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Review

Vacuolar (H^+)-ATPases in *Caenorhabditis elegans*: What can we learn about giant H^+ pumps from tiny worms?

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ABSTRACT

Vacuolar (H^+)-ATPases, also called V-ATPases, are ATP-driven proton pumps that are highly phylogenetically conserved. Early biochemical and cell biological studies have revealed many details of the molecular mechanism of proton pumping and of the structure of the multi-subunit membrane complex, including the stoichiometry of subunit composition. In addition, yeast and mouse genetics have broadened our understanding of the physiological consequences of defective vacuolar acidification and its related disease etiologies. Recently, phenotypic investigation of V-ATPase mutants in *Caenorhabditis elegans* has revealed unexpected new roles of V-ATPases in both cellular function and early development. In this review, we discuss the functions of the V-ATPases discovered in *C. elegans*.

The acidification of intracellular organelles is essential for various cellular functions, including protein degradation and vesicular trafficking. H^+ transport across membranes is mediated primarily by ATP-dependent proton pumps known as vacuolar H^+-ATPases (V-ATPases) [1–3]. These pumps are composed of two discrete domains. The V1 domain is a 650-kDa peripheral macromolecular complex that hydrolyzes ATP to generate the energy required for pumping protons. The Vo domain is a 260-kDa integral membrane protein complex that transports H^+ across the lipid bilayer.

The V1 domain is composed of subunits A to H, whereas the Vo contains the a, c, c″, d, e, and e′ subunits. Several subunits have different isoforms or alternatively spliced variants that are expressed in a tissue-specific manner and that appear to be specific for the function of V-ATPases in those tissues. The activity of V-ATPases is highly regulated by several mechanisms, including the reversible dissociation of the Vo and V1 domains. In addition to the acidification of intracellular compartments, V-ATPases are present in the plasma membranes of some specialized cells, where they perform coupled transport of molecules to carry out cell-specific functions, such as renal acidification, bone absorption, regulation of cytoplasmic pH, and spermatogenesis [1]. In addition, several lines of evidence from genetic studies and cellular analyses have suggested that the Vo domain is involved in membrane fusion, independent of the V1 domain. In this study, we review the main characteristics of the structure and functions of V-ATPases and discuss how several studies that used *Caenorhabditis elegans* as a model system contributed to our understanding of the various functions of V-ATPases.

1. Structure and functions of V-ATPases

V-ATPases are multi-subunit complexes organized into two functional domains that are operated by a rotary mechanism energized by ATP hydrolysis (Fig. 1, reviewed in [1–3]). In the V1 domain, there are eight different subunits, designated A to H. Three copies each of the A and B subunits are organized in an alternating fashion to form a hexamer. ATP hydrolysis occurs at the interface between the A and B subunits. The other V1 subunits constitute peripheral and central stalks that have distinct functions in the rotary mechanism by which the V-ATPases couple ATP hydrolysis to proton pumping. One copy each of the C and H subunits and three copies each of the G and E subunits form peripheral stalks that act as stators [4–8]. One D and one F subunit form a central stalk that serves as a rotor to couple the energy generated by ATP hydrolysis to the actual rotation of the proteolipid ring in Vo to transport protons.

The Vo domain is composed of six different subunits: a, c, c′, c″, d, and e. Higher organisms lack c′, which is universal only in fungi, but contain a type I transmembrane accessory subunit, Ac45 [9,10]. A single Vo domain comprises four or five copies of the c subunit and one copy of the "a" and "d" subunits, whereas the stoichiometry of the 9-kDa

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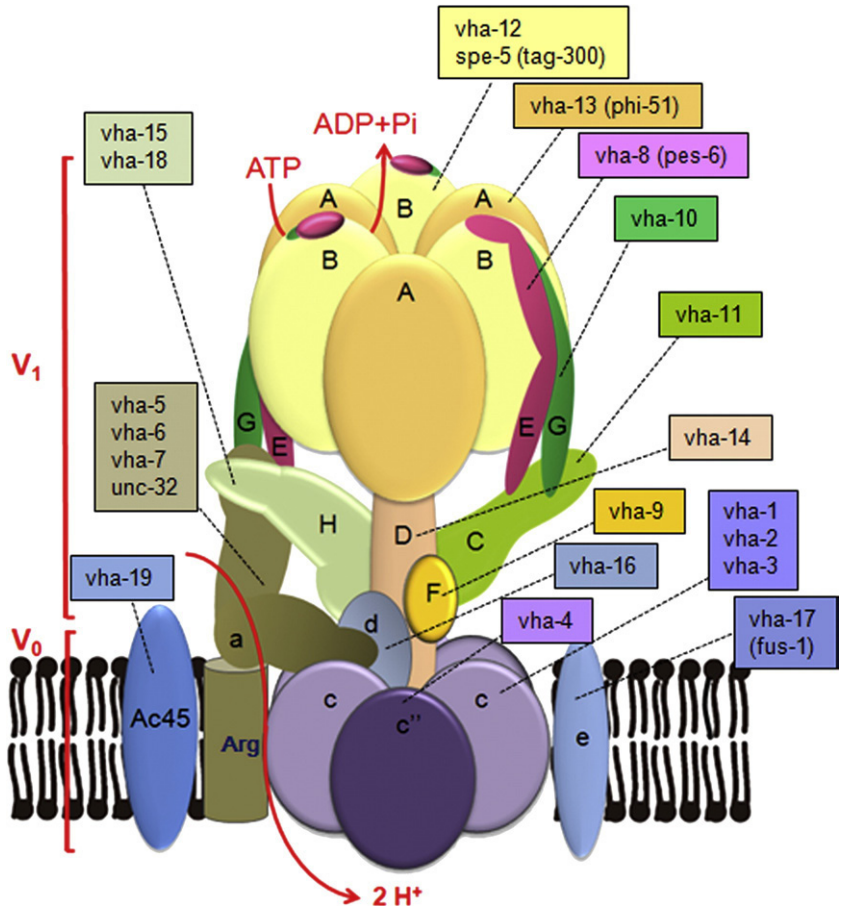

