CATION ANTIPORTS OF ANIMAL PLASMA MEMBRANES

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Introduction

Both organic and inorganic solutes can be transported across biological membranes against their electrochemical gradient, by coupling to the downhill movement of another species. In animal cells, the underlying mechanism most often involves symport (cotransport) or antiport (countertransport) with either sodium or hydrogen ions. The energy invested in the uphill translocation of solutes is ultimately provided by hydrolysis of ATP by Na^+- or H^+-transporting ATPases and is transiently stored as potential energy in the form of an electrochemical gradient of these ions. This introductory review describes the properties of systems that catalyze both Na^+- or H^+-driven countertransport of inorganic cations, present in vertebrates and invertebrates. Although great strides have been made towards the biochemical identification and characterization of vertebrate transporters, the molecular analysis of the invertebrate systems is still in its infancy. Only a brief comparative overview is provided for general reference in this introductory section. More specific descriptions of the individual systems and recent developments are described more fully in the following chapters. To shorten the reference list and to avoid redundancy with the following sections, comprehensive reviews were cited wherever possible and references to original work were kept to a minimum.

Cation antiport driven by the sodium-motive force

Two main types of cation exchangers (or antiports) will be discussed here: Na^+/Ca^{2+} exchangers and Na^+/H^+ exchangers. In their forward mode, both are driven by the inward gradient of Na^+ set up by the Na^+/K^+-ATPase. In vertebrates, the two exchangers share a number of functional properties: (1) they are allosterically regulated by the same species that normally functions as the intracellular ligand (i.e. by Ca^{2+} in the case of Na^+/Ca^{2+} exchange and by H^+ in the case of the Na^+/H^+ antiporter); (2) the rate of exchange is modulated by the intracellular concentration of ATP, despite the fact that hydrolysis of the nucleotide is not esssential for transport; and (3) their activity is influenced by vicinal lipids and/or by ancillary proteins, possibly cytoskeletal components. In addition, vertebrate Na^+/Ca^{2+} and Na^+/H^+ exchangers share some structural features as well: (1) they are glycoproteins that span the membrane 10–12 times; (2) they have a cleavable signal sequence, which directs the amino terminus of the mature protein to the

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extracellular aspect of the membrane; and (3) the cardiac Na^+/Ca^{2+} exchanger and at least some isoforms of the Na^+/H^+ exchanger have clusters of positively charged and hydrophobic residues that are exposed to the cytosol and conform to the predicted structure of calmodulin-binding domains. Schematic diagrams of these structures can be found in the reviews by Reeves *et al.* (1994) and Bianchini and Pouyssegur (1994). Despite these similarities in function and overall topology, however, there is virtually no homology between the primary structures of the Na^+/Ca^{2+} and Na^+/H^+ antiporters, suggesting that they evolved independently. In invertebrates, the same families of exchangers may also occur, but evidence has also emerged for a novel type of electrophoretic $2Na^+/H^+$ antiporter.

Na^+/Ca^{2+} exchangers in vertebrates

Na^+/Ca^{2+} exchange has been implicated in the regulation of smooth and cardiac muscle contraction, in retinal phototransduction, in epithelial membrane cross-talk and in a variety of other functions (Eisner and Lederer, 1985; Nicoll *et al.* 1990; Reeves, 1992). Two main types of Na^+/Ca^{2+} exchanger have been identified and characterized: the cardiac and retinal forms. As decribed briefly below, while both proteins translocate Ca^{2+} in exchange for Na^+, their stoichiometry differs, as does their structure.

The cardiac antiporter exchanges one Ca^{2+} for three Na^+. As a result, the exchange process is electrophoretic, i.e. it generates a current that may alter the membrane potential and can indirectly influence other processes that are voltage-sensitive. Moreover, the electrogenicity of the antiport implies that the rate of exchange will itself be dependent on the transmembrane potential.

