New insights into the regulation of V-ATPase-dependent proton secretion

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Breton S, Brown D. New insights into the regulation of V-ATPase-dependent proton secretion. Am J Physiol Renal Physiol 292: F1–F10, 2007. First published October 10, 2006; doi:10.1152/ajprenal.00340.2006.—The vacuolar H＋-ATPase (V-ATPase) is a key player in several aspects of cellular function, including acidification of intracellular organelles and regulation of extracellular pH. In specialized cells of the kidney, male reproductive tract and osteoclasts, proton secretion via the V-ATPase represents a major process for the regulation of systemic acid/base status, sperm maturation and bone resorption, respectively. These processes are regulated via modulation of the plasma membrane expression and activity of the V-ATPase. The present review describes selected aspects of V-ATPase regulation, including recycling of V-ATPase-containing vesicles to and from the plasma membrane, assembly/disassembly of the two domains (V0 and V1) of the holoenzyme, and the coupling ratio between ATP hydrolysis and proton pumping. Modulation of the V-ATPase-rich cell phenotype and the pathophysiology of the V-ATPase in humans and experimental animals are also discussed.

THE VACUOLAR H＋-ATPASE (V-ATPASE) is a multisubunit enzyme that mediates ATP-driven proton transport across membranes. It is expressed in all eukaryotic cells, where it participates in the acidification of intracellular organelles (11, 27, 46, 82, 125, 135). The V-ATPase is also found at high density in the plasma membrane of specialized cells that are involved in active proton transport and extracellular pH regulation. These include renal intercalated cells (1, 26, 29, 50, 110, 135), osteoclasts (13, 125), narrow and clear cells in the epididymis and vas deferens, and interdigital cells in the inner ear (64, 117). The V-ATPase in collecting duct intercalated cells is critical for the establishment of an acidic luminal pH via V-ATPase-dependent proton secretion (21, 30, 33). Low luminal pH and low bicarbonate concentration are important factors that contribute to maintaining spermatozoa in a quiescent state during their maturation and storage (5, 54). In osteoclasts, the V-ATPase is a key player in bone resorption (13, 69), and in the inner ear it participates in establishing a high K＋ level in the endolymph, which is essential for hearing (64, 117, 120). The critical roles of the V-ATPase in these specialized acidifying cells require the tight physiological regulation of its functional expression at the plasma membrane. The present review will examine selected mechanisms that are involved in the regulation of proton-pumping activity and plasma membrane expression of the V-ATPase. This minireview is not intended at describing the hormonal regulation of the V-ATPase, which has been extensively described in a previous review by Wagner et al. (135).

Structure of the V-ATPase

The V-ATPase is composed of many subunits and is divided into two distinct domains or sectors (27, 46, 63, 82, 85, 114, 135). The V0 sector is responsible for proton translocation and contains subunits traditionally designated by the lower case letters, a, c, c′, c", and d (Fig. 1). The V1 sector forms a large cytosolic complex composed of eight subunits, designated by the capital letters A–H. These subunits have recently been renamed according to the new official nomenclature shown in Table 1 (114). For simplicity, the abbreviated single-letter subunit identification will be used throughout the text. Three copies of the A subunits alternate with three copies of the B subunits to form part of the V1 sector, which is connected to the V0 sector via two or more stalks, composed of subunits C, D, E, F, G, and H (63, 65, 135). Subunit A is responsible for ATP hydrolysis, but subunit B also contains an ATP binding site and is thought to play a regulatory role (27, 46, 82, 85, 114, 135).