Fig. 1. Structure of the V-ATPase. A schematic showing the expression of V-ATPase in *C. elegans* is presented, and all known corresponding worm subunits are indicated. V-ATPase consists of a peripheral V1 domain that hydrolyzes ATP and an integral Vo domain that translocates protons across the membrane. The A and B subunits, which are the core subunits of the V1 domain, participate to form ATP binding and hydrolysis pockets. In the Vo domain, the a and e subunits are adjacent to a proteolipid ring, which is composed of the c, c′, and c″ subunits. The V1 and Vo domains are connected by a central stalk composed of the D, F, and d subunits and surrounded by a peripheral stalk composed of the C, E, F, G, and H subunits of the V1 domain. The *C. elegans* genome encodes orthologs of all subunits except c′, which is present in yeast but is absent in mammals. The proteolipid ring in the worm therefore is likely composed of only c and c″ subunits, and may be accessorized by Ac45, which has been reported to interact with the a, c, and d subunits in mammals [77]; however, the stoichiometry of the subunits is not known. Protons are translocated from the cytoplasm to the lumen through two hemi-channels in the "a" subunit by repetitive protonation and deprotonation of arginine, which is buried between the hemi-channels. Glutamates in the proteolipid ring of the V1 domain rotate in the lipid bilayer with a force driven by ATP hydrolysis.

polypeptide e subunit remains unknown [11,12]. The c, c′, and c″ subunits are highly hydrophobic and form a proteolipid ring in the membrane that rotates around the central stalk as protons are pumped. Each proteolipid subunit contains a single buried glutamic acid (Glu) in transmembrane 4 (TM4) of subunits c and c′ and in TM3 of subunit c″. The carboxyl groups of the buried glutamic acids undergo reversible protonation during proton transport [13]. The 100-kDa subunit "a" contains an N-terminal domain facing the cytoplasm, and transmembrane helices of the C-terminal domains are embedded in the lipid bilayer. Located in those TMs are two hemi-channels, one oriented towards the cytoplasm, and the other towards the lumen, through which protons bind and dissociate from the buried carboxyl groups in the proteolipid ring [2]. The essential residue for proton transport is an arginine (R735 in VPH1 of *Saccharomyces cerevisiae*) located in TM7 that appears to be identical across species. This arginine forms a luminal facing hemi-channel and promotes deprotonation of the buried glutamate in each of the proteolipid ring subunits [14]. Recent cryogenic EM studies have reported that the d subunit appears to form the top of the proteolipid ring and contact the "a" subunit [8,15]. Whether it serves as a rotating shaft itself is not known, although mutation of the "d" subunit affects the coupling efficiency [16].

V-ATPases acidify the lumen of various intracellular organelles, including endosomes, lysosomes, and secretory vesicles. The resulting high concentration of protons in these organelles is key to the biological functions of each organelle. First, the acidic pH generated in the lumen by V-ATPases facilitates the dissociation of protein complexes. In this mode, ligand–receptor complexes, such as the low density lipoprotein (LDL) receptor and the insulin receptor that have been internalized into endosomes, are dissociated by the low pH in the endosomes, and the released receptors can then be recycled back to the plasma membrane [17]. Another example is the newly synthesized lysosomal enzymes associated with the mannose-6 phosphate receptors in Golgi-derived vesicles [18]. When these vesicles fuse with a lysosome, those enzymes dissociate from the receptors because of the low pH in the lysosome [19]. In a further step, the low luminal pH activates those unloaded enzymes to catalyze biochemical reactions. In secretory vesicles, protease activity is required for conversion of the precursor forms of peptide hormones and growth factors, such as insulin and EGF, into mature forms [19,20]. The acidic environment of endosomes also facilitates invasion of enveloped viruses, such as influenza virus and vesicular stomatitis virus, as well as toxins, such as anthrax toxin and diphtheria toxin, into host cells by triggering the formation of membrane pores [21]. In certain cases, the low luminal pH of intracellular organelles is a prerequisite for proper vesicular trafficking. The budding of endosomal carrier vesicles containing released ligands from early to late endosomes is dependent upon luminal acidification by V-ATPases. In this process, V-ATPase itself may serve as a binding scaffold for