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CELL SCIENCE AT A GLANCE

# The vacuolar-type H$^+$-ATPase at a glance – more than a proton pump

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## ABSTRACT

The vacuolar H$^+$-ATPase (V-ATPase) has long been appreciated to function as an electrogenic H$^+$ pump. By altering the pH of intracellular compartments, the V-ATPase dictates enzyme activity, governs the dissociation of ligands from receptors and promotes the coupled transport of substrates across membranes, a role often aided by the generation of a transmembrane electrical potential. In tissues where the V-ATPase is expressed at the plasma membrane, it can serve to acidify the extracellular microenvironment. More recently, however, the V-ATPase has been implicated in a bewildering variety of additional roles that seem independent of its ability to translocate H$^+$. These non-canonical functions, which include fusogenicity, cytoskeletal tethering and metabolic sensing, are described in this Cell Science at a Glance article and accompanying poster, together with a brief overview of the conventional functions of the V-ATPase.


**KEY WORDS: Acidification, pH regulation, Proton pump, Proton motive, Vacuolar ATPase**

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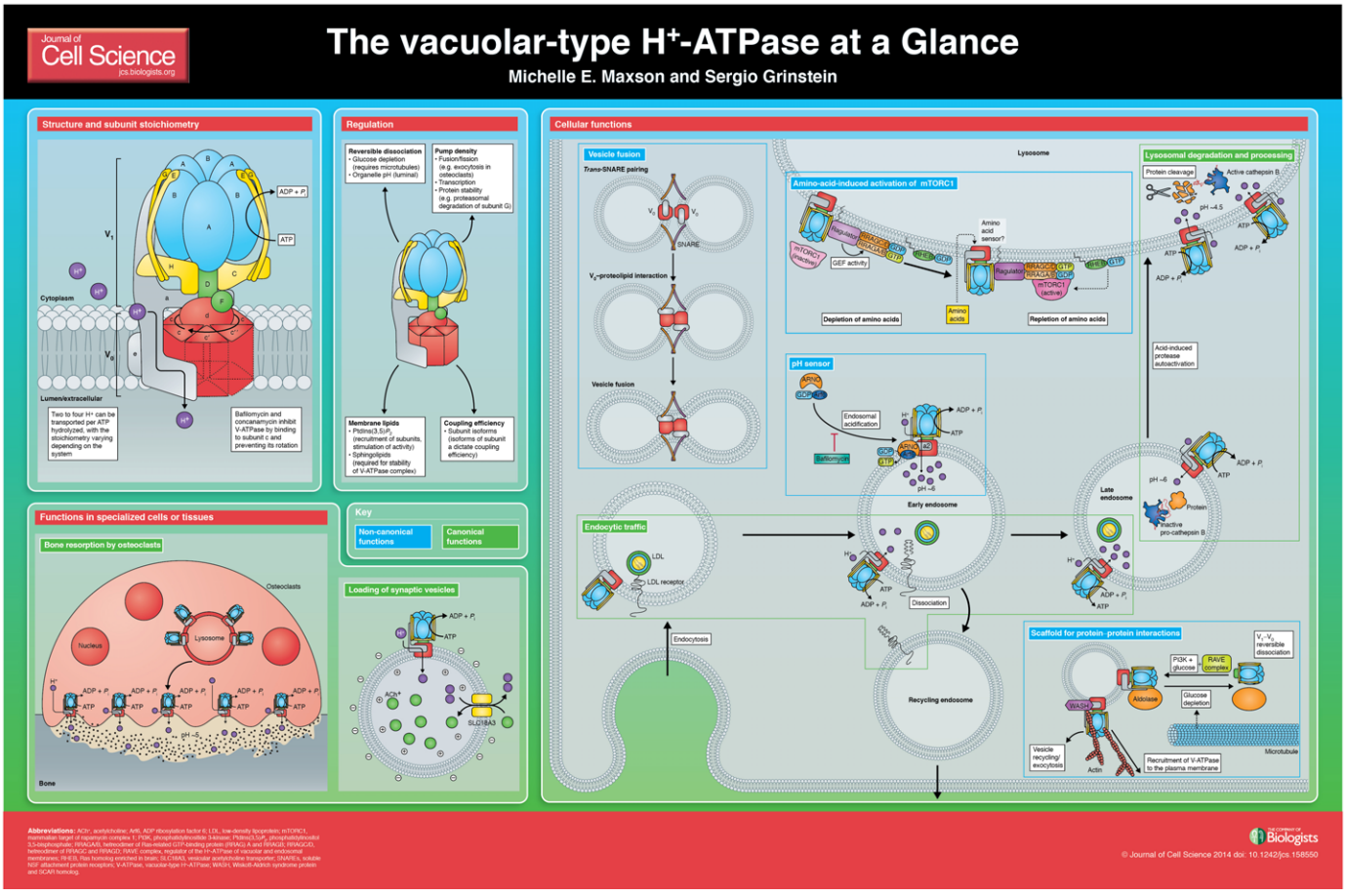
## Introduction