Some of the V-ATPase subunits are encoded by different genes and have more than one isoform (112, 113, 122, 123, 130, 135). The V0 sector contains subunits encoded by different genes and have more than one isoform (112, 113, 122, 123, 130, 135). These include subunits B, C, E, G, a, and d. Subunit B has two isoforms: the B1 isoform, originally called the kidney isoform of the 56-kDa subunit, and the B2 isoform, originally called the brain isoform of the 56-kDa subunit (83, 103). Two isoforms are also known for the C, E, and d subunits. C1 is ubiquitously expressed whereas C2 has been detected in kidney, lung, and epididymis (102, 112, 123). The E1 isoform is sperm specific and located on the acrosome of spermatozoa, and the E2 isoform (originally called the 31-kDa subunit or the E subunit) is ubiquitously expressed (60, 124). Subunit d1 is also ubiquitous, whereas d2 is present in kidney.
lungs, osteoclasts, and epididymis (102, 112, 116). Three G subunit isoforms have been described: G1 is ubiquitously expressed, G2 is brain specific and G3 has been localized in the kidney and epididymis (38, 78, 102, 112, 123). Four subunit isoforms have been identified (a1, a2, a3, and a4) and show different tissue and subcellular expression (135). Subunit a1 is the ubiquitously expressed isoform (24, 98). Subunit a2 was detected in the kidney, epididymis, lung, and spleen (58, 99, 102), and subunit a3 was localized in osteoclasts (127, 128) and in the kidney (58). Subunit a4 was detected in the kidney, inner ear, and the epididymis (43, 58, 88, 102, 115, 120).

Pathophysiology Associated with V-ATPase Dysfunction

**Mutations of genes coding for subunits of the V-ATPase.** The functional importance of the V-ATPase in humans was revealed in patients harboring mutations of some of its subunits. Mutations of the Atp6v1b1 and Atp6v0a4 genes coding for subunits B1 and a4, respectively, induce recessive distal renal tubular acidosis (dRTA) (64, 115, 120). Impairment of net proton secretion by collecting duct intercalated cells was proposed to be the leading cause of the disease. The exact mechanisms by which these mutations cause inhibition of the V-ATPase are still unknown. A recent study using a rat inner medullary collecting duct cell line indicated that the point load. Interestingly, the absence of B1 induced a concomitant increase in the apical membrane expression of the B2 isoform in medullary collecting duct intercalated cells (45). These results indicated that while the B1 subunit seems to be essential for maximal V-ATPase activity, the B2 subunit might partially compensate, at least in the medullary collecting duct, for the absence of functional B1 in these mice. However, compensatory mechanisms were not apparent in cortical collecting ducts, where intercalated cells exhibited a marked reduction in their rate of V-ATPase-dependent proton secretion (45). Because Atp6v1b1 −/− mice are fertile, it is possible that the B2 isoform, which is expressed in the apical pole of clear cells together with B1 (97), might compensate for the lack of the B1 subunit in their male reproductive tract. In contrast, in humans harboring single point mutations of the B1 isoform, the presence of this dysfunctional isoform seems to be sufficient to prevent the compensatory mechanism provided by the B2 isoform. In Atp6v1b1 −/− mice, the absence of the B1 subunit seems to allow for the assembly of the B2 isoform into the V-ATPase complex, at least in medullary collecting ducts.

**Inhibition of V-ATPase by the heavy metal cadmium.** The effect of environmental pollutants on various aspects of cellular

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<th>Subunit Name Abbreviations</th>
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V-ATPase, vacuolar H+-ATPase.
larity functions, including those related to the activity of the V-ATPase, has been the subject of previous studies. In particular, the heavy metal cadmium has been shown to induce damage to the kidney and male reproductive tract (68, 74, 92, 126). Cadmium is present in food and tobacco smoke, and it accumulates in the environment due to human contamination by mining, smelting, and industrial use. Compared with several organs, the kidney and epididymis are among those that accumulate cadmium most efficiently (89, 126). Cadmium nephrotoxicity in humans and experimental animals is accompanied by impaired reabsorptive functions of the proximal tubule, in a manner similar to the acquired Fanconi syndrome (126). Cadmium toxicity in the kidney and male reproductive tract has been partially attributed to inhibition of the V-ATPase. Cadmium directly inhibits the ATPase activity of the V-ATPase in brush-border membranes and endocytic vesicles isolated from the kidney, and cadmium-treated rats develop a mild systemic metabolic acidosis (52). In the male reproductive tract, cadmium toxicity is accompanied by a reduction in the size of testis, epididymis, and seminal vesicles, and a decrease in sperm concentration (68, 92). Cadmium exposure in rats results in a significant alkalinization of the luminal fluid of the epididymis (34). In addition, cadmium directly inhibits bafilomycin-sensitive ATPase activity in epididymal plasma membranes and inhibits bafilomycin-sensitive proton secretion in isolated vas deferens (53). It will be interesting to determine whether inhibition of the V-ATPase by cadmium may be linked to the oxidative effect of this heavy metal, as the activity of the V-ATPase is markedly reduced after oxidation of several of its cysteine residues (135). A significant increase in blood cadmium was observed in smokers (62), a condition known to impair male fertility (67, 140). Thus, while cadmium exerts a variety of toxic effects in cells, inhibition of the V-ATPase by this heavy metal may also participate in the pathophysiological states of the kidney and male reproductive tract that are associated with exposure to this major environmental hazard.

