Histol Histopathol (2010) 25: 1611-1620

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Vacuolar-type proton pump ATPases: roles of subunit isoforms in physiology and pathology

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Summary. The vacuolar-type H^+-ATPases (V-ATPases) are a family of multi-subunit ATP-dependent proton pumps involved in diverse cellular processes, including acid/base homeostasis, receptor-mediated endocytosis, processing of proteins and signaling molecules, targeting of lysosomal enzymes, and activation of various degradation enzymes. These fundamental cellular activities are naturally related to higher order physiological functions in multicellular organisms. V-ATPases are involved in several physiological processes, including renal acidification, bone resorption, and neurotransmitter accumulation. Both forward- and reverse-genetic approaches have revealed that V-ATPase malfunction causes diseases and/or pathophysiological states, demonstrating its diverse roles in normal physiology. Here, we focus on the recent insights into the function of mammalian V-ATPase in highly differentiated cells and tissues.

Key words: Proton pump, V-ATPase, Subunit isoform, Acidification, Human disease, Mouse model

Introduction

Vacuolar-type ATPases (V-ATPases) are large multisubunit, membrane-associated protein complexes that carry out the active transport of protons across the membrane bilayer. V-ATPases are involved in the acidification of intracellular compartments and of the extracellular environment. The proton gradient generated by V-ATPases contributes not only to the acidity of the intracellular compartments and the extracellular environment but also to the formation of ion motive force across the membranes. Corresponding to the diverse functions of the enzyme, V-ATPases are distributed in various cellular locations: endomembrane organelles, including the lysosomes, endosomes, the Golgi apparatus, secretory granules, and coated vesicles. They are also present in the plasma membrane of specialized cells, including osteoclasts and epithelial cells in the kidneys, male genital tracts, and ocular ciliary bodies. This ubiquitous and specific distribution of V-ATPase suggests that the enzyme is required for diverse cellular processes, including receptor-mediated endocytosis, protein processing, and degradation; targeting of lysosomal enzymes; and activation of various degradation enzymes and processing of signaling molecules.

V-ATPases are found in all eukaryotes, including fungi, plants, and animal cells, studied thus far. They are structurally conserved, regardless of kingdoms, i. e., Animalia, Plantae, or Fungi (Stevens and Forgac, 1997). V-ATPases are composed of two functional sectors known as V_1 and Vo (Fig. 1). The V_1 sector constitutes the catalytic sites for ATP hydrolysis and contains at least eight different types of subunits (*A-H*). The Vo part contains 4 to 6 subunits (*a, c, c', c", e and d*), which form a membrane embedded sector, and this sector is required for proton translocation across the membrane. This proton movement occurs without any other ion flux through this enzyme complex, making this process electrogenic in nature, and is coupled tightly to ATP hydrolysis at the catalytic sites of the V_1 sector.

As mentioned above, V-ATPases are distributed to various cellular membranes of the exocytic and endocytic pathways (Sun-Wada et al., 2004). From the viewpoint of cell biology, this is somewhat unusual, since most proteins and enzymes have their own cellular residents, implying that even if they appear in some subcellular compartments in transit, they eventually accumulate at one particular destination. However, V-ATPase is widely distributed in the post-Golgi compartments, including the *trans*-Golgi network,

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endosomes, lysosomes, secretory vesicles, and plasma membrane, and this proton pump in fact plays an important role in the function of these subcellular compartments. We raised a pertinent question more than ten years ago: what is the underlying mechanism enabling the diverse localization of V-ATPase within the cells and tissues? (Futai et al., 1998). More specifically, it is interesting to know whether the same enzyme shows various localizations within the cells or whether structurally distinctive pumps are distributed to the different subcellular compartments. In the last few decades, genomic studies, including cDNA surveys, reveal that V-ATPase complexes distributed to the different subcellular compartments exhibit structural differences.

The simplest of the V-ATPases is the yeast V-ATPase, in which only subunit *a* is encoded by more than one gene, *STV1* and *VPH1* (Kawasaki-Nishi et al., 2001). In contrast, in a protozoan counterpart, in *Paramecium* species, 17 distinctive genes are known to encode the subunit *a* (Wassmer et al., 2006). In the mammalian counterpart, *a* subunit has four isoforms. In addition to subunit *a,* subunits *B, E, G, H,* and *d* also contain isoforms (Sun-Wada et al., 2004) (Table 1). The expression pattern analyses together with both forward- and reverse-genetic approaches have demonstrated that specific V-ATPase complexes participate in highly differentiated cellular and tissue functions, including renal acidification, bone resorption, and neurotransmitter accumulation.

