

CLASSICS

The V-ATPase in insect epithelia



Mike O'Donnell discusses the impact of two classic papers, published by Helmut Wiczeorek in *Journal of Biological Chemistry* in 1989 and 1991, which report the discovery of the the insect midgut V-ATPase.

Ion motive ATPases are enzymes that catalyse the decomposition of ATP into ADP and use the energy associated with this reaction to drive ions across the cell membrane. Until the late 1980s it was thought that the ion motive ATPase that energizes transepithelial ion transport in insects differed fundamentally from that found in vertebrate tissues. In the basolateral (i.e. blood-facing) membrane of vertebrate epithelial cells, the Na^+/K^+ -ATPase acts to pump Na^+ out and K^+ in. This pump thus creates a sodium concentration gradient; the tendency for Na^+ to leak back into the cell is then used to energize multiple secondary epithelial transporters such as Na^+/H^+ exchangers, $\text{Na}^+/\text{glucose}$ transporters and $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters (Harvey et al., 2009). In contrast, insect epithelia were thought to pump potassium out of cells across the apical (i.e. lumen-facing) membrane. The insect potassium pump was insensitive to the drug ouabain, which blocks the actions of the Na^+/K^+ -ATPase, and it was strongly electrogenic; by transporting positive charge out of the cell, it made the lumen electrically positive with respect to the haemolymph in tissues such as the midgut, salivary glands, Malpighian tubules and sensory sensilla (Wiczeorek et al., 2009).

Arthur Ramsay was the first to show that K^+ transport in Malpighian tubules was active, as deduced from luminal K^+

concentrations that were above those consistent with electrochemical equilibrium (Ramsay, 1953). Active K^+ transport in insect epithelia was confirmed by measurement of ^{42}K fluxes in the midgut of lepidopterans (Harvey and Nedergaard, 1964). Harvey's group went on to postulate that 10 nm diameter particles in the apical membrane are the units of active K^+ transport by insect epithelia (Harvey et al., 1983). These particles, termed portosomes, form projections on the cytoplasmic surface of the membrane and in electron micrographs they resemble the $\text{F}_1\text{-F}_0$ -ATPase in mitochondria. Studies of Malpighian tubules and midguts showed that all alkali metal ions, not just potassium, were transported (Harvey and Zehran, 1972), leading to the adoption of the term 'common cation pump' for structures that are responsible for the active transport of positively charged ions (Maddrell, 1978; Maddrell and O'Donnell, 1992).

In the first of two classic papers that I discuss in this article, Wiczeorek and his team studied the means by which the insect potassium pump utilizes ATP (Wiczeorek et al., 1989). They needed a source of membranes from insect epithelial cells which transport K^+ at high rates and contain large numbers of portosomes (Wiczeorek et al., 1989). The goblet cells of the caterpillar midgut turned out to be ideal and Wiczeorek's group built upon an ingenious method developed for the preparation of highly purified goblet cell apical membranes (Cioffi and Wolfersberger, 1983) to investigate the pump. Through a series of elegant and painstaking biochemical steps they were able to produce relatively large quantities of membranes enriched in portosomes and they used these membranes to form vesicles (Wiczeorek et al., 1989). These vesicles were loaded with two fluorophores, one to measure the electrical potential difference across the vesicle membrane and the other to measure the pH inside the vesicle. Their interest in pH stemmed from an earlier biochemical study by the group (Schweickl et al., 1989), which reported an unexpected finding: the goblet cell

plasma membranes possessed an ATPase that was similar in size and functional properties to the vacuolar H^+ -ATPase found in intracellular organelles. Vesicle studies allowed them to investigate what this ATPase did when it was incorporated into membranes. In the presence of Mg and ATP, they found that both vesicle membrane potential generation and proton transport were insensitive to inhibitors of mitochondrial and P-type ATPases such as the Na^+/K^+ -ATPase, but sensitive to inhibitors of the vacuolar-type H^+ -ATPase, commonly referred to as the V-ATPase (Wiczeorek et al., 1989). In effect, this finding showed that the common cation pump was dependent in some way on the operation of a proton pump, the V-ATPase. In conjunction with the evidence for a plasma membrane V-ATPase in vertebrate renal epithelia (Al-Awqati et al., 1983; Brown et al., 2009), Wiczeorek's discovery of a plasma membrane V-ATPase in insect epithelia thus led physiologists to reassess the view that the V-ATPase was found exclusively in intracellular organelles such as lysosomes.

