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Diverse Roles of Single Membrane Organelles: Factors Establishing the Acid Lumenal pH[1]

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Eukaryotic cells have developed an array of endomembrane systems that have differentiated to carry out various functions. They are involved in the pathways of endocytosis and exocytosis, and have an acidic lumenal pH ranging from 4.5 to 6.5. This review describes recent studies on the animal cell organelles and how they relate to the well studied systems of yeast. We focus mainly on (i) the primary proton pump (vacuolar type H^+-ATPase) and other factors that establish acidic pH, and (ii) functions of the organelles as related to lumenal acidity.

Key words: acidification, endocytosis, exocytosis, H^+-ATPase, membrane traffic.

Membrane bound organelles involved in endo- and exocytic pathways are highly differentiated (*1-4*). They include endoplasmic reticulum, Golgi, secretory vesicles, synaptic vesicles, endosomes, coated vesicles, and lysosomes (Fig. 1). These single membrane organelles are cytoplasmic compartments different in function and lumenal contents. Basic but unanswered questions are: (a) by what mechanism are these diverse organelles formed, and (b) how are the unique lumenal contents of different organelles established. To answer these questions, we must first establish an understanding of the mechanisms of acidification and the roles of the acidic organellar interiors.

The vacuolar type proton pump (V-ATPase) is primarily responsible for lumenal acidification. In combination with the V-ATPase, ion channels and transporters, whose distribution varies depending on the organelle, establish the pH gradient and positive-inside membrane potential. The resultant electrochemical proton gradient provides the driving force for accumulation of hormones or transmitters into secretory granules or synaptic vesicles, whereas the acidic pH is essential for many organellar-specific functions such as recycling of receptors during receptor-mediated endocytosis, processing preproteins, and providing the optimal pH for lumenal enzymes.

In this article, we briefly summarize recent studies on animal cells aiming to understand the function of these diverse organelles. We refer to the results derived from studies of yeast vacuoles (*4-6*) in some detail because they provide the basis for understanding higher eukaryotes. This article is not meant to be comprehensive and studies on V-ATPases and acidic organelles not discussed here can be found in excellent recent reviews (*4-10*).

Factors establishing acidic lumenal pH

The V-ATPase is a primary proton pump found ubiquitously in various single membrane organelles of animal, plant, and fungal cells (*3-6*). In this section we discuss properties of the enzyme including genetics, structure, inhibitors, and kinetics. Other factors that contribute to establishing the organelle-specific lumenal pH are also discussed.

Structure of the V-ATPase. The V-ATPase represents one of the three major families of ion translocating ATPases (V-, F-, and P-type) (*11*). V- and F-type ATPases share similar structures and mechanisms and are different from P-type ATPases which form a phosphorylated enzyme intermediate of a specific aspartate residue. The V-ATPase has been purified from *Saccharomyces cerevisiae* (*5, 6, 12*), *Neurospora crassa* (*7*), bovine adrenal chromaffin granules (*13*), and coated vesicles (*14*). The enzyme has a complicated multi-subunit structure similar to F-type ATPase (F_oF_1 or ATP synthase) and can be similarly divided into two sectors referred to V_o and V_1 in analogy to F_o and F_1 (Fig. 2): V_o is the intrinsic membrane sector which mediates the proton pathway and V_1 is the peripheral membrane sector containing the catalytic domains. When studied by electron microscopy, the V_1 sector appears as a ball and stalk structure similar to F_1 but of slightly larger size (*15, 16*).

Distinct phenotypes of yeast lacking V-ATPase have been useful for identifying genes encoding protein subunits (*4-6*). Phenotypes include negative growth at pH 7.5 on non-respiratory carbon sources, failure to accumulate red pigment in an *ade2* cell, and sensitivity to calcium ion. Genetic studies indicate that V_1 and V_o sectors consist of six (69, 60, 54, 42, 32, and 27 kDa) and four (100, 13, 17, and 23 kDa) different subunits, respectively. Two more subunits are located at the interface between V_o and V_1.

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Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; EGF, epidermal growth factor; NEM, *N*-ethylmaleimide.

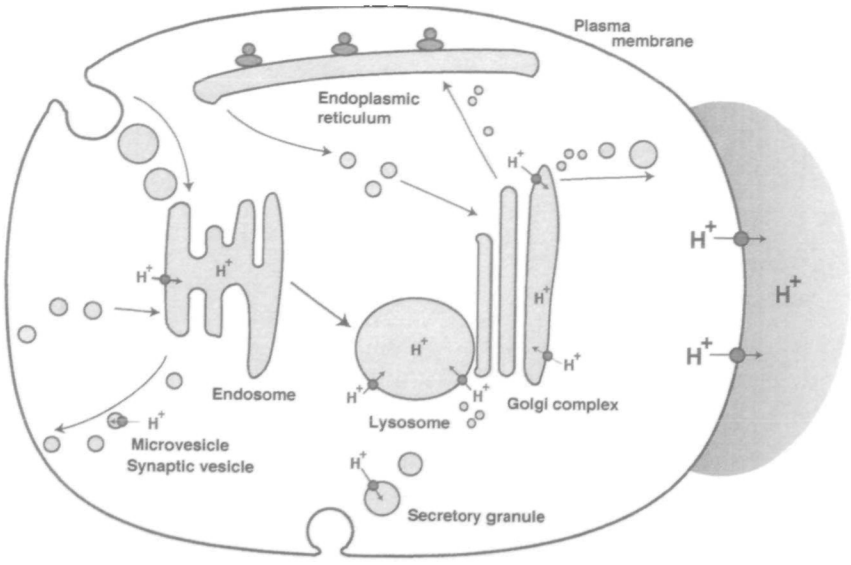


Fig. 1. **The acidic inside single membrane organelles in animal cells.** Extracellular acidification by plasma membrane V-ATPase is also shown.

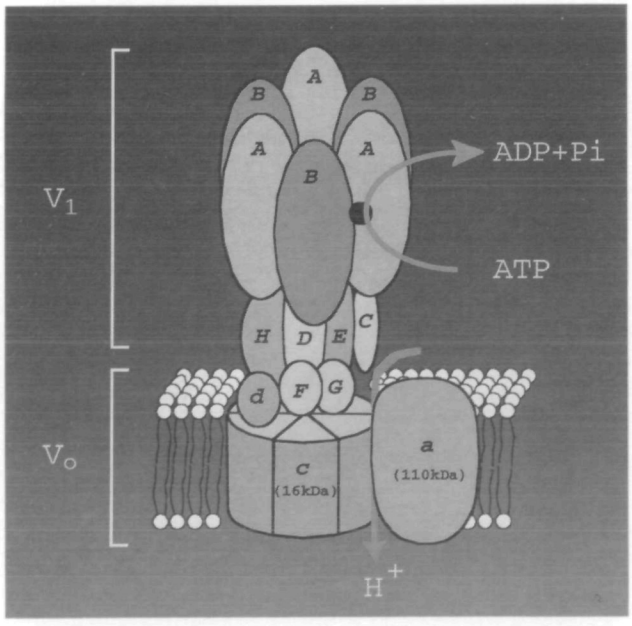


Fig. 2. **A model of the V-ATPase proton pump.** Subunit organization of the catalytic V_1 and membranous V_o. V_1 and V_o are designated by analogy to the F_1 and F_o sectors of F-type ATPases. Rotation of a core of subunits as a part of catalysis and transport is indicated by the arrow.

Mammalian enzymes have similarly complicated subunit structures, although unlike the yeast complex, not all have been unambiguously defined. Currently known V_1 subunits are *A* (72 kDa), *B* (57 kDa), *C* (41 kDa) (*32*), *D* (34 kDa); interface subunits *E* (33 kDa), *F* (14 kDa), and *G* (15 kDa); and V_o subunits are *a* (100-115 kDa), *c*″ (23 kDa), *d* (32-41 kDa), and *c* (16 kDa) (*4, 13, 14, 17*). Pairwise comparisons of plant and fungal sequences for subunits *A* or *B* show 60-70% identity (*7, 18-21*), indicating that this class of ATPase is evolutionary well conserved (*17*). Detailed discussions on structure, function, and assembly of subunits are found in a recent review from Stevens and Forgac (*4*).