The *a*3 isoform is associated with a lysosomal proton pump

Among the *a* subunit isoforms, the *a*3 isoform is characterized best in terms of its physiological relevance (Fig. 2). Genetic deficiency in this subunit results in autosomal recessive osteopetrosis (ARO) in humans and mice (Li et al., 1999, Frattini et al., 2000). Osteopetrosis is a bone disease characterized by impaired bone resorption. Bone is constructed by coordination of opposite actions, deposition of minerals by an osteoblast, and degradation by an osteoclast—a multinucleated cell derived from bone-marrow stem cells. Bone-resorption occurs in specific areas called the lacunae, where the osteoclasts attach to the bone surface, leaving a narrow space between the cell surface and the bone. The V-ATPase with the *a*3 isoform is specifically recruited to the cell surface facing the lacuna and transports protons to acidify the extracellular space to disintegrate the mineral and protein matrix (Toyomura et al., 2000). The absence of *a*3 thus results in reduced bone-resorbing activity, although osteoclast differentiation still occurs. The state of ARO has other etiologies, for instance, defective differentiation of osteoclasts and genetic loss of carbonic anhydrase, with the latter resulting in proton secretion.

Proton transport driven by V-ATPase generates membrane potential across the plasma membrane. Therefore, vectorial movements of ionic molecules that dissipate the membrane potential are also required to establish substantial acidification in bone resorption. In the bone-resorption lacunae, a chloride-conducting channel (Clc-7 protein) plays an essential role in canceling the membrane potential (outside positive) to allow the V-ATPase to pump out more protons for acidification. Through the collaboration of V-ATPase (proton pump) and Clc-7 (chloride channel), the osteoclasts secrete HCl into the lacuna. Genetic loss of either of these causes ARO (Kornak et al., 2001). *a*3 deficiency constitutes approximately half the ARO cases, and Clc-7 malfunction follows a close second. Collectively, genetic lesions in *a*3 and Clc-7 constitute 60-70 % of human ARO cases.

Although the *a*3 subunit is highly accumulated in osteoclasts, its presence is not restricted to this highly differentiated cell (Toyomura et al., 2000). This subunit isoform is expressed in a variety of cells, at various levels. The *a*3 subunit is predominantly associated with organelles in the late stages of the endocytic pathway in most cells; the V-ATPases with the *a*3 isoform act as the main proton pumps for acidification of the lumen of the endosomes and lysosomes. In undifferentiated osteoclasts, the *a*3 subunit is distributed in intracellular compartments scattered in the perinuclear region, showing lysosome and endosome characteristics (Toyomura et al., 2003). Upon receiving differentiation

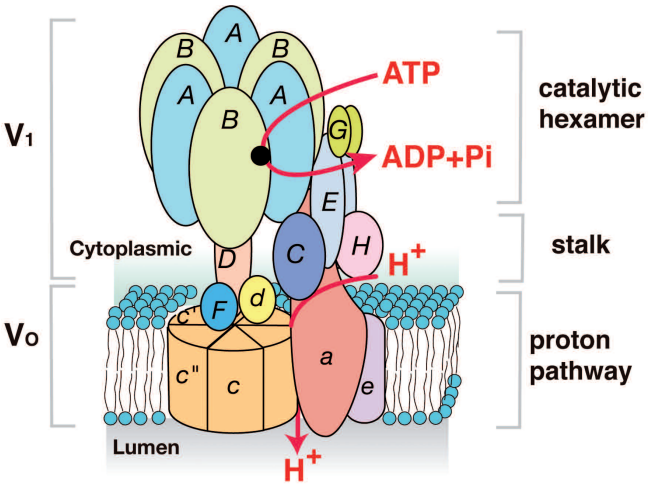


Fig. 1. Subunit organization of V-ATPase. V-ATPase is structurally and evolutionarily related to F-ATPase (ATP synthase), which is responsible for ATP synthesis in the mitochondria, chloroplast, and bacteria, although the physiological roles of these two enzymes are completely different. V-ATPase consists of two major functional sectors known as V_1 and Vo. The V_1 sector comprises at least eight different subunits (*A-H*). This sector contains three catalytic sites for ATP hydrolysis formed from the *A* and *B* subunits. The Vo sector contains up to 6 subunits (*a*, *c*, *c'*, *c''*, *e*, and *d*) and is responsible for proton translocation across the membranes. The subunits *C*, *E*, *G*, and H and the amino-terminal of the *a* subunit form a stalk-like structure connecting the ATP hydrolysis domain and the Vo sector.

stimuli, it relocates to the cell surface via a microtubule-dependent mechanism (Toyomura et al., 2000, 2003). In macrophages, the *a*3 subunit is mainly localized to the lysosomes, and during phagocytosis it is recruited to the nascent phagosomes via tubular extensions radiating from the lysosomes in the perinuclear region (Sun-Wada et al., 2009). This wide array of expression and localization profiles suggests that the function of *a*3 is not merely restricted to bone-resorption; it also participates in other vital functions. In fact, human ARO patients display diverse disorders, including defective immune response and hepatosplenomegaly (Del Fattore et al., 2007). The former may reflect malfunction in the bone marrow due to narrowing of the hollow interior of the bones, with subsequent compensation in the spleen resulting in splenomegaly. In addition, direct participation of the V-ATPase *a*3 subunit in innate immune functions has been suggested in mouse models (Sun-Wada et al., 2009). ARO patients often develop macrocephaly and sensorineural defects in vision and/or in hearing. These phenotypes could be explained by deformation of the foramina, which compresses the optic and auditory nerves and constricts brain-cerebrospinal fluid flow. Furthermore, the *a*3 isoform localizes on the membranes of secretory granules in pancreatic ß-cells (Sun-Wada et al., 2006). Although the mouse models and human patients do not display an apparent hyperglycemic state, loss of the *a*3 subunit reduces the efficiency of insulin secretion (Sun-Wada et al., 2006).