The pH of intracellular compartments varies widely: mitochondria are alkaline, whereas lysosomes are markedly acidic. The characteristic pH of each organelle is crucial for its function, impacting on diverse biological processes such as protein processing and degradation, intracellular transport, synaptic transmission and viral infection. Therefore, the luminal pH of



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each compartment must be controlled tightly. The membrane-associated vacuolar $H^+$-ATPase (V-ATPase) is primarily responsible for the establishment and maintenance of the acidic pH of endocytic and secretory organelles, pumping cytosolic $H^+$ into their lumen in an ATP-dependent manner (Forgac, 2007; Nishi and Forgac, 2002). The importance of the V-ATPase in cellular homeostasis has driven much research of its structure, stoichiometry and distribution, which dictate its function. Salient findings in these areas have been summarized briefly in Boxes 1 and 2 and are presented schematically in the poster. The main objective of this review is to describe the conventional (canonical) roles attributed to the V-ATPase, as well as its non-canonical functions that have been appreciated more recently.

## Canonical functions of the V-ATPase

The canonical functions of the V-ATPase all rely on its $H^+$-pumping ability, which has been reviewed in detail elsewhere (Holliday, 2014; Huynh and Grinstein, 2007; Mellman et al., 1986; Nishi and Forgac, 2002), and will only be summarized briefly here (see poster).

### Endocytic traffic

Perhaps the best-studied role of the V-ATPase is in endocytosis and vesicular traffic. In metazoan cells, internalized cargo includes nutrients and their carriers, receptor–ligand complexes, membrane proteins and even pathogens. Such cargo is sorted and processed as it traverses a tubulo-vesicular system of multiple stages, each displaying a characteristic and functionally relevant pH (Huotari and Helenius, 2011). The pH of components of the endocytic pathway ranges from ≤7 to ∼4.5, all more acidic than the physiological extracellular pH of ∼7.4 (see poster). The V-ATPase is largely responsible for the accumulation of $H^+$ in these vesicles (Huynh and Grinstein, 2007; Scott and Gruenberg, 2011). An acidified endosome lumen is important for the dissociation and correct targeting of receptors and their ligands (Maxfield and McGraw, 2004). Thus, low-density lipoprotein (LDL) dissociates in a pH-dependent manner from its receptor, facilitating recycling of unoccupied receptors to the plasma membrane. In a similar fashion, newly synthesized hydrolases tagged with mannose-6-phosphate are delivered by mannose receptors to late endosomes, where the acidic lumen induces dissociation, allowing for subsequent transport of the hydrolases to the lysosome, while the receptors recycle to the Golgi complex.

### Protein processing and degradation

The lysosome is the main catabolic organelle of the cell; materials are targeted to its lumen for degradation through the endocytic or autophagic pathways (Saftig and Klumperman, 2009). Intracellular protein degradation is essential for nutrition, protein turnover and antigen presentation. $H^+$ pumping by V-ATPases accounts for the uniquely acidic lysosomal pH of ∼4.5 that is essential for optimal enzyme function (see poster). This is best illustrated by the cathepsins, a family of proteases that are delivered to late endosomes in an inactive pro-cathepsin form. Progression to the acidic lysosome results in a conformational change of the cathepsin B pro-peptide, followed by autoactivation and cleavage that expose the active site of the mature enzyme (Pungerčar et al., 2009).

