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Recent Insights into the Structure, Regulation and Function of the V-ATPases

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Abstract

The V-ATPases are ATP-dependent proton pumps that acidify intracellular compartments and are also present at the plasma membrane. They function in such processes as membrane traffic, protein degradation, viral and toxin entry, bone resorption, pH homeostasis and tumor cell invasion. V-ATPases are large, multi-subunit complexes composed of an ATP-hydrolytic domain (V_1) and proton translocation domain (V_0) and operate by a rotary mechanism. This review focuses on recent insights into their structure and mechanism, the mechanisms that regulate V-ATPase activity (particularly regulated assembly and trafficking) and the role of V-ATPases in such processes as cell signaling and cancer. These developments have highlighted the potential of V-ATPases as a therapeutic target for a variety of human diseases.

Keywords

V-ATPase; acidification; proton transport; membrane traffic; cell signaling; cancer

The V-ATPases: Background on their structure, function and regulation

The vacuolar (H^+)-ATPases (V-ATPases) are ATP-driven proton pumps present in a variety of cellular membranes [1–5]. Within intracellular membranes, V-ATPases function in membrane trafficking processes such as receptor-mediated endocytosis and intracellular trafficking of lysosomal enzymes [1]. Acidification of endocytic compartments by the V-ATPases signals the release of internalized ligands (such as low density liporotein) from their receptors and allows these receptors to recycle to the cell surface. The released ligands

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are targeted to the lysosome, where the low pH facilitates their degradation by cathepsins. Acidification of endosomes is also involved in the vesicle budding that moves ligands along the endocytic pathway [1]. Lysosomal enzymes bound to mannose-6-phosphate receptors in the trans Golgi network similarly transit endocytic compartments, where the low pH causes their dissociation from the receptors and their subsequent targeting to lysosomes [1]. Thus, V-ATPase-dependent acidification plays an important signaling role in the cell. Various pathogenic agents, including envelope viruses like influenza and Ebola, and toxins from such organisms as diphtheria and anthrax, take advantage of this acidic endosomal environment to activate their entry into the cytoplasm of an infected cell [1]. Secretory pathway vesicles are also acidified by V-ATPases, facilitating the processing of prohormones like proinsulin and the packaging of small molecules, such as the neurotransmitter glutamate.

V-ATPases are also present at the plasma membrane of various cell types, including osteoclasts, renal intercalated cells and epididymal clear cells [3]. Osteoclasts employ plasma membrane V-ATPases to acidify the extracellular space between the cell and the bone, which is essential for bone resorption [4]. In the kidney, V-ATPases at the apical membrane of alpha intercalated cells secrete acid into the renal tubule, thus functioning in pH homeostasis, [3] whereas in the epididymus, they create a low luminal pH necessary for sperm maturation.

This review focuses on recent developments in the field of the V-ATPases, beginning with advances in our understanding of its structure and mechanism. Earlier work (including that described in the above paragraphs), is extensively discussed in references 1-5. While it has been known for some time that the V-ATPases are large, multi-subunit complexes composed of a peripheral V_1 domain that hydrolyzes ATP and an integral V_0 domain that translocates protons, and that they operate by a rotary mechanism, significant new insights have emerged from recent high-resolution studies. A second major topic that we review is the molecular mechanisms employed to regulate V-ATPase activity in cells, chief amongst which are regulated assembly of the V_1 and V_0 domains and regulated trafficking of V-ATPases, particularly to the plasma membrane of cells. The final section of this review focuses on recent insights into emerging functions of V-ATPases, including their role in cancer cell survival and metastasis, control of cell signaling pathways and other novel functions.

Structure and mechanism of V-ATPases

The V-ATPase is composed of fourteen subunits organized into two domains: the cytosolic V_1 domain and the integral V_0 domain [1](Fig. 1). V_1 is composed of subunits A-H and is responsible for ATP hydrolysis. The V_0 domain, which carries out proton transport, is composed of subunits a, d, e, c, and c″. Yeast contain an additional subunit, c′, while higher eukaryotes contain the accessory protein Ac45. The V-ATPase utilizes a rotary mechanism to drive proton transport across cell membranes [1]. Protons are thought to enter subunit a of the V_0 domain through a cytoplasmic hemichannel and protonate one of the conserved glutamic acid residues located on each subunit of the proteolipid ring (made up of subunits c, c′, and c″). ATP hydrolysis within the V_1 domain drives rotation of the central stalk of the V-ATPase (made up of subunits D, F, and d), which in turn causes rotation of the proteolipid

ring. This brings each protonated glutamic acid residue located on each subunit of the proteolipd ring into contact with a luminal hemichannel in subunit a. Here, the proton is released into the lumen as a result of stabilization of the deprotonated glutamic acid by a critical arginine residue located in subunit a [1]. Recent structural studies of the V-ATPase and the closely related families of F1F0 ATP synthases (F-ATPases) and archaebacterial ATPases (A-ATPases) have provided novel insights into subunit interactions and mechanism of the V-ATPase.