**Regulation of the V-ATPase**

V-ATPase-dependent proton extrusion is regulated by several mechanisms, which include 1) control of V-ATPase subunit expression in specialized cells; 2) intracellular targeting and recycling of V-ATPase-containing vesicles to and from the plasma membrane; 3) reversible dissociation of the V$_0$ and V$_1$ domains; and 4) modulation of the coupling ratio between ATP hydrolysis and proton pumping. This section will describe selected examples that illustrate the complexity of the cellular processes responsible for the regulation of the V-ATPase.

**Control of the V-ATPase-rich cell phenotype.** As mentioned above, renal collecting duct intercalated cells, epididymal clear cells, and osteoclasts all express very high levels of V-ATPase in their plasma membrane. These cells are part of the family of “mitochondria-rich” cells, which have common characteristics, including abundant mitochondria, high endocytic activity, abundant V-ATPase in intracellular vesicles and plasma membrane, and cytosolic carbonic anhydrase type II (CAII) (reviewed in Ref. 26). In the kidney, intercalated cells are divided into two groups: the proton-secreting type A intercalated cells and the bicarbonate-secreting type B intercalated cells. While type A and type B intercalated cells are both present in the cortical collecting duct, type B are rare in the outer stripe of the outer medulla and are absent from the inner stripe and the inner medulla. The marked depletion of intercalated cells that was observed in the collecting ducts of mice deficient in CAII (15) (Fig. 2) strongly suggested that this cytosolic carbonic anhydrase plays a key role in establishing or maintaining the intercalated cell phenotype. The reduction in the number of intercalated cells was more pronounced in the inner stripe of the outer medulla and the inner medulla, where almost no intercalated cells were observed, than in the cortex. The cause of this remarkable depletion of intercalated cells from CAII deficient mice is still unknown, but several possibilities exist. Is it, for example, a consequence of the chronic systemic acidosis that these animals develop, or does it result from a decrease in the intracellular concentrations of bicarbonate ions and protons secondary to the absence of cytosolic CAII? To help answer these questions, adult rats were treated chronically with the carbonic anhydrase inhibitor acetazolamide, using osmotic minipumps (6). A significant decrease in the number of type B intercalated cells with a corresponding increase in the number of type A intercalated cells was observed in cortical collecting ducts, while type A intercalated cells increased in number and appeared hyperplastic in the inner stripe of the outer medulla. Significant increases in the length of V-ATPase-
labeled apical membrane and AE1-labeled basolateral membranes of type A intercalated cells were measured in the acetazolamide-treated group, indicating that these cells were more active (Fig. 3). These results suggested that inhibition of carbonic anhydrase triggered a series of events within the collecting duct that would tend to increase net proton secretion, i.e., a decrease in the number of type B intercalated cells in the cortex, and increase in the number of more active type A intercalated cells in the outer medulla. In contrast, type A intercalated cells were less numerous in the inner medullary collecting ducts of treated animals. Several previous studies had shown that the collecting duct undergoes a significant morphological adaptation to systemic acid/base disturbances. Metabolic acidosis led to activation of type A intercalated cells and decreased activity of type B intercalated cells (8, 9, 75, 106, 109, 132), and metabolic alkalosis triggered the opposite response (8, 9, 75, 106, 132, 133). In view of these results, how might carbonic anhydrase inhibition alter the phenotypes of intercalated cells? Acetazolamide treatment induces systemic acidosis, but this effect is only transient (51, 76). Thus, if initial acidosis was the cause of the “activation” of type A intercalated cells on acetazolamide treatment, these cells would have to remain “activated” even after 2–4 wk, at which time a normal systemic acid/base status is reestablished (51, 76). Alternatively, the continued inhibition of carbonic anhydrase at the intracellular level might have been responsible for the establishment of the activated type A intercalated cell phenotype. Lack of cytosolic CAII activity would result in reduced production of intracellular protons and bicarbonate ions. Whether these factors would trigger the activated type A intercalated cell phenotype (and “inhibition” of the type B intercalated cell phenotype) still remains to be elucidated. In fact, it seems paradoxical that type A intercalated cells would develop morphological characteristics compatible with a higher rate of proton secretion (more extensive V-ATPase-labeled apical membrane domain and AE1-labeled basolateral membrane domain), under conditions where one of the key components of net acidification, CAII, is inhibited. However, this might also represent the response required to reestablish systemic acid-base balance on chronic carbonic anhydrase inhibition. For example, activation of type A intercalated cells might be the consequence of alterations in the transport capacity of kidney tubules located upstream of the collecting duct. Acetazolamide inhibits carbonic anhydrases other than CAII. Membrane-bound CAIV and cytosolic CAII are important players in bicarbonate reabsorption in the proximal tubules (44, 59, 129). Could an increased delivery of bicarbonate into the lumen of collecting ducts due to impaired proximal tubule bicarbonate reabsorption upon acetazolamide treatment be responsible for the activation of downstream intercalated cells? Further studies will be required to answer this question.