As mentioned briefly above, the rate of transport is additionally modulated by the availability of ATP, yet phosphorylation of the exchanger has not been detected. The nucleotide reduces the susceptibility to inhibition by Na^+, an aspect that is discussed in detail in the review by Reeves *et al.* (1994). These authors also consider the effect of cytoskeletal structures in the control of Na^+/Ca^{2+} exchange and review the evidence favouring the role of neighbouring lipids in activation of the antiport.

The cardiac exchanger was cloned and sequenced by Philipson and his colleagues (Nicoll *et al.* 1990). It has an open reading frame of 970 amino acids, with a predicted molecular size of 108 kDa. The molecular mass deduced from polyacrylamide gel electrophoresis is somewhat larger, probably as a result of glycosylation. Hydropathy plots predict 11 transmembrane domains and suggest the existence of a large cytosolic loop between transmembrane helices 5 and 6. The functional role of the loop remains uncertain, inasmuch as deletion of most of this domain has little effect on the rate of transport. However, data reviewed by Reeves *et al.* (1994) below suggest that regulation by ATP and calcium requires the cytosolic domain.

Cardiac-type exchangers have been cloned from other tissues, including brain and epithelia. Although they display extensive homology with the cardiac isoform, they can vary in the composition of the central hydrophylic region as a result of alternate splicing. These isoforms therefore derive from the same gene and have similar function. In this respect, they differ from the isoforms of the Na^+/H^+ exchanger discussed below, which are the product of separate genes.

The isoforms of the cardiac-type exchanger differ markedly both in structure and in function from the retinal form of the exchanger. The retinal rod exchanger was cloned by Reilander *et al.* (1992), who found it to have a predicted molecular mass of 130 kDa. As in the case of the cardiac exchanger, the predicted size falls short of that determined by electrophoresis (approximately 220 kDa) because of glycosylation and perhaps compounded by the prevalence of acidic residues that minimize binding of SDS. The general transmembrane topology of the rod exchanger resembles that of the cardiac type: 11 or 12 transmembrane domains with a large cytosolic loop near the middle of the molecule (see Reeves, 1992, for a comparative diagram). Yet, the primary structure of the two exchangers diverges markedly. In fact, only two regions of the retinal exchanger, each about 60 residues long, show sequence similarity with the cardiac isoforms. These are transmembrane regions and have between 28 and 38 % identity between the two exchanger types, which has prompted the suggestion that they may be essential for transport (Reeves, 1992). The marked structural differences between the retinal and cardiac Na^+/Ca^{2+} exchangers imply that, despite some superficial similarities, these molecules evolved independently or diverged from a common ancestor very early in evolution.

While Na^+ and Ca^{2+} are the sole substrates translocated by the cardiac-type isoforms, the retinal exchanger additionally requires K^+ on the same side at which Ca^{2+} is bound. Under physiological conditions, the stoichiometry of the system is believed to be 4 Na^+ in exchange for 1 Ca^{2+} plus 1 K^+. Hence, like the cardiac exchanger, the retinal antiport is electrogenic. However, unlike other antiporters discussed in this volume, the stoichiometry of the retinal system is not fixed and conditions have been described where electroneutral exchange of 3 Na^+ for 1 Ca^{2+} and 1 K^+ can occur.

It has been speculated that cotransport with K^+ is necessary to supplement the driving force for Ca^{2+} extrusion in the retinal rods. In the dark, these cells are comparatively highly permeable to Na^+ and Ca^{2+}, resulting in steady depolarization and tending to elevate the cytosolic concentrations of these ions. These conditions are not conducive to adequate Ca^{2+} extrusion by conventional (cardiac-type) exchangers. By coupling the efflux of Ca^{2+} to that of K^+, the cells derive sufficient energy to extrude Ca^{2+} effectively. In this instance, Na^+/K^+-ATPases contribute to Ca^{2+} extrusion not only by providing the inward Na^+ gradient but also by accumulating K^+ in the cells.