The V_1 catalytic subunit *A* shares about 25% sequence identity with the β and α subunits of F-type ATPase (*7*). Catalytic residues identified in the $F_1\beta$ subunit are conserved in subunit *A* such as those found in the P-loop (phosphate binding loop or glycine-rich sequence, Gly-X-X-X-X-Gly-Lys-Thr) and Gly-Glu-Arg-X-X-Glu (GERXXE) sequences (*22, 23*). Based on the conserved nature of these sequences, it was predicted that the lysine in the P-loop and the glutamate in the GERXXE sequence are catalytically important residues of the V-ATPase. This notion was confirmed by mutagenesis studies of the yeast enzyme (*24*). Structural predictions suggested that V_1 subunit *D* may be the counterpart of the $F_1\gamma$ subunit (*25*), the core of the $\alpha_3\beta_3$ hexamer (*26*). Other subunits of F_1 and V_1 do not exhibit statistically significant identities, but some of them contain structurally related domains. For example, V_1 subunit *G* is related to the hydrophilic domain of the *b* subunit of F_o suggesting that they have similar functions (*27*). In general, overall structural similarity suggests that the V- and F-type ATPases utilize the same mechanisms for ATP hydrolysis and proton translocation.

The V_o sector consists of at least five different subunits. The cDNA encoding the *c* subunit (16 kDa proteolipid) has been cloned from bovine (*28*), mouse (*29*), yeast (*30, 31*), and *Caenorhabditis elegans* (*32*). The sequences of each half of the V_o *c* subunit are homologous to F_o *c* subunit suggesting that the two proteins evolved from the same ancestral protein and that the V_o gene is a tandem repeat of the single F_o subunit gene (*18, 28*). Exon/intron organization of the human gene supports this view (*33*). The V_o *c* subunit has four hydrophobic transmembrane segments (I, II, III, IV) which are presumably α helices. Directed mutagenesis of the yeast gene suggests that a glutamate in the middle of domain IV is critical for proton translocation (*34*). Yeast V_o has another subunit (Ppa1p, Vma16p, or 23 kDa subunit) whose sequence can be aligned with other V_o *c* subunits except that an additional 50 amino terminal residues are present which result in a fifth transmembrane segment (*35*). In this case, mutagenesis studies suggest that the essential glutamate for transport is located in domain III. Recently, a similar five transmembrane seg-

ment subunit *c* sharing 52% identity has been identified in *C. elegans* (*32*).

ATPase activity and inhibitors. The catalytic mechanisms of both V- and F-type ATPases are highly cooperative. It is well known that the rate of product release by F_1 when all three sites participate in cooperative catalysis is 10^6-fold faster than that of a single site (*22, 23*). A similar mechanism has been suggested for V-ATPase (*36*). Estimated maximal turnover numbers for both enzymes are approximately the same. Consistent with this suggestion, bovine V-ATPase activity does not show simple Michaelis-Menten kinetics with respect to ATP concentration. Instead, there were three apparent K_ms ranging from micromolar to millimolar (*37*). The turnover rate for a single site was 10^3-fold lower than the maximal velocity and was similar to the rate estimated from the velocity of steady state hydrolysis from the lowest K_m (5 μM).

Several inhibitors and protein modifying reagents have been used to study properties of the enzyme and its functions. Early studies showed that V-ATPase is sensitive to *N*-ethylmaleimide (NEM) but most P- and F-type ATPases are not (*3, 11*). NEM was shown to modify a cysteine residue in the P-loop (GXXXCGKT) of V-ATPase subunit *A* (*38*). F-type ATPase and archae bacterial V-ATPases (*39-41*) (also called A-type ATPase, Ref. *41*) lack this cysteine, consistent with their insensitivity to NEM. Interestingly, *Escherichia coli* F_1 ATPase can be made NEM sensitive by introducing a cysteine into the P-loop (GGAGVGKT→GGAGCGKT) of the β subunit (*42*). Omeprazole is a potent inhibitor of the gastric P-type ATPase which reacts to cysteine residues exposed to the acidic apical surface of the parietal cell (*43*). The acid activated form of this compound also inhibits V-ATPase possibly by binding to the P-loop cysteine residue (*44*). Lipophilic cations, known to inhibit F-type ATPase by binding to the highly conserved β subunit DELSEED loop region (*45*), also bound to subunit *A* of V-ATPase and inhibited its activity (*46*). The aligned region of the V-ATPase is D/S/A·X·L·S/A·E/D·X·D/E.

Reversible oxidation of the P-loop cysteines may play an important role in regulating organelle acidification. The P-loop cysteine (Cys-254) of the bovine coated vesicle V-ATPase forms a disulfide bond with Cys-532 of the same subunit and results in inactivation of the enzyme (*47*). A regulatory role for oxidation of this cysteine was further suggested by genetic analysis in yeast. A *cys4* null allele in cysteine biosynthesis leads to low intracellular concentration of reduced glutathione and causes the loss of vacuolar acidification (*48*). Furthermore, the effects of *cys4* were suppressed by a mutation in the *A* subunit P-loop (Cys-261 to valine). These observations are compelling evidence that V-ATPase can be regulated by the redox-state of the catalytic subunit.

As first observed with the *N. crassa* enzyme (*49*), V-ATPase is almost completely inhibited by stoichiometric binding of bafilomycin A_1. The K_i was less than 10^{-8} M, whereas 10^{-4} M was required to achieve 60–80% inhibition of a P-type ATPase and 1 mM did not affect F-type ATPase. The inhibition of the V-ATPase was protected by the addition of V_o sector (*37*) or isolated *a* (100 kDa) subunit (*50*), suggesting that bafilomycin A_1 inhibits the enzyme by binding to the V_o *a* subunit. Structurally related concanamycins (concanolide, concanamycin A and C) are better inhibitors than bafilomycins (A1, D, or 21-*O*-acetyl bafilomycin) (*51*). Concanamycin B also inhibited V-ATPase, and its aglycon was 10 times more inhibitory. Significantly, its non-cyclic derivative was at least 10^3-fold less effective (*52*). These antibiotics, especially the bafilomycins, have been useful for analyzing the roles of acidic pH in vacuolar system organelles (see below).

Similar to F-type ATPase, dicyclohexylcarbodiimide (DCCD) potently inhibits V-ATPase by binding to the *c* subunit (*53, 54*). Bafilomycin A_1 had no effect on the binding of radioactive DCCD to the *c* subunit (*37*) suggesting that the binding sites for DCCD and bafilomycin A_1 are different.

Heterogeneity of V-ATPase subunits. Multiple isoforms of some of the V-ATPase subunits have been identified. Two cDNAs (bovine and human) coding for homologous but distinct *B* subunits have been cloned (*55*). They share 84% identity in their amino acid sequences, but one is expressed exclusively in the kidney while the other is widespread. From human genomic DNA, we have identified four genes coding for the *c* subunit but found that three of them were pseudogenes (*33*). At this time, the number of functional *c* subunit genes in mammals is still unclear.

Yeast genes encoding all V-ATPase subunits as well as those required for complex assembly have been identified (*4-6*). Two genes (*VMA3, VMA11*) code for the *c* subunit. The proteins are 56% identical in amino acid sequence but they do not represent genetic redundancy. Both gene products are essential for the V-ATPase activity indicating that they are subunits of the same vacuolar enzyme (*31*). Yeast also has two genes, *VPH1* and *STV1*, coding for isoforms of V_o *a* subunit (*56*). Vph1p and Stv1p are located in distinct compartments, probably vacuoles and endosomes, respectively. The mechanism for specific localization is currently not understood.