But how did the discovery of proton pumping by the vesicles explain the transport of K^+ and other cations? Answering this question in the first classic paper, Wiczeorek's research group showed that the addition of potassium to the vesicles dissipated the proton gradient, but not the membrane potential, leading to the brilliant insight that the enhanced proton efflux induced by potassium could be explained by the presence of a second ion transport protein in the vesicle membrane. This second transporter allowed protons to move out of the vesicle in exchange for inward movement of potassium. Cation selectivity of proton transport could thus be influenced not only by the specificity of the ATPase but also by the specificity of this proton/potassium antiporter (Wiczeorek et al., 1989).

Following on from this work in their second classic paper (Wiczeorek et al., 1991), the Wiczeorek group extended their vesicle studies to show that the V-ATPase does indeed energize exchange of K^+ for H^+ across the apical membrane. They

showed that the K^+/H^+ -antiporter that was driven by an outward-directed K^+ gradient was responsible for proton transport into the vesicle interior. This antiporter was not an ATPase because it did not require the presence of ATP for its activity and it could be inhibited by amiloride, a drug known to block the action of K^+/H^+ - and Na^+/H^+ -antiporters. Polyclonal antibodies against the V-ATPase blocked ATP-dependent proton transport but not K^+/H^+ -antiporter activity, thus providing unequivocal evidence that the V-ATPase and the antiporter are two distinct membrane proteins. The group also used separate fluorescent indicators for pH and membrane potential to show that more than one H^+ is exchanged per K^+ . Thus, the electrical component of the proton motive force generated by the V-ATPase drives electrogenic K^+ secretion through a K^+/nH^+ -antiporter (where nH^+ is a number of protons).

These two seminal papers by Wiczorek's research group thus revealed that the insect common cation pump is composed of not one but two distinct transporters on the apical membrane: the V-ATPase, which drives H^+ from cell to lumen, and the cation/ H^+ -antiporter, which recycles the protons and transfers K^+ (or Na^+) from cell to lumen. A pivotal feature of this mechanism is that the electrogenic nature of the H^+ pump can be used to drive Na^+ or K^+ uphill into the tubule lumen if the stoichiometry of the antiporter is $2H^+:1Na^+$ or $2H^+:1K^+$. As a consequence, an increase in lumen positive potential makes it easier to transport K^+ (or Na^+) across the apical membrane and increase its concentration in the lumen.

Wiczorek's discovery of the insect midgut V-ATPase (Wiczorek et al., 1989) stimulated many other researchers to consider the possible roles of plasma membrane V-ATPases in epithelia such as the Malpighian tubules and salivary glands. These studies showed that the apical V-ATPase could work in concert with the K^+/H^+ -antiporter to energize transepithelial fluid secretion in these tissues. The late 1980s and early 1990s were also a period of rapid developments in understanding the contributions of plasma membrane V-ATPases to urinary acidification and bicarbonate reabsorption by the mammalian kidney (Gluck and Nelson, 1992). In contrast to vertebrate renal tissues, in which the main consequence of the V-ATPase activity is

luminal acidification, in insect Malpighian tubules the cycling of protons from cell to lumen through the V-ATPase and from lumen to cell through the antiporter is associated with only modest changes in pH but large fluxes of cations (Na^+ , K^+) and, as a consequence, high rates of flow of osmotically obliged water.

