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Kidney Vacuolar H^+-ATPase: Physiology and Regulation

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The vacuolar H^+-ATPase is a multisubunit protein consisting of a peripheral catalytic domain (V_1) that binds and hydrolyzes adenosine triphosphate (ATP) and provides energy to pump H^+ through the transmembrane domain (V_0) against a large gradient. This proton-translocating vacuolar H^+-ATPase is present in both intracellular compartments and the plasma membrane of eukaryotic cells. Mutations in genes encoding kidney intercalated cell–specific V_0 a4 and V_1 B1 subunits of the vacuolar H^+-ATPase cause the syndrome of distal tubular renal acidosis. This review focuses on the function, regulation, and the role of vacuolar H^+-ATPases in renal physiology. The localization of vacuolar H^+-ATPases in the kidney, and their role in intracellular pH (pHi) regulation, transepithelial proton transport, and acid-base homeostasis are discussed.
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The vacuolar proton-translocating adenosine triphosphatases (ATPases) are a family of multisubunit ATP-driven proton pumps present in both intracellular compartments and the plasma membrane of eukaryotic cells.[1-3] They couple the energy released on ATP hydrolysis to the active transport of protons from the cytoplasm to either the lumen of various intracellular compartments or to the extracellular environment. Acidification of intracellular compartments is important for such processes as receptor-mediated endocytosis, intracellular trafficking of lysosomal enzymes, degradation of macromolecules, uptake of neurotransmitters, and the entry of various envelope viruses and toxins.[4-6] Vacuolar H^+-ATPases located in the cell membranes of many different cell types mediate proton extrusion from the cell.[7] This acidification of the extracellular environment often is linked to specialized cell function. Examples include osteoclasts where protons generated by the vacuolar H^+-ATPases are used to dissolve bone matrix, and macrophages where an acidic extracellular pH is involved in killing and digesting neighboring cells or pathogens.[8] In addition, vacuolar H^+-ATPases in other cells regulate the extracellular pH of closed extracellular compartments such as in the inner-ear endolymph fluid[9-11] or acidification of seminal fluid in the epididymis.[12] Perhaps the most significant function of vacuolar H^+-ATPases is in epithelia, where their role in acid/base transport and transepithelial transport is crucial for many physiologic processes.

This review focuses on the function and regulation of vacuolar H^+-ATPases in renal physiology and pathophysiology.

Structure and Molecular Organization of Vacuolar H^+-ATPases

Vacuolar H^+-ATPases belong to the large superfamily of ATPases, which is subdivided into 3 major subclasses: (1) P-type ATPases such as Na^+/K^+-ATPases, Ca^{2+}-ATPases, and H^+/K^+-ATPases, (2) mitochondrial F_1F_0-ATPases, and (3) V-type (vacuolar) H^+-ATPases (http://www.gene.ucl.ac.uk/nomenclature/).[13]

Mitochondrial F_1F_0-ATPases and vacuolar H^+-ATPases share many structural features in their subunit composition such as amino acid sequences and subunit arrangements.[14] Functionally, however, they are distinguished by the fact that F_1F_0-ATPases use a proton gradient for ATP synthesis whereas vacuolar H^+-ATPases use ATP hydrolysis to generate a proton gradient.[15]

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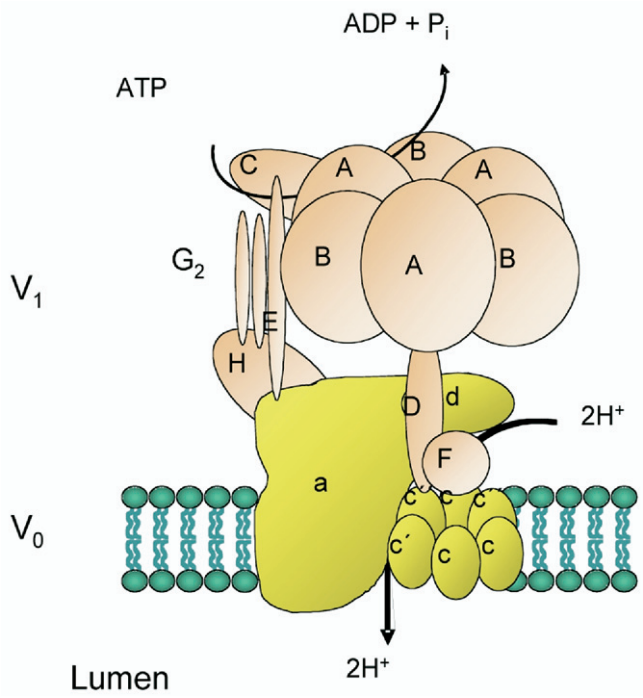

Figure 1 Structural model of vacuolar H^+-ATPase. Vacuolar H^+-ATPase is a multisubunit complex composed of a peripheral cytosolic domain V_1 and an integral domain V_0. The cytosolic V_1 domain consists of 8 subunits termed *A-H*. The membrane-bound V_0 domain is composed of the subunits a-d with several isoforms of the c subunits. Redrawn from Kawasaki-Nishi et al.[139] (Color version of figure is available online.)

The vacuolar H^+-ATPases are hetero-oligomeric complexes composed of 13 polypeptide types. These can be fractionated into a soluble cytoplasmically disposed V_1 domain of 640 kd, and a membrane-associated Vo domain of 240 kd, together forming a protein complex of approximately 900 kd (Fig 1). Both domains are connected through a stalk-like structure that belongs to the V_1 domain.[2,16] The peripheral domain (V_1) hydrolyzes ATP and the integral domain (V_0) conducts protons. Dissociation of V_1 and V_0 is an important in vivo mechanism of controlling vacuolar ATPase activity as described in detail later.

The names of the respective subunits are given according to the recently revised Human Genome Organization nomenclature (http://www.gene.ucl.ac.uk/ nomenclature/).[17] The subunit names consist of the family name (ATP6), followed by the domain name (V1 or V0), and then the specific polypeptide designation, (eg, *ATP6V1B1*). Thus, ATP6 designates all subunits of the vacuolar H^+-ATPase as a subfamily of the superfamily of ATPases. V_1 delineates subunits belonging to the peripheral catalytic V_1 domain whereas V_0 subunits belong to the membrane-bound V_0 domain. B1 designates the specific polypeptide subunit.

