol. Cell* **78**, 217–221.

DIXON, T. E., CLAUSEN, C., COACHMAN, D. AND LANE, B. (1986). Proton transport and membrane shuttling in turtle bladder. *J. Membr. Biol.* **94**, 233–243.

DOBYAN, D. C., MAGILL, P. A., FRIEDMAN, P. A., HEBERT, S. C. AND BULGER, R. E. (1982). Carbonic anhydrase histochemistry in rabbit and mouse kidneys. *Anat. Rec.* **204**, 185–197.

DORGE, A., BECK, F. X., RICK, R., NAGEL, W. AND THURAU, K. (1990). Effect of amiloride on electrolyte concentrations and rubidium uptake in principal and mitochondria-rich cells of frog skin. *Pflügers Arch.* **416**, 335–338.

DRENCKHAHN, D., SCHLUTER, K., ALLEN, D. P. AND BENNETT, V. (1985). Co-localization of Band 3 with ankyrin and spectrin at the basal membrane of intercalated cells in the rat kidney. *Science* **230**, 1287–1289.

DURHAM, J. H. AND NAGEL, W. (1986). Evidence for separate cellular origins of sodium and acid–base transport in the turtle bladder. *Am. J. Physiol.* **250**, C609–C616.

EHRENFELD, J., MASONI, A. AND GARCIA, R. F. (1976). Mitochondria-rich cells of frog skin in transport mechanisms: morphological and kinetic studies on transepithelial excretion of methylene blue. *Am. J. Physiol.* **231**, 120–126.

ELDRUP, E., MOLLGARD, K. AND BINDSLEV, N. (1980). Possible epithelial sodium channels visualized by freeze–fracture. *Biochim. biophys. Acta* **596**, 152–157.

EMMONS, C. AND KURTZ, I. (1993). H^+/base transport pathways in the cortical collecting duct. *Exp. Nephrol.* **1**, 325–333.

EMMONS, C. AND KURTZ, I. (1994). Functional characterization of three intercalated cell subtypes in the rabbit outer cortical collecting duct. *J. clin. Invest.* **93**, 417–423.

EVAN, A., HUSER, J., BENGELE, H. H. AND ALEXANDER, E. A. (1980). The effect of alterations in dietary potassium on collecting system morphology in the rat. *Lab. Invest.* **42**, 668–675.

FEJES-TOTH, G., CHEN, W. R., RUSVAI, E., MOSER, T. AND NARAY-FEJES-TOTH, A. (1994). Differential expression of AE1 in renal HCO_3-secreting and -reabsorbing intercalated cells. *J. biol. Chem.* **269**, 26717–26721.

FEJES-TOTH, G. AND NARAY-FEJES-TOTH, A. (1992). Differentiation of renal beta-intercalated cells to alpha-intercalated and principal cells in culture. *Proc. natn. Acad. Sci. U.S.A.* **89**, 5487–5491.

FEJES-TOTH, G. AND NARAY-FEJES-TOTH, A. (1993). Differentiation of intercalated cells in culture. *Pediatr. Nephrol.* **7**, 780–784.

FOSKETT, J. K. AND USSING, H. H. (1986). Localization of chloride conductance to mitochondria-rich cells in frog skin epithelium. *J. Membr. Biol.* **91**, 251–258.

FRAZIER, L. W. (1978). Cellular changes in the toad urinary bladder in response to metabolic acidosis. *J. Membr. Biol.* **40**, 165–177.

FRITSCHE, C. AND SCHWARTZ, J. H. (1985). Identification of the bicarbonate secretory cell of the turtle bladder. *Am. J. Physiol.* **249**, F858–F862.

FRITSCHE, C., SCHWARTZ, J. H., HEINEN, R. R., TIETZ, R. AND KLEINMAN, J. G. (1989). HCO_3^- secretion in mitochondria-rich cells is linked to an H^+-ATPase. *Am. J. Physiol.* **256**, F869–F874.

GLUCK, S. (1992). The osteoclast as a unicellular proton-transporting epithelium. *Am. J. med. Sci.* **303**, 134–139.

GLUCK, S., CANNON, C. AND AL-AWQATI, Q. (1982). Exocytosis regulates urinary acidification in turtle bladder by rapid insertion of H^+ pumps into the luminal membrane. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4327–4331.