The *a*4 isoform as a subclass of the renal proton pump

Atp6v0a4, previously known as *Atp6n1b*, encoding the *a*4 subunit, is the gene responsible for the autosomal recessive form of distal renal tubular acidosis (dRTA) (Smith et al., 2000). In contrast to the other *a*1, *a*2, and *a*3 isoforms, the *a*4 isoform is highly restricted to

Table 1. Characteristics of V-ATPase subunit isoforms.

Domain		Subunit	Yeast gene	Mouse isoforms (expression)	Mouse gene
V_1	Catalytic hexamer (*A*3*B*3)	A	*VMA1*		*Atp6a1*
		B	*VMA2*	*B*1 (renal, epididymis, otic)	*Atp6V1B1*
				*B*2 (ubiquitous)	*Atp6V1B2*
	Stalks	C	*VMA5*	*C*1 (ubiquitous)	*Atp6V1C1*
				*C*2-a (lung)	*Atp6V1C2a*
				*C*2-b (lung, kidney)	*AtpV1C2b*
		D	*VMA8*		*Atp6V1D1*
		E	*VMA4*	*E*1 (testis)	*Atp6V1E1*
				*E*2 (ubiquitous)	*Atp6V1E2*
		F	*VMA7*		*Atp6V1F1*
		G	*VMA10*	*G*1 (ubiquitous)	*Atp6V1G1*
				*G*2 (neural)	*Atp6V1G2*
				*G*3 (renal, epididymis)	*Atp6V1G3*
		H	*VMA13*	*H*1 (two alternatively spliced isoforms found in human)	*Atp6V1H1*
Vo	Proton pathway	*d*	*VMA6*	*d*1 (ubiquitous)	*Atp6Vod1*
				*d*2 (renal, epididymis)	*Atp6Vod2*
		e	*VMA9*		*Atp6v0e*
		a	*STV1* *VPH1*	*a*1(ubiquitous, synaptic vesicle localization)	*Atp6Voa1*
				*a*2 (ubiquitous, Golgi localization)	*Atp6Voa2*
				*a*3 (ubiquitous, lysosomal localization)	*Tcirg1 (Atp6Voa3)*
				*a*4 (renal, epididymis, optic)	*Atp6Voa4*
		c	*VMA3*		*Atlp6Voc*
		*c**	*VMA11*	No mammalian gene	
		c''	*VMA16*		*Atp6Vof*
		Ac45	*No yeast gene*		*ATP6AP1*
		Ac8-9	*No yeast gene*	Identical to (pro)renin receptor	*ATP6AP2*

*Ac45 and Ac8-9 are accessory subunits of V-ATPase (Feng et al., 2008; Kinouchi et al., 2010).

