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V-ATPases OF THE PLASMA MEMBRANE

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Summary

V-ATPases reside in high densities on the plasma membrane in specialized types of insect and vertebrate cells. They provide unique biochemical and electrophysiological properties that allow them to function in energizing the plasma membrane in insects, and in cellular acid excretion in vertebrates.

Introduction

The participation of V-ATPases in numerous aspects of endocytosis, secretion and sorting has been amply recognized (Forgac, 1989; Mellman *et al.* 1986). In fungi, plants and most animal cells, V-ATPases energize selected intracellular membrane compartments of the vacuolar system, providing an electrochemical driving force for the transport of solutes and acidifying the interior of several of these compartments.

The importance of the V-ATPases in the function of the plasma membrane is not as widely recognized. Several of the papers in this volume highlight our increasing awareness that, in certain cell types, V-ATPases serve in cellular proton transport and as the principal means for energizing secondary active transport proteins.

The plasma membrane V-ATPase of *Entamoeba histolytica*

As the papers by Mukohata and Gogarten in this volume discuss, the V-ATPases are most closely related to the archaebacterial H^+-ATPases (A-ATPases). Most bacterial species digest and absorb extracellular nutrients by secreting hydrolases and transporting solutes directly through the encapsulating bacterial membranes. At some remote point in evolution, a primordial prokaryote undoubtedly acquired the capacity to engulf extracellular material, allowing it to carry out the digestion and absorption of nutrients in an enclosed vacuole. In these primitive systems, the membrane composition of the vacuole was presumably the same as that of the plasma membrane. With subsequent evolution, a far more complex organization and structure of the vacuolar system has developed, in which the V-ATPase continues to function as the major electrochemical driving force energizing vacuolar transport functions.

In her paper, Bakker-Grunwald discusses *Entamoeba histolytica*, an organism providing a snapshot of the evolutionary origins of the vacuolar system. *E. histolytica* is a

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primitive eukaryote in which the vacuoles form directly and with little modification, from the plasma membrane. A V-ATPase resides in the plasma membrane of the organism and is internalized with the invaginating membrane during the formation of a vacuole. The V-ATPase then serves to acidify the vacuole interior, driving Na^+/H^+ exchange that removes sodium ions from the cytosol, and presumably promoting the action of any acid hydrolases that might be secreted into the vacuole. The vacuoles fuse, on a time-dependent probabilistic basis, with the plasma membrane, allowing the expulsion of undigested material and returning the V-ATPase to the cell surface.

E. histolytica is an important human pathogen. It is the principal cause of amoebic dysentery, and it may also produce liver and brain abscesses. The plasma membrane V-ATPase of the amoeba may allow it to survive under anaerobic conditions by excreting protons produced by glycolysis, contributing to its ability to form abscesses.

Why did fungi lose the plasma membrane V-ATPase?

Saccharomyces cerevisiae, Neurospora crassa and other fungi do not have a V-ATPase in the plasma membrane. Instead, their plasma membranes have an electrogenic proton pump belonging to the P-ATPase family (Slayman, 1985). This family of ATPases probably evolved from a precursor of the bacterial Kdp K^+-ATPase (Epstein *et al.* 1990). What conditions created an evolutionary advantage for these organisms to replace the plasma membrane V-ATPase with the P-type H^+-ATPase?