GRINSTEIN, S., NANDA, A., LUKACS, G. AND ROTSTEIN, O. (1992). V-ATPases in phagocytic cells. *J. exp. Biol.* **172**, 179–192.

HANCOX, N. M. AND KOMENDER, J. (1963). Quantitative and qualitative changes in the 'dark' cells of the renal collecting tubules in rats deprived of water. *Q. J. exp. Physiol.* **48**, 346–354.

HANSSON, H. (1960). The effect of carbonic anhydrase on the sense of taste, an unusual side effect of a drug. *Acta soc. med. Upsaliensis* **65**, v.

HARVEY, B. J. (1992). Energization of sodium absorption by the H^+-ATPase pump in mitochondria-rich cells of frog skin. *J. exp. Biol.* **172**, 289–309.

HARVEY, W. R. (1980). Water and ions in the gut. In *Insect Biology in the Future 'VBhl 80'* (ed. M. Locke and D. S. Smith), pp. 105–124. New York: Academic Press.

HARVEY, W. R., CIOFFI, M. AND WOLFERSBERGER, M. G. (1983). Potassium ion transport ATPase in insect epithelia. *J. exp. Biol.* **106**, 91–117.

HAYASHI, M., SCHUSTER, V. L. AND STOKES, J. B. (1988). Absence of transepithelial anion exchange by rabbit OMCD: evidence against reversal of cell polarity. *Am. J. Physiol.* **255**, F220–F228.

HINTON, B. T. AND KEEFER, D. A. (1985). Binding of [^{3}H]aldosterone to a single population of cells within the rat epididymis. *J. Steroid Biochem.* **23**, 231–233.

HINTON, B. T. AND PALLADINO, M. A. (1995). Epididymal epithelium: its contribution to the formation of a luminal fluid microenvironment. *Microsc. Res. Tech.* **30**, 67–81.

HOLTHOFER, H., SCHULTE, B. A., PASTERNACK, G., SIEGEL, G. J. AND SPICER, S. S. (1987). Immunocytochemical characterization of carbonic anhydrase-rich cells in the rat kidney collecting duct. *Lab. Invest.* **57**, 150–156.

HUMBERT, F., PRICAM, C., PERRELET, A. AND ORCI, L. (1975). Specific plasma membrane differentiations in the cells of the kidney collecting tubule. *J. Ultrastr. Res.* **52**, 13–20.

ILIC, V. AND BROWN, D. (1980). Modification of mitochondria-rich cells in different ionic conditions: changes in morphology and cell number in the skin of *Xenopus laevis*. *Anat. Rec.* **196**, 153–161.

JONAS, L. (1981). Histochemical localization of carbonic anhydrase in flask cells of the clawfrog mesonephros (*Xenopus laevis* Daudin). *Acta histochem.* **68**, 238–247.

JUST, F. AND WALZ, B. (1994). Immunocytochemical localization of Na^+/K^+-ATPase and V-H^+-ATPase in the salivary glands of the cockroach, *Periplaneta americana*. *Cell Tissue Res.* **278**, 161–170.

KAISSLING, B. (1982). Structural aspects of adaptive changes in renal electrolyte secretion. *Am. J. Physiol.* **243**, F211–F226.

KASHGARIAN, M., BIEMESDERFER, D., CAPLAN, M. AND FORBUSH, B. (1985). Monoclonal antibody to Na,K-ATPase: Immunocytochemical localization along the nephron. *Kidney Int.* **28**, 899–913.

KATZ, U. AND GABBAY, S. (1988). Mitochondria-rich cells and carbonic anhydrase content of toad skin epithelium. *Cell Tissue Res.* **251**, 425–431.

KATZ, U. AND SCHEFFEY, C. (1986). The voltage-dependent chloride conductance of toad skin is localized to mitochondria-rich cells. *Biochim. biophys. Acta* **861**, 480–482.

KELLER, A. R. (1963). A histochemical study of the toad urinary bladder. *Anat. Rec.* **147**, 367–377.

KERJASCHKI, D. AND HORANDNER, H. (1976). The development of mouse olfactory vesicles and their cell contacts: a freeze-etching study. *J. Ultrastruct. Res.* **54**, 420–444.

