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CLASSICS

# The V-ATPase in insect epithelia


**Mike O'Donnell discusses the impact of two classic papers, published by Helmut Wieczorek 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, $Na^+$/glucose transporters and $Na^+/K^+/2Cl^-$ 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 (Wieczorek 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 portasomes, form projections on the cytoplasmic surface of the membrane and in electron micrographs they resemble the $F_1$-$F_0$-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, Wieczorek and his team studied the means by which the insect potassium pump utilizes ATP (Wieczorek et al., 1989). They needed a source of membranes from insect epithelial cells which transport $K^+$ at high rates and contain large numbers of portasomes (Wieczorek et al., 1989). The goblet cells of the caterpillar midgut turned out to be ideal and Wieczorek'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 portasomes and they used these membranes to form vesicles (Wieczorek 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 (Schweikl 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 (Wieczorek 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), Wieczorek'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, Wieczorek'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 (Wieczorek et al., 1989).

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

Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

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 that 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 Wieczorek'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.

Wieczorek's discovery of the insect midgut V-ATPase (Wieczorek 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 (Jagadeshwaran 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).

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