We found three *C. elegans* genes coding for the *c* subunit and detected the corresponding transcripts. Two of them (*vha-1* and *vha-2*) form an operon on chromosome III (*32*), and the third gene *vha-3* is in a cluster with the subunit *C* gene on chromosome IV (Oka, T., Yamamoto, R., and Futai, M., *J. Biol. Chem.*, in press). The product of *vha1* is 66% identical to those of *vha-2* or *vha-3*, and 61% identical to *VMA3* of *S. cerevisiae*. *vha-2* and *vha-3*, whose nucleotide sequences are 85% identical, are predicted to produce identical proteins. To test whether these subunits are differentially expressed, we constructed transgenic worms carrying the control regions of these genes upstream of the green fluorescent protein (GFP) open reading frame. *vha-1* and *vha-2* were expressed similarly in most cells of the larvae (L1), and were expressed most highly in the H-shaped excretory cells in adults (Fig. 3). These results suggested that V-ATPase genes are strongly transcribed at specific stages in different cells. In contrast to three genes for the *c* subunit, we found only one gene (*vha-4* in chromosome II) encoding the *C. elegans* 23 kDa proteolipid subunit (*32*).

Because multiple isoforms of some V-ATPase subunits have been identified, it is tempting to speculate that the different V-ATPases result in different internal pH in the various organelles. Further work, including identification of multiple isoforms for the *c* and *a* subunits, is required to answer this question.

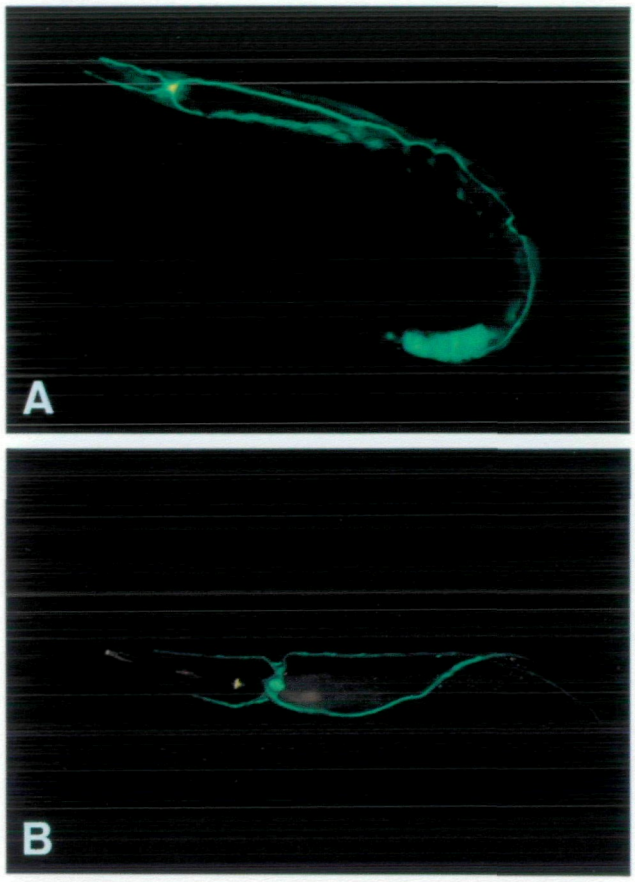


Fig. 3. **Specific expression of V-ATPase subunit genes in the H-shaped excretory cell of adult *Caenorhabditis elegans*.** Control regions of the *c* and *B* subunit genes were inserted upstream of the GFP (green fluorescent protein) coding region, and introduced into the *C. elegans* gonad (*32*). (A) The distribution of GFP fluorescence indicates specific expression of *c* subunit in the H shaped cell and rectum. (B) The head of a worm harboring the *B* subunit::GFP fusion expression plasmid. Note the fluorescence indicating the H-shaped cell.

Factors establishing organellar specific pH: Donnan-type equilibrium, ion channels, and transporters. Proton transport by V-ATPase is not the sole mechanism for establishing and maintaining ion gradients and membrane potentials across organelle membranes. Other factors include proton or ion movement through membranes and negatively charged molecules in the lumen. Understanding such factors are still at an early stage. A typical acidic organelle, the lysosomes, are able to maintain an acidic pH at least for two days in the absence of ATP (*57*). This result suggests that a Donnan-type equilibrium is formed with negatively charged molecules and protons.

Presence of Cl^- transporting system(s) in yeast vacuolar membrane was suggested from the kinetics of ATP-dependent acidification monitored by fluorescent dyes (*58*). Cl^- dissipates the membrane potential formed by the V-ATPase and is necessary for formation of a pH gradient. Moreover, radioactive chloride was taken up into vacuolar membrane vesicles upon ATP hydrolysis in a manner that was sensitive to the protonophore CCCP. Kinetic analysis suggests that two different Cl^- transport systems are present in the membrane. Furthermore, electrophysiological studies suggested that the yeast vacuole has at least one cation channel that is regulated by membrane potential and calcium (*59*).

Similarly, presence of Cl^- ion is required for forming a proton gradient in synaptic vesicles for shunting the membrane potential (*60*). ATP hydrolysis was still observed without Cl^- or electrical short circuiting although net proton transport into these vesicles was not observed. These results indicate that proton transport by V-ATPase is regulated by membrane potential (*61*) but the ATPase activity itself is not. This implies that ATPase activity in the vesicles is not obligatorily coupled with proton transport. In this regard, Nelson proposed "a proton slip" mechanism that prevents over-acidification of organellar lumens (*9*). Chromaffin granule V-ATPase hydrolytic rates were also found to be regulated by anions (*61, 62*).

Cl^- channel activity in coated vesicles was suggested by radioactive Cl^- uptake (*63*) and confirmed by reconstitution of the activity from purified channel protein (*64*). Regulation of Cl^- channel activity by phosphorylation/dephosphorylation in vesicles from proximal tubule and coated vesicles was shown (*63, 65*). Redhead *et al.* showed recently that p64, a Cl^- channel protein from bovine kidney microsomes, is specifically located in the membranes of secretory vesicles (*66*). A related Cl^- channel was found in the osteoclast plasma membrane (*67*).

The Na^+/K^+ ATPase is known to be incorporated into endosomes where it drives the inside positive membrane potential which apparently inhibits proton transport by V-ATPase. As expected, the pH of early endosomes of human cultured cells was decreased almost 1 pH unit upon addition of Na^+/K^+ pump inhibitor ouabain (*68*). This stimulation of acidification was found only in early endosomes (*69*). These observations suggest that Na^+/K^+ pump activity in specific internalized compartments is a part of the mechanism regulating organellar pH.

The electro-neutral Na^+/H^+ exchanger (NHE) was shown to be incorporated into the phagosomal membrane, but was not directly responsible for regulation of pH (*70*). Acidification of phagosomes was carried out by V-ATPase delivered from endosomes. Organellar acidification may also be regulated by controlling the density of V-ATPase, association/dissociation of V_1 and V_o, and a low molecular weight protein inhibitor or activator (*4*).

Single membrane organelles

The various functions of organelles are dependent upon the proper internal pH and electrochemical potential which are established by a combination of proton pumps, transporters, and channels. In this section we discuss physiological roles of single membrane organelles and their acidic lumens.

Acid extrusion to extracellular space. Proton secretion occurs in several physiological processes (*1, 2*). Bicarbonate is reabsorbed in kidney epithelia in a manner that is coupled to extrusion of protons (*10*). Acidic pH is essential for sperm maturation in the seminal duct (*71*), and for breakdown of bone matrix in regions of bone resorption (*72*). In each of these examples, the major proton transporter is the V-ATPase. Using immunological techniques, V-ATPase has been localized to the plasma membrane of epithelial cells in kidney proximal tubules (*10*), seminal duct (*73*), as well as superficial cells of

bladder epithelium (*74*). In the osteoclast, the acidifying enzyme found in the ruffled border is somewhat different from those in cytoplasmic organelles (*72*). Consistent with the role of the V-ATPase in acidification of urine in the mouse bladder, urinary pH is increased by the introduction of bafilomycin A_1 (*74*). Furthermore, when inside-out bladder was placed in dilute buffer, bafilomycin-sensitive acidification of the medium was observed. V-ATPases were also found in the urinary bladder of frog (*75*) and turtle (*76*) which is the counterpart of the proximal and distal tubules of mammalian kidney. In addition to these epithelial cells, V-ATPase found in the macrophage plasma membrane has been suggested to control cytoplasmic pH (*77*). The plasma membrane V-ATPase of insect midgut goblet cells transports protons to the goblet cavity. The resultant electrochemical gradient of protons drives a K^+/H^+ antiporter for transport of K^+ into the cavity and protons into the cell (*78*). This coupled process makes goblet cell cavity slightly more alkaline than the cytoplasm.