Perhaps the P-type H^+-ATPase offered an advantage for survival on land. Unlike animal cells, plant and fungal cells maintain a turgor pressure that drives cell growth and movement (Schaller and Sussman, 1987), and the enveloping cell wall acts as a bulwark to prevent bursting. Plant and fungal cells generate turgor pressure by using a membrane potential, generated by the P-type H^+-ATPase, to energize the accumulation of potassium. The concentration of potassium may be quite low in the fresh water of terrestrial environments. Since the stoichiometry of the P-type H^+-ATPase is $1H^+$ transported per ATP hydrolyzed (Slayman, 1985; Perlin *et al.* 1986), it can, in principle, generate membrane potentials of 350 mV or more, allowing potassium ions to be concentrated by passive influx to about 10^6-fold in the cytosol. Like their evolutionary brothers, the F-ATPases, V-ATPases probably have a stoichiometry of $3H^+/ATP$ (Dixon and Al-Awqati, 1980; Nelson, 1989). The V-ATPases can sustain membrane potentials of only 240 mV, and may not have provided fungi and plants with a driving force for ion uptake sufficient to survive in fresh water. In contrast, animal cells do not maintain turgor pressure, but maintain isosmotic ion gradients of sodium and potassium, usually using the Na^+/K^+-ATPase, an adaptation that afforded greater mobility by eliminating the requirement for a cell wall (Maloney and Wilson, 1985).

Fungi are relatively immotile cells; when they grow on a solid medium their secreted protons accumulate, creating an acidic microenvironment from which they cannot easily escape. As the papers by Nelson, Anraku, Stevens, Klionsky, Kane and Manolson emphasize, yeast grow well at pH 5 and below. Fungi live comfortably in acidic environments because the P-type H^+-ATPase continues to transport protons effectively against a pH gradient of this magnitude. In contrast, an external pH of 5 places the V-

ATPase close to its thermodynamic limit for proton transport, and its rate of proton transport approaches zero.

The acquisition of a P-type H^+-ATPase on the plasma membrane may therefore have provided several evolutionary advantages for fungi and plants.

Plasma membrane V-ATPases in animal cells

A major theme underscored in this symposium is the diversity of animal cells that retained (or secondarily evolved) and amplified V-ATPases on the plasma membrane as a generator of many different cell-specific transport and electrical functions. The papers in this volume by W. Harvey, Wieczorek, Klein, Dow, Moffett, Maddrell and Zeiske discuss the importance of the V-ATPase as a primary electromotive force in several specialized insect cells: the potassium-secreting goblet cells of the insect gut, the cells of the Malpighian tubule and the enveloping cells of the sensillum. The papers by B. Harvey, Baron, Grinstein, Gluck and Brown discuss the function of the V-ATPase in transcellular proton transport in specialized vertebrate cells: the proton-secreting mitochondria-rich cells of the frog skin and urinary bladder, the osteoclast, the macrophage and neutrophil, and the proton-transporting cells of the kidney. It is anticipated that an increasing number of cells employing plasma membrane V-ATPases, in phyla other than those discussed here, will be identified in the next several years.

What conditions created an evolutionary advantage for these animal cells to use V-ATPases instead of P-type H^+-ATPases to execute these functions? Some potential answers may emerge after a brief comparison of the functions subserved by the plasma membrane V-ATPase.

Properties of plasma membrane V-ATPases in animal cells

Plasma membrane V-ATPases in arthropod and vertebrate cells share several features that are not generally observed in the V-ATPases in intracellular membranes.

Plasma membrane V-ATPases are present at high densities, far greater than the densities on intracellular membranes

As the papers by Klein, Baron and Brown in this volume show, V-ATPases in the plasma membrane of insect midgut and sensillum-enveloping cells, osteoclasts and renal intercalated cells form dense two-dimensional arrays in the plasma membrane. The toad bladder mitochondria-rich cell and the renal intercalated cell have nearly 15 000 V-ATPases μm^{-2} (Brown *et al.* 1987), and densities of a similar magnitude are observed in the osteoclast (Baron, 1989; Blair *et al.* 1989; Vaananen *et al.* 1990; Baron, this volume), insect midgut (Klein *et al.* 1991) and insect sensillum (Klein and Zimmerman, 1991).

