A simple nomenclature for a complex proton pump: *VHA* genes encode the vacuolar H$^+$-ATPase

Heven Sze, Karin Schumacher, Mathias L. Müller, Senthilkumar Padmanaban and Lincoln Taiz

The vacuolar-type H$^+$-ATPase acidifies intracellular compartments and is essential for many processes, including cotransport, guard cell movement, development, and tolerance to environmental stress. We have identified at least 26 genes encoding subunits of the vacuolar-type H$^+$-ATPase in the *Arabidopsis thaliana* genome, although inconsistent nomenclature of these genes is confusing. The pump consists of subunits A through H of the peripheral V_1 complex, and subunits a, c, c″ and d of the V_o membrane sector. Most V_1 subunits are encoded by a single gene, whereas V_o subunits are encoded by multiple genes found in duplicated segments of the genome. We propose to name these genes *VHA-x*, where *x* represents the letter code for each subunit. Applying a consistent nomenclature will help us to understand how the expression, assembly and activity of this pump are integrated with plant growth, signaling, development and adaptation.

Published online: 11 March 2002

Plant growth and development depend on the uptake, translocation and sorting of 15 essential nutrients and countless metabolites to specific organs, cells and subcellular compartments. Furthermore, to survive, plants need to adapt to and tolerate changes in the environment such as toxic metals and excess salt. How plants sense and respond to changing nutrient levels and environmental stresses by up- or down-regulation of transporters and their activities is poorly understood. Proton pumps occupy a prominent position among all transporters. Without the primary proton motive force to energize coupled carriers or ion channels, all other transport, and thus life, would cease. In spite of this, we do not understand how proton pumps are integrated into the signal transduction networks that govern growth and adaptation.

Of three distinct proton pumps in plants, the vacuolar-type H-ATPase (V-ATPase) is the most complex in subunit composition [1–3]. The completion of the *Arabidopsis thaliana* genome gives the first glimpse of the number of proton pumps required by a higher plant to complete its life cycle. The plasma membrane H$^+$-ATPase extrudes H$^+$ from the cell and energizes the uptake and release of many nutrients across the plasma membrane of plant cells. *Arabidopsis* has 12 members of P-type H$^+$-ATPase genes (AHA1–AHA12), some of which are expressed in a tissue-specific manner [4,5]. Inside the cell, the V-ATPase and the H$^+$-PPase acidify intracellular compartments (Fig. 1), but it is unclear how the cellular roles of these two distinct pumps differ [6]. In *Arabidopsis*, we now know that three genes encode homologs of H$^+$-PPase (AVP1–AVP3) [7]. The aim of this article is to:

- Highlight briefly the importance of vacuolar ATPase for growth, development and adaptation of plants.
- Identify all the genes of this complex pump following completion of the sequencing of the *Arabidopsis* genome.
- Show the chromosome location of all the genes.
- Propose a simple name for the genes applicable to all plants.

A pump with diverse functions

V-ATPase is emerging as a pump with diverse and surprising functions in eukaryotes. In plants, V-ATPase has been localized to vacuoles and other membranes of the secretory system, including the endoplasmic reticulum (ER), Golgi and small vesicles as well as the plasma membrane (Fig. 1) [1,3]. A major role of this pump is to acidify the vacuole, provide the energy for transport of ions and metabolites, and so influence turgor and cell expansion. Other experiments have suggested that the proton electrochemical gradient is important for protein sorting [8]. Surprisingly, the V_o integral sector was recently shown to be required for vesicle–vesicle fusion in yeast [9,10], suggesting that the membrane sector has a role in membrane fusion during the formation of the central vacuole. The V-ATPase complex is conserved in all eukaryotes (Table 1) [2,11]. However, the unique physiology of plants, including nutrient transport, flowering, stress tolerance and the particular functions of guard cells, vascular and meristem tissues, indicates that this pump plays many roles specific to plants.