The Cytosolic V_1 Domain

The cytosolic V_1 domain of the vacuolar H^+-ATPase consists of 8 different subunits termed A-H. The A and B subunits show a high homology with the α and β subunits of the F_1F_0-ATPase. This high homology together with mutational studies using yeast vacuolar H^+-ATPases have been used to establish several functions for the A and B subunits. The sequence comparisons show that the vacuolar H^+-ATPase B subunits share approximately 20% to 25% amino acid identity not only with the vacuolar H^+-ATPase A subunit, but also with the α and β subunits of the mitochondrial F_1F_0-ATPase, suggesting that all 4 of these proteins evolved from a common ancestral nucleotide-binding protein.

The A subunit contains all of the consensus nucleotide-binding sequences found in the catalytic nucleotide binding subunit β of the F_1F_0-ATPase.[18] Mutations of conserved residues in these sequences in the A subunit lead to significant losses of activity,[19,20] as previously observed for the β subunit of the F_1F_0-ATPase. Unlike with the nucleotide-binding sites on the A subunit, the nucleotide binding sites on the B subunit appears to be noncatalytic. However, inhibition of vacuolar H^+-ATPase activity also occurred after modification of a single noncatalytic site of the B subunit by a photo-activated, nonhydrolyzable, nucleotide analog.[21] This raises the possibility that the nucleotide-binding site of the B subunit may participate in the regulation of H^+-ATPase activity.

Although only 1 isoform of the A subunit has been identified, 2 highly homologous B subunits (*ATP6V1B1* and *ATP6V1B2*) exist in many species. *ATPV1B1* (B1) and *ATPV1B2* (B2) show an expression pattern that is both tissue and cell specific.[22,23] Comparison of the B1 and B2 subunit isoform amino acid sequences reveals that the central 469 amino acids are highly conserved, whereas the 20 to 25 amino acids at both the amino and carboxy termini have diverged greatly, thus raising the possibility that these terminal regions provide specialized isoform-specific functions of the B subunit.[24] It has been speculated that the 2 B isoforms may confer differences in enzymatic activities of the vacuolar H^+-ATPases or in vacuolar H^+-ATPase–sorting capacities.

The B1 subunit of the vacuolar H^+-ATPase has a C-terminal D-T-A-L sequence. This zinc-binding protein domain (PDZ)-binding motif is recognized by so-called *PDZ proteins*, and mediates protein-protein interactions, which could mediate targeting or trafficking of the complex.[25] In contrast, the B2 isoform lacks the C-terminal PDZ-binding motif. Alterations in the PDZ potentially could affect insertion and function of the vacuolar H+ ATPase, although none of the *ATP6V1B1* missense mutations identified to date in distal renal tubular acidosis (dRTA) kindreds (see later) are located in the carboxy terminal region of the protein.[26]

The Membrane-Associated V_0 Domain

The V_0 domain is a 260-kilodalton integral complex composed of 5 different subunits (subunits a, d, c, c′, and c″, with molecular masses of 17-100 kilodaltons) that function in proton translocation. A number of these subunits have been shown to have multiple isoforms in a variety of species.[27]

Two isoforms of the a subunit are of special interest because of their links to human disease (see later).

The c subunit(s), including the c′ and c″ isoforms, all are expressed in the V_0 sector and are known as proteolipids because of their extreme hydrophobicity. Proton translocation through the proton channel is potently inhibited in the nanomolar range by the macrolide antibiotics bafilomycin A_1 and concanamycin.[28] These selective vacuolar H^+-ATPase inhibitors may act through interactions with the proteolipid c′ and c″ subunits.[29,30] It also recently has been shown that the a subunit of vacuolar H^+-ATPase participates in binding bafilomycin A1 along with the c subunits.[31]

Site-directed and random mutagenesis have been used to discern the function of 4 of the 5 V_0 subunits. Each of the 3 proteolipid subunits (c, c′, and c″) contains a single buried glutamate residue that is essential for proton translocation.[32] In addition, the a subunit contains a number of buried charged residues that influence proton transport. The a subunit also plays critical roles in intracellular targeting of the vacuolar H^+-ATPase, in controlling assembly with V_1, the coupling of proton transport with ATP hydrolysis, and reversible dissociation of the V_1 and V_0 domains.[33]

The a subunit recently received special attention because at least 2 human diseases are caused by mutations in 2 isoforms of this subunit (*ATP6V0A3* and *ATP6V0A4*). Loss-of-function mutations in the *ATP6V0A4* gene cause autosomal-recessive distal renal tubular acidosis.[34] Mutations in the *ATP6V0A3* gene result in one type of infantile malignant autosomal-recessive osteoporosis.[35]

ATP6V0A4 (a4) is expressed only in the kidney and epididymis.[34,36] Murine a4 has 833 residues and shows 85% amino acid identity to the human kidney-specific isoform. The human and murine genes also have similar genomic organization. Moreover, *ATP6V0A4* maps to a region of mouse chromosome 6 that is syntenic with the segment of human 7q33 to 34 containing *ATP6V0A4*. Together these findings establish the 2 genes as orthologs.[36] The mouse a4 protein is 61%, 52%, and 47% identical to the other isoforms, a1, a2, and a3, respectively. *ATP6V0A4* shares 40% amino acid identity with a subunit homologs as evolutionary distant as Neurospora and yeast, indicating the biological importance of this gene family.[36] The transmembrane topology of the a subunits of the vacuolar H^+-ATPase, however, still is a controversial issue and additional structural/crystallography studies are needed to clarify its transmembrane arrangement.[37,38]

Recently, the gene-encoding d subunit has been cloned and sequenced from human beings and plants. Two isoforms of the d subunits have been described, d1 is ubiquitous whereas d2 expression is limited to kidney and bone.[39] The d subunit is classified as a component of the V_0 domain through protein-protein interactions rather than by integration into the membrane itself, and is proposed to be present at the cytoplasmic side of the membrane.[40] Its precise contribution to vacuolar H^+-ATPase function remains unclear, although Nishi et al[41] suggested that the d subunit may play a role in coupling of ATP hydrolysis to proton transport.