By contrast with the Na^+/H^+ exchanger, little is known about regulation of Na^+/Ca^{2+} exchange by hormones or growth factors, nor has modulation by phosphorylation been documented. Regulation by the cytoskeleton has been intimated and association of the cardiac Na^+/Ca^{2+} exchanger with ankyrin *in vitro* has been reported (Li *et al.* 1993). The full significance of this interaction remains to be established.

Na⁺/H⁺ exchangers in vertebrates

Almost all animal cells are endowed with at least one type of molecule capable of exchanging intracellular H^+ for external Na^+. As in the case of Na^+/Ca^{2+} countertransport, the ultimate source of energy indirectly driving this process can be traced to the hydrolysis of ATP by the Na^+/K^+-ATPase. Unlike Na^+/Ca^{2+} exchange, however, the exchange of Na^+ for H^+ occurs with a one-to-one stoichiometry and hence is

electroneutral and potential-insensitive. Details of the structure of the Na^+/H^+ antiporter are provided in the reviews by Bianchini and Pouyssegur (1994) and Demaurex and Grinstein (1994). To obviate repetition, this section will concentrate on the multiplicity of functions and on the control of Na^+/H^+ antiport activity.

The Na^+/H^+ exchangers are a family of multitalented molecules. Although their basic mechanism is simple and invariant, they have been implicated in an astonishing array of divergent functions. These include the regulation of the intracellular pH (pHi), which is of paramount importance to cellular homeostasis. Generation of metabolic acid is continuously counteracted by extrusion of H^+ in exchange for Na^+. In addition, Na^+/H^+ exchange is central to the uptake of Na^+ in the gastrointestinal tract, to the reabsorption of NaCl in the proximal tubule and to the recovery of bicarbonate in the renal medulla (Fliegel and Frohlich, 1993; Tse *et al.* 1993). In these instances, exchangers located on the apical side of epithelial cells mediate the vectorial transport of Na^+ and acid equivalents and indirectly promote the translocation of Cl^- in exchange for bicarbonate. Moreover, there is convincing evidence that Na^+/H^+ exchange plays an important role in the maintenance and control of the cellular volume (Grinstein *et al.* 1989). Indeed, cells shrunk in hypertonic media can be restored to near-normal size by activating the antiport. Finally, it has been widely speculated that Na^+/H^+ exchange is also involved in cellular proliferation and perhaps in cellular adherence and motility. These suggestions stem from the observations that growth promoters and cross-linking of adherence receptors activate cation exchange (Grinstein *et al.* 1989; Schwartz *et al.* 1990). Inhibition of cell growth and of chemotaxis by agents that inhibit the antiport provides supportive, yet circumstantial, evidence for these hypotheses.

Four distinct isoforms of the Na^+/H^+ exchanger (NHE), termed NHE-1 to NHE-4, have been cloned (Sardet *et al.* 1989; Orlowski *et al.* 1992). All are integral membrane proteins with 10–12 membrane-spanning domains and a sizable (approximately 300 residues long) cytosolic tail near the carboxy terminus of the protein. NHE-1 is a ubiquitous phosphoglycoprotein of between 815 and 820 amino acids. It is thought to be the 'housekeeping' isoform of the antiport, mainly responsible for pHi and possibly cell volume regulation. In epithelial cells, NHE-1 is found predominantly, if not exclusively, on the basolateral membrane. NHE-2 is 809–813 amino acids long and is abundant in the stomach, intestine, kidney and uterus and is also detectable in skeletal muscle. Overall, the functional properties of NHE-2 resemble, but are not identical to, those of NHE-1. The precise physiological role of NHE-2 and the reason for its selective tissue distribution remain to be defined.