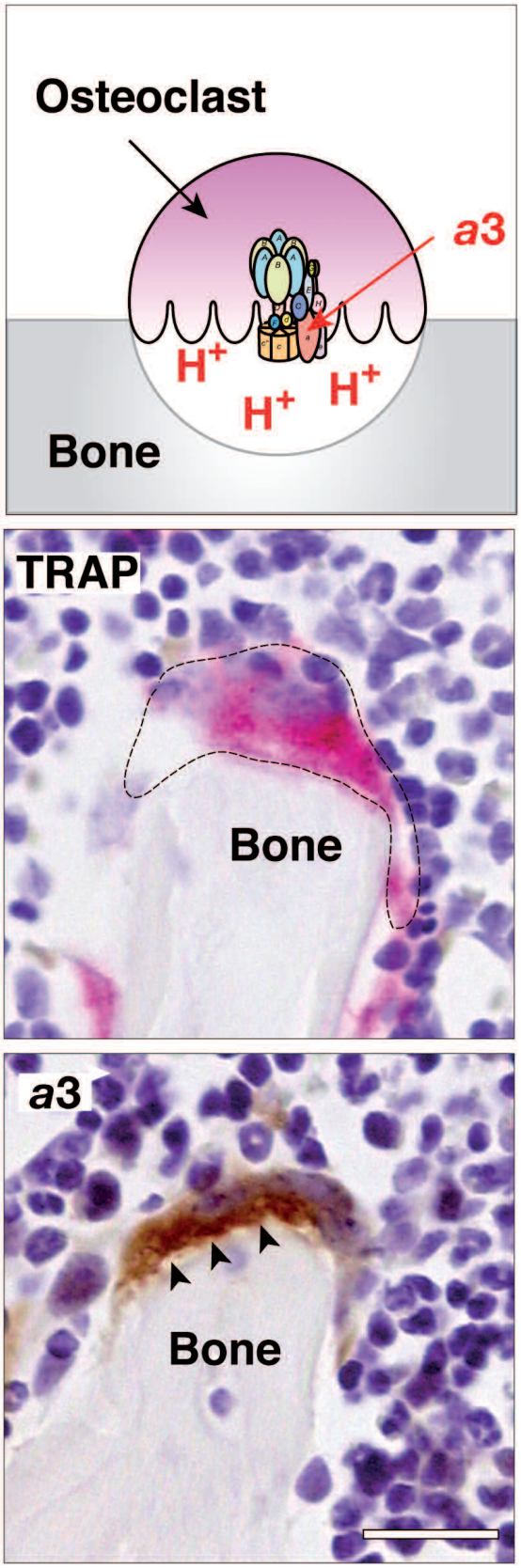
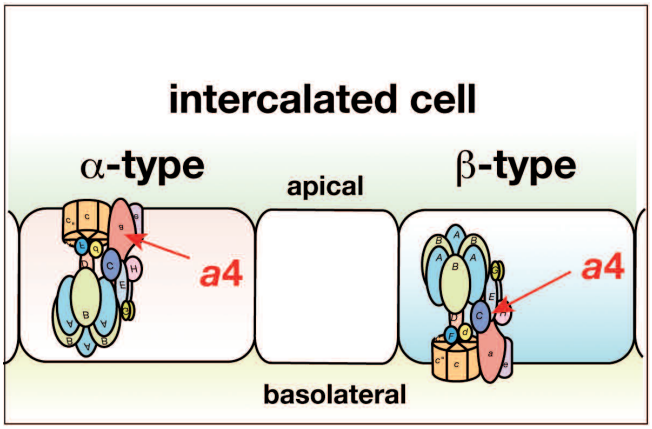


Fig. 2. Localization of the V-ATPase *a*3 isoform in the osteoclast plasma membrane. The upper panel, the model of an osteoclast, is shown together with a resorption lacuna formed between the plasma membrane and the bone surface. The presence of V-ATPase in the plasma membrane is schematically shown. The lower two panels show the histochemistry of the areas around the bone surface. Mouse tibiae were fixed, decalcified, and embedded. Succesive paraffin sections (4 μm) were stained for tartrate-resistant acid phosphatase (TRAP) (a marker for osteoclasts) and the *a*3 isoform (*a*3) of V-ATPase. The outline of the multinuclear osteoclast is indicated by a broken line. The localization of *a*3 on the plasma membranes of osteoclasts is indicated by arrowheads. Bar: 25 μm.



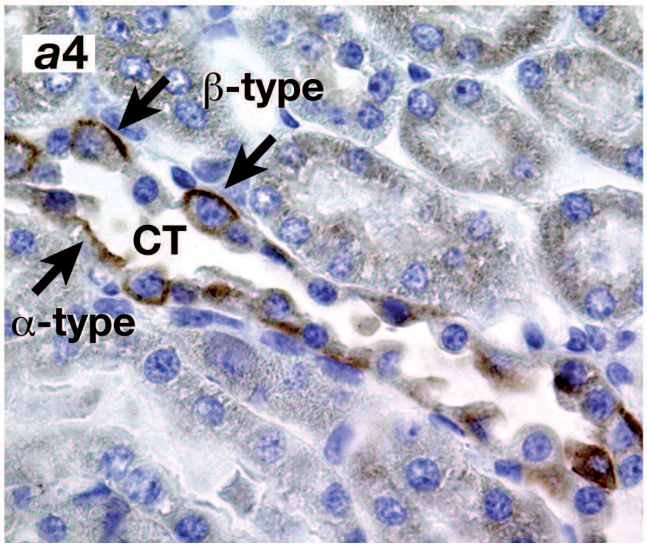


Fig. 3. Localization of the *a*4 isoform on the two major subtypes of intercalated cells in the cortical collecting duct. The upper panel is a schematic representation of the two major subtypes of intercalated cells in the cortical collecting duct. The lower panel shows the histochemistry of the areas around the renal cortical region, using paraffin sections. The α-cells, proton-secreting cells, have an apical V-ATPase with an *a*4 isoform, and ß-cells have *a*4 containing V-ATPase of the opposite polarity.