The V_1 domain is divided into several subdomains: the A3B3 cylinder, the central stalk, and the peripheral stalks. The A3B3 subdomain is made up of alternating A and B subunits, forming a hexamer that carries out ATP hydrolysis. The 3 ATP hydrolytic sites are located at one of the two A/B interfaces, where the A subunit contributes the majority of the residues to this site [1,6]. Recent evidence from crystallization of the *Thermus thermophilus* A3B3 complex suggests that hydrophobic clusters within this region are critical for ATP hydrolysis, possibly to facilitate the transition between open and closed catalytic sites [6]. Furthermore, electron cryomicroscopy (cryo-EM) of the yeast V-ATPase has visualized three different rotational states of the enzyme [7]. Energy from ATP hydrolysis is then coupled to central stalk movement, which serves as a rotor for the complex. The central stalk is composed of single copies of subunits D, F, and d. Subunit d is present on top of the proteolipid ring, providing the connection between the ring and the central stalk in V_1 [1]. X-ray analysis of the DF complex from *Enterococcus hirae* revealed a β-hairpin region of subunit D that may interact with the A3B3 subdomain to increase the rate of ATP hydrolysis [8]. Subunit F binds to subunit D through its C-terminal region, an interaction required for binding to subunit d.

It has previously been observed that upon reversible dissociation of the V_1 and V_0 domains (an important mechanism of regulating V-ATPase activity, as discussed later), the ATPase activity of V_1 is inhibited. Moreover, it was shown that subunit H is essential for this inhibition [9]. Based upon the observed cross-linking between subunit H in the i peripheral stalk and subunit F in the central stalk in isolated V_1, but not intact V_1V_0, it i was proposed that subunit H inhibited ATP hydrolysis by free V_1 by interacting with subunit F in the central rotor and preventing rotation [10]. This model has now been supported by the low resolution structure of the yeast V-ATPase [11]. A loop region of subunit F has been proposed as the site of this interaction [12]. Nevertheless, subunit H i may also contribute to inhibition of activity by a mechanism independent of tethering [9].

The peripheral stalks are made up of a core EG heterodimer that is connected to single ; copies of the C and H subunits and the N terminus of subunit a [11,13]. Eukaryotic V-ATPases contain three EG heterodimers [11,13], bacterial V-ATPases and A-ATPases i each contain two [14], and F-ATPases contain a single homo- or heterodimer [15]. The peripheral stalks serve to tether the A_3B_3 hexamer to the N terminus of subunit a, and prevent rotation of the stator during ATP hydrolysis [1,13]. Both the H and C subunits are composed of two globular domains connected by a flexible linker, and together with the N terminus of subunit a, form a collar at the interface of V_1 and V_0. The C subunit i contacts two of the three EG heterodimers, one through its head domain and the other through its foot domain [16]. The head domain of subunit C forms a high affinity interaction with one of the EG heterodimers

[16,17] while the foot domain binds the N terminus of subunit a and a second EG heterodimer with lower affinity [18,19]. The H subunit also contacts one of the EG heterodimers and the N terminus of subunit a i [11,19]. Importantly, these contacts between V_1 and V_0 must be broken during *in vivo* dissociation of the complex (discussed later), such that subunit C is released from both domains. A unique bulge in the coiled-coil region of the G subunit may aid in this dissociation by spring-loading the EG/C interaction to facilitate release *in vivo* [17]. Cryo-EM images of the *Manduca sexta* V_1 domain suggest that the peripheral stalks maintain considerable rigidity after dissociation of V_1 and V_0 [20].

Recently the N terminus of subunit I of *Meiothermus ruber*, homologous to subunit a, was crystallized [21] and residues critical for V_1/V_0 interaction in the yeast subunit a were identified [22]. The N terminus of subunit a is essential for V-ATPase function as it serves to tether V_1 to the membrane and must withstand the torque generated by V_1 during rotor movement. Additionally, this region contains the information necessary for correct targeting of V-ATPases to various cellular destinations [23]. Subunit a exists as two isoforms in yeast (Vphlp and Stvlp) and four isoforms in mammalian cells (a1-a4) [1]. Vphlp targets the pump to the vacuole while Stvlp targets it to the Golgi. In mammals, a1 and a2 are primarily intracellular (a1 has been identified in synaptic vesicles while a2 is present in endosomes), whereas a3 and a4 are capable of targeting V-ATPases to the plasma membrane of osteoclasts and renal intercalated cells, respectively [1,3,4].