The presence of numerous and activated type A intercalated cells in rats treated chronically with acetazolamide was in apparent contradiction to the marked depletion of intercalated cells that was observed in CAII-deficient mice. These results might indicate a more general role for CAII in regulating the intercalated cell phenotype. In agreement with this notion was the discovery that CAII forms a “transport metabolon” by interacting directly with acid/base transporters, including the anion exchanger AE1, the sodium bicarbonate cotransporter NBC1, and the sodium-hydrogen exchanger NHE1 (77, 118, 119). Thus, in addition to providing proton and bicarbonate ions from the hydration of CO2 within intercalated cells, and other CAII-expressing cells, CAII might play a more general, regulatory role for a variety of transporters. It is, therefore, possible that the absence of this regulatory protein might have more profound consequences to the cell than the simple inhibition of its enzymatic activity by acetazolamide. Newborn CAII-deficient mice have both type A and B intercalated cells in their kidneys (Breton S, Alper S, and Brown D, unpublished observations). Thus the disappearance of intercalated cells is the result of a progressive process that occurs after birth. Further studies will be required to determine whether intercalated cell depletion occurs following the systemic acidosis that the mice quickly develop after birth, or is the consequence of the absence of CAII as a regulatory protein for the function of other acid/base transporters that are critical to the generation and maintenance of the intercalated cells phenotype.
Targeting and recycling of the V-ATPase. Previous studies, mainly in yeast and osteoclasts have shown that the targeting of the V-ATPase is controlled by the assembly of different subunit isoforms into the holoenzyme (63, 66, 127, 135). In kidney and epididymal V-ATPase-rich cells, the a2 isoform is generally expressed in intracellular structures and the a4 isoform is located in the plasma membrane (58, 102). Coimmunoprecipitation assays from kidney extracts demonstrated that isoforms a1, a2, and a4 all bind to the B2 isoform, while only a4 can associate with B1 (122). This might explain the predominant localization of the B1 isoform in the plasma membrane (29, 30) and the B2 expression in subapical vesicles (97) in kidney intercalated cells and epididymal clear cells.

In acidifying mammalian V-ATPase-rich cells, proton secretion is regulated via recycling of V-ATPase-containing vesicles to and from the plasma membrane (1, 9, 20, 25, 26, 36, 75, 95, 110). An increase in V-ATPase plasma membrane expression closely correlates with an increase in proton secretion. In kidney intercalated cells, systemic acidosis causes the accumulation of V-ATPase in the apical membrane of type A intercalated cells, and conversely, alkalosis induces the retrieval of V-ATPase molecules from the membrane via endocytosis. While these processes were described a while ago, the molecular entities responsible for this response are still largely unknown. V-ATPase-containing vesicles possess an extensive cytoplasmic coat that is devoid of clathrin (28, 31) and caveolin (29, 30) and the B2 expression in subapical vesicles (97) in kidney intercalated cells and epididymal clear cells.