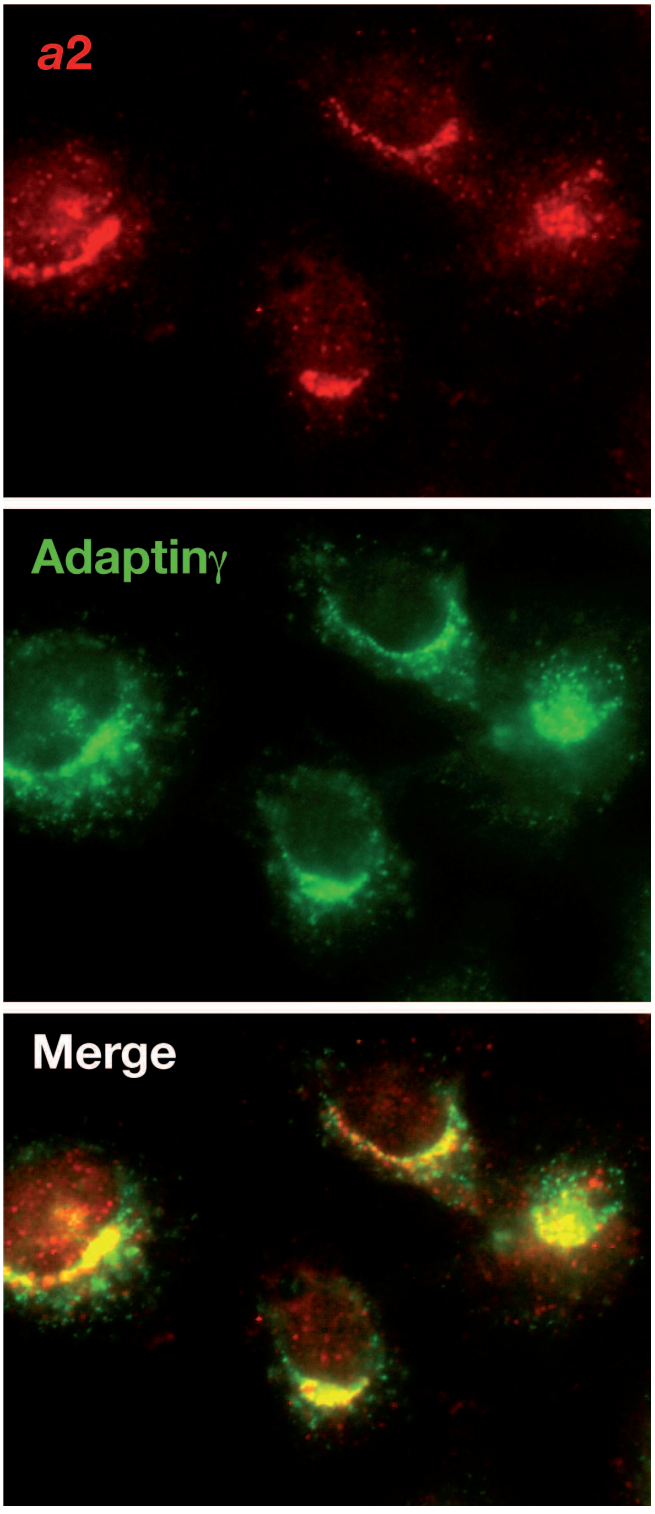


Fig. 4. Golgi localization of the *a2* isoform. The B16 cells were fixed and stained with antibodies against the *a2* isoform and adaptin γ, a marker protein of the Golgi apparatus.

epithelial cells in certain tissues, including the renal tubules, epididymis, and cochlea. This tight tissue specificity is regulated by a transcription factor Foxi1, which is also responsible for distal renal acidosis (Vidarsson et al., 2009).

The kidney-specific expression of the *a*4 and *B*1 isoforms then raised the speculation that they assemble to form a kidney-specific V-ATPase complex. This is partially true since *B*1-specific antibodies precipitate the *a*4 subunit isoform but not the other a subunits (*a*1, *a*2, and *a*3) under non-denatured conditions, showing that the *B*1 subunit is specifically composed of V-ATPase and the *a*4 subunit. However, the situation is complicated further when antibodies against the *B*2 subunit, the ubiquitously expressed isoform, are used in immunoprecipitation experiments. In this case, the *a*1, *a*2, and *a*3 isoforms are precipitated as expected, but in addition to these "ubiquitous" a subunits, the *a*4 subunit is also found in the precipitates. This suggests that *a*4 is associated with *B*1 and is able to form a V-ATPase complex with the *B*2 subunit isoforms. This molecular nature of *a*4 is noteworthy in terms of functional redundancy.

Another complication is with respect to the functional phenotype due to genetic loss. We recently found altered expression of the *a*4 subunit in the ocular tissues lacking the *a*3 subunit. The transport epithelium-specific *a*4 subunit is highly expressed in the retinal pigmented epithelial cell layer, while the *a*3 subunit is mainly localized to uveoscleral tissues where the *a*4 subunit is expressed below detection levels. However, in the mutant ocular system lacking the *a*3 subunit, the *a*4 subunit becomes positive in the scleral/uveal tissues, probably compensating for the lack of the *a*3 subunit (submitted). This ectopic expression of the *a*4 subunit implies the presence of a regulatory mechanism to compensate for the loss of one of the subunits of the V-ATPase complex by substitution with the other subunit *a*, most likely at the level of gene expression. Similar upregulation of the other subunit isoforms was observed in pancreatic Langerhans islets lacking the *a*3 subunit (Sun-Wada et al., 2006). In gene knock-out experiments of the *a*3 locus, we found that the *a*2 subunit became more abundant in the islets of both α- and ß-cells, suggesting that the amount of V-ATPase complex is regulated in the cells, and reduced supply of one of the *a* subunit isoforms turns on the production of the other isoform(s) for compensation. The mechanism by which the cells sense the amount of proton pump, or more likely, the levels of acidification, and transmit such information to the gene expression machinery remains to be discovered.