Acidic pH in organelles of endocytic processes. In receptor-mediated endocytosis, small regions of the plasma membrane called coated pits form clathrin-coated vesicles. After shedding the clathrin coat, these vesicles form early endosomes and fuse with lysosomes which have a lumenal pH ranging between 6.5 and 4.5 (*1-3*). The endocytic process is important for metabolic regulation such as uptake of low density lipoprotein and ferrotransferin. Elucidation of the roles of acidic lumenal pH in endocytosis and exocytosis has been analyzed with the help of ionophores or acidotrophic agents (*1, 2*). A caveat, however, is that these agents often alter morphology of endomembrane compartments and cause extensive vacuolization (*79, 80*). Importantly and fortuitously, bafilomycin A_1 does not cause such effects and specifically inhibits acidification. Within 30 min, presence of bafilomycin caused the lysosome lumenal pH to rise at least to pH 6.3 in cultured cells. Acid-inside pH is recovered after removing the antibiotic (*79*). Clearly, bafilomycin provides a specific inhibitor that can be used to isolate the effects of altered V-ATPase activity.

With bafilomycin, the roles of endosome acidification have been reevaluated. For example, in the presence of bafilomycin A_1, EGF (epidermal growth factor) was internalized by receptor-mediated endocytosis but not degraded (*79*). Similarly, bafilomycin A_1 caused accumulation of diphtheria toxin mainly in the endosomes but not the lysosomes (*80*). This result suggested that the acidic pH was required for toxin transport from endosomes to lysosomes. Bafilomycin was then found to inhibit formation of intermediate vesicles between early and late endosomes (*81*) and vesicular transport from endosomes to lysosomes (*82*). On the other hand, bafilomycin only slightly slowed internalization and recycling of the transferin receptor from early endosomes (*82, 83*). Taken together, these results are consistent with the hypothesis that the acidic lumenal pH is required at the later stages of endocytosis.

Small membrane vesicles are involved in secretory pathways such as vesicular transport between the endoplasmic reticulum and the Golgi apparatus, or Golgi and secretory vesicles (*1, 2*). The inside acidic pH of these vesicles is believed to be essential for maturation of secretory proteins. The proteolytic cleavage of preproteins occurs during the formation of mature secretory vesicles. Inhibition of lumenal acidification in the trans-Golgi network caused blocked processing of prohormone (*84*). Similarly, bafilomycin A_1 also affected processing of β-amyloid precursor proteins (*85, 86*).

Vesicles participating in exocytosis or endocytosis contain proteins necessary for proper trafficking (*1, 2, 87*). In this regard, Forgac and coworkers (*88*) showed that purified coated vesicle V-ATPase contained the 50 kDa subunit of AP-2 clathrin assembly complex which distinguishes between proteins to be transported from plasma membrane and those left behind. Interestingly, the AP2 subunit was required for reconstitution of ATP-dependent proton conduction by coated vesicle V-ATPase, indicating that the protein is an integral part of the V-ATPase (*89*). Their results are consistent with an interesting possibility that the V-ATPase and vesicle forming machinery functionally interact (*4*).

Secretory granules and neural synaptic vesicles. Secretion of hormones and neurotransmitters are carried out *via* an exocytotic process. Chromaffin granules in the adrenal medulla accumulate monoamines such as serotonin and adrenaline (*90*), while neurosecretory granules also accumulate monoamines as well as other transmitters (*91*). The electrochemical proton gradient established by the V-ATPase provides the driving force for accumulating transmitters into vesicles. Transport is inhibited by nigericin but not by valinomycin-K^+ indicating that the pH gradient, and not membrane potential, is used to drive uptake. This observation strongly suggests that transport is mediated by a proton/monoamine antiporter. It is noteworthy that acidic lumenal pH is likely to be maintained up to the exocytotic event because dissipation of the electrochemical gradient causes efflux of transmitters. Whether the gradient itself is required for the process of exocytosis remains unknown.

Neural synaptic vesicles accumulate glutamate in a membrane potential-dependent manner while monoamine accumulation depends upon a ΔpH (*60, 92, 93*). Transmitter transport coupled with an electrochemical gradient can be artificially reconstituted in liposomes with the specific transmitter transporter and an F-type ATPase (*94*). These experiments confirmed that the role of the V-ATPase is to establish an electrochemical gradient of protons and that the activity can be replaced by other proton pumps.

Pharmacologically important compounds often alter lumenal content and pH of secretory vesicles. 1-Methyl-4-phenylpyridinium (MPP^+), which is known to cause selective degradation of dopaminergic neurons, is transported into synaptic vesicles and chromaffin granules through ΔpH-coupled monoamine transporters (*95*). Other agents act less specifically. For example 2-(4-phenylpiperidino)-cyclohexanol and bromocriptin dissipate electrochemical gradients in the same manner as protonophores and inhibit transmitter transport indirectly (*96*). Neuronal blockers, usually lipophilic amines, penetrate vesicles in nonprotonated lipophilic forms and accumulated inside as protonated forms. This accumulation results in dissipating the pH gradient. These compounds inhibit monoamine or GABA transport which are proton gradient driven but give a slight stimulation to glutamate transport which is driven by membrane potential (*97*). Anti-neoplastic agents such as daunomycin and doxorubicin also accumulate into acidic vesicles following the same mechanism (*98*).

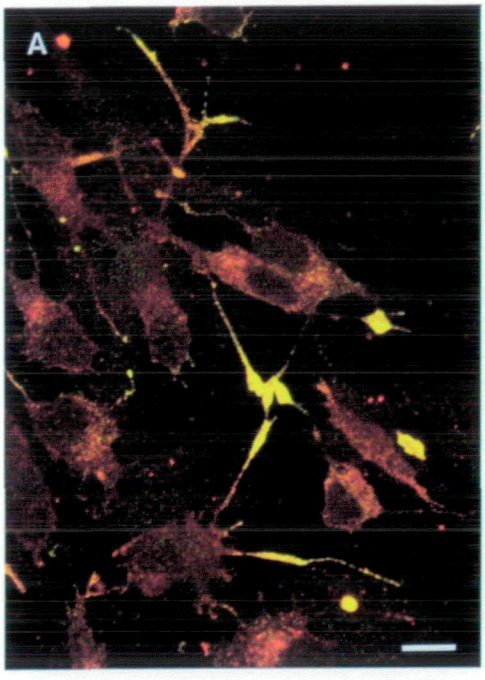
Microvesicles from endocrine cells. Microvesicles

(synaptic vesicle-like) are small organelles of 50–70 nm diameter morphologically that are similar to synaptic vesicles but not directly derived from the Golgi apparatus (*87*). They are found in exocrine cells such as pinealocytes, posterior pituitary, adrenal chromaffin cells, and pancreatic β cells. Secretory granules (different from microvesicles) in the same cells may contain different transmitters. For example, pancreatic β cells have both microvesicles and secretory granules containing GABA or insulin, respectively (*99, 100*) while in the rat pheochromocytoma PC12 cells, microvesicles accumulate acetylcholine (*101*) and secretory granules store dopamine (*102*). Microvesicles are purified by differential centrifugations from posterior pituitary (*103*) and pinealocytes (*104*). *In vitro*, the isolated pituitary vesicles transport norepinephrine (*103*) and vesicles from pinealocytes accumulate L-glutamate (*104, 105*), both uptake systems are coupled to a V-ATPase-driven ΔpH (Fig. 4). To release glutamate in the pineal gland thus eliciting a cell-to-cell signal, microvesicles fuse with the plasma membrane by a mechanism triggered by calcium (*106*). Similar to synaptic vesicles, microvesicles have proteins necessary for vesicle fusion (*87*); however, membrane proteins of the two vesicles are not the same. Microvesicles have synaptophysin but the electrophoretic mobility is slightly faster than that found in synaptic vesicles, and pinealocyte microvesicles do not have synapsins at all (*102*).