NHE-3 has 831–832 amino acid residues and is found exclusively in the stomach, intestine and kidney, suggesting an epithelial localization and function. Indeed, recent immunocytochemical evidence has localized NHE-3 to the apical membrane of polar epithelia in these tissues. These findings are entirely consistent with earlier pharmacological observations in epithelial layers and in transfected cells. Antiport activity on the luminal (apical) side of epithelial monolayers was reported to be less susceptible to inhibition by amiloride and its analogues than its basolateral counterpart. It was separately demonstrated that, when transfected into antiport-deficient cells, NHE-3 is much less sensitive to amiloride than is NHE-1. These data are therefore compatible with

the hypothesis that NHE-3 is the luminal isoform, whereas NHE-1 is the basolateral equivalent.

NHE-4 is somewhat shorter than the other isoforms (717 amino acids in the rat), and mRNA encoding this isoform is abundant in stomach, with smaller amounts found in skeletal muscle, uterus, brain and kidney. It is noteworthy that, unlike NHE-1, NHE-2 and NHE-3, NHE-4 has proved to be refractory to heterologous expression. It thus remains unclear whether this protein in fact catalyzes Na^+/H^+ exchange activity.

The complexity introduced by the coexistence of multiple isoforms is further accentuated by the variety of agents and conditions that regulate antiport activity. Protein kinases have repeatedly been invoked in the regulation of Na^+/H^+ exchange (for reviews, see Fliegel and Frohlich, 1993; Tse *et al.* 1993). However, not all kinases affect antiport activity in a comparable manner, nor do all isoforms react to the same kinase with identical responses. Thus, although protein kinase C agonists stimulate NHE-1 and NHE-2, they appear to inhibit NHE-3 (Tse *et al.* 1993). Similarly, cyclic-AMP-dependent kinases stimulate or have little effect on some isoforms yet markedly inhibit others. Phosphatase antagonists can also stimulate at least some isoforms of the antiporter, ostensibly by elevating the level of phosphoproteins due to the presence of constitutively active kinases. To add to the complexity, both serine/threonine phosphatase antagonists (e.g. okadaic acid) and tyrosine phosphatase antagonists (e.g. vanadate) can promote Na^+/H^+ exchange. These different inhibitors may stimulate distinct steps of one common signalling cascade, but multiple convergent pathways could also exist. Clearly, important aspects of the regulation of Na^+/H^+ exchange by phosphotransferase reactions remain to be defined. In fact, even the early indications that phosphorylation of the antiporter (NHE-1) was responsible for stimulation of transport have more recently been questioned. Hence, phosphorylation of elements distal to the antiporter itself may ultimately be more important.

Calcium was one of the earliest mediators suggested to activate antiport activity. Despite great progress in the field, the mode of action of calcium remains unclear. Phosphorylation of the antiporter or of regulatory proteins by Ca^{2+}-calmodulin-dependent kinases is certainly conceivable. It is noteworthy that all four isoforms contain multiple sites with the consensus motif recognized by Ca^{2+}-calmodulin-dependent kinase II (for a review, see Fliegel and Frohlich, 1993). Alternatively, the Ca^{2+}-calmodulin complex may bind directly to the cytosolic tail of the antiporter, modifying its conformation. The primary structures of some of the isoforms, including NHE-1, possess regions of hydrophobic and positively charged residues that conform to the predicted composition of calmodulin-binding domains, as defined in other Ca^{2+}-sensitive proteins. However, as discussed in the review by Reeves *et al.* (1994), the presence of such domains does not necessarily imply that calmodulin binding indeed occurs, and binding to other complementary regions (negatively charged and hydrophobic) is equally possible.

Other levels of regulation of Na^+/H^+ exchange have also been described. As discussed in detail in the review by Demaurex and Grinstein (1994), the intracellular concentration of ATP dictates the properties of the exchanger, with subtle but significant differences detected in the behaviour of the individual isoforms, at least in heterologous expression

systems. Additionally, the antiporter is modulated by the cellular volume. NHE-1 and NHE-2 are stimulated in hypertonically stimulated cells and inhibited in hypotonically swollen cells. In contrast, NHE-3 is inhibited by hypertonicity; this may be of importance in the kidney medullary cells, which are exposed to concentrated filtrate.