The C-terminal domain of subunit a is composed of eight transmembrane helices (TMs) [24,25] and is thought to form proton conducting hemichannels that allow protons to reach and leave the buried carboxylate side chains on the proteolipid ring [1]. Mutagenesis data suggest that TM3, TM4 and TM7 comprise the luminal hemichannel while TM7 and TM8 make up the cytoplasmic hemichannel [24]. Interestingly, residues on TM7 of subunit a involved in forming the luminal hemichannel are located on the same helical face as residues involved in forming the cytoplasmic hemichannel, suggesting that there is helical swiveling of critical TMs in subunit a during proton transport [24]. Recent studies of the bacterial F-ATPase support the importance of helical swiveling in proton transport [26]. Interestingly, all of the specific V-ATPase inhibitors that have thus far been identified bind to the proteolipid ring of the V_0 domain, including bafilomycin and concanamycin [27,28], archazolid [29], apicularen [30] and PA1b, the first peptide inhibitor, whose binding site appears to also include subunit e, and whose specificity suggests it as a promising insecticide [31]. Many of these inhibitors may work by intercalating into the proteolipid ring and preventing helical swiveling required for proton transport [24,32]. The recent cryo-EM structure of the yeast V-ATPase has shown that the key transmembrane helices in subunit a that participate in proton translocation are oriented with a high degree of tilt with respect to the plane of the membrane [7]. Similar observations have been made for the F-type ATPase from Polytomella [33]. This suggests that interpretation of the results supporting the role of helical swiveling in proton transport may need to be modified to accommodate this novel structure of subunit a. Structural studies of the V-ATPase have thus provided information into how this complex functions as a rotary motor in proton transport, and may also provide insights relevant to how V-ATPase activity is regulated.

Regulation of V-ATPase activity

Regulated assembly of the V-ATPase

An important mechanism of regulating V-ATPase activity in response to various stimuli is control of assembly of the V_1 and V_0 domains (Fig.2). In yeast, dissociation of the complex occurs rapidly and reversibly in response to glucose depletion and does not require new protein synthesis [2]. While assembly has most often been measured by immunoprecipitation and Western blot or by cell fractionation, immunocytochemical studies using fluorescently tagged subunits suggest that the C subunit of the V_1 domain may be the only subunit to completely dissociate from the complex *in vivo* [34]. This likely results in dissociation of the remainder of the V_1 domain from V_0 following cell disruption and fractionation since the holoenzyme is destabilized by the loss of any subunit.

Multiple factors have been shown to be important in controlling V-ATPase assembly in yeast. Activation of the Ras/cyclic AMP (cAMP)/protein kinase A (PKA) pathway, which normally occurs upon readdition of glucose to glucose-starved yeast, was shown to increase V-ATPase assembly [35]. Disassembly is prevented at a neutral extracellular pH, possibly due to the requirement for functional V-ATPases to maintain acidic vacuoles under these conditions [36]. Assembly has also been suggested to be under control of cytosolic pH, with elevated cytosolic pH leading to increased V-ATPase assembly [37]. Because increased cytosolic pH was also shown to lead to increased PKA activity, a positive feedback loop between PKA and V-ATPase assembly may occur in yeast.

Interaction with either of two glycolytic enzymes, aldolase or phosphofructokinase, appears to enhance V-ATPase assembly in yeast [38,39]. In the case of aldolase, catalytic activity is unnecessary, but direct interaction with subunits of both the V_1 and V_0 domains is required for assembly to occur. In the case of phosphofructokinase, the effect is partial and appears to involve another V-ATPase assembly factor, RAVE (Regulator of the ATPase of Vacuolar and Endosomal membranes). RAVE is a heterotrimeric complex that interacts with the V_1 domain of the V-ATPase and stabilizes it in an assembly competent state [2]. Interestingly, RAVE preferentially binds to the vacuolar a subunit isoform (Vphlp) and appears to be involved in assembly of only complexes that are targeted to the vacuole, but not those that are targeted to the Golgi [40]. In higher eukaryotes, rabconnectin 3 may play a similar role [41-43]. Salt stress also regulates V-ATPase assembly, and binding of the signaling lipid PI (3,5) P2 to the N-terminal domain of Vphlp is important in controlling this process [44].

Regulated assembly of the V-ATPase is not restricted to yeast, but occurs in higher eukaryotes as well. In insect cells, assembly of the V-ATPase is under control of PKA, although PKA-independent assembly has also been demonstrated [45]. Upon maturation of dendritic cells, increased assembly of the V-ATPase on lysosomes promotes the low pH required for antigen processing [46]. This increased assembly was recently shown to occur upon induction of a semi-mature state of dendritic cells associated with immune tolerance [47]. In this case, phosphatidylinositol-3-kinase (PI-3 kinase) and mechanistic target of rapamycin (mTOR) were shown to be required for assembly. PI-3 kinase, along with extracellular signal-regulated kinase (ERK), has also been shown to activate V-ATPase assembly following infection of cells with influenza virus [48]. The resulting enhanced

endosomal acidification aids in entry of the virus through hemagglutinin (HA)-mediated pore formation. Glucose regulated assembly in mammalian cells is also PI-3 kinase dependent [49], and may function to rid the cell of the additional metabolic acid generated by higher rates of glycolysis. EGF (epidermal growth factor) stimulation of V-ATPase assembly [50] is thought to be important in stimulating protein degradation in lysosomes, thus providing adequate levels of amino acids necessary for stimulation of mTORC1 (see later)..