The Stalk

The A and B subunits of the V_1 domain are connected to the V_0 domain through several protein subunits termed the *stalk*, which also belongs to the V_1 domain. Electron microscopy studies indicate that similar to the F_1F_0-ATPases,[42] the vacuolar H^+-ATPases also contain multiple stalks connecting the peripheral and integral domains.[43] The D subunit is located in the central stalk whereas E and G subunits form part of the peripheral stalk connecting V_1 and V_0. These assignments have been confirmed by rotation experiments showing that subunits D and F are present in the central rotor[44] whereas subunit G is located in the peripheral stator.[45]

The C subunit of the *Thermus thermophilus* vacuolar H^+-ATPase is homologous to the eukaryotic subunit D of vacuolar H^+-ATPases. Analysis of the crystal structure of the C subunit provides further evidence that it is part of the stalk and may help to attach the central stalk to the V_0 domain.[46] This subunit C is of particular interest because of its putative role in regulating dissociation of the V-ATPase complex in vivo.[47] Dissociation of V_1 and V_0 represents an important mechanism of controlling vacuolar H^+-ATPase activity in cells[48,49] and occurs with release of subunit C from both the V_1 and V_0 domains. By interacting with key subunits of the peripheral stalk (including subunits E, G, and a), subunit C may be able to disrupt interactions between these subunits that otherwise would lead to a stably assembled peripheral stalk.[50] In addition, at least the NH_2-terminus of the a subunit (Vph1 in yeast) of the V_0 domain also interacts with the A and H subunits, forming a stator-like structure. The stator-like structure may be important for coupling ATP hydrolysis to proton translocation.[51] Moreover, other proteins associated to the subunits of the vacuolar H^+-ATPase have been identified. These proteins probably are involved in the targeting of the pump to specific intracellular structures.[52]

Kidney-Specific Subunits of the Vacuolar H^+-ATPase

The ubiquitous and intercalated cell-specific isoforms of the vacuolar H^+-ATPase subunits are expressed differentially in mature kidney and during mouse nephrogenesis. Ubiquitous A, B2, E1, G1, and C1 show an early and stable expression throughout nephrogenesis, followed by a slight increase around birth. This expression pattern contrasts with the later and progressive expression of the intercalated cell-specific isoforms B1, G3, C2, and a4. Subcellular fractionation studies indicated a preferential location of both ubiquitous and intercalated cell–specific subunits to endosomes in both developing and mature kidneys.[53]

Little is known about the expression patterns of these intercalated cell–specific isoforms in the kidney with the exception of the B1 isoform, *ATP6V1B1*, and a4 subunit, *ATP6V0A4*. Mutations in *ATP6V1B1* and *ATP6V0A4* genes, encoding intercalated cell–specific B1 and a4 subunits, respectively, have been associated with early onset cases of dRTA, and may suggest that the segmental distribution of

intercalated cellδspecific isoforms of vacuolar H^+-ATPase could be acquired during early infancy.

Distribution and Role of the Vacuolar H^+-ATPase in the Kidney

The kidney plays a major role in the regulation of acid-base homeostasis. This is achieved by several mechanisms that include the reabsorption of bicarbonate and the secretion of H^+ and the excretion of ammonium and phosphate and other titratable acids.

The vacuolar H^+-ATPase is one of several acid-base regulating proteins that are involved in this process.[54] The vacuolar H^+-ATPase is essential for acidification of diverse intracellular compartments in eukaryotic cells, including endosomes, lysosomes, clathrin-coated, and synaptic vesicles. In addition to its intracellular distribution, vacuolar H^+-ATPase is located in the plasma membrane of specialized epithelial cells of the kidney, and epididymis as well as in the ruffled border membrane of osteoclasts, where it is involved in urinary or semen acidification and in bone resorption, respectively.

Proximal Tubule

A large amount of evidence supports the concept that a bafilomycin and N-ethylmaleimide (NEM)-sensitive vacuolar H^+-ATPase is located in the apical membrane and in intracellular organelles of proximal tubule epithelial cells.[55,56] This includes numerous functional studies using intact tubules, isolated brush-border membrane vesicles, and immunocytochemical analysis using specific antibodies raised against various subunits of the vacuolar H^+-ATPase. Specifically, vacuolar H^+-ATPase is present in apical invaginations at the base of the microvili, distinct from clathrin-coated regions.[57] The presence of the 31-kd E subunit of the vacuolar H^+-ATPase along with the brain or B2 (*ATP6V1B2*) isoform of the 56-kd subunit have been shown in rat proximal tubule segments. The B2 subunit of the vacuolar H^+-ATPase is expressed in the kidney proximal convoluted tubule and in the thick ascending limb (TAL), distal convoluted tubule (DCT), connecting segment, and in both α- and β-type intercalated cells of the collecting ducts.[57,58] The functional relevance of the B2 isoform expression in proximal tubules is unknown, but it may allow the vacuolar H^+-ATPase to function and recycle without interference from the complex PDZ and PDZ-binding protein interactions that otherwise would occur in this region of the cell.[15] Under baseline conditions, the B2 isoform was found predominantly on intracellular vesicles.[59] However, α-intercalated cells show high levels of B2 subunit expression in the apical membrane, where it colocalized with E and B1. These findings indicate that in addition to its role in the acidification of intracellular organelles, the B2 isoform could contribute to transepithelial proton secretion.[58]

The proximal tubule reabsorbs 70% to 80% of the filtered bicarbonate, which occurs mainly in the initial segments. Two main transport systems mediate H^+-secretion on the apical side, the first step in bicarbonate reabsorption, Na^+/H^+ exchange, and H^+ secretion via the vacuolar H^+ ATPase. Several isoforms of Na^+/H^+-exchangers, localized in the brush-border membrane of the proximal tubule, including NHE-2, NHE-3, and NHE-8 are involved in proton secretion. About 50% of the overall apical NHE activity may be mediated by NHE-3, the remainder by another isoform(s).[60] Up to 40% of proximal tubule bicarbonate reabsorption is Na^+ independent. It is sensitive to the vacuolar H^+-ATPase inhibitor bafilomycin and is mediated by vacuolar H^+-ATPases expressed in the brush-border membrane.