The acid-dependent activity of proteases is also important for processing a variety of precursor proteins into their mature form

### Box 1. V-ATPase structure and stoichiometry

The V-ATPase is composed of the two multi-subunit domains $V_1$ and $V_0$ (Benlekbir et al., 2012; Forgac, 2007; Inoue et al., 2005). The $V_0$ domain is membrane embedded; the $V_1$ domain associates with the cytosolic part of $V_0$ (see poster). Generally, hydrolysis of one ATP by the $V_1$ domain provides the energy that is necessary for one 360° rotation of the $V_0$ ring, propelling 2-4 cytosolic $H^+$ across the membrane against their electrochemical gradient (Cross and Müller, 2004; Kettner et al., 2003). $V_1$ is made up of eight subunits (A–H) with stoichiometry: $A_3$ $B_3$ C D $E_3$ F $G_3$ H (Muench et al., 2009). The three catalytic ATP-binding sites are located at each A/B interface. ATP hydrolysis drives a conformation change of the three A/B subunits, causing the rotation of the central stalk that consists of subunits D and F. Subunits C, E, G and H form a peripheral stalk with a stator function that prevents the movement of the $(A/B)_3$ portion during ATP hydrolysis.

The central stalk couples the energy of ATP hydrolysis to the rotation of the membrane-embedded $V_0$ domain that comprises nine subunits (a–e), with a stoichiometry in yeast of subunits a $c_4$ c′ c″ d e. In other eukaryotes, the yeast subunit c′ has been replaced by an additional subunit c (Toei et al., 2010). Subunits c c′ and c″ form a proteolipid ring, rotation of which, past the stationary subunits, enables $H^+$ translocation. Cytosolic $H^+$ pass through a hemichannel and protonate glutamine residues of the rotating c c′ c″ proteolipid ring. Exiting $H^+$ are then transferred to a crucial arginine residue in subunit a and, ultimately, expelled into the organellar lumen/extracellular space through a second hemichannel. The function of subunit e of the $V_0$ domain remains unknown. Specific inhibitors of the V-ATPase, including bafilomycin, concanamycin, lobatamides and salicylihalamides, bind to and inhibit the rotation of $V_0$ (Huss and Wieczorek, 2009).

### Box 2. Distribution of the V-ATPase

The V-ATPase is ubiquitous in eukaryotes, distributed mostly on intracellular tubulo-vesicular membranes, but found also at the plasma membrane in specialized cell types (Forgac, 2007; Nishi and Forgac, 2002). Acidification of the lumen of endocytic and secretory organelles is crucial for progress of cargo through transport pathways, receptor-ligand dissociation and protein degradation. In addition, in cell types such as osteoclasts, renal proximal tubule and intercalating cells, and epididymal clear cells, V-ATPases that are present at the plasma membrane secrete $H^+$ into the extracellular space, thereby mediating specialized functions that require acidification of the external microenvironment or changes in membrane potential.

Multiple isoforms and splice variants of the V-ATPase exist, which are believed to contribute to the specialized roles the pump plays in various intracellular locations and tissues. The most diverse subunit of the V-ATPase is subunit a, which exists in two isoforms in yeast and four isoforms in mammalian cells. In yeast, the two a isoforms Vph1p and Stv1p are localized to the vacuole and the Golgi/endosome, respectively (Kawasaki-Nishi et al., 2001; Manolson et al., 1994). In mammalian cells, isoform a1 is generally associated with neurons, isoform a2 with renal proximal cells, isoform a3 with osteoclasts, and isoform a4 with renal intercalating cells and epididymal cells, although some isoform a2 and a3 splice variants are ubiquitously expressed (Marshansky and Futai, 2008; Toei et al., 2010). Intracellular localization studies revealed greater overlap between isoforms, with isoforms a2 and a3 being associated with both endocytic and secretory organelles (Marshansky and Futai, 2008; Toei et al., 2010). In addition to the four isoforms of subunit a, mammalian cells can express two isoforms of subunits B, H, E and d, and three isoforms of C and G, which – although not fully characterized – undoubtedly contribute to the diversity of V-ATPase function.