Regulated trafficking of the V-ATPase

A second major mechanism of controlling V-ATPase activity is through regulating trafficking. This is particularly important in controlling proton secretion by epithelial cells, including those in the kidney and epididymus. Alpha intercalated cells in the collecting duct of the kidney respond to a low cytoplasmic pH by increasing proton secretion across the apical membrane [3]. This is accomplished by fusion of intracellular V-ATPase-containing vesicles with the apical membrane, thus increasing pump density at the cell surface. This increase is mediated by a bicarbonate-dependent, soluble adenylate cyclase which, in response to decreased cytoplasmic pH, increases cAMP [51]. Increased cAMP levels in turn increase PKA activity, which has been shown to directly phosphorylate subunit A of the V-ATPase and result in apical localization of the pump [52]. AMP kinase (AMPK) also regulates V-ATPase assembly by phosphorylation of the A subunit (albeit at a different site), with increased AMPK activity leading to decreased proton pumping [53]. V-ATPase trafficking is also regulated by PKA and AMPK in proximal tubular cells of the kidney [54].

In epididymal clear cells, the V-ATPase functions in sperm maturation. As in the kidney, activation of a soluble, bicarbonate-sensitive adenylate cyclase leads to elevated cAMP, PKA activity and translocation of pumps to the apical membrane [3]. The actin cytoskeleton appears to be important in this process as actin depolymerization with inhibitors of RhoA/ROCK II leads to increased apical V-ATPase [55]. In addition, luminal acidification is under control of aldosterone, which is known to regulate sodium reabsorption and proton secretion in the kidney [56].

Other regulatory mechanisms

There is also evidence for direct modulation of V-ATPase activity without changes in V-ATPase assembly or localization. The Cdc42 effector Ste20, which regulates filamentous growth in yeast in response to nutrient depletion, modulates V-ATPase activity through direct interaction with the H subunit of V_1 [57]. In mammalian cells, the heme-binding protein HRG-1, which regulates endocytosis and motility, was shown to enhance V-ATPase activity without altering assembly [58]. Prohormone processing may in part be controlled by regulation of V-ATPase activity through association with the accessory subunit Ac45 [59]. Interestingly, the intracellular parasite Legionella pneumophila secretes a factor (SidK) which inhibits the host V-ATPase present in the host phagosome to ensure a neutral phagosomal environment for the pathogen [60]. Regulation of expression of V-ATPase genes is at least partly under control of TFEB, which is in turn controlled by mTORC1 [61]. Regulation of V-ATPase activity is thus emerging as a highly complex process that employs

a variety of disparate mechanisms, in keeping with the diversity of cellular functions served by V-ATPases.

Emerging functions of V-ATPases

Intracellular signaling

The V-ATPase has important roles in intracellular signaling. Both the Wnt and Notch pathways rely upon V-ATPases for full activation across multiple organisms and cell types (Fig.3). In both pathways, trafficking and processing of cell surface receptors requires an active and intact V-ATPase. Specifically, in the case of canonical Wnt signaling, the cell surface Wnt receptor LRP6 (low density lipoprotein receptor-related protein 6) cannot be activated when the V-ATPase is inhibited pharmacologically or genetically. Therefore, inhibition of V-ATPase activity blocks Wnt-induced gene transcription during development [62-65]. Inhibiting the V-ATPase also blocks the non-canonical branch of Wnt signaling, which controls planar cell polarity, as demonstrated during *Drosophila* development [65]. In Notch signaling, proper Notch receptor processing, which releases the transcriptionally active Notch intracellular domain (NICD), requires V-ATPase function [66–69]. After ligand binding at the cell surface, the Notch receptor undergoes successive cleavage events that release the NICD and allow its translocation to the nucleus. When the V-ATPase is perturbed (genetically, [66,67] chemically [69] or by alteration of a regulator [68]) Notch cleavage by γ-secretase in endosomes, which is the final step in NICD release, is greatly diminished. This effect is conserved and observed *in vivo* in invertebrates [66,67,69] and vertebrates, [68,69] as well as in human cell lines derived from normal and cancerous tissues [69].

In contrast to signaling that primarily relies on V-ATPase-generated proton gradients, the pump directly contributes, by an as yet undefined mechanism, to the complex signaling of metabolic control. It was found to be critical for sensing of amino acids and subsequent activation of mTOR complex 1 (mTORC1) [70,71](Fig.3). Many signals relaying cellular status are integrated by mTORC1 to properly coordinate cellular growth with nutrient and energy availability. Activation of mTORC1 cannot occur without amino acids. Therefore, blocking this input blocks positive growth signals from multiple pathways and in multiple contexts [70-73]. Amino acids stimulate recruitment of mTORC1 to the lysosomal surface, where its direct activator, Rheb, is localized. This recruitment is carried out by the RagGTPases, a family of four GTPases that are related to Ras. The RagGTPases are themselves held at the lysosome by a heteropentameric complex termed the Ragulator [74]. The Ragulator undergoes amino acid-dependent associations with the V-ATPase [70]. During amino acid starvation, association with the V_1 domain is strengthened, as demonstrated by co-IP experiments [70]. V-ATPase inhibition blocks amino acid signal transmission to the Ragulator, which can be rescued by expression of constitutively active Rags [70]. Further work discovered that the Ragulator and V-ATPase are also critical for activation of AMPK, another important node of cellular metabolic control in response to energy stress [75]. This finding establishes the V-ATPase as an essential component of the switch between anabolic and catabolic processes in the cell.