After proton secretion, H^+ combines with the filtered HCO_3^- to produce H_2O and CO_2, a process catalyzed by the membrane-bound carbonic anhydrase IV. CO_2 then diffuses into the proximal tubule cells where it reacts with H_2O (catalyzed by the cytosolic carbonic anhydrase II isoform) to form H^+ and HCO_3^-. The generated HCO_3^- is exported into blood via the basolateral electrogenic Na^+/HCO_3^- (kNBC-1, SLC4A4) whereas the proton is recycled back into the tubule lumen by the Na^+/H^+-exchangers and vacuolar H^+-ATPase where it can react with another HCO_3^- ion.[54]

Loop of Henle

The TAL reabsorbs 15% to 20% of filtered bicarbonate. Proton secretion and bicarbonate transport in the TAL of the loop of Henle are mediated by both the vacuolar H^+-ATPase and other transporters including the Na^+/H^+-exchanger, NHE-3.[61] As in the proximal tubule, the apical mechanism responsible for this transport is predominantly via the Na^+-H^+ exchanger. Vacuolar H^+-ATPase in this segment is located in numerous cytoplasmic vesicles, which are concentrated at the apical pole of the cell. Immunogold electron microscopy has shown clearly that a part of the vacuolar H^+-ATPase is located in the apical plasma membrane, and another part of it is associated with subapical vesicles.[57] Both the electroneutral Na^+-HCO_3^- cotransporter, NBC(N)1, and electrogenic Na^+-HCO_3^- cotransporter, NBC4, are expressed in the TAL. The Cl^-/HCO_3^- exchanger, AE2, also has been localized in the basolateral membrane of the TAL in rat and mouse kidney,[62] and might provide a potential route for bicarbonate reabsorption in this membrane domain.

Distal Tubule and Connecting Segment

The vacuolar H^+-ATPase (containing the 56-kd B1 subunit) is present at relatively high levels on the apical plasma membrane of DCT cells, where it forms a very sharp line at the level of the membrane by immunostaining.[57] In the late DCT, intercalated cells begin to appear interspersed with the H^+-ATPase expressing DCT cells. Intercalated cells express very high levels of H^+-ATPase. Relatively little staining of intracellular vesicles is seen in the DCT and the capacity of the vacuolar H^+-ATPase to recycle in this segment is unknown.

The connecting segment joins the DCT with the cortical collecting duct and contains a larger percentage of intercalated cells than the DCT. Intercalated cells are subclassified (α type, β type, or non-α, non-β) based on the presence or absence of the anion exchanger, AE1 (SLC4A1), and the subcellular distribution of the vacuolar H^+-ATPase. The connecting segment contains a large percentage of β cells and more of the subclass of intercalated cells that have apical vacuolar H^+-ATPase but no basolateral AE1 as compared with the collecting duct. In addition, connecting tubule cells have a distinct apical band of vacuolar H^+-ATPase staining similar to that of DCT cells.[57]

Cortical Collecting Duct

Intercalated cells of the collecting duct express the highest levels of vacuolar H^+-ATPases among all acid-base transporting cells in the kidney. The subcellular localization of the vacuolar H^+-ATPase within an intercalated cell subtype is dependent on whether the cell secretes or absorbs net H^+ equivalents. α-type intercalated cells mediate secretion of net H^+ equivalents through an apical H^+-ATPase, which functions in series with the basolateral Cl^-/HCO_3^- exchanger, AE1 (Figs 2A and 2B). β-type intercalated cells have an apical Cl^-/HCO_3^- exchanger that functions in series with a basolateral H^+-ATPase to mediate secretion of OH^- equivalents, particularly during metabolic alkalosis (Fig 2A and 2B).

β cells have basolateral vacuolar H^+-ATPase, but, in addition, many cells have a diffuse or even a bipolar vacuolar H^+-ATPase distribution.[63] α intercalated cells can be distinguished from β-intercalated cells by the presence of the anion exchanger, AE-1 (band 3 protein), on their basolateral plasma membrane (Fig 2C). This protein is undetectable in typical β-intercalated cells.[64] Two other Cl^-/HCO_3^- exchangers have been identified more recently in β-intercalated cells: pendrin[65] and AE4.[66]

Non-α, non-β-intercalated cells have an apical Na^+-independent Cl^-/HCO_3^- exchanger, similar to β-type intercalated cells, but express the H^+-ATPase along the apical membrane, similar to α-intercalated cells. All intercalated cells contain a large amount of cytoplasmic carbonic anhydrase, which is also a key protein in supporting the proton-transporting capacity of these cells by providing membrane-associated pumps and transporters with a supply of proton and bicarbonate. The 56-kd B1 subunit of the vacuolar H^+-ATPase, which contains a C-terminal PDZ-binding DTAL amino acid motif that interacts with Na^+/H^+ exchanger regulatory factor (NHERF1), is expressed in intercalated cells.[25]

In the cortical collecting duct, all subtypes of intercalated cells are detectable. In the outer stripe of the outer medulla, α cells predominate but a few residual β cells can be found. In the inner stripe of the outer medulla, only α-intercalated cells are present, which represent about 40% of the epithelial cell population of the collecting duct. Principal cells make up the remaining 60% of the tubule epithelium. In the inner medulla, the epithelium initially contains between 5% and 10% of α-intercalated cells. These cells disappear from the epithelium in the middle and terminal portions of the inner medullary collecting duct.[57]