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(Nishi and Forgac, 2002). Secreted hormones, such as insulin, gastrin and thyroxine that are essential for physiological homeostasis, are produced as pro-hormones in the Golgi and packaged into secretory vesicles (Fisher and Scheller, 1988). Processing of the pro-hormones to their biologically active form requires cleavage by proteases that are stimulated by the acidity of the secretory vesicle lumen (~pH 5), which is generated by active V-ATPases.

#### Plasma membrane functions

In certain specialized cells, the V-ATPase is localized also to the plasma membrane, where it is involved in $H^+$ extrusion to the extracellular space (Nishi and Forgac, 2002). Examples include renal intercalated cells, epididymal clear cells, phagocytes and osteoclasts. Plasmalemmal V-ATPase function in renal cells is important for the systemic acid-base balance, through secretion of acid into the urine (Brown and Breton, 2000). Epididymal clear cells pump $H^+$ across their membrane to maintain the seminal fluid at a low pH, required for normal maturation and storage of sperm (Brown and Breton, 2000). Activated neutrophils and macrophages use plasma membrane V-ATPases to maintain a neutral cytoplasmic pH, i.e. extruding $H^+$ are generated by a metabolic respiratory burst (Brisseau et al., 1996).

The process of bone resorption by osteoclasts has provided great insight into the function and targeting of plasma membrane V-ATPases (see poster). Osteoclasts are large multinucleated cells of the monocyte family that function in bone remodeling (Edwards and Mundy, 2011; Teitelbaum and Ross, 2003). When not resorbing bone tissue, osteoclasts contain many intracellular vesicles that are rich in V-ATPases. Upon maturation, osteoclasts attach to bone matrix through $\alpha_v\beta_3$ integrins and form an actin-rich sealing zone that segregates the area that is in contact with the bone. They then target V-ATPase-containing vesicles to the sealed-off area, where they undergo exocytosis, thereby generating a ruffled border (Toyomura et al., 2003). $H^+$ pumped into the extracellular resorption lacuna establish an acidic microenvironment that favors solubilization of $CaCO_3$ and $PO_4^{3-}$, and is required for the activity of secreted acid hydrolases that break down the organic bone matrix.

#### Synaptic vesicle loading and coupled transport

In the brain, the V-ATPase is functional in the synaptic vesicles of nerve cells (Moriyama and Futai, 1990). Neurotransmitter uptake into these vesicles can be driven by the electrochemical gradient that is established by the V-ATPase (see poster panel 'Synaptic vesicle loading'). Some vesicular transporters rely on the $H^+$-concentration gradient (ΔpH), whereas others utilize primarily the electrical potential ($\Delta\psi$) that is generated by the V-ATPase. Thus, monoamine transporters that import serotonin and dopamine rely mostly on ΔpH, while glutamate transporters are driven primarily by the $\Delta\psi$ (El Mestikawy et al., 2011; Van Liefferinge et al., 2013). The uptake of the positively charged acetylcholine by the vesicular acetylcholine transporter SLC18A3 is propelled by the efflux of two $H^+$, thereby utilizing both components of the electrochemical gradient, and γ-aminobutyric acid (GABA) transporters also rely on ΔpH and $\Delta\psi$. Once loaded with neurotransmitter, synaptic vesicles tether to the plasma membrane where they fuse, thereby releasing the neurotransmitters into the synaptic space (Moriyama et al., 1992).

The electrochemical gradient generated by the V-ATPase can also power ion flux through a variety of cellular ion and organic molecule transporters. The endocytic antiporter CLC-7 exchanges two $Cl^-$ for one $H^+$, facilitating continued $H^+$ pumping while inducing the accumulation of $Cl^-$ in the vesicular lumen (Graves et al., 2008). The $H^+$-coupled amino acid transporter PAT2, which is found in the endoplasmic reticulum and recycling endosomes, mediates the 1:1 transport of small neutral amino acids in exchange for $H^+$ (Rubio-Aliaga et al., 2004). Clearly, the electrochemical $H^+$ gradient created by the V-ATPase – and not solely the luminal acidification – is important for the function of a variety of intracellular compartments.