Cancer

Recent work has supported the concept that the V-ATPases play critical roles in cancer cells (Fig.4). Overexpression of V-ATPase subunits has been observed in various cancer cell lines and tumor samples [76-89]. Furthermore, a number of studies have found that the V-ATPase is expressed at the plasma membrane of invasive cancer cells, including breast, prostate, pancreatic, liver, lung, ovarian, and esophageal cancers as well as melanoma and Ewing sarcoma [76,78,79,82–84,86,87,90–93].

Tumor cells are more sensitive to V-ATPase-inhibition-dependent cell death than non-tumor cells, [94] and loss of V-ATPase activity reduces cell growth in a number of cancers, likely because of the important role the plays in regulating cytoplasmic pH [83,85,89,91]. Whereas cancer cell apoptosis and anoikis occurs after extended inhibition of V-ATPase activity [94–97], shorter treatment with V-ATPase inhibitors induces a cellular stress response and autophagy [94]. One study has found that intracellular acidosis caused by V-ATPase inhibition in breast cancer cells stabilizes expression of the pro-apoptotic protein Bnip3, resulting in cell death [95]. It has also recently been demonstrated that treatment of breast cancer cells with the V-ATPase inhibitor archazolid disrupts internalization of the transferrin receptor, leading to iron deprivation and subsequent apoptosis [98]. Further work is needed to better elucidate how the pump is involved in cell growth and survival. The V-ATPase has also been implicated in promoting drug resistance, possibly by generating pH gradients across intracellular or plasma membranes that drive drug transport. Specifically, V-ATPase expression is correlated with drug resistance in human lung cancer samples and its inhibition restores drug sensitivity in colon cancer cell lines [86,99].

Loss of V-ATPase activity reduces both *in vitro* invasion and migration of a variety of cancer cell lines and reduces *in vivo* breast cancer and melanoma metastasis [76,78,79,84,85,87,89,91,92,95,96,100]. Specific ablation of plasma membrane V-ATPase activity is sufficient to reduce *in vitro* invasion and migration of invasive breast cancer cells, suggesting that these V-ATPases are critical in the invasiveness of at least some cancers [92]. Isoforms of subunit a are likely involved in trafficking pumps to different cellular membranes, including the plasma membrane of cancer cells. The a3 isoform of subunit a, which targets V-ATPases to the plasma membrane of osteoclasts, is overexpressed in breast cancer cells and melanoma cells, and is critical for their invasiveness *in vitro* and *in vivo*, respectively [76,79,84]. Importantly, a3 overexpression in noninvasive breast cells enhances invasiveness and plasma membrane V-ATPase expression [84]. The mechanisms by which the V-ATPase contributes to migration and invasion are not fully understood. Acidification of intracellular compartments or the extracellular space may promote pH-dependent activation and activity of secreted proteases, such as cathepsins, that participate in digestion of extracellular matrix, thus aiding in invasion [78,79,101,102]. The V-ATPase may also be involved in the organization and localization of factors critical for cell motility, such as Rac1, actin and EGF Receptor [100,103]. Overall, the diverse functions of the V-ATPase in tumor survival and metastasis make it an attractive potential target in the development of anti-cancer drugs.

Other novel functions

Amongst the more novel and controversial functions proposed for the V-ATPase is membrane fusion. Homotypic vacuole fusion in yeast requires acidification of the vacuole, mediated by the V-ATPase. Mutants of the proteolipid c subunits of the V_0 domain have been identified that support acidification but are defective in vacuolar fusion, supporting an acidification-independent role of V_0 in fusion [104]. The highly hydrophobic proteolipid subunits of V_0 are proposed to form a pore that promotes lipid mixing between two membranes brought together by trans-SNARE complex formation. Similarly, an inactive mutant of the a1 subunit is able to restore synaptic vesicle release in a Drosophila strain in which a1 has been disrupted, suggesting an acidification-independent role of V_0 in synaptic vesicle fusion [105]. In fact, V_0 has been suggested to serve as a pH sensor that helps control vesicle fusion [106]. By contrast, whereas an inactive mutant of the yeast a subunit isoform Vphlp is not able to rescue homotypic vacuole fusion in a Vph1p-null yeast strain, introduction of a proton pumping pyrophosphatase from plant at least partially restored fusion, suggesting vacuolar acidification is the primary role of the V-ATPase in this system [107]. Additional studies will be required to resolve the role of V_0 in membrane fusion.