A great deal of subsequent research has focused on how the V-ATPase is regulated. The fly salivary gland, in particular, has proved to be a potent and flexible model for understanding the regulation of V-ATPase activity. The functional V-ATPase is formed by the assembly of multiple smaller protein subunits, and the intracellular second messenger cAMP has been shown to promote the reversible assembly of these subunits into the complete enzyme, resulting in activation of V-ATPase in the gland (Baumann and Walz, 2012). In Malpighian tubules, multiple diuretic peptides enhance both fluid secretion and the lumen-positive transepithelial electrical potential difference and are thus thought to stimulate V-ATPase activity, but the mechanisms by which peptide second messengers such as cAMP and cGMP interact with the ATPase are unclear. Applied research has shown that peptides which inhibit the insect V-ATPase, including the depsipeptide fungal toxins known as destruxins (Liu and Tzeng, 2012) and pea albumin 1, subunit b (PA1b), a peptide isolated from legume seeds (Gressent et al., 2011), offer promise as bioinsecticides for control of pest species.

There have also been extensive studies of how the V-ATPase regulates the pH of the insect gut. Paradoxically, the apical V-ATPase can be coupled to processes that cause alkalization of the midgut in Lepidoptera to pH values above 10 (Azuma et al., 1995). Subsequent research has also shown that the V-ATPase is also located on the basal membrane of the dipteran gut, where it also drives luminal alkalization of the anterior midgut (Onken and Moffett, 2009; Shanbhag and Tripathi, 2005). By contrast, an apical V-ATPase in the posterior dipteran midgut has been implicated in acid secretion and alkali recovery (Jagadeeswaran et al., 2010).

Although the V-ATPase has come to dominate our thinking of how insect epithelial transport is energized, we have in some ways come full circle, with recent

evidence that the Malpighian tubules and gut in some species are equipped with not just an apical V-ATPase but also a basolateral Na^+/K^+ -ATPase. The importance of the Na^+/K^+ -ATPase in tubules was missed because a co-localized toxin extruder protects the ATPase from the archetypal inhibitor ouabain (Torrie et al., 2004). Although the V-ATPase does the 'heavy lifting' in energizing transepithelial ion transport, the Na^+/K^+ -ATPase in the tubules and gut may play an ancillary role in adjusting Na and K levels within the cells and lumen (D'Silva et al., 2017a,b; Linton and O'Donnell, 1999; Patrick et al., 2006).

Mike O'Donnell
McMaster University
odonnell@mcmaster.ca

References

- Al-Awqati, Q., Gluck, S., Reeves, W. and Cannon, C. (1983). Regulation of proton transport in urinary epithelia. *J. Exp. Biol.* **106**, 135-141.
- Azuma, M., Harvey, W. R. and Wiczorek, H. (1995). Stoichiometry of K^+/H^+ antiport helps to explain extracellular pH 11 in a model epithelium. *FEBS Lett.* **361**, 153-156.
- Baumann, O. and Walz, B. (2012). The blowfly salivary gland - a model system for analyzing the regulation of plasma membrane V-ATPase. *J. Insect. Physiol.* **58**, 450-458.
- Brown, D., Paunescu, T. G., Breton, S., Marshansky, V. (2009). Regulation of the V-ATPase in kidney epithelial cells: dual role in acid-base homeostasis and vesicle trafficking. *J. Exp. Biol.* **212**, 1762-1772.
- Cioffi, M. and Wolfersberger, M. G. (1983). Isolation of separate apical, lateral and basal plasma membrane from cells of an insect epithelium. A procedure based on tissue organization and ultrastructure. *Tissue and Cell* **15**, 781-803.
- D'Silva, N. M., Donini, A. and O'Donnell, M. J. (2017a). The roles of V-type H^+ -ATPase and Na^+/K^+ -ATPase in energizing K^+ and H^+ transport in larval *Drosophila* gut epithelia. *J. Insect Physiol.* **98**, 284-290.
- D'Silva, N. M., Patrick, M. L. and O'Donnell, M. J. (2017b). Effects of rearing salinity on expression and function of ion motive ATPases and ion transport across the gastric caecum of *Aedes aegypti* larvae. *J. Exp. Biol.* **220**, 3172-3180.
- Gluck, S. and Nelson, R. (1992). The role of the V-ATPase in renal epithelial H^+ transport. *J. Exp. Biol.* **172**, 205-218.
- Gressent, F., Da Silva, P., Eyraud, V., Karaki, L. and Royer, C. (2011). Pea Albumin 1 subunit b (PA1b), a promising bioinsecticide of plant origin. *Toxins* **3**, 1502-1517.
- Harvey, W. R., Boudko, D. Y., Rheault, M. R. and Okech, B. A. (2009). NHE(VNAT): an H^+ V-ATPase electrically coupled to a Na^+ :nutrient amino acid transporter (NAT) forms an Na^+/H^+ exchanger (NHE). *J. Exp. Biol.* **212**, 347-357.
- Harvey, W. R., Cioffi, M., Dow, J. A. and Wolfersberger, M. G. (1983). Potassium ion transport ATPase in insect epithelia. *J. Exp. Biol.* **106**, 91-117.
- Harvey, W. R. and Nedergaard, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the *Cecropia* silkworm. *PNAS* **51**, 757-765.
- Harvey, W. R. and Zehran, K. (1972). Active transport of potassium and other alkali metals