KIM, J., CHA, J. H., TISHER, C. AND MADSEN, K. M. (1996). Role of apoptotic and nonapoptotic cell death in the removal of intercalated cells from the medullary collecting duct of the developing rat kidney. *Am. J. Physiol.* (in press).

KIM, J., TISHER, C. C. AND MADSEN, K. M. (1994). Differentiation of intercalated cells in developing rat kidney: an immunohistochemical study. *Am. J. Physiol.* **266**, F977–F990.

KLINEFELTER, G. R., LASKEY, J. W., KELCE, W. R., FERRELL, J., ROBERTS, N. L., SUAREZ, J. D. AND SLOTT, V. (1994). Chloroethylmethanesulfonate-induced effects on the epididymis seem unrelated to altered Leydig cell function. *Biol. Reprod.* **51**, 82–91.

KOICHI, Y., SATLIN, L. M. AND SCHWARTZ, G. J. (1992). Adaptation of rabbit cortical collecting duct to *in vitro* acidification. *Am. J. Physiol.* **263**, F749–F756.

LARSEN, E. H., USSING, H. H. AND SPRING, K. R. (1987). Ion transport by mitochondria-rich cells in toad skin. *J. Membr. Biol.* **99**, 25–40.

LEPIER, A., AZUMA, M., HARVEY, W. R. AND WIECZOREK, H. (1994). K^+/H^+ antiport in the tobacco hornworm midgut: the $K(^+)$-transporting component of the K^+ pump. *J. exp. Biol.* **196**, 361–373.

LEVINE, N. AND MARSH, D. J. (1971). Micropuncture studies of the electrochemical aspects of fluid and electrolytes transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. *J. Physiol., Lond.* **213**, 557–575.

LIU, X., TEMPLE, S. P. AND RISBRIDGER, G. P. (1993). Acute effects of ethane dimethane sulfonate (EDS) on the structure of the cauda epididymidis in the rat: selective destruction of clear cells in the proximal cauda region. *Reprod. Fert. Dev.* **5**, 295–306.

LONNERHOLM, G. (1977). Carbonic anhydrase in the intestinal tract of the guinea-pig. *Acta physiol. scand.* **99**, 53–61.

LONNERHOLM, G. (1983). Carbonic anhydrase in the monkey stomach and intestine. *Acta physiol. scand.* **117**, 273–279.

LONNERHOLM, G., SELKING, O. AND WISTRAND, P. J. (1985). Amount and distribution of carbonic anhydrases CA I and CA II in the gastrointestinal tract. *Gastroenterology* **88**, 1151–1161.

LONNERHOLM, G. AND WISTRAND, P. (1983). Carbonic anhydrase in the human fetal gastrointestinal tract. *Biol. Neonate* **44**, 166–176.

LUDENS, J. H. AND FANESTIL, D. D. (1972). Acidification of urine by the isolated urinary bladder of the toad. *Am. J. Physiol.* **223**, 1338–1344.

MACHEN, T. AND ERLIJ, D. (1975). Some features of hydrogen (ion) secretion by the frog skin. *Biochim. biophys. Acta* **406**, 120–130.

MADSEN, K. M., CLAPP, W. L. AND VERLANDER, J. W. (1988). Structure and function of the inner medullary collecting duct. *Kidney Int.* **34**, 441–454.

MADSEN, K. M. AND TISHER, C. C. (1986). Structure–function relationships along the distal nephron. *Am. J. Physiol.* **250**, F1–F15.

MADSEN, K. M., VERLANDER, J. W., KIM, J. AND TISHER, C. C. (1991). Morphological adaptation of the collecting duct to acid–base disturbances. *Kidney Int.* **40** (Suppl. **33**), S57–S63.

MATSUMOTO, T., FEJES-TOTH, G. AND SCHWARTZ, G. J. (1993). Developmental expression of acid–base-related proteins in the rabbit kidney. *Ped. Nephrol.* **7**, 792–797.

MATSUMOTO, T., WINKLER, C. A., BRION, L. P. AND SCHWARTZ, G. J. (1994). Expression of acid–base-related proteins in mesonephric kidney of the rabbit. *Am. J. Physiol.* **267**, F987–F997.

MEMBRE, H., HATIER, R. AND GRIGNON, G. (1985). Activité de l'anhydrase carbonique dans l'épididyme de rat au cours du développement. *Reprod. Nutr. Dévelop.* **25**, 867–872.