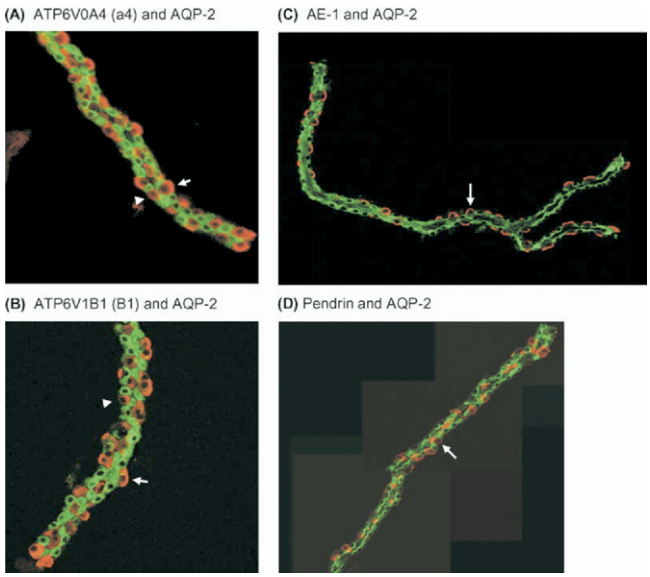

Figure 2 Localization of acid-base transporting proteins in cortical and medullary collecting ducts. Isolated mouse cortical and medullary collecting ducts were labeled with antibodies against the principal cell-specific water channel aquaporin-2 (AQP-2, green), and then colabeled with antibodies against the *ATP6V0A4 (a4)* or *ATP6V1B1 (B1)*subunits of the vacuolar H^+-ATPase, or against anion exchanger-1 (*AE-1*) or against pendrin, and viewed with confocal microscopy. (A and B) Expression of both vacuolar H^+-ATPase subunits in cortical collecting duct fragments was restricted to cells negative for AQP-2, thus representing intercalated cells. In many cells expression of the vacuolar H^+-ATPase subunits was basolateral (arrow) or diffuse. Some cells also showed apical staining of the vacuolar H^+-ATPase (arrowhead). (C) Localization of the Cl^-/HCO_3^- exchanger, AE1, in cortical and medullary collecting duct. The expression of AE1 is restricted to the basolateral side of intercalated cells, thus marking these cells as α-intercalated cells. (D) Apical localization of the chloride anion exchanger, pendrin, in intercalated cells. In the intact kidney this protein is expressed apically in non–α-intercalated cells. The basolateral and apical localization of AE1 and pendrin in intercalated cells, respectively, suggests polarity of expression of transport proteins. Original magnification: 400x. Reprinted from Wagner et al.[140]

The late distal tubule, connecting segment, and cortical collecting duct together reabsorb 5% of the filtered bicarbonate. In addition to the reabsorption of filtered bicarbonate, the kidney also must generate new bicarbonate to buffer approximately 70 mEq of acid produced by daily metabolism. In the H^+-secreting α type intercalated cells, net excretion of protons occurs through apically localized vacuolar H^+-ATPases, where the H^+ is produced by the cytosolic carbonic anhydrase II. The HCO_3^- generated by the intracellular hydration of CO_2 exits the cell across the basolateral membrane by the kidney-specific isoform of the Cl^-/HCO_3^- exchanger AE-1 (SLC4A1).[67] On the contrary, under conditions of bicarbonate secretion such as in metabolic alkalosis, β-type intercalated cells are activated.[68] These are found only in the connecting segment and cortical collecting

duct. β-type intercalated cells secret bicarbonate into the tubular fluid via an apically located Cl^-/HCO_3^- exchanger, whereas basolaterally expressed vacuolar H^+-ATPases extrude protons into the interstitium. This apical Cl^-/HCO_3^- exchanger is insensitive to 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) in the presence of Cl^-, but becomes DIDS sensitive in its absence.[69] Two Cl^-/HCO_3^- exchangers have been identified in β-intercalated cells: pendrin[65] and AE4.[66] Pendrin localizes in the apical membrane of all non-α-intercalated cells,[65] and is regulated by acid-base status[70] (Fig 2D). Genetic loss of pendrin function (PDS knock-out mice) leads to a reduced capacity of bicarbonate secretion in isolated perfused cortical collecting ducts.[71] Pendrin seems to have some sensitivity to DIDS.[72] AE4, another Cl^-/HCO_3^- exchanger, is DIDS insensitive and also is expressed in non–α-intercalated cells. However, its physiologic role is uncertain.[66]

Medullary Collecting Duct

The medullary collecting duct only possesses H^+-secreting cells. There are no HCO_3^--secreting cells in the medullary collecting duct. However, in addition to α-intercalated cells,[64] the outer medullary collecting duct (OMCD) contains principal cells that are involved in water, Na^+, and K^+ transport. In vitro perfusion studies of isolated OMCDs demonstrated that proton secretion is mediated by vacuolar H^+-ATPases and bicarbonate absorption is Cl^- dependent due to the presence of the basolateral Cl^-/HCO_3^- exchanger, AE-1 (band 3 protein) (Fig 2C). The activity of the vacuolar H^+-ATPase in this segment is under the control of several hormones. α-intercalated cells likely have other physiologic roles in addition to regulating acid-base balance. For example, intercalated cells secrete Cl^-, which may participate in the regulation of fluid and electrolyte balance.[73] In α-intercalated cells of the rat OMCD, basolateral Cl^- uptake occurs through the $Cl^-/HCO3^-$ exchanger AE-1, and the $Na^+/K^+/2Cl^-$ exchanger, NKCC1.[74] Moreover, the $Cl^-/HCO3^-$ exchanger, Slc26a7, is expressed on the basolateral membrane of α-intercalated cells of OMCD and is greatly upregulated with water restriction in vivo.[75]

An important feature of the medullary collecting duct is the presence of titratable acids: phosphate, citrate, and ammonia, which buffer excreted protons.[76] The buffering of protons is necessary to maintain a favorable proton gradient across the apical membrane to facilitate vacuolar H^+-ATPase activity. Excretion of ammonium is a major regulatable component of net acid secretion and is thought to occur through secretion of H^+ in parallel with nonionic diffusion of NH_3. NH_3 and H^+ together form NH_4^+, a nonpermeable ion that is trapped in the collecting duct lumen.