While these processes were described a while ago, the molecular entities responsible for this response are still largely unknown. V-ATPase-containing vesicles possess an extensive cytoplasmic coat that is devoid of clathrin (28, 31) and caveolin-1 (19) but consists mainly of the V-ATPase subunits themselves (26). Therefore, it appears that the V-ATPase relies on clathrin- and caveolin-independent mechanisms for the regulation of its recycling. Is the V-ATPase involved in its own trafficking/recycling? Interestingly, some of its subunits are homologous to the family of so-called soluble N-ethyl-maleimide-sensitive fusion protein attachment protein (SNAP) receptor (SNARE) proteins. Subunits a and c of the V-ATPase interact with synaptobrevin and synaptophysin on synaptic vesicles (47). The V-ATPase also associates with syntaxin 1A and SNAP23 in inner medullary collecting duct cultured cells (7, 70, 84). Another member of the SNARE family, cellubrevin, is highly expressed in epididymal clear cells (20) and kidney intercalated cells (18), and cleavage of cellubrevin by tetanus toxin significantly inhibits bafilomycin-dependent net proton secretion in clear cells of the male reproductive tract (20). SNAP23 is also highly expressed in clear cells, where it partially colocalizes with the V-ATPase in the apical pole (41), but its participation in proton secretion has not yet been demonstrated.

Another indication that the V-ATPase may be part of the trafficking machinery that is involved in its own targeting, or the targeting of other proteins, was provided by a study showing that the V0 domain of the V-ATPase may be involved in membrane fusion (100). In addition, subunit H interacts with the AP-2 adaptor protein involved in clathrin-mediated endocytosis and the Nef protein, which mediates internalization of human immunodeficiency virus via its receptor CD4 (48, 79). More recently, a role for the V0 domain of the V-ATPase in sensing the pH of endosomes and recruiting “coat” proteins was described (58). Subunit c interacts with Arf6 and isoform a2 interacts with ARNO (ADP-riboseylation factor nucleotide site opener), which acts as a GDP-GTP exchange factor (GEF) for Arf6. Recruitment of these proteins to endosomes strongly depends on V-ATPase-induced endosomal acidification. Arf6 and ARNO are involved in vesicle coat formation and regulate endocytosis (40, 42, 91, 101). In proximal tubules, disturbance of V-ATPase-ARNO-Arf6 interactions significantly impairs the degradative pathway by preventing the delivery of endocytosed proteins from early endosomes to late endosomes (58).

This study identified a novel function for the V-ATPase as a pH sensor that enables recruitment of cytosolic proteins and subsequent trafficking processes in the endosomal degradative pathway. Whether the pH-dependent recruitment of small G proteins is specific for the a2 isoform, or whether the other isoforms, a1, a3, and a4, might also participate in this process, perhaps at the level of other intracellular organelles or even at the plasma membrane, will require further studies.