- by the isolated midgut of the silkworm. *Curr. Topics Membr. Transp.* **3**, 367-410.
- Jagadeshwaran, U., Onken, H., Hardy, M., Moffett, S. and Moffett, D.** (2010). Cellular mechanisms of acid secretion in the posterior midgut of the larval mosquito (*Aedes aegypti*). *J. Exp. Biol.* **213**, 295-300.
- Linton, S. M. and O'Donnell, M. J.** (1999). Contributions of K^+Cl^- cotransport and Na^+/K^+ -ATPase to basolateral ion transport in Malpighian tubules of *Drosophila melanogaster*. *J. Exp. Biol.* **202**, 1561-1570.
- Liu, B.-L. and Tzeng, Y.-M.** (2012). Development and applications of destruxins: A review. *Biotechnol. Adv.* **30**, 1242-1254.
- Maddrell, S.** (1978). Transport across insect excretory epithelia, *Transport Across Multi-Membrane Systems*. Springer, 239-271.
- Maddrell, S. H. and O'Donnell, M. J.** (1992). Insect Malpighian tubules: V-ATPase action in ion and fluid transport. *J. Exp. Biol.* **172**, 417-429.
- Onken, H. and Moffett, D. F.** (2009). Revisiting the cellular mechanisms of strong luminal alkalization in the anterior midgut of larval mosquitoes. *J. Exp. Biol.* **212**, 373-377.
- Patrick, M. L., Aimanova, K., Sanders, H. R. and Gill, S. S.** (2006). P-type Na^+/K^+ -ATPase and V-type H^+ -ATPase expression patterns in the osmoregulatory organs of larval and adult mosquito *Aedes aegypti*. *J. Exp. Biol.* **209**, 4638-4651.
- Ramsay, J.** (1953). Active transport of potassium by the Malpighian tubules of insects. *J. Exp. Biol.* **30**, 358-369.
- Schweikl, H., Klein, U., Schindlbeck, M. and Wiczorek, H.** (1989). A vacuolar-type ATPase, partially purified from potassium transporting plasma membranes of tobacco hornworm midgut. *J. Biol. Chem.* **264**, 11136-11142.
- Shanbhag, S. and Tripathi, S.** (2005). Electrogenic H^+ transport and pH gradients generated by a V- H^+ -ATPase in the isolated perfused larval *Drosophila* midgut. *J. Membr. Biol.* **206**, 61-72.
- Torrie, L. S., Radford, J. C., Southall, T. D., Kean, L., Dinsmore, A. J., Davies, S. A. and Dow, J. A. T.** (2004). Resolution of the insect ouabain paradox. *PNAS* **101**, 13689.
- Wiczorek, 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.
- Wiczorek, H., Beyenbach, K. W., Huss, M. and Vitavska, O.** (2009). Vacuolar-type proton pumps in insect epithelia. *J. Exp. Biol.* **212**, 1611-1619.
- Wiczorek, 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.