In addition to vacuolar H^+-ATPase, a H^+/K^+-ATPase is expressed in the OMCD. The contribution of H^+/K^+-ATPases to proton secretion in this segment and thus to bicarbonate reabsorption is relatively minor compared with the vacuolar H^+-ATPase. H^+/K^+-ATPases rather may play an important role during systemic potassium depletion.[77]

The inner medullary collecting duct (IMCD) is composed of 2 separate segments, the initial portion and the terminal region, which comprise approximately the last two thirds of the IMCD.[78] From the initial third of the IMCD, intercalated cells gradually disappear, and only cells called *IMCDs* are found in the terminal portion of this tubule segment. These cells express AQP-2 and are involved in the final concentration of urine.

Function of the Vacuolar H^+-ATPase: Chloride Dependence

Vacuolar H^+-ATPase is an electrogenic pump that contributes to the translocation of H^+ ions across the cell membrane, thus rendering the cell interior negative with respect to the exterior. The generation of both a transmembrane potential and a chemical gradient results in self-limitation of the vacuolar H^+-ATPase. The presence of a parallel Cl^- conductance at cell plasma membranes and in many intracellular organelles provides an electric shunt that compensates for the positive charge transferred by the pump. It thus dissipates the electrical gradient and permits the continued pumping of H^+.[79] The chloride dependence of plasma membrane H^+-ATPase is a complex issue because of the expression of numerous other conductances or electrogenic transporters in apical and basolateral membrane domains. In the kidney, vacuolar H^+-ATPase–dependent acidification is Cl^- dependent in endosomal fractions and in brush-border membrane vesicles.[15] Vacuolar H^+-ATPase–dependent H^+ extrusion was reduced after preincubation in Cl^--free media in isolated rat proximal tubules.[80] Angiotensin II stimulates H^+-ATPase–dependent proton extrusion via a process involving a Cl^--dependent insertion of vesicles into the brush-border membrane.[81] Moreover, in mouse proximal tubules, the insertion of vacuolar H^+-ATPase–containing vesicles was delayed in the absence of chloride.[82]

To our knowledge, there have been no studies reporting on the Cl^- dependence of vacuolar H^+-ATPase activity in the thin loop of Henle. In vivo microperfusion experiments in the late distal tubule showed a reduction of electrogenic H^+ secretion by the Cl^- channel blocker, 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), indicating coupling between H^+-ATPase and Cl^- transport.[83]

The mechanisms for the apparent differences in the requirement for chloride in the different nephron segments have remained elusive to date, and there remains some controversy as to the molecular identity of the associated Cl^--conducting proteins for each segment. Several promising candidates have been identified, including: ClC-5, AQP-6, and cystic fibrosis transmembrane conductance regulator (CFTR).[15] An order of anion selectivity with I^-, NO_3^-, and SCN^- over Cl^- and Br^-[84] is suggestive of the involvement of members of the ClC Cl^- channel family.

Early reports indicated the presence of a protein kinase A–activated Cl^--conductive pathway, CFTR, in brush-bor-

der membrane vesicles, suggesting that CFTR protein was involved in protein kinase A–activated Cl^- fluxes in these vesicles.[85,86] However, in brush-border membranes prepared from CFTR knock-out mice a similar Cl^- conductance still was found, excluding CFTR as an important contributor to the observed Cl^- conductance.

Endocytosis and Acidification of Intracellular Vesicles

Regulation of vacuolar H^+-ATPase function may occur at several levels, such as assembly or disassembly of V_1 and V_0 domains, trafficking and sorting to and from the membrane, interactions with other (activator or inhibitory) proteins, alteration of the number of cells expressing vacuolar H^+-ATPases (polarity of intercalated cells), or by increased transcription/translation of vacuolar H^+-ATPases.

There is ample in vivo and in vitro evidence for regulation of H^+-ATPase activity by trafficking/exocytosis. Likewise, H^+-ATPase–dependent acidification is required for exocytosis to occur. The secretory and endocytotic pathways in membrane-bound compartments of epithelial and nonepithelial cells are acidified via the action of a vacuolar H^+-ATPase. In early endosomes isolated from proximal tubules, 3 proteins (V-type ATPase, Cl^- channel, Na^+/H^+ exchanger) may regulate the formation, maintenance, and dissipation of the proton gradient. Defects in either the vacuolar H^+-ATPase or the chloride conductance pathway can result in a failure of organelles and vesicles to acidify appropriately.[15] Many of the acidification-dependent steps involve vesicle fission and fusion via specialized coat protein (COP)-coated transport vesicles.[87] Association of COP proteins, in particular β COP and small guanosine 5′-tri-phosphate (GTP)ases of the ADP-ribosylation factor (ARF) [adenosine 5′-diphosphate (ADP)-ribosylation factor] family, with some vesicles depends on the generation of an acidic luminal pH.[88] Thus, neutralization of vesicle luminal pH may inhibit some steps of the intracellular trafficking pathway by preventing the recruitment of coat proteins that are required for vesicle formation and budding.

Kidney proximal tubule epithelial cells have an extensive apical endocytotic apparatus that is involved in the reabsorption of low molecular weight proteins that traverse the glomerular filtration barrier. They also are involved in the extensive recycling of functionally important apical plasma membrane transporters. The molecular mechanisms responsible for the regulation of endocytic processes in transporting epithelia are still not understood completely. The Arf-nucleotide exchange factor, ADP-ribosylation factor nucleotide site opener (ARNO), Arf6, and Arf1, small GTPases, are part of the kidney proximal tubule receptor–mediated endocytosis pathway. ARNO, Arf1, and Arf6 recruitment from the cytosol to endosomes is pH-dependent.[89] Although the apical membrane of the proximal tubule is highly specialized for clathrin-mediated endocytosis of cell-surface and filtered proteins, there is no direct evidence that endocytosis-derived clathrin-coated vesicles from renal epithelia contain a functional vacuolar H^+-ATPase. Because intracellular vesicle trafficking involves the sequential passage of transported molecules through a series of acidified compartments, it is to be expected that perturbation of the acidification process will lead to tubule dysfunction and potentially to pathophysiologic states.