The V-ATPase appears to play additional roles in membrane trafficking. Budding of vesicles from endosomes requires, among other factors, Arf6 (a low molecular weight G protein) and cytohesin, a GTP/GDP exchange factor for Arf6. Cytohesin was recently shown to interact in a pH-dependent manner with the N terminus of subunit a of the V_0 domain, which acts as a pH sensor in controlling budding [108]. The V-ATPase was shown to play an indirect role in clathrin coated vesicle formation by its involvement in recycling of internalized cholesterol from endosomes back to the plasma membrane [109]. V-ATPase-dependent acidification is also important for correct sorting of proteins in the secretory pathway [110].

The V-ATPase has a number of additional functions in yeast. These include overall pH homeostasis [111], and adaption to osmotic stress [112]. The former is similar to one role proposed for plasma membrane V-ATPases in cancer cells, where these pumps aid in ridding the cell of the large amount of metabolic acid generated by the cell's reliance on glycolysis (see above). The latter function accounts for the increased assembly of the V-ATPase observed on exposure of yeast cells to high salt. A particularly intriguing observation is the link between vacuolar acidification and lifespan, where enhanced vacuolar acidity helps preserve mitochondrial function [113,114].

Concluding remarks—V-ATPase-dependent acidification is now established as a central regulator of many cellular and organismal processes, including membrane traffic, protein degradation, pH homeostasis and bone resorption. What is still emerging is the importance of the V-ATPase in controlling the activity of various signaling pathways, including Wnt, Notch, mTOR and AMPK. As we come to better understand the mechanisms of regulating V-ATPase assembly and trafficking, new possibilities for therapeutic intervention in these signaling pathways arise. Moreover, the important and emerging role of the V-ATPase, and in particular V-ATPase isoforms, in diseases such as cancer, osteoporosis and viral infection, makes the development of novel drugs targeting subclasses of V-ATPase involved in these diseases an exciting possibility.

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Trends

Recent structural studies of the V-ATPase have identified several novel features, including the presence of highly tilted alpha helices in subunit a of the integral V_0 domain that participate in proton translocation across the membrane.

Regulated assembly of the V-ATPase from its peripheral V_1 and integral V_0 domains represents an important mechanism of controlling its activity *in vivo* and has been shown to be under control of various signaling pathways, including protein kinase A (PKA) and PI-3 kinase.

The V-ATPase has been found to function in a variety of cellular signaling pathways, including the mechanistic target of rapamycin complex 1 (mTORC1), AMP-activated protein kinase (AMPK), Wnt and Notch.

The V-ATPase is emerging as important for cancer cell survival and invasion, making it a potential target in the development of anti-cancer drugs.

Outstanding Questions

What is the precise mechanism by which proton translocation occurs through the integral V_0 domain of the V-ATPase and how is this driven by rotary motion of initiated by ATP hydrolysis in the peripheral V_1 domain?

What is the mechanism by which signaling pathways such as PI-3 kinase and PKA modulate V-ATPase assembly in response to various stimuli, including changes in nutrient concentrations or the presence of growth factors?

How do V-ATPases participate in the activation of cellular signaling molecules, including mTORCl and AMPK?

How do V-ATPases promote the survival and invasion of tumor cells?

Can V-ATPases be exploited as a therapeutic target in the treatment of diseases such as cancer, osteoporosis and viral infection, in which they participate.