The last layer of complexity is introduced not by the antiporters themselves but by the cellular context in which they are expressed. Thus, when present in two cell types (and conceivably also in two distinct membranes within one cell type), the same isoform may display differing properties. This peculiar behaviour is becoming increasingly apparent in experiments in which the same isoform is expressed heterologously in a variety of cells. On the one hand, responses anticipated from the reported behaviour in the native tissue are often found not to occur in a different cellular context. For instance, the sensitivity to cyclic AMP reported in epithelia that express NHE-3 is not always found when this isoform is transfected into non-polar, antiport-deficient cells. Moreover, the kinetic properties of a single isoform seemingly vary widely depending on the cell type used as recipient for transfection (J. Orlowski and D. Warnock, personal communication). Finally, the inability to demonstrate functional NHE-4 in heterologous transfection systems probably reflects the absence in the transfectants of an essential cofactor required for the exchanger to become active. It is not known what features of the cells are important determinants of antiport function, but some indications can be derived from other transport systems. Cytosolic proteins that function as activators or inhibitors of the V-type H^+-ATPase have been isolated, and analogous molecules could conceivably modulate the antiporters. Cytoskeletal components attach to and regulate a variety of ion channels, raising the possibility that a similar control is exerted in the case of the antiporters. Finally, vicinal lipids modulate the Na^+/Ca^{2+} exchanger and other transporters, and preliminary evidence suggests that a similar relationship exists for the Na^+/H^+ exchangers.

In summary, the study of vertebrate Na^+/H^+ exchange and its regulation is rapidly becoming an area of much greater scope and complexity than originally perceived. It is, in fact, quite likely that the full magnitude of the problem is not yet apparent. Additional isoforms of the antiport will probably be discovered and novel regulatory processes will be identified. The interaction of antiporters with extraneous proteins is an area of great interest that remains totally unexplored, yet it is likely to yield answers regarding the control of antiport topology and regulation. Only when these parameters are defined will the multiplicity of functions of antiporters be fully understood.

Na^+/H^+ exchange in invertebrates

Although invertebrate systems have been investigated by far less thoroughly than their vertebrate counterparts, electroneutral Na^+/H^+ antiport, similar to that of vertebrates, may also occur in invertebrates. For example, leech or crayfish neurones and leech glial cells possess electroneutral Na^+/H^+ exchange mechanisms (Schlue and Thomas, 1985; Gaillard and Rodeau 1987; Deitmer and Schlue, 1987). There are also rare reports of electroneutral Na^+/H^+ exchange in epithelia: basolateral membranes of the crustacean hepatopancreatic epithelium as well as apical membranes of crayfish gills appear to have an electroneutral Na^+/H^+ antiporter (Ahearn *et al.* 1994; Strauss and Graszynski, 1992).

In contrast, there is increasing evidence for a novel type of electrophoretic Na^+/H^+ antiport in invertebrates that is located in the apical membrane of epithelia and exchanges two extracellular sodium ions for one intracellular proton (for a review, see Ahearn *et al.* 1994). $2Na^+/H^+$ antiport was first analyzed in brush-border membrane vesicles of the hepatopancreatic epithelium from the freshwater prawn *Macrobrachium rosenbergii* and in plasma membrane vesicles of posterior gills from the green crab *Carcinus maenas*. It has also been characterized in brush-border membrane vesicles of the hepatopancreatic epithelium and of the antennal gland labyrinth from the Atlantic lobster *Homarus americanus* and in brush-border membrane vesicles of the pyloric caecal epithelium from the starfish *Pycnopodia helianthoides*. Invertebrate $2Na^+/H^+$ exchangers serve such different functions as, for example, acidification in the crustacean gut and Na^+ absorption in crab gills and may also play a role in Ca^{2+} absorption in crustaceans and echinoderms. A monoclonal antibody to crustacean hepatopancreatic brush-border elements has been developed in the laboratory of Greg Ahearn; it inhibited $2Na^+/H^+$ antiport in the same membrane and recognized a single protein band with a molecular mass of 185 kDa in Western blots of hepatopancreas, antennal gland and gill epithelia. Immunostaining using this antibody revealed locations in all those tissues where vesicle studies had detected $2Na^+/H^+$ antiport (Kimura *et al.* 1994). The occurrence of $2Na^+/H^+$ antiport with similar characteristics in crustaceans and echinoderms, two representative groups of protostomia and deuterostomia, respectively, prompted the suggestion that $2Na^+/H^+$ antiport may be a widely distributed invertebrate antiporter and that the electroneutral mammalian Na^+/H^+ exchanger may be a departure from the animal norm (Ahearn and Franco, 1991).