Interaction of the Vacuolar H^+-ATPase With *N*-Ethylmaleimide–Sensitive Factor Attachment Protein Receptors

Soluble *N*-ethylmaleimide–sensitive factor attachment protein receptor (SNARE) complexes are a class of vesicle-associated membrane proteins. SNARE complexes bridge opposing membrane bilayers that appear to mediate specific membrane fusion in the endomembrane system.[90] Although SNARE complexes appear to represent a universal membrane fusion machine, their role in determining the specificity of intracellular membrane fusion still is being established. Each SNARE complex characterized to date appears to consist of a thermostable parallel helix bundle composed of 4 heptad repeat-containing SNARE motifs. These proteins, involved in the trafficking and fusion of synaptic vesicles, recently have been renamed as *arginine-containing SNAREs* (R-SNAREs) or *glutamine-containing SNAREs* (Q-SNAREs).[91] More than 30 mammalian SNARE proteins have been identified and classified into 3 distinct groups: the syntaxin, vesicle-associated membrane protein (VAMP), or 25-kDa synaptosome-associated protein (SNAP)-25 families, based on their sequence homology and domain structure.

Certain subunits of the vacuolar H^+-ATPase have the capacity to bind to SNARE proteins, suggesting that the SNARE machinery may participate in vacuolar H^+-ATPase trafficking. Clostridial toxins, which are specific SNARE proteases, inhibit acid-induced vacuolar H^+-ATPase exocytosis in cultured IMCD cells.[92]Subunit E of the vacuolar H^+-ATPase co-immunoprecipitates with α-SNAP, SNAP-23, syntaxin, and VAMP2. Interaction between syntaxin-1A and the vacuolar H^+-ATPase also is involved in the exocytosis of the pump to the apical membrane of IMCD cells.[93] Although SNARE proteins clearly have been implicated in the attachment of vesicles to their target membranes, the subsequent process of membrane fusion still remains largely uncharacterized. The V_0 domain of the vacuolar H^+-ATPase, particularly subunit c, was proposed to be a major player in the lipid bilayer mixing that occurs during membrane fusion.[94]

Interaction of the Vacuolar H^+-ATPase With PDZ Domain–Binding Protein and its Actin Anchorage

In addition to SNARE proteins, the vacuolar H^+-ATPase has the capacity to interact with various other proteins, indicating its role in a multitude of regulatory functions. The B1 (*ATP6V1B1*) subunit of the vacuolar H^+-ATPase possesses a COOH-terminal DTAL motif typical of a PDZ-

interacting domain.[95,96] The association of the B1 subunit with the PDZ protein NHERF-1 in rat kidney β-intercalated cells has been shown.[25] NHERF-1 colocalizes with the vacuolar H^+-ATPase in either the apical or basolateral pole. NHERF-1 is not detectable in α-intercalated cells, suggesting that interaction of the vacuolar H^+-ATPase with NHERF-1 might play a role in modulating the variable vacuolar H^+-ATPase polarity that characterizes the β-cell phenotype. Although NHERF-1 is located in the apical brush border in the proximal tubule, the vacuolar H^+-ATPase is located in a distinct membrane domain at the base of the brush border consistent with the expression of the truncated B2 subunit isoform, lacking the DTAL motif, in this tubule segment. Association between the B1 subunit and another PDZ binding protein, the Na^+/bicarbonate transporter, NBC3, also has been shown in kidney lysates.[97] Thus, PDZ binding may prove a mechanism for the functional binding of the vacuolar H^+ ATPase with other transport proteins in the kidney. Indirect interaction between the vacuolar H^+-ATPase and the actin cytoskeleton occurs via NHERF binding. In addition, the V_1 complex can bind actin directly. The amino termini of both the B1 and B2 subunits of the vacuolar H^+-ATPase contain high-affinity F-actin binding sites. Moreover, direct interaction between F-actin and subunit C has been reported. Therefore, it is possible that C subunit plays a crucial role in controlling binding of the vacuolar H^+-ATPase to the actin cytoskeleton when the pump is inserted into the plasma membrane.[98] Direct binding of the vacuolar H^+ ATPase to actin can confer stability of the pump in the membrane and also may impart a signal transduction pathway between focal adhesion points and the vacuolar H^+ ATPase.

Regulation of the Vacuolar H^+-ATPase

Mechanisms of Regulation of Vacuolar H^+-ATPase Activity

The distribution and phenotypic expression of intercalated cells varies in the different regions of the connecting segment and collecting duct. Although all intercalated cells can modulate their cell surface expression of vacuolar H^+-ATPase by vesicle trafficking, the situation is especially complex in the cortical collecting duct and the connecting segment of the urinary tubule. Intercalated cells of the collecting duct express high levels of the vacuolar H^+-ATPase in intracellular vesicles and/or on their plasma membrane. The cortical collecting duct has 2 distinct functional subtypes of intercalated cells: the H^+-secreting or α type has an apical H^+-ATPase and basolateral Cl^-/HCO_3^- exchanger and shows vigorous apical endocytosis. The HCO_3^- secreting or β type has its polarization reversed compared with α cells. That is, it has an apical Cl^-/HCO_3^- exchanger and a basolateral H^+-ATPase. β cells further are characterized easily in that they bind peanut lectin on the apical membrane but have no apical endocytosis. Also, α- and β-intercalated cells have been distinguished by a differential expression of other proteins, including NHERF-1.[25]

During metabolic acidosis, the number of β-intercalated cells is reduced whereas that of α-intercalated cells increases without a change in the total number of intercalated cells. This suggests conversion of one cell type to another; this is a process termed *plasticity of functional epithelial polarity*.[99] By using an immortalized intercalated cell line, Schwartz et al[100] found that this adaptation was mediated by an extracellular protein, hensin. Hensin is secreted as a monomer that then is polymerized in the extracellular environment by a complex process. Hensin is expressed in most epithelial cells, but in the kidney it is found only in collecting ducts where it is expressed by both principal and intercalated cells.[101] Hensin can reverse the functional phenotype of cultured intercalated cells. Thus, bicarbonate-secreting intercalated cells in culture could be converted to proton-secreting cells by growing them on a matrix that contains hensin.[102] In this model, the HCO_3^--secreting β-intercalated cells removed Cl^-/HCO_3^- exchangers from the apical membrane and began to insert Cl^-/HCO_3^- exchangers on the basolateral membrane. This complex process required protein synthesis. Cyclophilin, via its cis/trans prolyl isomerase activity, is required for hensin polymerization, galectin-3 is needed to aggregate the protein and activation of integrins for the development of the hensin fiber.[103,104] An intact cytoskeletal network of actin and tubulin, and degradation and internalization of the apically located peanut lectin–binding protein also are required in this process. More recently, it has been shown using collecting ducts incubated in vitro that antihensin antibodies applied to the basolateral bathing medium can inhibit the induction of acid secretion and bicarbonate reabsorption that normally occurs after incubation of the tubules in acidic medium.[100] Clearly, the change in phenotype between α- and β-intercalated cells involves more than a simple change in the polarity of membrane transporters. Rather it requires restructuring of the entire sorting machinery.