Twenty years after its discovery [12], we are just beginning to understand the broader implications of the roles that the V-ATPase pump plays in plants. V-ATPase-deficient mutants are powerful tools for dissecting V-ATPase functions *in vivo*. The first V-ATPase mutant in plants, *det3*, shows a reduction in subunit C and in V-ATPase activity, and is de-etiolated when grown in the dark [13]. Moreover, stomatal closure induced by high levels of

 1360-1385/02/$ – see front matter PII: S1360-1385(02)02240-9

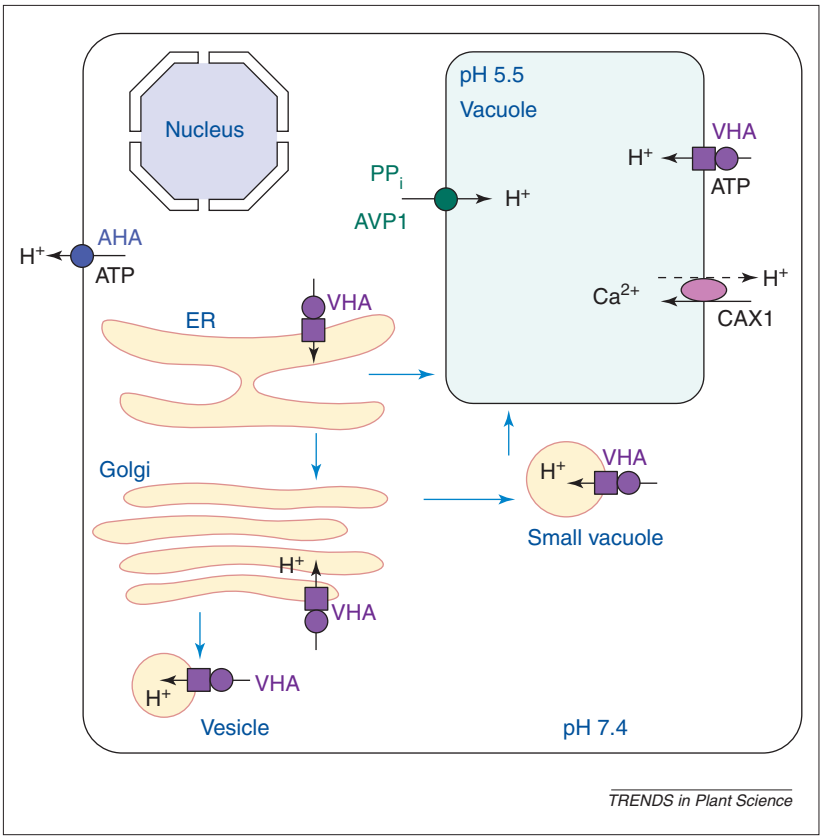

Fig. 1. A pump with diverse functions. Vacuolar-type H^+-ATPase (VHA) has been localized to vacuoles, endoplasmic reticulum (ER), Golgi, small vacuoles, vesicles and the plasma membrane in plants. Acidification of intracellular compartments provides energy for the release or uptake of many ions (e.g. Ca^{2+}) and metabolites. The resulting changes in pH, ion gradient or activities and osmotic potential of these compartments conceivably affect diverse cellular functions [1], including protein sorting, growth and guard cell signaling. Abbreviations: AHA, *Arabidopsis* plasma membrane H^+-pumping ATPase; AVP1, vacuolar H^+-pumping PPase; CAX1, Ca^{2+}/H^+ antiporter.

external calcium ions was abolished in the mutant [14]. These studies show that V-ATPase functions are closely integrated with growth, development and guard cell signaling.

Recognizing genes encoding V-ATPase subunits

Although the *Arabidopsis* genome is completed, the genes encoding vacuolar H^+-ATPase subunits are often hard to distinguish according to the existing databases for several reasons. First, unlike the single polypeptide of the plasma membrane H^+-ATPase and the H^+-PPase, the V-ATPase has more than ten different subunits. Second, similarities between many V-ATPase subunits and those of the mitochondrial and the chloroplast ATP synthases (F-ATPases) have resulted sometimes in mislabeling in the gene or protein databases. Third, there is no consistent nomenclature of gene names among the various laboratories working on plant or animal V-ATPases. Names used to denote plant V-ATPase genes include Dc69 (carrot A) [15], At57 (*Arabidopsis* B) [16], vatpD (D) [17], TpP31 (barley E) [18], Vag (tobacco G) [19], AVA-P1 (c) [20] and DET3 (C) [13]. Here, we propose that plant biologists adopt the name '*VHA*' for genes encoding subunits of the vacuolar H^+-pumping ATPase. For example, genes from *Arabidopsis* would be identified as '*AtVHA*'; and those from rice (*Oryza sativa*) would be named *OsVHA*.