MOORE, H. D. AND BEDFORD, J. M. (1979). The differential absorptive activity of epithelial cells of the rat epididymis before and after castration. *Anat. Rec.* **193**, 313–327.

MURPHY, C. R., SWIFT, J. G., MUKHERJEE, T. M. AND ROGERS, A. W. (1979). Effects of ovarian hormones on cell membranes in the rat uterus. I. Freeze fracture studies of the apical membrane of the luminal epithelium. *Cell Biophys.* **1**, 181–193.

MURPHY, C. R., SWIFT, J. G., MUKHERJEE, T. M. AND ROGERS, A. W. (1982). Changes in the fine structure of the apical plasma membrane of endometrial epithelial cells during implantation in the rat. *J. Cell Sci.* **55**, 1–12.

MUTO, S., YASOSHIMA, K., YOSHITOMI, K., IMAI, M. AND ASANO, Y. (1990). Electrophysiological identification of a and b intercalated cells and their distribution along the rabbit distal nephron segments. *J. clin. Invest.* **86**, 1829–1839.

NARBAITZ, R., BASTANI, B., GALVIN, N. J., KAPAL, V. K. AND LEVINE, D. Z. (1995). Ultrastructural and immunocytochemical evidence for the presence of polarised plasma membrane $H(^+)$-ATPase in two specialised cell types in the chick embryo chorioallantoic membrane. *J. Anat.* **186**, 245–252.

NELSON, R. D., GUO, X. L., MASOOD, K., BROWN, D., KALKBRENNER, M. AND GLUCK, S. (1992). Selectively amplified expression of an isoform of the vacuolar $H(^+)$-ATPase 56-kilodalton subunit in renal intercalated cells. *Proc. natn. Acad. Sci. U.S.A.* **89**, 3541–3545.

NEUTRA, M. R. (1979). Linear arrays of intramembrane particles on microvilli in primate large intestine. *Anat. Rec.* **193**, 367–382.

ORCI, L., HUMBERT, F., AMHERDT, M., GROSSO, A., DESOUSA, R. C. AND PERRELET, A. (1975). Patterns of membrane organization in toad bladder epithelium: a freeze–fracture study. *Experientia* **31**, 1335–1338.

ORCI, L., HUMBERT, F., BROWN, D. AND PERRELET, A. (1981). Membrane ultrastructure in urinary tubules. *Int. Rev. Cytol.* **73**, 183–242.

ORDONEZ, N. G. AND SPARGO, B. H. (1976). The morphologic relationship of light and dark cells of the collecting tubule in potassium-depleted rats. *Am. J. Pathol.* **84**, 317–326.

PEACHY, L. D. AND RASMUSSEN, H. (1961). Structure of the toad's urinary bladder as related to its physiology. *J. biophys. biochem. Cytol.* **10**, 529–553.

REID, B. L. AND CLELAND, K. W. (1957). The structure and function of the epididymis. I. The histology of rat epididymis. *Aust. J. Zool.* **5**, 223–246.

RICHET, G. AND HAGEGE, J. (1975). Dark cells of the distal convoluted tubules and collecting ducts. II. Physiological significance. *Fortschr. Zool.* **23**, 299–306.

RODRIGUEZ, M. H., EKSTEDT, E. AND EINARSSON, S. (1990). Acidification of epididymal fluid in the boar. *Int. J. Androl.* **13**, 238–243.

ROSEN, S. (1972*a*). Localization of carbonic anhydrase activity in the vertebrate nephron. *Histochem.* **4**, 35–48.

ROSEN, S. (1972*b*). Localization of carbonic anhydrase in turtle and toad urinary bladder mucosa. *J. Histochem. Cytochem.* **20**, 696–702.

ROSEN, S. AND FRIEDLEY, N. J. (1973). Carbonic anhydrase activity in *Rana pipiens* skin: biochemical and histochemical analysis. *Histochem.* **36**, 1–4.

ROSEN, S., OLIVER, J. A. AND STEINMETZ, P. R. (1974). Urinary

acidification and carbonic anhydrase distribution in bladders of Dominican and Colombian toads. *J. Membr. Biol.* **15**, 193–205.