Unanswered questions and the future direction

We have herein reviewed the extensive studies directed towards single membrane organelles and V-ATPases. The major remaining challenge is to understand the diversity of acidic organelles: what is the mechanism that such an array of organelles can develop and differentiate? Our understanding of the roles of the V-ATPase subunit isoforms, the channels and the transporters is quite limited, although it is clear that these activities play critical roles establishing specific degrees of lumen acidity. As discussed above, dissipation of membrane potential is necessary for establishing proton gradients in synaptic vesicles suggesting that proton transport by V-ATPase is regulated by membrane potential. A fascinating possibility is that one (or a combination) of subunits of the V-ATPase functions as a ΔpH or $\Delta\Psi$ sensor and regulator of pump activity. In this regard, a brain protein of the degenerin family is a proton-gated cation channel and functions in acid sensing (*107*). These questions will be answered by combined biochemical electrical physiological and molecular approaches.

We recently found an organelle with a extremely acidic lumenal pH. The giant blood cell of the marine *Acidian* has



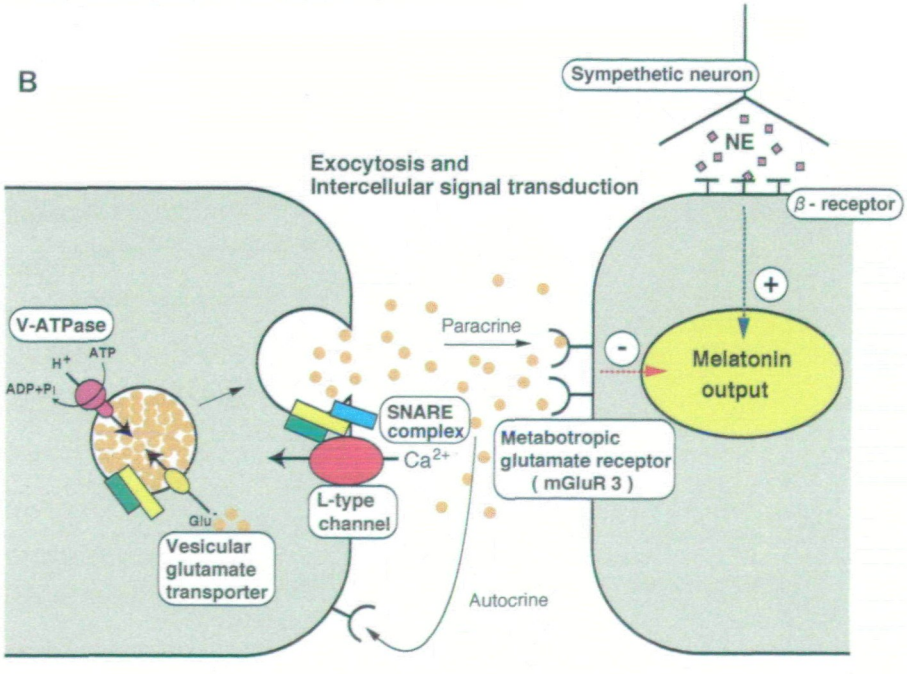


Fig. 4. **Roles of microvesicles in pinealocytes.** A: Confocal fluorescence micrograph of cultured rat pinealocytes double-stained with antibodies against SV2B (green) and synaptophysin (red). Co-localization of these proteins are seen in microvesicles, especially in the process terminals. See Refs. *106, 108,* and *109* for more details. B: A regulatory scheme for melatonin synthesis. Mammalian pinealocytes are glutaminergic paraneurons. Upon depolarization, glutamate is released *via* an exocytotic process. Glutamate binds to the metabotropic glutamate receptor type 3 (mGluR3), signaling decreased melatonin output mediated through an inhibitory cAMP cascade. In contrast, innervating sympathetic neurons release norepinephrine (NE) activating beta receptors and stimulate increased levels of melatonin (*87*).

a single large vacuole with an internal pH of 0.1 (unpublished observations). This pH, 10-fold more acidic than the mammalian gastric lumen, is the most acidic organelle in biology so far described. This organelle is an excellent model for studying mechanisms of acidification. The recent discovery of the *Ascidian* blood cell and mammalian microvesicles makes it likely that unknown acidic organelles with unique functions in cellular homeostasis will continue to be found.