We have identified all the genes encoding putative VHA proteins in the completed *Arabidopsis* genome using several methods. Blast searches of deduced proteins from *Arabidopsis* ESTs and the genome-sequencing project were performed

Table 1. Proposed nomenclature of V-ATPase subunit genes and proteins in plants[a]

Subunit	Function[b]	Yeast reference[c]		New name	*Arabidopsis*	
		kDa	Gene	Gene or protein	kDa	Protein
V_1 peripheral sector						
A	Catalytic ATP-binding	69	*VMA1*	VHA-A	68	Peripheral
B	Noncatalytic ATP binding	60	*VMA2*	VHA-B	54	Peripheral
C	V_1 stability, activity	42	*VMA5*	VHA-C	42	Peripheral
D	Central stalk, coupling [29]	32	*VMA8*	VHA-D	29	Peripheral
E	Peripheral or central stalk [22,24]	27	*VMA4*	VHA-E	26	Peripheral
F	Bridge V_1–V_o contacts	14	*VMA7*	VHA-F	14	Peripheral
G	Coupling V_1 and V_o	13	*VMA10*	VHA-G	12	Peripheral
H	Regulatory	54	*VMA13*	VHA-H	50	Peripheral
V_o membrane sector						
a	Coupling, assembly [26]	95–101	*VPH1/STV1*[d]	VHA-a	89–95	Integral, 6–7 TM
c	Proton translocation	16	*VMA3*	VHA-c	16	Integral, 4 TM
c′	Proton translocation	17	*VMA11*	–	ND	–
c″	Proteolipid similar to c	23	*VMA16*	VHA-c″	18	Integral, 5 TM
d	V_o assembly, stability	36	*VMA6*	VHA-d	40	Peripheral
e		–	ND	VHA-e	~8	Integral 2 TM

[a]Yeast gene names and subunits are listed for reference only. Suggested new name can be applied to any plant and animal V-ATPases. *Arabidospsis thaliana* genes would be denoted as AtVHA-x.
[b]Data from Refs [11,21].
[c]Data from Ref. [11].
[d]*VPH1* and *STV1* are homologs of subunit a in yeast [21].
Abbreviations: ND, not detected. TM, transmembrane domain.

Heven Sze*
Senthilkumar Padmanaban
University of Maryland, Cell Biology and Molecular Genetics, HJ Patterson Hall, College Park, MD 20742-5815, USA.
e-mail: hs29@umail.umd.edu

Karin Schumacher
ZMBP-Pflanzenphysiologie, Universitaet Tübingen, Auf der Morgenstelle 1, 72076 Tübingen, Germany.

Mathias L. Müller
Maxygen Inc., 515 Galveston Drive, Redwood City, CA 94063, USA.

Lincoln Taiz
Molecular, Cellular and Developmental Biology, University of California, Santa Cruz, CA 95064, USA.

Table 2. Most V_1 subunits are encoded by a single gene in *Arabidopsis*

Subunit name(s)[a]	Gene no.	Accession no. Locus	(protein)	DNA exons	Protein aa	MW	pI
A	**1**						
VHA-A		At1g78900	(AAC83021)	20	623	68812	5.1
B	**3**						
VHA-B1/At57 [16]		At1g76030	(AAC36485)	12	486	54107	5.0
VHA-B2		At4g38510	(CAB37507)	14	487	54304	5.0
VHA-B3		At1g20260	(AAF88162)	14	485	54250	5.1
C	**1**						
VHA-C/Det3 [13]		At1g12840	(AAF78489)	11	375	42619	5.4
D	**1**						
VHA-D/vatpD [17]		At3g58730	(CAB88290)	1	261	29057	9.5
E	**3**						
VHA-E1		At4g11150	(AAC35545)	6	230	26060	6.1
VHA-E2		At3g08560	(AAG51352)	5	235	26852	9.2
VHA-E3		At1g64200	(AAF24559)	6	237	27085	5.8
F	**1**						
VHA -F		At4g02620	(AAC78269)	4	128	14259	6.1
G	**3**						
VHA-G1/Vag1 [19]		At3g01390	(AAF24609)	3	110	12396	5.8
VHA-G2/Vag2		At4g23710	(CAB81289)	3	106	11741	5.5
VHA-G3		At4g25950	(CAB39660)	3	108	12115	5.1
H	**1**						
VHA-H		At3g42050	(CAB91576)	11	441	50284	6.6