Cation antiport driven by the proton-motive force

Most secondary transport mechanisms across animal plasma membranes are energized by the sodium-motive force that is generated by the primary Na^+/K^+-ATPase; by contrast, the primary energization of plant, fungal or bacterial plasma membranes is, in general, mediated by H^+-ATPases that produce a proton-motive force driving H^+-dependent secondary transport (Harold, 1986). However, H^+-dependent symports and antiports are well known for animal plasma membranes, too. For example, H^+ symport and H^+ antiport have been reported in vertebrate epithelia (organic cation antiport in human placenta, Ganapathy *et al.* 1988; Prasad *et al.* 1992; organic cation antiport in canine kidney, Sokol *et al.* 1988; Holohan *et al.* 1992). However, in all these cases, the proton-motive force is not produced by primary H^+ transport but by secondary Na^+/H^+ antiport that is itself energized by the Na^+/K^+-ATPase.

The larval midgut of the tobacco hornworm *Manduca sexta* was the first animal tissue in which the proton-motive force generated by a primary proton pump was established as the energy source of secondary transport across the plasma membrane (for reviews, see Wieczorek, 1992; Lepier *et al.* 1994). Unlike most animal epithelia, the midgut epithelium of the tobacco hornworm lacks a basolateral Na^+/K^+-ATPase. Instead, all secondary transport processes across the midgut, including the absorption of amino acids and the regulation of the high luminal pH, are energized by a primary proton-pumping V-ATPase located in the apical membrane. The electrical component of the proton-motive
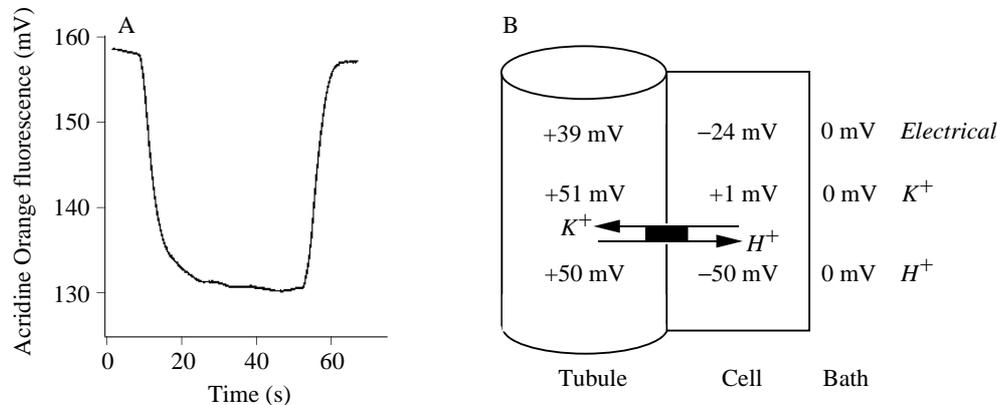
Fig. 1. (A) K⁺/H⁺ antiport as determined by the fluorescence quenching of Acridine Orange. The assay was started by the addition of 5 μl of vesicles (approximately 20 μg of protein) to 795 μl of a K⁺-free solution (pH 8), and the pH gradient was dissipated by the addition of 20 mmol l⁻¹ K⁺ (final concentration). For further experimental details, see Wieczorek et al. (1989). Brush-border membrane vesicles were isolated from Malpighian tubules of the tobacco hornworm by a Mg^{2+} precipitation method according to Wolfersberger et al. (1987). (B) Voltages and electrochemical potentials for H⁺ and K⁺ across the epithelium of Formica polyctena Malpighian tubules. Bath saline: 51 mmol l⁻¹ K⁺, pH 7.2. Data were taken from Van Kerkhove (1994).