Role of soluble adenyl cyclase in V-ATPase recycling. To identify the sensor responsible for the response of proton-secreting cells to acid/base variations of their extracellular environment, the male reproductive tract was used as a model system. This tissue allows for the study of V-ATPase surface expression and its regulation by luminal pH and bicarbonate by using in vivo perfusion techniques. V-ATPase apical membrane expression in clear cells is strongly dependent on alkalinization of the epididymal lumen to levels above its physiologic acid pH of 6.6 (95). Addition of bicarbonate or a permeant cAMP analog into the luminal perfusate also induces a marked increase in the apical accumulation of V-ATPase, together with a significant reduction in the endocytosis of V-ATPase from the cell surface. We have also shown that soluble adenyl cyclase (sAC) is highly expressed in clear cells (95). sAC is a chemoceptor that mediates bicarbonate-dependent elevation of cAMP (37). Interestingly, inhibition of sAC abolished the pH- and bicarbonate-induced apical accumulation of the V-ATPase, indicating that this enzyme serves as a bicarbonate sensor responsible for the clear cell response. We proposed that clear cells have the ability to respond to rises in luminal bicarbonate concentration by increasing their rate of proton secretion, following bicarbonate-induced sAC activation and cAMP production. This response would help to reestablish the luminal bicarbonate concentration (and low pH value) to its resting low value. A candidate protein responsible for the apical entry of bicarbonate is the sodium-bicarbonate cotransporter NBC3, which is expressed in the apical membrane of intercalated cells (105) and clear cells (104). Because sAC is also expressed in other proton-secreting cells, including kidney thick ascending limbs, distal tubules, and intercalated cells (95), we propose that this enzyme may represent a common sensor by which acidifying cells can sense and modulate the pH of their environment. For example, it is possible that the activation of intercalated cells that we observed upon chronic acetazolamide treatment (6) was the consequence of higher luminal delivery of bicarbonate due to impairment of proximal tubule reabsorption, followed by activation of sAC and subsequent elevation of intracellular cAMP in these cells. In addition, sAC might play a role in the response of intercalated cells to systemic CO2 concentrations. Schwartz and Al-Awqati (108) previously showed that CO2 induces the exocytosis of V-ATPase-containing vesicles in intercalated cells from collecting ducts perfused in vitro. Because an entry of CO2 into the cells would favor the formation of intracellular bicarbonate, this effect might have been secondary to activation of sAC, followed by cAMP elevation.
Role of the actin cytoskeleton in V-ATPase recycling. Another important component of the cell that mediates V-ATPase recycling is the cytoskeleton. Microtubule disruption induces a marked loss of V-ATPase polarity together with the appearance of numerous V-ATPase-containing vesicles scattered throughout the cytoplasm (16, 20, 32). The actin cytoskeleton also regulates the subcellular localization of the V-ATPase. Actin filaments are very dynamic structures, and their role in modulating membrane protein trafficking is complex and depends on the cell type or protein examined (reviewed in Refs. 2 and 56). Modulation of the actin cytoskeleton by gelsolin was shown to control the apical membrane expression of the V-ATPase in clear cells (10). Gelsolin is an actin-capping and -severing protein that is abundantly expressed in clear cells, intercalated cells, and osteoclasts (10, 12, 73). The severing activity of gelsolin is strongly dependent on calcium (61, 139). BAPTA-AM and the PLC inhibitor U-73122 were also shown to abolish the pH-induced apical accumulation of the V-ATPase (10). We postulated that maintenance of the actin cytoskeleton in a depolymerized state by gelsolin facilitates calcium-dependent accumulation of the V-ATPase in response to luminal pH alkalization.

Interestingly, some subunits of the V-ATPase can interact either indirectly (subunit B1) (23) or directly (B1, B2, and C subunits) (36, 57, 134) with the actin cytoskeleton. The V-ATPase binds to F-actin but not G-actin, and increased interaction between the V-ATPase and actin coincides with internalization of the pump in osteoclasts (36). Interaction of the B1 subunit with the PDZ protein NHERF1 might play a role in stabilizing the holoenzyme in the basolateral membrane of type B intercalated cells, where the V-ATPase colocalizes with NHERF1 (23). Whether direct interaction between V-ATPase and actin is modulated in intercalated cells and clear cells and might regulate the recycling of the pump still remains to be investigated.

Association/dissociation of the V-ATPase. The reversible assembly/disassembly of the V-ATPase holoenzyme has been mainly studied in yeast. Upon glucose deprivation, inactivation of the V-ATPase occurs in Saccharomyces cerevisiae via disassembly of the V1 from the V0 domain (reviewed in Refs. 63, 65, and 85). After dissociation, the free V1 complex remains stable in the cytosol and competent for rapid reassembly with the V0 domain on readdition of glucose. This mechanism ensures a concerted balance between cytosolic ATP levels and the pH homeostasis of intracellular structures. While these studies showed that dissociated V0 and V1 domains of the V-ATPase are in dynamic equilibrium with the fully assembled holoenzyme, the mechanisms responsible for glucose-dependent assembly of the pump remain largely unknown. None of the known glucose-induced signaling pathways are involved in the disassembly of the V-ATPase in yeast, indicating the participation of an unconventional glucose-deprivation mechanism (93). Glucose-induced activation of the V-ATPase was shown to be modulated by protein kinase C and calcium-calmodulin-dependent protein kinases (14). Assembly/disassembly of the V-ATPase complex was also shown in Manduca sexta (reviewed in Ref. 63) and more recently in renal epithelial cells (107).