[a]Published names of genes are also shown.
Abbreviations: aa, amino acid; MW, molecular weight.

Table 3. Integral V_o subunits are encoded by multiple genes in *Arabidopsis*

Subunit name(s)	Gene no.	Accession no. Locus	(Protein)	DNA exons	Protein aa	MW	pI
a	**3**						
VHA-a1		At2g28520	(AAD21487)	17	780	89335	7.3
VHA-a2		At2g21410	(AAD23686)	18	821	93105	5.4
VHA-a3		At4g39080	(CAB38828)	18	843	95160	5.9
c	**5**						
VHA-c1/AVA-P1 [20]		At4g34720	(CAA18851)	3	164	16571	8.6
VHA-c2/AVA-P2		At1g19910	(AAA99937)	3	165	16642	8.6
VHA-c3/AVA-P3		At4g38920	(CAB80555)	3	164	16571	8.6
VHA-c4/AVA-P4		At1g75630	(AAF87129)	3	166	16685	8.6
VHA-c5/AVA-P5		At2g16510	(AAD26493)	3	164	16571	8.6
c′′	**2**						
VHA-c′′1		At4g32530	(CAB79970)	4	180	18374	7.8
VHA-c′′2		At2g25610	(AAD31363)	4	178	18218	7.8
d	**2**						
VHA-d1		At3g28710	(BAB02186)	10	351	40791	5.0
VHA-d2		At3g28715	Q9LHA4	10	351	40784	5.0
e [= M9.7]	**2**						
VHA-e1		At5g55290	(BAB08598)	3	70	7725	6.7
VHA-e2		At4g26710	(CAB36517)	3	70	7685	6.7

Abbreviations: aa, amino acid; MW, molecular weight.