In addition to such regulation in terms of the production of the V-ATPase complex, their subcellular localization might be regulated by the sensing mechanism as well. The *a*4-containing V-ATPase complex is usually found on the plasma membranes of cells with epithelial characteristics. The *a*2 subunit, in contrast, is mostly localized in the Golgi apparatus (see

next section). The *a*3 subunit resides in the endosomes and lysosomes. In the event of functional compensation between these subunit isoforms, the V-ATPase containing each isoform should be transported toward distinct destinations upon the loss of one of these isoforms. This implies that there is a cellular mechanism that regulates the subcellular localization of the V-ATPase complex by sensing the extent of acidification. The acidification of endomembrane systems are considered as a regulatory parameter in protein sorting along the endocytic and exocytic compartments (Mellman et al., 1986), although discovering the molecular basis of this sorting has only started recently (Hurtado-Lorenzo et al., 2006). The functional compensation among the subunit isoforms suggests that the regulatory circuit must be operating in the opposite direction within the cells.

The *a*2 subunit: a Golgi resident

Most recently, mutations at the *ATP6V0A2* locus encoding the *a*2 subunit are shown to be responsible for an inherited skin disease *cutis laxa* (Kornak et al., 2008). The loss of *a*2 function causes defective post-translational glycosilation of the secreted proteins, resulting in abnormal assembly of the extracellular matrix and skin. At the cellular level, defective sugar processing is consistent with the previous observation that the *a*2 subunit is predominantly localized in the Golgi apparatus, where sugar moieties are added to proteins targeted to the cell surface (Toyomura et al., 2000). The Golgi localization of *a*2 has also been reported in that V-ATPase with the *a*2 and *E*2 subunit is expressed at high levels in the acrosome, a specialized form of the Golgi apparatus in sperm (Sun-Wada et al., 2002). The acidification inside the Golgi apparatus is considered to be rather mild compared to other exocytic and endocytic compartments that are more distally localized. Loss of the *c* subunit, a common component of all the V-ATPases, severely affects Golgi morphology, especially the *trans*-Golgi network (Sun-Wada et al., 2000). The *a*2 subunit has also been shown to recruit proteins that regulate vesicular trafficking (Hurtado-Lorenzo et al., 2006). These facts underscore the physiological importance of V-ATPase function in the Golgi apparatus.

Because the *a*2 subunit is expressed ubiquitously at various levels in all tissues, a rather restricted phenotype associated with the human mutations suggested that the mutations associated to the *cutis laxa* represent hypomorphic alleles. However, this is not the case because the mutations are shown to occur at splicing sites resulting in frame shifts or premature termination of the translation. The truncated forms of the protein are most likely non-functional since the *a* subunit has transmembrane segments at the C-terminal half of the molecule; thus, premature termination of translation produces proteins lacking some transmembrane segments. These abnormal proteins are expected to be mislocalized and be degraded even if they could leave the endoplasmic reticulum. Therefore, the rather mild phenotype associated with the mutations on *ATP6V0A2* suggests that there may be functional compensation by the other *a* subunit isoforms.

The *a*1 subunit isoform is distributed in most tissues

At present, no phenotypes associated with the dysfunction of the V-ATPase *a*1 subunit have been reported in human or other mammalian models. The *a*1 subunit is highly expressed in neuronal cells. V-ATPase is a component of the synaptic vesicles where the proton pump provides a pH gradient and membrane potential required for the accumulation of various neurotransmitters into the vesicles (Moriyama and Futai, 1990; Takamori et al., 2006). This subunit isoform is also expressed in various other tissues as seen in the *a*2 and *a*3 subunit isoforms. The lack of identification of genetic phenotypes in natural mutations may indicate the importance of the *a*1 subunit in the general cellular physiology since mutations in these "essential" components will threaten cell viability, resulting in lethality in the earliest stages of embryonic development. This has been proven in the case of the V-ATPase *c* subunit mutation. The *c* subunit is the component of the Vo part of V-ATPase. This 16-kDa protein is extremely hydrophobic and constitutes the Vo ring unit with the a subunit. Unlike the *a* subunit, the c subunit is encoded by a single gene, *ATP6V0C* (Hanada et al., 1991); thus, loss of this one gene affects all the V-ATPases. Indeed, mouse embryos homozygous for the null allele of the *Atp6v0c* cannot gastrulate but degenerate at the egg cylinder stage during early development. The defects are obvious at cellular levels. The mutant cells are not able to acidify the intracellular compartments, as expected, and in addition to this phenotype, they exhibited swollen Golgi compartments, defects in endocytosis, and defective proliferation. These observations suggest that V-ATPase plays essential roles in mammalian cells (Sun-Wada et al., 2000). We speculate that the V-ATPase *a*1 subunit is required for various cellular functions, and this will be examined by creating a model mouse with a conditional knock-out genetic modification.