### Regulation of V-ATPase function

The luminal pH typically decreases in a graded, accurately regulated fashion as vesicles progress along the endocytic or secretory pathways. This requires continuous and accurate control of the rate of $H^+$ pumping, which is accomplished by adjusting V-ATPase activity or concentration (Forgac, 2007; Jefferies et al., 2008; Nishi and Forgac, 2002).

The $V_1$ and $V_0$ domains of the V-ATPase (see Box 1) can dissociate reversibly in response to stimuli. Reversible dissociation was first observed in the *Manduca sexta* larval midgut during molting (Sumner et al., 1995). In yeast, $V_1$ and $V_0$ dissociate in response to glucose withdrawal (Kane, 1995). In the latter system the dissociation is dependent on an interaction between the V-ATPase with microtubules (Xu and Forgac, 2001). Recent evidence suggests that the subunit C of the $V_1$ domain (see Box 1) directly interacts with microtubules and is the sole component that dissociates during glucose withdrawal (Tabke et al., 2014). The mechanisms underlying the dissociation and its implications as a possible metabolic sensor are discussed below in the section describing non-canonical functions of the V-ATPase. In mammalian cells, V-ATPase dissociation and association has been observed in certain cell types. Renal cells, like yeast cells, are responsive to changes in glucose levels, although the physiological significance of this remains unknown (Sautin et al., 2005). In neuroendocrine cells, $V_1$ and $V_0$ domains have been observed to associate on secretory granules in response to neutral pH and to dissociate under acidified condition to aid in exocytosis (Poëa-Guyon et al., 2013). Baby hamster kidney cells show increased dissociation of domains $V_1$ and/or $V_0$ in less acidic endocytic organelles, which is thought to be regulated by local lipid composition and membrane fluidity (Lafourcade et al., 2008). Finally, dendritic cells regulate the assembly of the V-ATPase on late endosomes and/or lysosomes to optimize the degree of acidification required for antigen processing (Trombetta et al., 2003). Thus, dissociation and association of the $V_1$ and/or $V_0$ domains represent a rapid and effective means of controlling V-ATPase activity. It is, therefore, puzzling that reversible dissociation has not been detected in plants (Schnitzer et al., 2011).

Changes in pump density can also regulate the pH of vesicles or of the extracellular space. Endocytic vesicles quickly acquire V-ATPases through fusion with early endosomes and begin acidification, whereas more alkaline recycling endosomes lose V-ATPases through selective tubulation and fission of the sorting compartment (Huotari and Helenius, 2011). In addition, in renal and epididymal cells, exocytosis can deliver V-ATPases from internal vesicular stores to the plasma membrane, whereas endocytosis can retrieve them. These cell types respond to increased cytoplasmic bicarbonate levels by activating adenylyl cyclase, thereby increasing cAMP-induced exocytosis of V-ATPase-laden vesicles to the plasma membrane, resulting in stimulation of $H^+$ extrusion from the cells (Forgac, 2007; Pastor-Soler et al., 2003).

The number of $H^+$ pumped per ATP hydrolyzed, i.e. the coupling efficiency, has been observed to vary between

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intracellular organelles and proposed as yet another means to regulate V-ATPase function (see poster). The wild-type V-ATPase is not optimally coupled; therefore, efficiency can be increased or decreased to modulate $H^+$ transport. In yeast, the coupling ratio varies between two and four $H^+$ per ATP (Cross and Müller, 2004; Kettner et al., 2003), with the more alkaline Golgi complex showing a lower coupling ratio than the more acidic vacuole (Kawasaki-Nishi et al., 2001). Although the exact mechanisms remain to be delineated, differences in the isoforms of the $V_0$ domain subunit have been proposed to account for the differences in V-ATPase coupling efficiency.