The high plasma membrane densities of the V-ATPase could be the result of directed targeting to the plasma membrane. An alternative possibility is that most cells efficiently internalize any plasma membrane V-ATPase, but cells with plasma membrane V-ATPases have a reduced internalization efficiency. Studies in this volume presented by Kane, Stevens and Manolson suggest that the 100×10^3 M_r subunit in yeast (equivalent to the 100×10^3 M_r or 116×10^3 M_r subunit of the coated vesicle V-ATPase; see Forgac and

Stone, this volume) may have a role in targeting to the vacuole. Since this polypeptide has not been detected on plasma membrane V-ATPases from the kidney (Wang and Gluck, 1990), a possible role for the 100 (or 116)$\times 10^3$ M_r subunit may be in internalization into vacuoles.

Amplification is limited to specific cell types

In the insect, high densities of V-ATPase on the plasma membrane are observed in the midgut goblet cell and the enveloping cells of sensilla, but not in other cells in the same tissues (Klein *et al.* 1991; Klein and Zimmerman, 1991). Similarly, high densities of plasma membrane V-ATPase are found in the mitochondria-rich cell of toad bladder (Brown *et al.* 1987) and frog skin (B. Harvey, this volume) and in the intercalated cells of the mammalian kidney collecting tubule (Brown *et al.* 1988; Brown, this volume; Gluck, this volume), but V-ATPases are not immunocytochemically detectable in the principal cells of the same tissues. Osteoclasts have the only immunocytochemically detectable plasma membrane V-ATPase in bone (Baron, 1989; Blair *et al.* 1989; Baron, this volume).

The basis for cell-type-specific amplification remains unclear. An important clue has been found recently in the kidney, where intercalated cell-specific amplified expression of one isoform of the 56×10^3 M_r (*B*) subunit was found (Nelson *et al.* 1992). It is not yet known whether cell-specific amplification of subunits of the V-ATPase occurs in other cells with plasma membrane V-ATPases.

Plasma membrane V-ATPases are polarized

In the insect gut, V-ATPases are present only in the apical membrane. V-ATPases also reside in the specialized apical plasma membrane of the sensillum-enveloping cells. In the intercalated cell, dense arrays of V-ATPases reside in the plasma membrane and in a pool of cytoplasmic vesicles capable of fusing with the plasma membrane. In the osteoclast, a cell derived from the monocyte–macrophage lineage that lacks the zona occludens characteristic of classical epithelial cells, V-ATPase is polarized to the ruffled membrane in cells that are in a state of active bone resorption. It is not yet known whether the plasma membrane V-ATPase of macrophages and neutrophils resides in a polarized distribution.

The activity of plasma membrane V-ATPases is regulated to respond to physiological stimuli

In the Malpighian tubule of *Rhodnius prolixus*, the rate of V-ATPase-driven potassium, sodium and water secretion are minimal under fasting conditions, but are stimulated several hundred-fold by ingestion of blood, an effect controlled by 5-hydroxytryptamine and a peptide hormone (Maddrell, this volume). Goblet cells in the midgut of *Manduca sexta* have an apical plasma membrane V-ATPase energizing potassium secretion that is highly active during the eating cycle of the caterpillar, but shuts off during molting (Dow, this volume). Macrophages have a plasma membrane V-ATPase, nearly quiescent under control conditions, but activated by acidification of the cytosol and maintaining cytoplasmic pH. Neutrophils also have a normally silent plasma membrane V-ATPase;

phorbol esters, which activate NADPH oxidase and accelerate cytosolic proton generation, activate a plasma membrane V-ATPase that participates in maintaining cytoplasmic pH (Grinstein, this volume). Osteoclasts may regulate their bone-resorptive activity by controlling hydrogen ion secretion by the ruffled membrane (Baron, this volume; Baron, 1989; Gluck, 1992). The proton-transporting mitochondria-rich cells of frog skin and the intercalated cells of the kidney regulate hydrogen ion transport by changing the kinetic activity and the number of V-ATPases in the plasma membrane with changes in the intake or production of acid or CO_2 (B. Harvey, Gluck, Brown, this volume), a response that maintains the acid–base homeostasis of the organism.