ROTHBERG, K. G., HEUSER, J. E., DONZELL, W. C., YING, Y.-S., GLENNEY, J. R. AND ANDERSON, R. G. W. (1992). Caveolin, a protein component of caveolae membrane coats. *Cell* **68**, 673–682.

RUDNEFF, M. (1865). Ueber die epidermoidale Schicht der Froschaut. *Arch. mikrosk. Anat.* **1**, 295–298.

RUSSELL, V. E., KLEIN, U., REUVENI, M., SPAETH, D. D., WOLFERSBERGER, M. G. AND HARVEY, W. R. (1992). Antibodies to mammalian and plant V-ATPases cross react with the V-ATPase of insect cation-transporting plasma membranes. *J. exp. Biol.* **166**, 131–143.

SABOLIC, I., WUARIN, F., SHI, L.-B., VERKMAN, A. S., AUSIELLO, D. A., GLUCK, S. AND BROWN, D. (1992). Apical endosomes isolated from kidney collecting duct principal cells lack subunits of the proton pumping ATPase. *J. Cell Biol.* **119**, 111–122.

SAPIRSTEIN, V. S. AND SCOTT, W. N. (1975). Binding of aldosterone by mitochondria-rich cells of the toad urinary bladder. *Nature* **257**, 241–243.

SATLIN, L. M. AND SCHWARTZ, G. J. (1989). Cellular remodelling of HCO_3^- secreting cells in rabbit renal collecting duct in response to an acidic environment. *J. Cell Biol.* **109**, 1279–1288.

SCHACHOWA, S. (1876). Untersuchungen uber die Niere. Medical thesis dissertation, Berne, Switzerland.

SCHIEFFERDECKER, P. (1881). Zur Kenntnis des Baues der Schleimdrusen. *Arch. mikrosk. Anat.* **23**, 382–412.

SCHILLER, A. AND TIEDEMANN, K. (1981). The mature mesonephric nephron of the rabbit embryo. III. Freeze–fracture studies. *Cell Tissue Res.* **221**, 431–442.

SCHULZE, F. E. (1876). Epithel- und Drusen-Zellen. *Arch. mikrosk. Anat. EntwMech.* **3**, 137–203.

SCHUSTER, V. L. (1993). Function and regulation of collecting duct intercalated cells. *A. Rev. Physiol.* **55**, 267–288.

SCHUSTER, V. L., FEJES-TOTH, G., NARAY-FEJES-TOTH, A. AND GLUCK, S. (1991). Colocalization of H^+ATPase and band 3 anion exchanger in rabbit collecting duct intercalated cells. *Am. J. Physiol.* **260**, F506–F517.

SCHUSTER, V. L. AND STOKES, J. B. (1987). Chloride transport by the cortical and outer medullary collecting duct. *Am. J. Physiol.* **253**, F203–F212.

SCHWARTZ, G. J., BARASCH, J. AND AL-AWQATI, Q. (1985). Plasticity of functional epithelial polarity. *Nature* **318**, 368–371.

SCHWARTZ, J. H., BETHENCOURT, D. AND ROSEN, S. (1982). Specialized function of carbonic anhydrase-rich and granular cells of turtle bladder. *Am. J. Physiol.* **242**, F627–F633.

SCHWARTZ, J. H., ROSEN, S. AND STEINMETZ, P. R. (1972). Carbonic anhydrase function and the epithelial organization of H^+ secretion in turtle urinary bladder. *J. clin. Invest.* **51**, 2653–2662.

SPICER, S. S., STOWARD, P. J. AND TASHIAN, R. E. (1979). The immunolocalization of carbonic anhydrase in rodent tissues. *J. Histochem. Cytochem.* **27**, 820–831.

STEINMETZ, P. R. (1985). Epithelial hydrogen ion transport. In *The Kidney: Physiology and Pathophysiology* (ed. D. W. Seldin and G. Giebisch), pp. 1441–1470. New York: Raven Press.

STEINMETZ, P. R. (1986). Cellular organization of urinary acidification. *Am. J. Physiol.* **251**, F173–F187.

STETSON, D. L., BEAUWENS, R., PALMISANO, J., MITCHELL, P. P. AND STEINMETZ, P. R. (1985). A double-membrane model for urinary bicarbonate secretion. *Am. J. Physiol.* **249**, F546–F552.