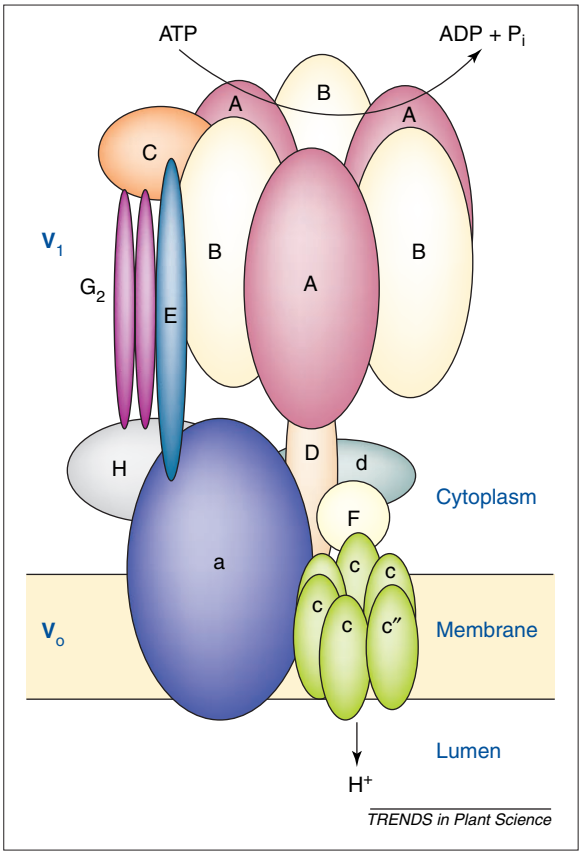

Fig. 2. A revised model of the vacuolar-type ATPase adapted from Ref. [22]. The model is based mostly on topology studies of the vacuolar-ATPase (V-ATPase) from yeast and the bovine clathrin-coated vesicle [21]. Electron micrographs of coated vesicle V-ATPase showed a peripheral stalk that runs from the top of the V_1 to the V_o sector with space between the central and the peripheral stalk [23]. Subunit E is thought to form part of the peripheral stalk, linking the outer surface of V_1 with V_o [22], rather than part of the central stalk [24]. Evidence suggests E is in contact with G, C and H. The cytosolic N-terminal domain of subunit 'a' is localized in the cytosol and associates with H and A [25]. The integral C-terminal domain of a subunit affects coupling of ATP hydrolysis to proton transport [26]. In yeast, each V_o complex contains all three types of c subunits [27]. Because subunit c′ is not found in the *Arabidopsis* genome, we propose each V_o has one subunit c′′ and five copies of subunit c. V-ATPase has been proposed to operate by a rotary mechanism similar to the F-ATPase [28]. In this model, ATP hydrolysis by the A_3B_3 hexamer held stationary by subunit a and the peripheral stalk drives rotation of the central core (D), which, in turn, causes the ring of c subunits to rotate. Subunit c carries the protons and the rotation of the c subunit ring is thought to be essential in driving proton transport.

initially using protein sequences of previously characterized V-ATPase subunits from yeast, plants and other eukaryotes. *Arabidopsis* Genome Initiative gene codes were obtained from the Membrane Transport Systems site of Ian Paulsen (http://www.biology.ucsd.edu/~ipaulsen/transport/). Predicted protein sequences were later obtained from the *Arabidopsis* Membrane Protein library (AMPL) site (http://www.cbs.umn.edu/arabidopsis), and the Munich Information Center for Protein sequences (MIPS) (http://mips.gsf.de/proj/thal/db/index.html). The information was verified independently with translated cDNAs and EST sequences whenever possible. A few genes encoding F-type ATP synthase subunits, but classified under V-ATPase, were deleted. These revisions are being incorporated in the public databases.

Genes for at least 12 distinct subunits have been identified (Tables 1–3). The pump consists of two main sectors: the peripheral V_1 binds and hydrolyses ATP and the integral V_o provides the pathway for