force drives electrophoretic K⁺/2H⁺ exchange, resulting in net K⁺ secretion and thus constituting an electrogenic K⁺ pump. The K⁺ pump of M. sexta midgut belongs to a group of unique insect alkali metal ion pumps (Harvey et al. 1983). In salivary glands and in Malpighian tubules, these ion pumps drive fluid secretion and regulate ionic composition, and in cuticular sensilla they are the main energy source for the generation of receptor currents. Wieczorek et al. (1989) hypothesized that there may be a general mechanism for this type of cation transport, involving a primary proton pump and secondary cation/proton antiport similar to that found in M. sexta midgut.

The only direct evidence for cation/H⁺ antiport other than that found in M. sexta midgut has been obtained from the Malpighian tubules of the same species (A. Lepier and H. Wieczorek, unpublished results). Using Acridine Orange as a fluorescent pH indicator, ATP-independent K⁺/H⁺ antiport could be demonstrated in purified brush-border membranes of Malpighian tubules: vesicles which had been preloaded with 20 mmol l⁻¹ K⁺ developed a pH gradient, inside acidic, when they were diluted into a solution containing less than 1 mmol l⁻¹ K⁺ (Fig. 1A); the pH gradient could be dissipated by the addition of extravesicular K⁺ or by NH₄⁺.

Indirect but convincing evidence for the existence of cation/proton antiport in insect plasma membranes emerged from studies on Malpighian tubules of Formica, Drosophila and Aedes (for a review, see Nicolson, 1993). In isolated Malpighian tubules of larval Drosophila hydei, amiloride inhibited transepithelial fluid secretion and decreased the luminal pH, bafilomycin A₁ inhibited secretory activity and caused a decrease in intracellular pH, and the removal of K⁺ from the bathing saline caused luminal acidification. These findings are all consistent with the hypothesis that, as in the M. sexta

midgut, the K^+ pump in *D. hydei* Malpighian tubules is a proton-pumping V-ATPase which works in parallel with a K^+/H^+ antiporter (see also Wessing *et al.* 1993). In line with this hypothesis, transepithelial secretion of NaCl and KCl in Malpighian tubules isolated from the adult mosquito *Aedes aegypti* was found to be sensitive to amiloride and to bafilomycin A_1 (Hegarty *et al.* 1992; Pannabecker and Beyenbach, 1993), as was luminal alkalization in the midgut of larval *Aedes aegypti* (T. Anraman and W. R. Harvey, unpublished results), indicating a cation (Na^+ or K^+)/proton exchange.

The most compelling indirect evidence for the existence of a cation/proton antiport in Malpighian tubules was delivered by studies with isolated tubules of the ant *Formica polyctena*. Emmy Van Kerkhove and Paul Steels, together with their coworkers, analyzed the mechanism of K^+ extrusion across the luminal membrane by measuring intra- and extracellular K^+ and H^+ activities and voltages across the plasma membranes of the isolated tubules (Van Kerkhove, 1994; see also Zhang *et al.* 1994). They showed (i) that the V-ATPase inhibitors bafilomycin A_1 and *N*-ethylmaleimide inhibited fluid secretion and depolarized the luminal membrane; (ii) that luminal acidification blocked the short-circuit current; and (iii) that an H^+ concentration gradient builds up across the luminal membrane. Taken together, these data indicate that the electrochemical H^+ gradient across the luminal plasma membrane is high enough to drive K^+ extrusion into the lumen by secondary electroneutral K^+/H^+ antiport (Fig. 1B).