Therefore, the V-ATPase function sustains basic physiology at a cellular level, and its loss causes lethality. Despite this essential function, at least in the case of the *a*2, *a*3, and *a*4 subunits, their losses do not result in a severe phenotype akin to embryonic lethality. This might imply that there can be compensation among subunit isoforms, although they exhibit distinctive subcellular localization and tissue specificity in normal animals.

Diversity in the V-ATPase catalytic sector (V_1)

The V_1 domain of the V-ATPases are composed of 3 *A* and 3 *B* subunits that assemble alternately to form a ring, and the *C, E, D, F, G, and H* subunits constitute a

stalk structure that connects V_1 and Vo (the membrane embedded part of the enzyme). This structural model was derived from an analogy between the V- and F-type ATPases, the latter being well defined at an atomic level (Abrahams et al., 1994). The F- and V-ATPases are composed of multiple subunits and show limited sequence similarity in their primary structure; furthermore, they display a similar catalytic mechanism for ATP hydrolysis and proton translocation; the two chemical and osmotic mechanisms are connected with mechanical rotation of the subunit complex (Sambongi et al., 1999; Nishio et al., 2002; Hirata et al., 2003).

The V_1 subunits show limited diversity as compared to those of the Vo membrane sectors. All mammalian V-ATPases possess *A* subunits containing the nucleotide binding and hydrolysis sites. The *A* subunit contains the nucleotide-binding site and constitutes the catalytic site for ATP hydrolysis along with the *B* subunit. In the mammalian genome, there is only one structural gene for this subunit; thus, all the V-ATPases that are distributed to various cellular locations share the single gene, *Atp6v1a*. The *B* subunits have two isoforms, *B*1 and *B*2; the former is expressed ubiquitously, while the latter is limited to certain types of epithelial cells, including the kidney-collecting duct, epididymis, and auditory system. This tissue-specific distribution pattern of *B*1 is consistent with the expression of the *a*4 subunit. Loss of the V-ATPase *B*1 subunit leads to distal renal acidosis and auditory defects in human patients. However, while renal acidosis occurs in both humans and mice, the auditory defects are restricted to humans.

With respect to the subcellular localization of the enzyme complex, it is still unclear whether any V_1 subunit(s) contributes to this process, excluding the *B* subunit isoforms. In several epithelia, including the renal epithelium and the epididymis, the *B*1 subunit is concentrated in the apical plasma membrane of the polarized epithelial cells, while the *B*2 subunit is mainly localized in the subapical cytoplasm (Da Silva et al., 2007). The *B* subunits are known to interact with filamentous actin (F-actin). This enzyme-cytoskeleton interaction plays important roles in the recruitment of V-ATPase to the cell surface of osteoclasts (Holliday et al., 2000; Zuo et al., 2006). However, the *B*1 and *B*2 subunits both interact with F-actin indistinguishably (Holliday et al., 2000); therefore, this interaction alone is not sufficient to explain the distinctive localization of the proton pumps, i.e., intracellular for *B*2 and cell surface for *B*1.

*C*1 and *C*2 are expressed from two distinctive genomic loci, *Atp6v1c1* and *Atp6v1c2*. The *C*1 isoform is ubiquitously distributed in all the tissues in mammals. The *Atp6v1c2* locus yields two distinctive products by alternative splicing: *C*2-a and *C*2-b, the expression of which is restricted to the lung. Although these subunits (*C*1, *C*2-a, and *C*2-b) are all structurally different, the functional differences have not been well documented (Sun-Wada et al., 2003). Similar to the *C* subunit isoforms, the functional differences among the *G* subunit isoforms (*G1, G2,* and *G3*) (Murata et al., 2002) await future model systems lacking the isoform genes. It is noteworthy that the *G*2 and *G*3 subunit isoforms also show strict tissue specificity in the brain and in the renal and inner ear epithelium, respectively, whereas the *G*1 isoform is expressed ubiquitously. However, the epithelium-specific *G*3 isoform can interact with the ubiquitous *a*1 subunit, while *G*1 is able to do so with the renal-enriched *a*4 isoform (Norgett et al., 2007). This nature of *G-a* interaction is similar to *B-a* interaction, in which no preference between tissue-restricted isoforms has been identified.

In contrast, V-ATPases with the different *E* subunits *E*1 and *E*2 show apparent differences in their biochemical properties (Sun-Wada et al., 2002; Hayashi et al., 2008). *E*1 is a ubiquitous subunit expressed across all the tissues studied thus far, while expression of the *E*2 subunit is highly restricted to sperm. Interestingly, V-ATPases containing the *E*1 and *E*2 subunit isoforms show different temperature sensitivities (Sun-Wada et al., 2002; Hayashi et al., 2008). The *E*1 and *E*2 subunits reside in the stalk connecting V_1 and Vo and function as a rotor or stator during rotation, as well as during the V_1 and Vo assembly. To date, no genetic mammalian models are available for either *E* subunit isoform.