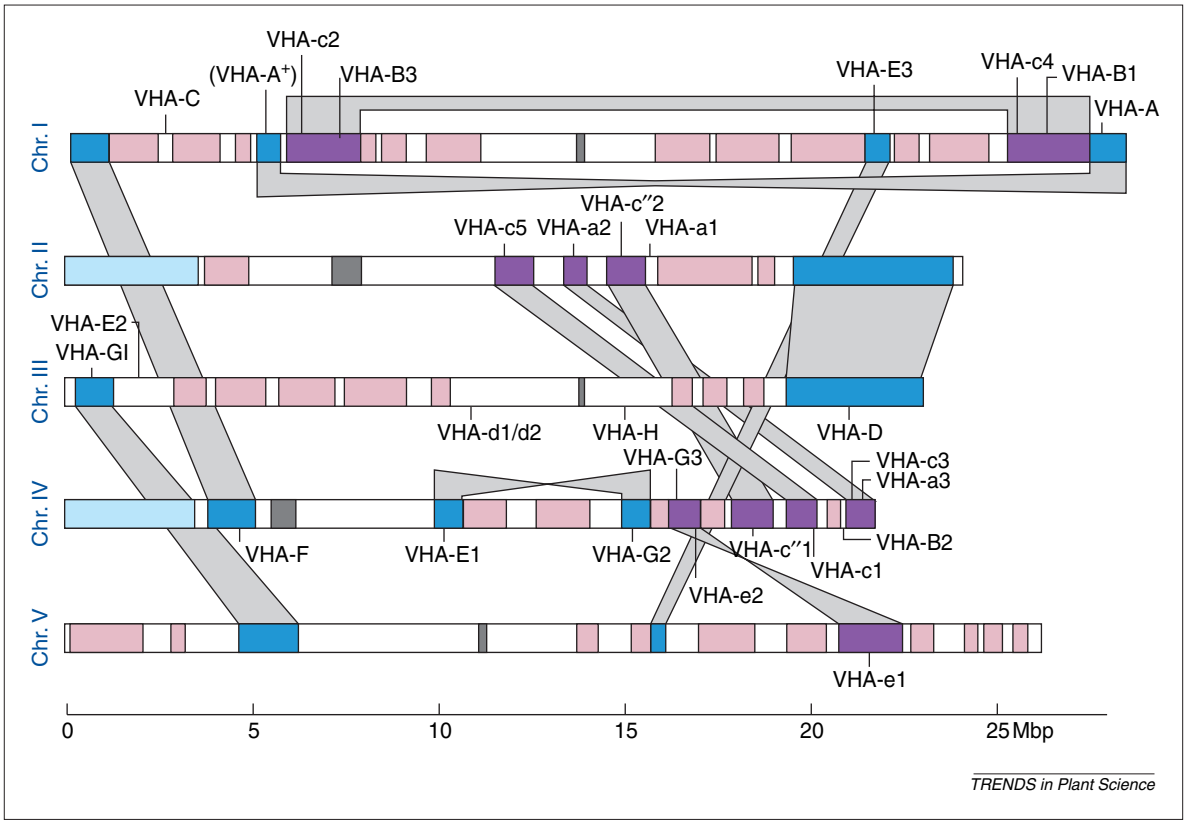

Fig. 3. Chromosomal locations of vacuolar-type H⁺-ATPase (VHA) genes. The five chromosomes (Chr. I–V) are depicted as horizontal bars, centromeres are indicated in dark gray. Dark-blue and purple bands represent duplicated regions in which VHA genes are located, pink and light-blue bands represent other duplicated segments [32]. Light-gray bands are twisted if the corresponding segments have reversed orientation.

proton conductance [1,11,21]. A revised model adapted from Ref. [22] and based on recent studies [21,23–28] is shown in Fig. 2. V_1 subunits include A, B, C, D, E, F, G and H. The function of each is briefly summarized in Table 1 based on studies using yeast, plants and other eukaryotes. The major body of evidence comes from experiments using yeast mutants and a combination of chemical modification, site-directed mutagenesis and *in vitro* assembly [11,21,22,26,29]. V_o subunits include a, c, c″ and d. Unlike yeast, a homolog of 17 kDa subunit c′, or Vma11, was not detected in the *Arabidopsis* genome or in the *Drosophila* genome [30]. Instead there is another integral protein of 8 kDa (sometimes referred to as 'M9.7'), which is tentatively named VHA-e.

We propose that each subunit be named 'VHA' followed by the subunit letter code (Table 1). This proposal was sent electronically to several investigators in March 2001 and discussed at the 12th International Workshop on Plant Membrane Biology in August 2001 to get a consensus. We urge the plant research community to adopt the proposed names as a step towards more accurate information exchange, and so facilitate progress. The VHA-x name has several advantages:

- The name VHA is applicable to all eukaryotes.
- The gene name is followed by a letter code, such as VHA-B1, to identify the predicted subunit.
- A letter code for each subunit is more definitive than a molecular mass that varies among isoforms within one plant and among different species.
- Mutant alleles can be named consistently as *vha-B1–1*.

The nomenclature of 'VHA' has been used to denote the V-ATPase in *Caenorhabditis elegans* [31] and *Drosophila* [30], although a subunit number (e.g. Vha-2) or molecular mass (e.g. vha16–2) can be confusing even to workers in the field.

Single and multiple genes encode V_1 and V_o subunits

Unlike the P-type proton pumps, which are encoded by multigene families in *Arabidopsis* [4], most of the subunits in the V_1 sector (A, C, D, F and H) are encoded by single-copy genes (Table 2). The exceptions are subunits B, E and G.

By contrast, all the V_o subunits are encoded by at least two genes (Table 3), and as many as five genes code for VHA-c [20]. Considering that extensive duplications cover most of the *Arabidopsis* genome [32], it is surprising that only five of the 14 V_1 genes are found in non-duplicated regions. The other nine genes are found in duplicated regions but their respective counterparts have been lost. In the case of VHA-A, remnants of its duplicate can still be found. By contrast, 11 of the 12 V_o genes are found in duplicated regions and in only one case the duplicate is lost (Fig. 3). Intriguingly, the completely

sequenced genomes of the fly and worm show that subunits 'c' and 'a' of the V_o sector are also encoded by multiple genes (from 3 to 5) [30,33], whereas most V_1 subunits are not (http://www.wormbase.org/; http://www.fruitfly.org/). The significance of this is not understood. We speculate that (i) pump activity is regulated by the expression, synthesis and assembly of the integral V_o subcomplex [1], and (ii) V_o subcomplexes can perform functions independently of V_1, such as in membrane fusion [10].