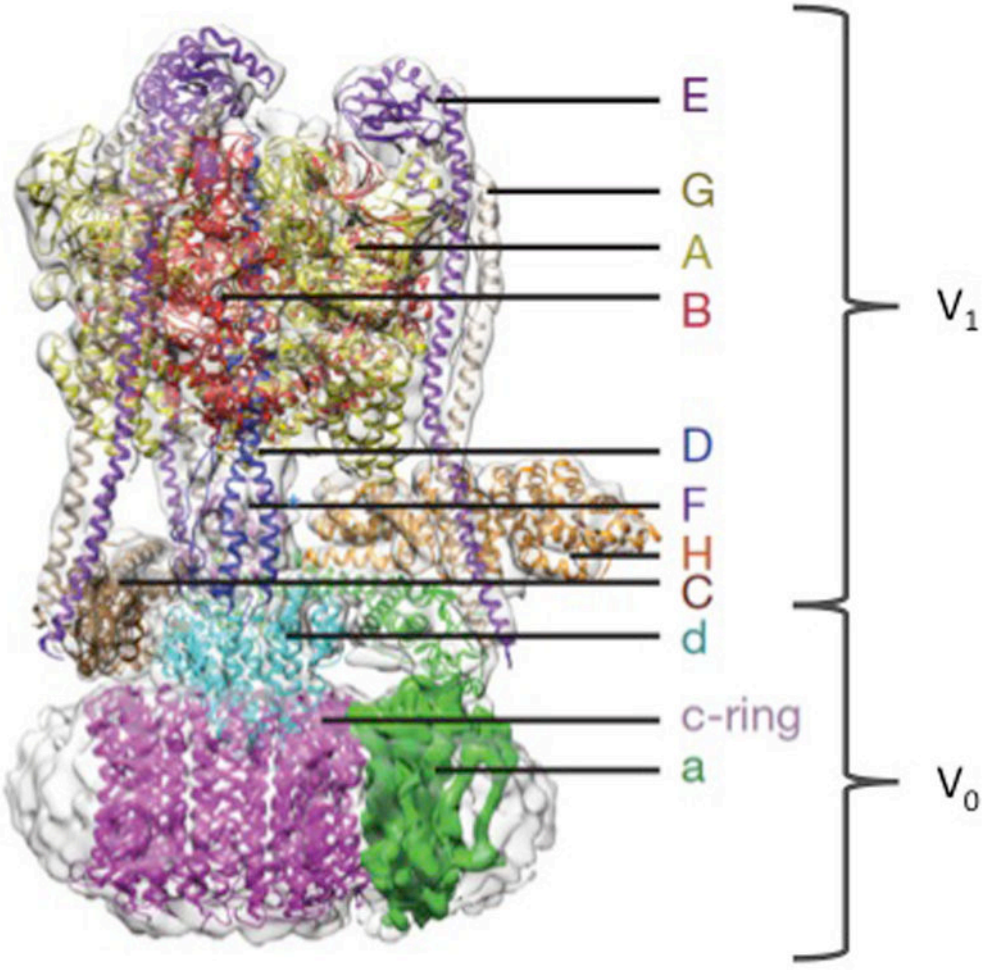

Figure 1. Structural model of the V-ATPase

Electron cryomicroscopy structure of the yeast V-ATPase with known crystal structures of individual subunits from *S.cerevisiae* and *T. thermophilus* fitted into the map. The V_1 cytosolic domain, made up of subunits A-H, is involved in ATP hydrolysis, while the integral V_0 domain, made up of subunits a, d, e, c, and c″, conducts protons across the membrane. ATP hydrolysis in the A3B3 complex drives central stalk movement (subunits D, F, and d), which in turn rotates the proteolipid ring to allow proton transport across the membrane through hemichannels located in subunit a. Subunits E, G, C and H and the N-terminal domain of subunit a make up the peripheral stalks and serve to tether V_1 to V_0. Note that subunit e is absent from the preparation used for this cryo-EM structure due to its loss during detergent extraction [7]. Figure adapted from [7].

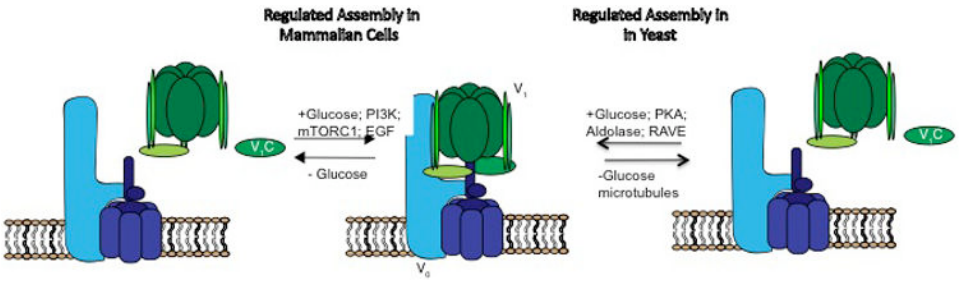

Figure 2. Regulation of V-ATPase assembly in yeast and mammalian cells

Regulated assembly represents an important mode of regulating V-ATPase activity in cells. The reversible dissociation of the V_1 and V_0 domains occurs in response to glucose depletion in both yeast and mammalian cells and in response to molting in insect cells, likely as a means to conserve cellular stores of ATP. Assembly of V_1 and V_0 occurs during maturation of dendritic cells to aid in antigen processing and in response to EGF (epidermal growth factor). Assembly in yeast is promoted by activation of PKA (protein kinase A), interaction with aldolase and the heterotrimeric complex RAVE (Regulator of the ATPase of Vacuolar and Endosomal memranes), whereas dissociation requires catalytic activity and intact microtubules. Assembly in mammalian cells is promoted by activation of PI3K and, in dendritic cells, activation of mTORC1 (mechanistic target of rapamycin complex 1).