STETSON, D. L. AND STEINMETZ, P. R. (1983). Role of membrane fusion in CO_2 stimulation of proton secretion by turtle bladder. *Am. J. Physiol.* **245**, C113–C120.

STETSON, D. L. AND STEINMETZ, P. R. (1985). A and B types of carbonic anhydrase-rich cells in turtle bladder. *Am. J. Physiol.* **249**, F553–F565.

STETSON, D. L., WADE, J. B. AND GIEBISCH, G. (1980). Morphologic alterations in the rat medullary collecting duct following potassium depletion. *Kidney Int.* **17**, 45–56.

SUN, E. L. AND FLICKINGER, C. J. (1979). Development of cell types and of regional differences in the postnatal rat epididymis. *Am. J. Anat.* **154**, 27–55.

VAANANEN, H. K., KARHUKORPI, E. K., SUNDQUIST, K., WALLMARK, B., ROININEN, I., HENTUNEN, T., TUUKKANEN, J. AND LAKKAKORPI, P. (1990). Evidence for the presence of a proton pump of the vacuolar $H^{(+)}$-ATPase type in the ruffled borders of osteoclasts. *J. Cell Biol.* **111**, 1305–1311.

VAN ADELSBERG, J., EDWARDS, J. C. AND AL-AWQATI, Q. (1993). The apical Cl/HCO_3 exchanger of beta intercalated cells. *J. biol. Chem.* **268**, 11283–11289.

VAN ADELSBERG, J., EDWARDS, J. C., TAKITO, J., KISS, B. AND AL-AWQATI, Q. (1994). An induced extracellular matrix protein reverses the polarity of band 3 in intercalated epithelial cells. *Cell* **76**, 1053–1061.

VAN HILLE, B., RICHENER, H., SCHMID, P., PUETTNER, I., GREEN, J. R. AND BILBE, G. (1994). Heterogeneity of vacuolar $H^{(+)}$-ATPase: differential expression of two human subunit B isoforms. *Biochem. J.* **303**, 191–198.

VERLANDER, J. W., MADSEN, K. M., STONE, D. K. AND TISHER, C. C. (1994). Ultrastructural localization of H^+ATPase in rabbit cortical collecting duct. *J. Am. Soc. Nephrol.* **4**, 1546–1557.

WADE, J. B. (1976). Membrane structural specialization of the toad urinary bladder revealed by the freeze–fracture technique. II. The mitochondria-rich cell. *J. Membr. Biol.* **29**, 111–126.

WEINER, I. D., WEILL, A. E. AND NEW, A. R. (1994). Distribution of Cl^-/HCO_3^- exchange and intercalated cells in rabbit cortical collecting duct. *Am. J. Physiol.* **267**, F952–F964.

WHITEAR, M. (1975). Flask cells and epithelial dynamics in frog skin. *J. Zool., Lond.* **175**, 107–149.

WIECZOREK, H., CIOFFI, M., KLEIN, U., HARVEY, W. R., SCHWEIKL, H. AND WOLFERSBERGER, M. G. (1990). Isolation of goblet cell apical membrane from tobacco hornworm midgut and purification of its vacuolar-type ATPase. *Meth. Enzymol.* **192**, 608–616.

WIECZOREK, H., PUTZENLECHNER, M., ZEISKE, W. AND KLEIN, U. (1991). A vacuolar-type proton pump energizes K^+/H^+ antiport in an animal plasma membrane. *J. biol. Chem.* **266**, 15340–15347.

WIECZOREK, H., WEERTH, S., SCHINDLBECK, M. AND KLEIN, U. (1989). A vacuolar-type proton pump in a vesicle fraction enriched with potassium transporting plasma membranes from tobacco hornworm midgut. *J. biol. Chem.* **264**, 11143–11148.

WILLUMSEN, N. J., VESTERGAARD, L. AND LARSEN, E. H. (1992). Cyclic AMP- and beta-agonist-activated chloride conductance of a toad skin epithelium. *J. Physiol., Lond.* **449**, 641–653.

WINGO, C. S. AND SMOLKA, A. J. (1995). Function and structure of H–K-ATPase in the kidney. *Am. J. Physiol.* **269**, F1–F16.

YORIO, T., PAGE, R. D. AND FRAZIER, L. W. (1991). Prostaglandin regulation of H^+ secretion in amphibian epithelia. *Am. J. Physiol.* **260**, R866–R872.