Future directions

The information emerging from the completed *Arabidopsis* genome raises many questions. How do cells coordinate the expression of 12 or more genes scattered among five chromosomes? How do cells coordinate the assembly of 12 subunits to form a functional pump complex? What particular roles does each subunit perform? Do they participate in catalytic, regulatory or assembly functions, or interact with other cellular components, such as the cytoskeleton or scaffolding proteins? How does increased expression of certain subunits aid in plant adaptation to environmental stresses [2,3]? What is the role of V-ATPase in signaling networks that determine morphogenesis [13] or guard cell movement [14]? Identifying the complement of V-ATPase genes in a higher plant was an important first step, and the tools available in this era of functional genomics offer the unique possibility to answer some of these questions. These tools include *Arabidopsis* mutants deficient in each V-ATPase subunit, DNA microarrays to study expression of V-ATPase subunits in plants subjected to different environmental cues, and proteomics to determine V-ATPase function and regulation through protein–protein interactions and protein modifications. Applying a consistent nomenclature to V-ATPase genes will help unify the information from these studies.

Acknowledgements
We want to thank Karl-Jozef Dietz, Masayoshi Maeshima and Stephen Mount for their input, suggestions and discussion: We also thank all participants in the session on the Nomenclature of Transporters at the 12th International Workshop of Plant Membrane Biology in Wisconsin.