V-ATPase assembly: an essential process for the expression of function

V-ATPase is a multisubunit enzyme embedded in various cellular membranes, and its biogenesis involves complicated processes that have not been fully understood yet. The *a, c, c', e* and *d* subunits are synthesized on the ER membrane, and they are assembled into the Vo membrane sector at that site; the subcomplex is then dispatched from the ER towards its subcellular destination, where it meets the V_1 catalytic sector to assemble into the functional proton pump. By extensive genetic screening using a unicellular organism, yeast, Stevens and colleagues have found a set of assembly factors for the Vo subcomplex, the effective functioning of which is required for proper expression of the V-ATPase function; gene products are not found in the final VoV_1-ATPase complex.

Recently, a human homologue of Vma21p was shown to be responsible for the assembly of the Vo subcomplex in the ER. Reduced functioning of this gene causes X-linked myopathy with excessive autophagy (XMEA). The onset of XMEA occurs during childhood and results in weakness of the skeletal muscles and proximal lower extremities, but other organs, including the heart and the brain, remain unaffected. The skeletal muscle of these patients shows vacuolation of the lysosome/endosome systems and increased autophagic markers, reflecting the defective clearance of endocytic and autophagic substrates for degradation (Ramachandran et al., 2009).

All the mutations found in human patients are thought to be attributable to a hypomorphic allele, since

most of them occur in introns or in the regulatory regions. One mutation caused an amino acid change, Gly->Ala, and no severe mutations have been found yet. This might reflect the fact that Vma21p function is critical for cell survival. Interestingly, muscles probably show high dependence on V-ATPase function: chloroquine, a common anti-malarial drug, dissipates pH gradients of lysosomes and endosomes, causing myopathy and heart failure as side effects. It is possible that muscles are more sensitive to a decrease in amino acids as compared to other tissues. Macroautophagy, a V-ATPase-dependent process that breaks down the cytoplasm to supply amino acids, is active in the muscle (Mizushima et al., 2004). However, this is still not sufficient to explain the lack of myopathy in the null mutant of the V-ATPase *a*3 subunit, which is a major V-ATPase complex in the lysosomes, where the final degradation of the cytoplasm, being engulfed by the autophagosome, takes place. Therefore, the pathology underlying the lack of Vma21p might involve some other physiological relevance of this protein.

Yeast genetics identified other components involving the assembly of the V-ATPase complex. Even though their function as the molecular chaperone in the biogenesis of the proton pump in the ER compartments in yeast cells has been well established, the cognate homologues have not been found in mammals, suggesting that the mechanism of complex assembly may be somewhat different in yeast and mammalian cells. Indeed, the subunits comprising the V_1 segment are interchangeable with the mammalian protein in yeast cells (Hayashi et al., 2008; Sun-Wada et al., 2002); we were unable to rescue the yeast Vo mutants by heterologous expression of the mammalian Vo subunits (our unpublished observation). One speculation is that this is due to the defective assembly of mammalian subunits with the yeast Vo complex because of a lack of molecular chaperones for the assembly, although this possibility remains to be experimentally proven in the future.

In this study, we focused on select subjects concerning the physiological consequences of the loss of V-ATPase function in mammalian systems. Accumulating knowledge on these subjects in non-mammalian organisms, including nematodes, fruit fly, and zebrafish, which are reinforced by amenable genetics, thus contributes greatly to our understanding of the physiological relevance of V-ATPase in higher organisms. Although it might be possible to predict the subunit correlation between the mammalian and non-mammalian isoforms (Lee et al., 2010) based on the primary sequence comparison (Allan et al., 2005), we would like to reserve such predictions at present because of the lack of information on the subcellular distribution of V-ATPase subunits in non-mammalian systems. The structural motifs determining the localization of V-ATPases are still under investigation; therefore, it is premature to predict or interpret the intracellular locations from the primary sequences of V-ATPase subunits in different species.

Perspectives

Individual V-ATPase subunits and their isoforms contribute directly to the diversity of roles served by the enzyme in normal physiological processes. However, the regulatory mechanism underlying the unique combinations of isoforms and the information necessary for targeting the enzyme to different cellular destinations are still not clearly understood. This knowledge would be useful in the therapy of V-ATPase-related diseases.

Acknowledgements. The original work from our laboratories described in this article was carried out in collaboration with H, Tabata, N. Kawamura, M. Aoyama, and other coworkers, whose names appear in the references. This study was supported by grants-in-aids from the Ministry of Education, Culture, Science and Technology Japan, Japan Science and Technology Agency, Hayashi Foundation, and Noda Foundation.

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Accepted June 21, 2010