The factors regulating the activity of plasma membrane V-ATPases differ from those regulating the acidification of intracellular compartments

The activity of the V-ATPases in intracellular compartments is inherently transient and limited by the proton electrochemical gradient across the vacuolar membrane. Various compartments of the vacuolar system maintain different steady-state intravesicular pH values (Yamashiro and Maxfield, 1988) that may be controlled by the vacuolar membrane potential (Mellman *et al.* 1986). The potential difference across the vacuolar membrane may be influenced by the Na^+/K^+-ATPase (Fuchs *et al.* 1989), and the chloride conductance of the vacuole (Mulberg *et al.* 1991; Grinstein and Al-Awqati, this volume). Evidence for direct regulation of V-ATPases in vacuoles is also emerging (Forgac, Stone and Gluck; this volume).

The activity of V-ATPases in the plasma membrane is also affected by the proton electrochemical gradient across the membrane, but a wider variety of influences may affect the potential across the plasma membrane than those affecting the vacuole. For example, a rise in insect hemolymph potassium concentration may depolarize the goblet cell, stimulating the apical V-ATPase by reducing transiently the potential difference across the apical membrane (Moffet, this volume). Regulation of the membrane potential controlling proton secretion may also occur through the collective transport functions of different cell types in an epithelium (W. Harvey, Moffett, B. Harvey and Gluck; this volume). The anion conductance of the plasma membrane, unlike the vacuolar membrane, is not necessarily a primary determinant of the rate of proton transport (Gluck, this volume). As indicated above, several examples of systems exhibiting direct regulation of the plasma membrane V-ATPase are presented in this volume; it is unlikely (although direct evidence has not been obtained) that the mechanisms controlling the plasma membrane V-ATPases affect the acidification of the intracellular vacuolar compartments required for constitutive cell function.

Structural diversity and different enzymatic properties of the V-ATPase may allow selective regulation in different membrane compartments

V-ATPases isolated from several mammalian organs and membrane fractions have remarkably similar structures, but recent studies demonstrate functional and structural heterogeneity. Differences have been found in the enzymatic properties of V-ATPases isolated from different kidney membrane fractions (Wang and Gluck, 1990; Gluck, this volume). Structural heterogeneity has been found in the mammalian 56×10^3 M_r subunit

(Bernasconi *et al.* 1990; Südhof *et al.* 1989; Puopolo *et al.* 1992), and in the $31\times10^3 M_r$ subunit (Wang and Gluck, 1990; Hemken *et al.* 1992). Although prior studies suggested that the V-ATPase of the osteoclast resembled that in the kidney and other tissues (Blair *et al.* 1989; Bekker and Gay, 1990), evidence for a pharmacologically unique type of V-ATPase containing an isoform of the $70\times10^3 M_r$ subunit in osteoclasts is presented by Baron in this volume. Structural heterogeneity of the insect V-ATPase has not been reported, but the isolation and generation of antibodies to the V-ATPase from *Manduca* midgut (Wieczorek, this volume) should allow this problem to be examined shortly.

Plasma membrane V-ATPases in insects and vertebrates differ in their physiological roles

The plasma membrane V-ATPases of insects and vertebrates share several properties discussed above, but differ importantly in their physiological functions. The plasma membrane V-ATPases of insects generate a membrane potential, used secondarily to drive drive potassium secretion, resulting in extreme alkalization of the lumen to pH>11 (Dow, 1984). In the midgut, electrophysiological studies presented by W. Harvey, Wolfersberger, Zeiske, Moffett and Dow, and vesicle studies presented by Wieczorek and Klein, all discussed in this volume, have prompted a model with an apical V-ATPase generating a potential difference of up to 240 mV across the apical membrane of the goblet cell (Dow and Peacock, 1989). This potential difference drives an electrogenic K^+/H^+ antiporter operating in parallel in the same membrane, resulting in the net inward movement of protons and the secretion of potassium. Antithetically, the midgut lumen of *Manduca* larvae is extremely alkaline despite the presence of an apical plasma membrane V-ATPase. Similarly, V-ATPases are the primary driving force generating a membrane potential and salt and water fluxes in the Malpighian tubule and the rectum; the V-ATPase-generated membrane potential in the enveloping cells of the sensillum drives the signalling currents initiated by activation of the sensory cells (Klein, this volume).