References

1 Sze, H. *et al.* (1999) Energization of the plant cell membranes by H^+-pumping ATPases: biosynthesis and regulation. *Plant Cell* 11, 677–689
2 Lüttge, U. and Ratajczak, R. (1997) The physiology, biochemistry, and molecular biology of the plant vacuolar ATPase. *Adv. Bot. Res.* 25, 253–296
3 Dietz, K. *et al.* (2001) Significance of the V-type ATPase for the adaptation to stressful growth conditions and its regulation on the molecular and biochemical level. *J. Exp. Bot.* 52, 1969–1980
4 Axelsen, K. and Palmgren, M. (2001) Inventory of the superfamily of P-type ion pumps in *Arabidopsis*. *Plant Physiol.* 126, 696–706
5 Palmgren, M.G. (2001) Plant plasma membrane H^+-ATPases: powerhouses for nutrient uptake. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52, 817–845
6 Maeshima, M. (2001) Tonoplast transporters: organization and function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52, 469–497
7 Drozdowicz, Y.M. and Rea, P.A. (2001) Vacuolar H^+ pyrophosphatases: from the evolutionary backwaters into the mainstream. *Trends Plant Sci.* 6, 206–211
8 Matsuoka, K. *et al.* (1997) A vacuolar-type H^+-ATPase in a nonvacuolar organelle is required for sorting of soluble vacuolar protein precursors in tobacco cells. *Plant Cell* 9, 533–546
9 Wickner, W. and Haas, A. (2000) Yeast homotypic vacuole fusion: a window on organelle trafficking mechanisms. *Annu. Rev. Biochem.* 69, 247–275
10 Peters, C. *et al.* (2001) Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. *Nature* 409, 581–588
11 Stevens, T.H. and Forgac, M. (1997) Structure, function, and regulation of the vacuolar H^+-ATPase. *Annu. Rev. Cell Dev. Biol.* 13, 779–808
12 Sze, H. (1985) H^+-translocating ATPases: advances using membrane vesicles. *Annu. Rev. Plant. Physiol.* 36, 175–208
13 Schumacher, K. *et al.* (1999) The *Arabidopsis det3* mutant reveals a central role for the vacuolar H^+-ATPase in plant growth and development. *Genes Dev.* 13, 3259–3270
14 Allen, G.J. *et al.* (2000) Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis det3* mutant. *Science* 289, 2338–2342
15 Zimniak, L. *et al.* (1988) The cDNA sequence of the 69-kDa subunit of the carrot vacuolar H^+-ATPase. Homology to the beta-chain of F_0F_1-ATPases. *J. Biol. Chem.* 263, 9102–9112
16 Manolson, M. *et al.* (1988) cDNA sequence and homologies of the '57-kDa' nucleotide-binding subunit of the vacuolar ATPase from *Arabidopsis*. *J. Biol. Chem.* 263, 17987–17994
17 Kluge, C. *et al.* (1999) Subunit D of the vacuolar H^+-ATPase of *Arabidopsis thaliana*. *Biochim. Biophys. Acta* 1419, 105–110
18 Dietz, K. *et al.* (1995) Subunit E of the vacuolar H^+-ATPase of *Hordeum vulgare* L.: cDNA cloning, expression and immunological analysis. *Plant J.* 8, 521–529
19 Rouquie, D. *et al.* (1998) Cloning of the V-ATPase subunit G in plant: functional expression and sub-cellular localization. *FEBS Lett.* 437, 287–292
20 Perera, I. *et al.* (1995) Several genes encode nearly identical 16 kD proteolipids of the vacuolar H^+-ATPase from *Arabidopsis thaliana*. *Plant Mol. Biol.* 29, 227–244
21 Forgac, M. (2000) Structure, mechanism and regulation of the clathrin-coated vesicle and yeast vacuolar H^+-ATPases. *J. Exp. Biol.* 203, 71–80
22 Arata, Y. *et al.* (2002) Cysteine-directed crosslinking to subunit B suggests that subunit E forms a part of the peripheral stalk of the V-Tpase. *J. Biol. Chem.* 277, 3357–3563
23 Wilkens, S. *et al.* (1999) Structure of the vacuolar ATPase by electron microscopy. *J. Biol. Chem.* 274, 31804–31810
24 Gruber, G. *et al.* (2000) Three-dimensional structure and subunit topology of the V_1 ATPase from *Manduca sexta* midgut. *Biochemistry* 39, 8609–8616
25 Landolt-Marticorena, C. *et al.* (2000) Evidence that the NH_2 terminus of vph1p, an integral subunit of the V_0 sector of the yeast V-ATPase, interacts directly with the Vma1p and Vma13p subunits of the V_1 sector. *J. Biol. Chem.* 275, 15449–15457
26 Kawasaki-Nishi, S. *et al.* (2001) The amino-terminal domain of the vacuolar proton-translocating ATPase a subunit controls targeting and *in vivo* dissociation, and the carboxyl-terminal domain affects coupling of proton transport and ATP hydrolysis. *J. Biol. Chem.* 276, 47411–47420
27 Powell, B. *et al.* (2000) Molecular characterization of the yeast vacuolar H^+-ATPase proton pore. *J. Biol. Chem.* 275, 23654–23660
28 Junge, W. *et al.* (1997) ATP synthase: an electrochemical transducer with rotatory mechanics. *Trends Biochem. Sci.* 22, 420–423
29 Xu, T. and Forgac, M. (2000) Subunit D (Vma8p) of the yeast vacuolar H^+-ATPase plays a role in coupling of proton transport and ATP hydrolysis. *J. Biol. Chem.* 275, 22075–22081
30 Dow, J.A. (1999) The multifunctional *Drosophila melanogaster* V-ATPase is encoded by a multigene family. *J. Bioenerg. Biomembr.* 31, 75–83
31 Oka, T. *et al.* (1997) Three vha genes encode proteolipids of *Caenorhabditis elegans* vacuolar-type ATPase. Gene structures and preferential expression in an H-shaped excretory cell and rectal cells. *J. Biol. Chem.* 272, 24387–24392
32 Blanc, G. *et al.* (2000) Extensive duplication and reshuffling in the *Arabidopsis* genome. *Plant Cell* 12, 1093–1101
33 Oka, T. *et al.* (2001) Four subunit a isoforms of *Caenorhabditis elegans* vacuolar H^+-ATPase. Cell-specific expression during development. *J. Biol. Chem.* 276, 33079–33085