Electrophoretic cation/proton antiport: evolutionary constraints

Electroneutral cation/proton antiport appears to occur in virtually all mammalian cells and may be the standard exchange mode in vertebrates. Although electroneutral cation/proton antiport has also been reported for various invertebrate cells, there are prominent examples of electrophoretic cation/proton antiport in invertebrates, e.g. $2Na^+/H^+$ antiport in crustacean gastrointestinal epithelia and in crustacean gills, and $K^+/2H^+$ antiport in the tobacco hornworm midgut (see above). Furthermore, all known prokaryotic cation/proton antiporters are electrophoretic, exchanging one intracellular Na^+ for two extracellular H^+ (Padan and Schuldiner, 1993). What is the common denominator for the expression of electrophoretic cation/proton antiporters?

All of these examples share the property that the membranes face extreme environments that may be unstable, since the ionic composition of the extracellular medium may be subject to wide variations (e.g. in the gut depending on the meal, or in crab gills depending on the water salinity). Regarding pH, the crustacean hepatopancreas faces an acid extracellular medium (see Ahearn and Clay, 1989). By contrast, the pH in the midgut lumen of the tobacco hornworm can be higher than 11 (Dow, 1984) and alkalophilic bacteria inhabit environments with pH values ranging from 10 to 12 (Padan and Schuldiner, 1993). Regarding salinity, halophilic bacteria live in a milieu with sodium concentrations reaching $4\,mol\,l^{-1}$, whereas the gills of shore crabs may be surrounded by a medium of dilute salinity (Shetlar and Towle, 1989). In all these cases, the chemical component of the ion-motive force may not be sufficient or may be in the wrong direction to drive ion exchange by means of electroneutral antiport. Since, however, the antiport is electrophoretic, the total ion-motive force including the electrical

component can be used to energize secondary transport and may even be utilized for acidification or alkalization of the extracellular environment. Thus, $K^+/2H^+$ antiport appears to be a prerequisite for the alkalization of the midgut lumen in the tobacco hornworm (see Lepier *et al.* 1994) and $2Na^+/H^+$ antiport may be involved in the gastric luminal acidification in crustaceans (Ahearn and Clay, 1989).

Electroneutral Na^+/H^+ antiport as it is found in mammalian cells may be an adaptation to the constant environment with a high extracellular Na^+ concentration and a neutral and well-buffered pH. Evidently, electrogenicity of cation/proton antiport renders the system more versatile, allowing the use of both the chemical and electrical components of the primary ion-motive force and enabling the organism to face unfavourable or inconstant extracellular environments. Since electrophoretic cation/proton antiport may be widely distributed in prokaryotes and in lower animals, it is tempting to speculate, in accordance with Ahearn and Franco (1991), that electroneutral cation/proton antiport is an evolutionary apomorphy whereas electrophoretic cation/proton antiport represents the ancestral condition.

Concluding remarks

In summary, it is becoming apparent that the study of cation antiporters is an emerging, complex field. Many groups of antiporters evidently exist, some with multiple isoforms. Transport can be driven by Na^+, coupled to the Na^+/K^+ pump, or by H^+, with coupling to H^+ pump activity. In addition, exchange can be either electrogenic or electroneutral. Finally, antiport activity can in some instances be regulated by second messengers, by the cellular content of adenine nucleotides, by cell shape and volume and, possibly, by associated molecules such as lipids or cytoskeletal elements. Full understanding of the function of cation antiporters will require their molecular identification, which has already been accomplished for some of the vertebrate systems (Sardet *et al.* 1989; Orlowski *et al.* 1992), but is only in its preliminary stages in invertebrates. It is hoped that the relationship between the different families of antiporters and their phylogenetic links will be clarified by the analysis of their detailed structural and mechanistic features.

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