The V-ATPase can also be regulated at the level of transcriptional or protein stability (Merzendorfer et al., 1997). An interesting recent report has identified a new connection between the late endosoma Rab-interacting lysosomal protein (RILP) and regulation of the V-ATPase (De Luca et al., 2014). RILP was observed to recruit subunit G of $V_1$ to the late endosome, an interaction that is apparently necessary for endocytic acidification. RILP overabundance in the cytosol was associated with proteasomal degradation of subunit G, providing a second mechanism of V-ATPase regulation.

There is also evidence that V-ATPase activity is regulated by the lipid composition of the membrane. Sphingolipids containing a 26 carbon acyl chain are seemingly required for ATP hydrolysis by $V_1$ (Chung et al., 2003). Moreover, lack of these sphingolipids is associated with instability of the $V_1$–$V_0$ complex and impaired endocytic acidification. Recently, another membrane lipid species, phosphatidylinositol (3,5)-bisphosphate (PtdIns(3,5)$P_2$), has been identified to be important for V-ATPase activity (Li et al., 2014). Subunit a of the $V_0$ domain (Vph1p in yeast) interacts with PtdIns(3,5)$P_2$ and is recruited from the cytosol to the membrane when PtdIns(3,5)$P_2$ levels peak in endocytic compartments. Decreased V-ATPase activity and $H^+$ pumping in the absence of PtdIns(3,5)$P_2$ are associated with reduced assembly of $V_1$ subunits in the endocytic pathway.

## Non-canonical functions of the V-ATPase

Although the functional consequences of the acidification that is generated by the $H^+$ pump are well documented, there are other effects of the V-ATPase that are not readily attributable to its proton-motive activity. These non-canonical functions, which are still poorly understood, are discussed below.

### Membrane fusion

Several studies have pointed to a role for the V-ATPase in membrane fusion (see poster). Early *in-vitro* experiments using purified vesicles or vacuoles revealed that homotypic fusion requires a transmembrane $H^+$ gradient and is, therefore, sensitive to proton translocators (protonophores) or V-ATPase inhibitors (Hampe et al., 1990; Ungermann et al., 1999). These observations were supported by studies that used intact cells; in *Drosophila* and mammalian neurons, as well as in mammalian pancreatic β-cells, the V-ATPase is required for exocytosis of synaptic and/or secretory vesicles (Hiesinger et al., 2005; Poëa-Guyon et al., 2013; Sun-Wada et al., 2006). Although *prima facie* this suggested energetic coupling of the electrochemical $H^+$ gradient with the fusion process, subsequent studies concluded instead that the role of the V-ATPase is structural. Specifically, it was proposed that, following soluble NSF attachment protein receptor (SNARE)-mediated docking, the $V_0$ domains of two adjacent membranes form a *trans*-complex, thereby creating a fusion pore that aids vesicle fusion (Bayer et al., 2003; Peters et al., 2001). The hydrophobic proteolipid core of $V_0$ is well-suited to generate the fusion pore (Clare et al., 2006; Peters et al., 2001). Recent studies suggest that even a single subunit of $V_0$ can promote fusion between two membranes (Baars et al., 2007; Takeda et al., 2008), but the precise mechanism remains unresolved (Strasser et al., 2011). Moreover, the mode of action of the V-ATPase remains controversial. Several studies argue that the physical presence of the $V_0$ domain – and not the $H^+$-pumping ability of the V-ATPase – is required for fusion (Baars et al., 2007; Bayer et al., 2003). However, a very recent study supports the original conclusion, namely that acidification, rather than forming a fusion pore is the main role of the V-ATPase in vesicle fusion (Coonrod et al., 2013). Regardless of the ultimate outcome of this controversy, the importance of the V-ATPase in fusion appears unquestionable.