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REFERENCES

1. Mellman, I., Fuchs, R., and Helenius, A. (1986) Acidification of the endocytic and exocytic pathway. *Annu. Rev. Biochem.* **55**, 663-700
2. Goldstein, J.L., Brown, M.S., Anderson, R.G.W., Russel, D.W., and Schneiden, W.J. (1985) Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. *Annu. Rev. Cell Biol.* **1**, 1-39
3. Forgac, M. (1989) Structure and function of vacuolar class of ATP driven proton pumps. *Physiol. Rev.* **69**, 765-796
4. Stevens, T.H. and Forgac, M. (1997) Structure, function and regulation of the vacuolar (H^+)-ATPases. *Annu. Rev. Cell Dev. Biol.* **13**, 779-808
5. Anraku, Y. (1996) Structure and function of the yeast vacuolar membrane H^+ ATPase in *Handbook of Biological Physics* Vol. 2 (Konings, W.N., Kaback, H.R., and Lolkema, J.S., eds.) pp. 93-109, Elsevier, Amsterdam
6. Kane, P.M. and Stevens, T.H. (1992) Subunit composition, biosynthesis, and assembly of the yeast vacuolar proton-translocating ATPase. *J. Bioenerg. Biomembr.* **24**, 383-393
7. Bowman, B.J., Vázquez-Laslop, N., and Bowman, E.J. (1992) The vacuolar ATPase of *Neurospora crassa*. *J. Bioenerg. Biomembr.* **24**, 361-370
8. Sze, H., Ward, J.M., and Shoupeng, L. (1992) Vacuolar H^+-translocating ATPases from plants: structure, function and isoforms. *J. Bioenerg. Biomembr.* **24**, 371-381
9. Nelson, N. (1992) Structural conservation and functional diversity of V-ATPases. *J. Bioenerg. Biomembr.* **24**, 407-414
10. Gluck, S.L. (1992) The structure and biochemistry of the vacuolar H^+ ATPase in proximal and distal urinary acidification. *J. Bioenerg. Biomembr.* **24**, 351-359
11. Pedersen, P.L. and Carafoli, E. (1987) Ion motive ATPases. I. Ubiquity, properties and significance to cell function. *Trends Biochem. Sci.* **12**, 146-150
12. Uchida, E., Ohsumi, Y., and Anraku, Y. (1985) Purification and properties of H^+-translocating, Mg^{2+}-adenosine triphosphatase from vacuolar membranes of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **260**, 1090-1095
13. Moriyama, Y. and Nelson, N. (1987) The purified ATPase from chromaffin granule membranes in an anion-dependent proton pump. *J. Biol. Chem.* **262**, 9175-9180
14. Forgac, M. (1992) Structure and properties of the coated vesicle (H^+)-ATPase. *J. Bioenerg. Biomembr.* **24**, 341-350
15. Moriyama, Y., Yamamoto, A., Tashiro, Y., and Futai, M. (1991) Chromaffin granule H^+-ATPase has F_1-like structure. *FEBS Lett.* **291**, 92-96
16. Taiz, S.L. and Taiz, L. (1991) Ultrastructural comparison of the vacuolar and mitochondrial H^+-ATPase of *Dacus carota*. *Bot. Acta* **104**, 117-121
17. Nelson, N. (1989) Structure, molecular genetics and evolution of vacuolar ATPases. *J. Bioenerg. Biomembr.* **21**, 553-571
18. Gogarten, J.P., Kibuk, H., Ditlrich, P., Taiz, L., Bowman, E.J., Bowman, B.J., Manolson, M.F., Poole, R.J., Date, T., Oshima, T., Konishi, J., Denda, K., and Yoshida, M. (1989) Evolution of the vacuolar H^+-ATPase: Implications for the origin of eukaryotes. *Proc. Natl. Acid Sci. USA* **86**, 6661-6665
19. Bowman, E.J., Tenney, K., and Bowman, R.J. (1988) Isolation of genes encoding the *Neurospora* vacuolar ATPases. Analysis of *vma-1* encoding the 67 kDa subunit reveals homology to other ATPases. *J. Biol. Chem.* **263**, 13994-14001
20. Hirata, R., Ohsumi, Y., Nakano, A., Kawasaki, H., Suzuki, K., and Anraku, Y. (1990) Molecular structure of a gene, *VMA1*, encoding the catalytic subunit of H^+-translocating adenosine triphosphatase from vacuolar membrane of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **265**, 6726-6733
21. Puopolo, K., Kumamoto, C., Adachi, I., and Forgac, M. (1991) A single gene encodes the catalytic "*A*" subunit of the bovine vacuolar H^+ ATPase. *J. Biol. Chem.* **266**, 24564-24572
22. Futai, M. and Omote, H. (1996) F-type H^+ ATPase (ATP synthase): catalytic site and energy coupling in *Handbook of Biological Physics* (Konings, W.N., Kaback, H.R., and Lolkema, J.S., eds.) Vol. 2, pp. 47-74, Elsevier, Amsterdam
23. Futai, M., Noumi, T., and Maeda, M. (1989) ATP synthase (H^+-ATPase): results by combined biochemical and molecular biological approaches. *Annu. Rev. Biochem.* **58**, 111-136
24. Liu, Q., Leng, X.-H., Neuman, P.R., Vasilyeva, E., Kane, P.M., and Forgac, M. (1997) Site-directed mutagenesis of the yeast V-ATPase *A* subunit. *J. Biol. Chem.* **272**, 11750-11756
25. Nelson, H., Mandian, S., and Nelson, N. (1995) A bovine cDNA and yeast gene (*VMA8*) encoding the subunit D of vacuolar H^+ ATPase. *Proc. Natl. Acad. Sci. USA* **92**, 497-501
26. Abrahams, J.P., Leslie, A.G.W., Lutter, R., and Walker, J.E. (1994) Structure at 2.8 Å resolution of F_1 ATPase from bovine heart mitochondria. *Nature* **370**, 621-628
27. Supeková, L., Supek, F., and Nelson, N. (1995) The *Saccharomyces cerevisiae VMA10* is an intron-containing gene encoding a novel 13-kDa subunit of vacuolar H^+-ATPase. *J. Biol. Chem.* **270**, 13726-13732
28. Mandel, M., Moriyama, Y., Hulmes, J.D., Pan, Y.-C.E., Nelson, H., and Nelson, N. (1988) cDNA sequence encoding the 16-kDa proteolipid of chromaffin granule implies gene duplication in the evolution of H^+-ATPases. *Proc. Natl. Acad. Sci. USA* **85**, 5521-5524
29. Hanada, H., Hasebe, M., Moriyama, Y., Maeda, M., and Futai, M. (1991) Molecular cloning of cDNA encoding the 16 kDa subunit of vacuolar H^+-ATPases from mouse cerebellum. *Biochem. Biophys. Res. Commun.* **176**, 1062-1067
30. Nelson, H. and Nelson, N. (1989) The progenitor of ATP synthases was closely related to the current vacuolar H^+-ATPase. *FEBS Lett.* **247**, 147-153
31. Umemoto, N., Ohya, Y., and Anraku, Y. (1991) *VMA11*, a novel gene that encodes a putative proteolipid, is indispensable for expression of yeast vacuolar membrane H^+ ATPase activity. *J. Biol. Chem.* **266**, 24526-24532
32. Oka, T., Yamamoto, R., and Futai, M. (1997) Three *vha* genes encode proteolipids of *Caenorhabditis elegans* vacuolar-type ATPase. Gene structure and preferential expression in an H-shaped excretory cell and rectal cells. *J. Biol. Chem.* **272**, 24387-24392
33. Hasebe, M., Hanada, H., Moriyama, Y., Maeda, M., and Futai, M. (1992) Vacuolar type H^+-ATPase genes: presence of four genes including pseudogenes for the 16 kDa proteolipid subunit in human genome. *Biochem. Biophys. Res. Commun.* **183**, 856-863
34. Noumi, T., Beltrán, C., Nelson, H., and Nelson, N. (1991) Mutational analysis of yeast vacuolar H^+-ATPase. *Proc. Natl. Acad. Sci. USA* **88**, 1938-1942
35. Hirata, R., Graham, L.A., Takatuki, A., Stevens, T.M., and Anraku, Y. (1997) *VMA11* and *VMA16* encodes second and third proteolipid subunits of the *Saccharomyces cerevisiae* vacuolar membrane H^+-ATPase. *J. Biol. Chem.* **272**, 4795-4803
36. Uchida, E., Oshima, Y., and Anraku, Y. (1988) Characterization and function of catalytic subunit *A* of H^+-translocating adenosine triphosphatase from vacuolar membrane of *Saccharomyces cerevisiae*. A study with 7-chloro-4-nitrobenzo-2-oxa,1,3-diazole. *J. Biol. Chem.* **263**, 45-51
37. Hanada, H., Moriyama, Y., Maeda, M., and Futai, M. (1990) Kinetic studies of chromaffin H^+-ATPase and effects of bafilo-