In contrast, vertebrate cells use the plasma membrane V-ATPase primarily for proton transport. The mitochondria-rich cell of the frog skin and toad bladder and the 'A-type' intercalated cell of the kidney collecting tubule are proton-secreting cells that have a V-ATPase on the apical plasma membrane (B. Harvey, Gluck, Brown, this volume). Electrogenic apical proton secretion results in the generation of bicarbonate inside the cell that exits from the basolateral membrane through an electroneutral Cl^-/HCO_3^- antiporter. Chloride then exits *via* a basolateral chloride channel, maintaining charge balance in the cell during transport. Electrogenic proton secretion without transepithelial charge balance would rapidly produce a large adverse lumen-positive (or pond-positive) potential difference across the apical membrane, which would limit the proton secretory rate. In the intact epithelium, the potential difference of the lumen is normally either negative, as a result of the sodium absorption potential generated by the neighboring principal cells, or only slightly positive owing to a paracellular chloride conductance. Macrophages and neutrophils also use the plasma membrane V-ATPase to excrete hydrogen ions rather than to generate a membrane potential (Grinstein, this volume.)

Why did animal cells maintain V-ATPases as plasma membrane proton pumps?

No electrogenic P-type plasma membrane H^+-ATPases have yet been identified in animal cells, although some animal cells do have electroneutral P-ATPase proton pumps in the plasma membrane. For example, both the stomach (Sachs and Munson, 1991) and the intercalated cells in the kidney (Wingo *et al.* 1990; Verlander *et al.* 1991) have an electroneutral H^+/K^+-ATPase in the plasma membrane. In the kidney, this enzyme appears to function primarily in active potassium reabsorption rather than in controlling net acid secretion. Thus, the choice of V-ATPases for electrogenic plasma membrane proton transport in animal cells was probably not a haphazard accident of evolution, and may have provided several physiological advantages.

In vertebrate cells that use the plasma membrane V-ATPases mainly for acid excretion, an obvious advantage is the difference in the stoichiometry between P-ATPases and V-ATPases. More protons are transported per ATP consumed in V-ATPases. The remarkable ability of plasma membrane V-ATPases to pack at high densities would also enhance the proton-pumping capacity per area of membrane. Another potential advantage stems from the limitation in the magnitude of the external pH gradient that V-ATPases can sustain. A P-type H^+-ATPase producing an external pH of 2 could result in denaturation of surrounding proteins and protonation of organic acids, which would then ferry protons back into the cell.

There are also several possible advantages of a V-ATPase in cells using a proton pump mainly to generate a membrane potential. The high potential differences generated under some conditions by the P-type H^+-ATPase in plant and fungal cells would not be tolerated in animal cells because of dielectric breakdown of the lipid bilayer. Dielectric breakdown often occurs at potentials above 200 mV in animal cells, approximately the limiting potential difference that V-ATPases are capable of sustaining. The foldings and invaginations of the plasma membrane observed on most animal cells, forming structures such as microvilli, would also increase the susceptibility to dielectric breakdown by providing foci for charge accumulation. The absence of a substantial anion conductance in the same membrane as the proton pump, as is observed in most of the animal cells discussed above, would contribute to the likelihood of potentials approaching the limit for dielectric breakdown if a P-type H^+-ATPase were present in the plasma membrane. High potential differences are endured in plant and fungal cells because the cell wall limits the size of any pores formed transiently by dielectric breakdown; plant protoplasts are nearly as susceptible to dielectric breakdown as animal cells (D. Tsong, personal communication).

Finally, V-ATPases may offer the cell unique mechanisms for regulation. The nature of their interactions with the cytoskeleton and of their intercompartmental traffic in the vacuolar system is only at the earliest stages of understanding.

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