### pH sensor

It is generally acknowledged that dissipation of the $H^+$ gradient disrupts normal traffic along the endocytic and secretory pathways. Yet, it is not entirely clear how the fission and fusion machineries that are located on the cytosolic part of the endomembrane vesicles and tubules perceive the luminal pH. Interestingly, some evidence supports a role of the V-ATPase itself as a sensor and transmembrane transducer of the luminal pH (see poster). Intravesicular acidification was shown to promote the recruitment of Arf1, a GTPase involved in vesicle genesis and coat protein complex (COP) coating, to the cytosolic leaflet of the membrane of pancreatic membrane vesicles (Zeuzem et al., 1992a; Zeuzem et al., 1992b). Subsequent *in-vivo* studies confirmed that Arf1 and COP components associate with endosomes in a pH-dependent manner (Aniento et al., 1996; Gu and Gruenberg, 2000; Gu et al., 1997). These studies suggested that the V-ATPase itself senses the luminal pH and conveys the information across the membrane but no direct evidence was provided at the time. Subsequent work in renal cells addressed specifically this possibility, showing that subunit isoform a2 of the V-ATPase recruits the Arf6 nucleotide-exchange factor (ARNO, also known as CYTH2) (Hurtado-Lorenzo et al., 2006; Merkulova et al., 2010). Remarkably, this recruitment is sensitive to uncouplers or inhibitors of the acidification, implying that the luminal pH mediates (or is required for) binding of subunit isoform a2 to ARNO. This interaction is required for endosome recycling. Luminal acidification is thought to induce a change of conformation in the V-ATPase in order to facilitate docking of ARNO (Hosokawa et al., 2013). Because steps other than endosome recycling are also affected by dissipation of this acidification, other effectors are likely to be recruited and/or activated by the pH in a similar way, either through the V-ATPase or other transducers.

### Amino-acid-induced activation of mTORC1

The mammalian target of rapamycin (mTOR) is a Ser/Thr protein kinase that is involved in nutrient- and growth-factor-sensitive biosynthetic pathways. mTOR can function as part of two similar complexes, mTORC1 or mTORC2 (Betz and Hall, 2013; Jewell et al., 2013). mTORC1 couples the availability of amino acids to cell growth and autophagy, whereas mTORC2 – which is less well-characterized – contributes to rearrangements of the actin cytoskeleton. Components of the mTORC1 pathway – the Ragulator complex (comprising LAMTOR1, LAMTOR2, LAMTOR3, LAMTOR4 and LAMTOR5) and the Ras-related GTP-binding proteins (RRAGA, RRAGB, RRAGC and RRAGD; hereafter referred to as Rag GTPases) – localize to the lysosomal membrane in an inactive form (see poster panel 'Amino-acid-induced activation of mTORC1', left V-ATPase complex).

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Accumulation of amino acids activates the GEF activity of Ragulator, which mediates the GDP–GTP exchange of RRAGA/B (Bar-Peled et al., 2012). In addition, the recently identified tumor suppressor complex of folliculin (FLCN) and the folliculin-interacting proteins 1 and 2 (FNIP1 and 2, respectively) – which displays GTPase-activating protein (GAP) activity – mediates GTP-GDP hydrolysis of RRAGC/D (Bar-Peled et al., 2013; Panchaud et al., 2013; Petit et al., 2013; Tsun et al., 2013). These processes convert the Rag GTPases to the active complex that is required for the recruitment of mTORC1. Upon localization of mTORC1 to the lysosome, mTORC1 kinase activity is activated by the GTP-binding protein RHEB. Importantly, the recruitment and activation of mTORC1 requires the lysosomal V-ATPase (Zoncu et al., 2011), and several recent studies suggest that the V-ATPase itself senses the amino acid levels (Bar-Peled et al., 2012; Xu et al., 2012; Zoncu et al., 2011) (see poster). Because the V-ATPase also interacts with Ragulator (Bar-Peled et al., 2012; Zoncu et al., 2011) it is well-suited to convey information of amino acid availability to mTORC1. Whether the V-ATPase senses the levels of cytosolic or luminal amino acids remains debatable. Curiously, whereas ATP hydrolysis and rotation of the V-ATPase $V_0$ domain are required for activation of mTORC1, it is not clear whether luminal acidification is, in fact, required. The *in-vitro* model suggests that the pH gradient is dispensable (Zoncu et al., 2011) but a second report concluded that lysosomal acidity is involved, if only indirectly, by facilitating the breakdown of luminal proteins into amino acids (Xu et al., 2012). Regardless of the source of signaling amino acids, these studies suggest a tantalizing model whereby the V-ATPase links an amino acid-generated signal to the activation of mTORC1 and cell proliferation.