In mammalian cells, some of the V-ATPase subunits interact with enzymes of the glycolytic pathway including aldolase (71, 72) and phosphofructokinase-1 (121). Interestingly, glucose stimulates V-ATPase-dependent acidification of intracellular compartments in the kidney cell lines HK-2 and LLC-PK1 via activation of the phosphatidylinositol 3-kinase (PI3K) pathway (107). In osteoclasts, the V-ATPase colocalizes with PI3K (80), and inhibition of PI3K by wortmannin abolishes bone resorption (81), further indicating that PI3K might be involved in the regulation of the V-ATPase in mammalian cells. In addition, glucose deprivation in HK-2 cells induced the dissociation of the V1 from the V0 domain of the V-ATPase, in a manner similar to that seen in yeast (107). However, in contrast to yeast, the regulation of V-ATPase by glucose requires PI3K, and expression of constitutively active PI3K under glucose-free conditions mimics the effect induced by glucose. The factors linking glucose levels and PI3K to regulate the V-ATPase are still unknown. Interaction between subunit B of the V-ATPase and F-actin is dependent on active PI3K (36), and the PI3K signaling pathway modulates the actin cytoskeleton via the small GTPase Rac (131). Therefore, it was proposed that the regulation of V-ATPase by PI3K might take place via modulation of actin filaments (107).

The role of V-ATPase subunits themselves in regulating the assembly of the holoenzyme has been studied extensively in yeast (39, 66, 85, 111). Deletion of any of the V-ATPase subunits, except subunit H, results in the loss of assembly of the V1 and V0 domains. However, while assembly can occur in the absence of subunit H, the assembled V-ATPase is inactive (55) (reviewed in Refs. 63 and 85). The G subunit associates closely with E and, together with subunits C and H and the soluble part of subunit a, forms the peripheral stalk that connects the V1 and V0 domains (3, 4, 87, 137). Several point mutations of subunit G have been shown to increase the stability of the holoenzyme and to partially block glucose-dependent dissociation of the V1 and V0 domains in yeast (35).

Control of coupling between ATP hydrolysis and proton-pumping activity. In yeast, various subunits of the V-ATPase, including subunits a, d, A, and C, control the activity of the V-ATPase by modulating the coupling between ATP hydrolysis and proton pumping (39, 63, 65, 66, 86, 90, 111). Whereas mutations of subunit C cause destabilization of the V-ATPase, the rates of ATPase activity and proton transport by the holoenzymes that remain assembled are significantly increased (39). Subunit D has also been identified as a regulator of the coupling between proton transport and ATPase activity (136). Subunit H inhibits the ATPase activity of the dissociated V1 domain, thereby preventing its nonproductive ATP hydrolysis (94). Whether the differential associations of specific subunit isoforms in different intracellular compartments of mammalian cells, including kidney intercalated cells and epididymal clear cells, can modulate the activity of the V-ATPase at these different locations will require further investigation.

Conclusion

The complexity of the V-ATPase holoenzyme, which is composed of several subunits, some having more than one isoform, may reflect the need for differential regulation of the various functions that the enzyme performs in a variety of cell types and subcellular locations. Specific targeting of the pump to distinct membrane domains and intracellular organelles, trafficking and recycling of the holoenzyme, dissociation-association of the two domains of the pump, and coupling
efficiency of proton-pumping activity are complex processes that are being actively studied and might be governed by the assembly of a specific set of subunit isoforms and accessory proteins. Interestingly, the participation of the V-ATPase in functions other than proton pumping across membranes is slowly emerging in a variety of cell types, including kidney epithelial cells.

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