mycin A1. *Biochem. Biophys. Res. Commun.* **170**, 873-818
38. Feng, Y. and Forgac, M. (1992) Cysteine 254 of the 73 kDa *A* subunit is responsible for inhibition of the coated vesicle (H^+)-ATPase upon modification by sulfhydryl reagents. *J. Biol. Chem.* **267**, 5817-5822
39. Denda, K., Konishi, J., Oshima, T., Date, T., and Yoshida, M. (1988) The membrane-associated ATPase from *Sulfolobus acidocaldarius* is distantly related to F_1 ATPase as assessed from the primary structure of its alpha subunit. *J. Biol. Chem.* **263**, 6012-6015
40. Inatomi, K., Eya, S., Maeda, M., and Futai, M. (1989) Amino acid sequence of the α and β subunits of *Methanosarcina barkeri* ATPase deduced from cloned genes: similarity to subunits of eukaryotic vacuolar and F_oF_1-ATPases. *J. Biol. Chem.* **264**, 10954-10959
41. Ihara, K. and Mukohata, Y. (1991) The ATP synthase of *Halobacterium salinarium* (*halobium*) is an archaebacterial type as revealed from the amino acid sequences of its two major subunits. *Arch. Biochem. Biophys.* **286**, 111-116
42. Iwamoto, A., Orita, Y., Maeda, M., and Futai, M. (1994) *N*-Ethylmaleimide-sensitive mutant (βVal-153→Cys) *Escherichia coli* F_1 ATPase: cross-linking of the mutant β subunit with the α subunit. *FEBS Lett.* **352**, 243-246
43. Besancon, M., Shin, J.M., Mercier, F., Munson, K., Miller, M.F., Hersey, S., and Sachs, G. (1993) Membrane topology and omeprazole labeling on the gastric H^+,K^+-adenosine triphosphatase. *Biochemistry* **32**, 2345-2355
44. Moriyama, Y., Patel, V., Ueda, I., and Futai, M. (1993) Evidence for a common binding site for omeprazole and *N*-ethylmaleimide in subunit *A* of chromaffin granule vacuolar-type H^+ ATPase. *Biochem. Biophys. Res. Commun.* **196**, 699-706
45. Zhuo, S., Paik, S.R., Register, J.A., and Allison, W.S. (1993) Photoinactivation of the bovine heart mitochondrial F_1-ATPase by (^{14}C) dequalinium cross-links phenylalanine-403 of an α subunit to a site or sites contained within residues 440-457 of a β subunit. *Biochemistry* **32**, 2219-2227
46. Moriyama, Y., Patel, V., and Futai, M. (1995) Quinacrine mustard and lipophilic cations inhibitory to both vacuolar H^+-ATPase and F_oF_1-ATP synthase. *FEBS Lett.* **359**, 69-72
47. Feng, Y. and Forgac, M. (1994) Inhibition of vacuolar H^+-ATPase by disulfide bond formation between cysteine 254 and cysteine 532 in subunit *A*. *J. Biol. Chem.* **269**, 13224-13230
48. Oluwatosin, Y.E. and Kane, P.M. (1997) Mutations in the *CYS4* gene provide evidence for regulation of the yeast vacuolar H^+-ATPase by oxidation and reduction *in vivo*. *J. Biol. Chem.* **272**, 28149-28157
49. Bowman, E.J., Siebers, A., and Altendorf, K. (1988) Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells and plant cells. *Proc. Natl. Acad. Sci. USA* **85**, 7972-7976
50. Zhang, J., Fengm, Y., and Forgac, M. (1994) Proton conduction and bafilomycin binding by the V_o domain of the coated vesicle V-ATPase. *J. Biol. Chem.* **269**, 23518-23523
51. Dröse, S., Bindseil, K.U., Bowman, E.J., Siebers, A., Zeeck, A., and Altendorf, K. (1993) Inhibitory effect of modified bafilomycins and concanamycins on P- and V-type adenosinetriphosphatases. *Biochemistry* **32**, 3902-3906
52. Ito, K., Kobayashi, T., Moriyama, Y., Toshima, K., Tatsuta, K., Kakiuchi, T., Futai, M., Ploegh, H.L., and Miwa, K. (1995) Concanamycin B inhibits the expression of newly-synthesized MHC class II molecules on the cell surface. *J. Antibiotics* **48**, 488-494
53. Sutton, R. and Apps, D.K. (1981) Isolation of a DCCD-binding protein from bovine chromaffin-granule membranes. *FEBS Lett.* **130**, 103-106
54. Umemoto, N., Yoshihisa, T., Hirata, R., and Anraku, Y. (1990) Roles of the *VMA3* gene product, subunit *c* of the vacuolar membrane H^+-ATPase, on vacuolar acidification and protein transport. *J. Biol. Chem.* **265**, 18447-18453
55. Nelson, R.D., Guo, X.L., Masood, K., Brown, D., Kalkbrenner, M., and Gluck, S. (1992) Selectively amplified expression of an isoform of the vacuolar H^+-ATPase 56-kilodalton subunit in renal intercalated cells. *Proc. Natl. Acad. Sci. USA* **89**, 3541-3545
56. Manolson, M.F., Wu, B., Proteau, D., Taillon, B.E., Roberts, B.T., Hoyt, M.A., and Jones, E.W. (1994) *STV1* gene encodes functional homologue of 95 kDa yeast vacuolar H^+ ATPase subunit vph1p. *J. Biol. Chem.* **269**, 14064-14074
57. Moriyama, Y., Maeda, M., and Futai, M. (1992) Involvement of a non-proton pump factor (possibly Donnan-type equilibrium) in maintenance of an acidic pH in lysosomes. *FEBS Lett.* **302**, 18-29
58. Wada, Y., Ohsumi, Y., and Anraku, Y. (1992) Chloride transport of yeast vacuolar membrane vesicles: a study of *in vitro* vacuolar acidification. *Biochim. Biophys. Acta* **1101**, 296-302
59. Wada, Y., Ohsumi, Y., Tanifuji, M., Kasai, M., and Anraku, Y. (1987) Vacuolar ion channel of the yeast, *Saccharomyces cerevisiae*. *J. Biol. Chem.* **262**, 17260-17263
60. Moriyama, Y. and Futai, M. (1990) H^+-ATPase, a primary pump for accumulation of neurotransmitters, is a major constituent of brain synaptic vesicles. *Biochem. Biophys. Res. Commun.* **173**, 443-448
61. Moriyama, Y. and Nelson, N. (1987) Internal anion binding sites and membrane potential dominate the regulation of proton pumping by chromaffin granule ATPase. *Biochem. Biophys. Res. Commun.* **149**, 140-144
62. Moriyama, Y. and Nelson, N. (1987) Nucleotide binding sites and chemical modification of the chromatin granule proton ATPase. *J. Biol. Chem.* **262**, 14723-14729
63. Mulberg, A.E., Tulk, B.M., and Forgac, M. (1991) Modulation of coated vesicle chloride channel activity and acidification by reversible protein kinase A-dependent phosphorylation. *J. Biol. Chem.* **266**, 20590-20593
64. Xie, X.-S., Crider, B.P., and Stone, D.K. (1989) Isolation and reconstitution of the chloride transporter of clathrin-coated vesicles. *J. Biol. Chem.* **264**, 18870-18873
65. Bae, H.-R. and Verkman, A.S. (1990) Protein kinase A regulates chloride conductance in endocytic vesicles from proximal tubule. *Nature* **348**, 637-639
66. Redhead, C., Sullivan, S.K., Koseki, C., Fujiwara, K., and Edwards, J.C. (1997) Subcellular distribution and targeting of the intracellular chloride channel p64. *Mol. Biol. Cell* **8**, 691-704
67. Schlesinger, P.H., Blair, H.C., Teitelbaum, S.L., and Edwards, J.C. (1997) Characterization of the osteoclast ruffled border chloride channel and its role in bone resorption. *J. Biol. Chem.* **272**, 18636-18643
68. Cain, C.C., Sipe, D.M., and Murphy, R.F. (1989) Regulation of endocytic pH by the Na^+,K^+-ATPase in living cells. *Proc. Natl. Acad. Sci. USA* **86**, 544-548
69. Fuchs, R., Schmid, S., and Mellman, I. (1989) A possible role for Na^+,K^+-ATPase in regulating ATP-dependent endosome acidification. *Proc. Natl. Acad. Sci. USA* **86**, 539-543
70. Hackam, D.J., Rotstein, O.D., Zhang, W.-J., Demaurex, N., Woodside, M., Tsai, O., and Grinstein, S. (1997) Regulation of acidification. Differential targeting of Na^+/H^+ exchanger, Na^+/K^+ ATPase and vacuolar-type H^+ ATPases. *J. Biol. Chem.* **272**, 29810-29820
71. Caflisch, C.R. and Duboss, T.D. Jr. (1990) Direct evaluation of acidification by rat testis and epididymis: role of carbonic anhydrase. *Am. J. Physiol.* **258**, E143-E150
72. Chatterjee, D., Chakraborty, M., Leit, M., Neff, L., Jamsakello-Kumpu, S., Fuchs, R., Bartkiewicz, M., Hernando, N., and Baron, R. (1992) The osteoclast proton pump differs in its pharmacology and catalytic subunit from other vacuolar H^+-ATPase. *J. Exp. Biol.* **172**, 193-204
73. Breton, S., Smith, P.J.S., Lui, B., and Brown, D. (1996) Acidification of the male reproductive tract by a proton pumping (H^+)-ATPase. *Nature Med.* **2**, 470-472
74. Tomochika, K.-I., Shinoda, S., Kumon, H., Mori, M., Moriyama, Y., and Futai, M. (1997) Vacuolar-type H^+-ATPase in mouse bladder epithelium is responsible for urinary acidification. *FEBS Lett.* **404**, 61-64
75. Steinmetz, P.R. (1986) Cellular organization of urinary acidification. *Am. J. Physiol.* **251**, F173-F187
76. Harvey, B.J., Lacoste, I., and Ehrenfeld, J. (1991) Common channels for water and protons at apical and basolateral cell