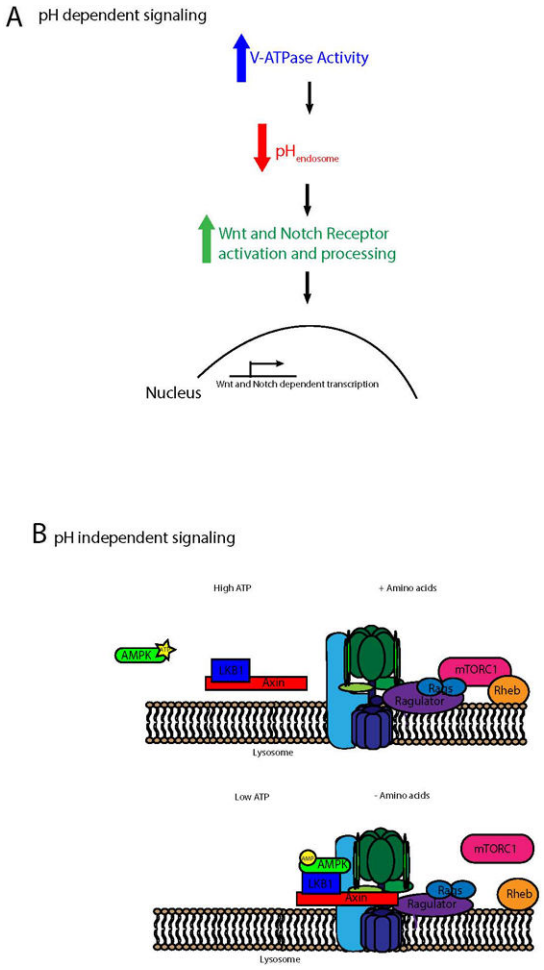

Figure 3. V-ATPase dependent cell signaling

A, Signaling pathways that rely on the low pH generated by the V-ATPase. Wnt and Notch signaling rely on the V-ATPase to maintain a proper pH environment in vesicles of the endocytic pathway for activation and subsequent signaling and control of gene transcription. *B*, Signaling pathways that rely on the V-ATPase, independent of its role in proton pumping. The V-ATPase-Ragulator complex is necessary for activation of mTORC1 and AMPK (AMP-activated protein kinase) downstream of cellular amino acids and ATP levels, respectively.

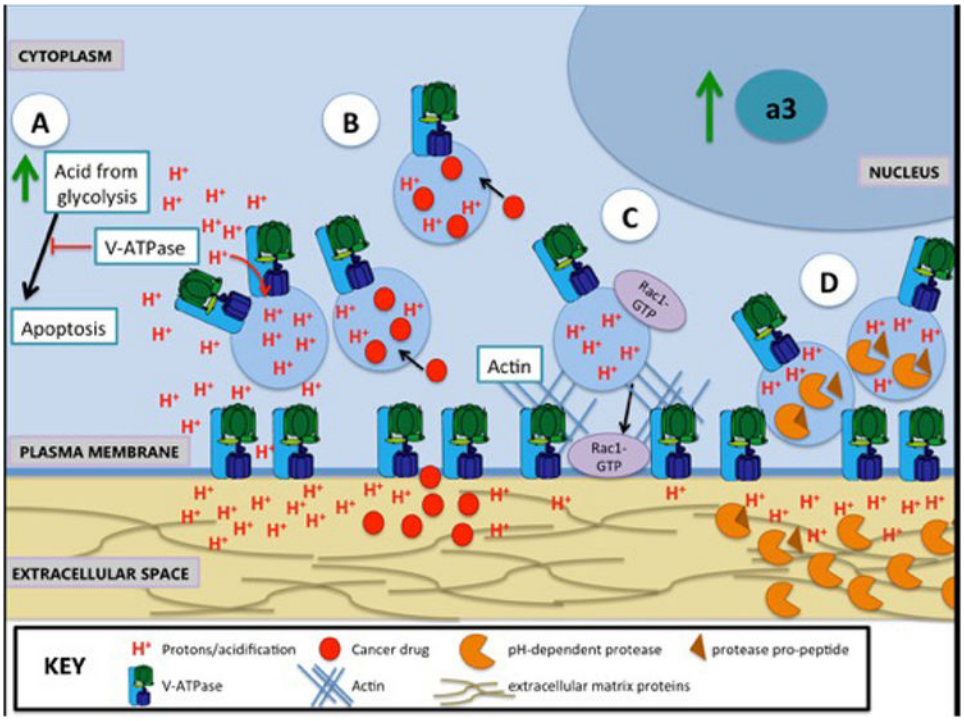

Figure 4. Functions of V-ATPase in cancer

A, The V-ATPase aids cancer cell survival, likely by its role in regulating cytoplasmic pH. Cancer cells experience an increased acid load due to enhanced glycolysis that can induce apoptosis. By transporting protons out of the cytosol, the V-ATPase helps prevent apoptosis due to this increased acid production. *B*, The V-ATPase may contribute to the development of drug resistance, either by promoting the transport of drugs into intracellular compartments or into the extracellular space or by preventing drug entry. *C*, The V-ATPase is involved in cancer cell migration, possibly through direct interaction with cytoskelet al proteins, such as actin, or by promoting the trafficking of proinvasive factors, such as EGF receptor, to the leading edge. *D*, V-ATPases contribute to cancer cell invasion. Upregulation of the a3 isoform occurs in several invasive cancer cell lines and is believed to localize V-ATPases to the plasma membrane, where they contribute to an acidic extracellular microenvironment that promotes the activation and activity of pH-dependent proteases, such as the cathepsins, that allow cancer cells to invade through extracellular matrix. Intracellular V-ATPases may also contribute to the activation of proteases involved in invasion.