### Scaffold for protein-protein interactions

Interactions with seemingly unrelated cellular components also contribute to the functionality of the V-ATPase, which serves as a scaffold that supports cytoskeletal and metabolic platforms (see poster). The V-ATPase has long been known to interact with actin. This association, originally observed in osteoclasts (Lee et al., 1999; Nakamura et al., 1997), involves subunits B and C of the $V_1$ domain (Chen et al., 2004; Holliday et al., 2000; Ma et al., 2012; Vitavska et al., 2003). The C subunit contains 'head' and 'foot' structural motifs similar to those found in actin-binding proteins, such as gelsolin (Drory et al., 2004). It has, therefore, been speculated that the V-ATPase promotes the regulation of cytoplasmic G-actin pools, as well as crosslinking and stabilization of actin into filaments (Vitavska et al., 2005). By connecting vesicles to actin filaments, the V-ATPase is thought to play a role in vesicular sorting and transport during exocytosis and recycling. Moreover, because the $V_1$ domain – including its B and C subunits – dissociates from the membrane upon changes in glucose levels (Kane, 1995), actin association with subunit C might regulate cytoskeletal tethering in response to metabolic cues (Vitavska et al., 2003). In addition to binding actin directly, the V-ATPase appears to bind to the Wiskott-Aldrich and scar homolog (WASH) complex (Carnell et al., 2011). Because WASH promotes actin polymerization, its presence in the vicinity of the V-ATPase should have a synergistic effect on the formation and attachment of actin filaments.

V-ATPase also interacts with the metabolic enzymes aldolase and 1-phosphofructokinase. Aldolase interacts with the V-ATPase subunits a, B and E, and this interaction is required to stabilize the assembled V-ATPase on the membrane (Lu et al., 2007; Lu et al., 2001; Lu et al., 2004; Merkulova et al., 2010; Merkulova et al., 2011). Indeed, aldolase separates from the V-ATPase upon glucose starvation (Lu et al., 2004), underlying the reported dissociation of the $V_1$ and $V_0$ domains in a microtubule-dependent manner (Tabke et al., 2014; Xu and Forgac, 2001). Interestingly, although it does stabilize the $V_1$–$V_0$ complex, aldolase is not required for the reassembly of the dissociated domains when glucose is reintroduced; the regulator of the $H^+$-ATPase of vacuolar and endosomal membranes (RAVE) complex (comprising Skp1, Rav1 and Rav2) is responsible for the reassembly process, which also requires phosphatidylinositol 3-kinase (Seol et al., 2001; Smardon et al., 2002).

The a subunit of V-ATPase also interacts with 1-phosphofructokinase, an enzyme that catalyzes the rate-limiting step in glycolysis (Su et al., 2008; Su et al., 2003). Although effects of 1-phosphofructokinase on the assembly of V-ATPase have not been completely established, there are indications that it also contributes to the stability of the V-ATPase (Su et al., 2008). The combined system – consisting of the V-ATPase and the glycolytic enzymes – is positioned to serve as a metabolic sensor and regulator, coupling the generation of ATP with its utilization, and with the regulation of intracellular pH.

## Concluding remarks

Until recently, proteins performing two disparate functions were considered an oddity and were regarded with suspicion. It is becoming increasingly clear that multi-tasking is a common feature of many proteins, and the V-ATPase is no exception. In addition to its canonical role as a generator of electrochemical $H^+$ gradients that can activate enzymes or drive substrate transport, we now also consider the V-ATPase as being a fusogen, as a transducer of information, and an anchorage site for the cytoskeleton and important metabolons. We should remain open-minded and welcome other, so far not appreciated, new functional roles of this fascinating enzyme.

### Competing interests

The authors declare no competing interests.

### Funding

M.E.M. is a fellow of the Heart and Stroke Foundation of Canada. Research in the authors' laboratory is supported by the Canadian Institutes for Health Research grants MOP7075, MOP102474 and MOP4665.

### Cell science at a glance

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