membranes of frog skin and urinary bladder epithelia. Effects of oxytocin, heavy metals, and inhibitors of H^+-adenosine triphosphatase. *J. Gen. Physiol.* **97**, 749-776
77. Swallow, C.J., Grinstein, S., and Rotstein, O.D. (1990) A vacuolar type H^+-ATPase regulates cytoplasmic pH in murine macrophages. *J. Biol. Chem.* **265**, 7645-7654
78. Wieczorek, H. (1992) The insect V-ATPase, a plasma membrane proton pump energizing secondary active transport: molecular analysis of electrogenic potassium transport in the tobacco horn worm midgut. *J. Exp. Biol.* **172**, 335-343
79. Yoshimori, T., Yamamoto, A., Moriyama, Y., Futai, M., and Tashiro, Y. (1991) Bafilomycin A1, a specific inhibitor of vacuolar-type H^+-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. *J. Biol. Chem.* **266**, 17707-17712
80. Umata, T., Moriyama, Y., Futai, M., and Mekada, E. (1990) The cytotoxic action of diphtheria toxin and its degradation in intact Vero cells are inhibited by bafilomycin A_1, a specific inhibitor of vacuolar-type H^+-ATPase. *J. Biol. Chem.* **265**, 21940-21945
81. Clague, M.J., Urbé, S., Aniento, F., and Gruenberg, J. (1994) Vacuolar ATPase activity is required for endosomal carrier vesicle formation. *J. Biol. Chem.* **269**, 21-24
82. Van Weert, A.W.M., Dunn, K.W., Geuze, H.J., Maxfield, F.R., and Stoorvogel, W. (1995) Transport from late endosomes to lysosomes, but not sorting of integral membrane proteins in endosomes, depends on vacuolar proton pump. *J. Cell Biol.* **130**, 821-834
83. Johnson, L.S., Dunn, K.W., Pytwski, B., and McGraw, T.E. (1993) Endosome acidification and receptor trafficking: bafilomycin A1 slows receptor externalization by a mechanism involving the receptor's internalization motif. *Mol. Biol. Cell* **4**, 1251-1266
84. Xu, H. and Shields, D. (1994) Prosomatostatin processing in permealized cells. Endoproteolytic cleavage is mediated by a vacuolar ATPase that generates an acidic pH in the trans-Golgi network. *J. Biol. Chem.* **269**, 22875-22881
85. Haass, C., Capell, A., Citron, M., Teplow, D.B., and Selkoe, D.J. (1995) The vacuolar H^+ ATPase inhibitor differentially affects proteolytic processing of mutant and wild-type β-amyloid precursor protein. *J. Biol. Chem.* **270**, 6186-6192
86. Knops, J., Suomensaari, S., Lee, M., McConlogue, L., Seubert, P., and Sinha, S. (1995) Cell-type and amyloid precursor protein-type specific inhibition of Aβ release by bafilomycin A1, a selective inhibitor of vacuolar ATPases. *J. Biol. Chem.* **270**, 2419-2422
87. Moriyama, Y., Yamamoto, A., Yamada, H., Tashiro, Y., and Futai, M. (1996) Role of endocrine cell microvesicles in inter-cellular chemical transduction. *Biol. Chem. Hoppe-Seyler* **377**, 155-165
88. Myers, M. and Forgac, M. (1993) The coated vesicle vacuolar (H^+)-ATPase associates with and is phosphorylated by the 50 kDa polypeptide of the clathrin assembly protein AP-2. *J. Biol. Chem.* **268**, 9184-9186
89. Liu, Q., Feng, Y., and Forgac, M. (1994) Activity and *in vitro* reassembly of the coated vesicle (H^+)-ATPase requires the 50-kDa subunit of the clathrin assembly complex AP-2. *J. Biol. Chem.* **269**, 31592-31597
90. Johnson, R.G. Jr. (1988) Accumulation of biological amines into chromaffin granules: a model for hormone and neurotransmitter transport. *Physiol. Rev.* **68**, 232-307
91. Moriyama, Y. and Futai, M. (1990) Presence of 5′-hydroxyltryptamine (serotonin) transport coupled with vacuolar type H^+-ATPase in neurosecretory granules from bovine posterior pituitary. *J. Biol. Chem.* **265**, 9165-9169
92. Moriyama, Y., Maeda, M., and Futai, M. (1990) Energy coupling of L-glutamate transport and vacuolar H^+-ATPase in brain synaptic vesicles. *J. Biochem.* **108**, 689-693
93. Schuldiner, S., Shirvan, A., and Linial, M. (1995) Vesicular neurotransmitter transporters: from bacteria to human. *Physiol. Rev.* **75**, 369-392
94. Moriyama, Y., Iwamoto, A., Hanada, H., Maeda, M., and Futai, M. (1991) One step purification of *Escherichia coli* (F_oF_1) and its reconstitution into liposomes with neurotransmitter transporters. *J. Biol. Chem.* **266**, 22141-22146
95. Moriyama, Y., Amakatsu, K., and Futai, M. (1993) Uptake of the neurotoxin, 1-methyl-4-phenylpyridinium, a neurotoxin causing Parkinsonism, into chromafin granules and synaptic vesicles through monoamine transporter. *Arch. Biochem. Biophys.* **305**, 271-277
96. Moriyama, Y., Amakatsu, K., Yamada, H., Park, M., and Futai, M. (1991) Inhibition of neurotransmitter and hormone transport into secretory vesicles by 2-(4-phenylpiperadino)cyclohexanol and 2-bromo-α-ergocriptine: both compounds act as uncouplers and dissipate electrochemical gradient of protons. *Arch. Biochem. Biophys.* **290**, 233-238
97. Moriyama, Y., Tsai, H.L., and Futai, M. (1993) Energy-dependent accumulation of neuron blockers causes selective inhibition of neurotransmitter uptakes by brain synaptic vesicles. *Arch. Biochem. Biophys.* **305**, 278-281
98. Moriyama, Y., Manabe, T., Yoshimori, T., Tashiro, Y., and Futai, M. (1994) ATP-dependent uptake of anti-neoplastic agents by acidic organelles. *J. Biochem.* **115**, 213-218
99. Thomas-Reetz, A., Hell, J.W., During, M.J., Walch-Solimena, C., Jahn, R., and De Camilli, P. (1993) A γ-aminobutyric acid transporter driven by a proton pump is present in synaptic-like microvesicles of pancreatic β-cells. *Proc. Natl. Acad. Sci. USA* **90**, 5317-5321
100. Thomas-Reetz, A. and De Camilli, P. (1994) A role of synaptic vesicles in non-neuronal cells: clues from pancreatic β cells. *FASEB J.* **8**, 209-216
101. Bauerfeind, R., Regnier-Vigonronx, A., Flatmasrk, T., and Huttner, W.B. (1993) Selective storage of acetylcholine, but not catecholamines, in neuroendocrine synaptic-like microvesicles of early endosomal origin. *Neuron* **11**, 105-121
102. Rebois, R.V., Reynolds, E.E., Toll, L., and Howard, B.D. (1980) Storage of dopamine and acetylcholine in granules of PC12, a clonal pheochromocytoma cell line. *Biochemistry* **19**, 1240-1248
103. Moriyama, Y., Yamamoto, A., Yamada, H., Tashiro, Y., Tomochika, K., Takahashi, M., Maeda, M., and Futai, M. (1995) Microvesicles isolated from bovine posterior pituitary accumulate norepinephrine. *J. Biol. Chem.* **270**, 11424-11429
104. Moriyama, Y. and Yamamoto, A. (1995) Microvesicles isolated from bovine pineal gland specifically accumulate L-glutamate. *FEBS Lett.* **367**, 233-236
105. Moriyama, Y. and Yamamoto, A. (1995) Vesicular L-glutamate transporter in microvesicles from bovine pineal glands: driving force, mechanism of chloride anion-activation, and substrate specificity. *J. Biol. Chem.* **270**, 2314-2320
106. Yamada, H., Yamamoto, A., Yodozawa, S., Kozaki, S., Takahashi, M., Michibata, H., Morita, M., Furuichi, T., Mikoshiba, K., and Moriyama, Y. (1996) Microvesicle-mediated exocytosis of glutamate is a novel paracrine-like chemical transduction mechanism and inhibits melatonin secretion in rat pinealocytes. *J. Pineal Res.* **21**, 175-191
107. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C., and Lazdunski, M. (1997) A proton-gated cation channel involved in acid sensing. *Nature* **386**, 173-176
108. Hayashi, M., Yamamoto, A., Yatsushiro, S., Yamada, H., Futai, M., Yamaguchi, A., and Moriyama, Y. (1998) Synaptic vesicle protein SV2B, but not SV2A, is predominantly expressed and associated with microvesicles in rat pinealocytes. *J. Neurochem.* in press
109. Yamada, H., Yatsushiro, S., Zshio, S., Hayashi, M., Nishi, T., Yamamoto, A., Futai, M., Yamaguchi, A., and Moriyama, Y. (1998) Metabotropic glutamate receptors negatively regulate melatonin synthesis in rat pinealocytes. *J. Neurosci.* **18**, 2056-2062