Wagner et al[15] stated, that the notion that α and β cells are phenotypic variants of the same cell type that can remodel rapidly requires further information. Intercalated cells in the medulla retain their α-cell phenotype under all experimental conditions. This could indicate that their α-cell phenotype is fixed irreversibly, or that in experimental conditions in vivo conversion to β cells, does not occur. In addition, in the cortex, the relative numbers of α versus β cells are constantly changing in response to variations in acid-base status. Thus, the model of one plastic intercalated cell phenotype would require that the hensin-induced terminal differentiation of β cells into α-intercalated cells be a reversible phenomenon so that the appropriate response to alkalosis could occur (ie, the production of more β-type intercalated cells by α-cell dedifferentiation).

The apical anion exchanger in β cells was not detectable using antibodies against AE-1. Pendrin has now been identified as at least one major player responsible for apical anion exchange in β-intercalated cells.[99] Furthermore, apical pen-

drin is relocated to the cytosol of β-intercalated cells in acid-loaded mouse kidney, consistent with previous reports of acid-induced apical anion exchanger internalization in rabbit collecting ducts.[100]

Metabolic Regulation of the Vacuolar H+-ATPase Activity

Metabolic Acidosis

The vacuolar H+-ATPase plays an important role in the adaptive response of the kidney to altered acid-base or electrolyte status.[105] In metabolic acidosis and in respiratory acidosis (hypercapnia), upregulation of H^+ secretion occurs in the cortical collecting duct (CCD), OMCD, and initial IMCD.[106,107] In experimental models, this acid/base disturbance, often induced by the addition of NH_4Cl to the drinking water, increases expression of vacuolar H+-ATPase subunits in the luminal membrane of α-type intercalated cells in the late distal tubule, the connecting segment, and the collecting duct. Either acidosis or high CO_2 leads to redistribution of H+-ATPases to the membrane involving microtubular trafficking from intracellular stores.[108] In isolated perfused rabbit proximal tubules and collecting ducts an increase in CO_2 leads to the exocytotic insertion of vacuolar H+-ATPases, thus increasing its activity in the plasma membrane. This translocation of vacuolar H+-ATPase into the membrane is paralleled by increased activity of NEM-, bafilomycin-, or concanamycin-sensitive ATPase activity and proton extrusion.[109]

Metabolic Alkalosis

Transport processes are regulated in a mirror-like manner to metabolic alkalosis. H^+ secretion through the H+-ATPase is downregulated along the collecting duct by internalization of proton pumps. This is characterized by shifts of vacuolar H+-ATPase immunoreactivity from the apical pole to subapical compartments in α-type intercalated cells and a more pronounced basolateral staining in β-type intercalated cells.[63] The activation of HCO_3^- secretion in β-type intercalated cells by metabolic alkalosis involves the apical pendrin protein (described in detail previously).[70] Metabolic alkalosis as a result of hypokalemia is caused in part by the shift of intracellular K^+ to the extracellular space, resulting in the uptake of protons. Hypokalemia can be caused by low K^+ in the diet or more acutely by the application of loop diuretics. In the proximal tubule, the Na^+/HCO_3^- cotransporter NBC-1 is stimulated.[110] Intercalated cells found in the distal tubule and cortical collecting duct show hypertrophy.[111] This is accompanied by a more pronounced apical location of the vacuolar H+-ATPase and its increased activity.[112] These transport mechanisms contribute to the development of metabolic alkalosis under these conditions. In contradistinction to metabolic alkalosis, acute and chronic hypocapnia (respiratory alkalosis) has no effect on NEM-sensitive ATPase activity along the nephron.[106]

Regulation of the Vacuolar H+ ATPase by the Renin-Angiotensin Aldosterone System

Stimulation of vacuolar H+-ATPase activity by both angiotensin II and aldosterone has been reported for various nephron segments and cell types. Angiotensin II is a potent vasoconstrictor and, in addition to its effects on Na^+-homeostasis and blood pressure regulation, plays a role in the regulation of acid-base balance. Intrarenal angiotensin II is regulated by several complex processes, involving formation of both systemically delivered and intrarenally formed substrate, as well as receptor-mediated internalization. Angiotensin II signals mainly through 2 receptor subtypes: AT_1 and AT_2.[113] Although both receptors are expressed in the kidney, AT_1 is the predominant subtype found in blood vessels and almost ubiquitously along the nephron.[114]

Angiotensin II is a potent activator of bicarbonate reabsorption and proton secretion along the nephron. However, different results regarding the effect of angiotensin II on bicarbonate reabsorption and proton secretion have been reported at the functional level, depending on the angiotensin II concentration and tubule segment studied.[115] The target transport mechanisms include the Na^+/HCO_3^- cotransporter and Na^+/H^+ exchanger (NHE-3) in the proximal tubule, and the vacuolar H+-ATPase in the proximal tubule, distal convoluted tubule, and intercalated cells of the cortical collecting tubule. Luminal perfusion of angiotensin II stimulates Na^+/H^+ exchange in both early and late distal tubule and the vacuolar H+-ATPase in the late distal tubule.[116] In all instances, stimulation occurred through AT_1 receptors. Consistent with these observations, Wagner et al[117] recently found that angiotensin II (10^{-8} M) activates H+-ATPase in intercalated cells via the AT_1 receptor by stimulating the trafficking of the H+-ATPase into the membrane. In addition to acute effects of angiotensin II on vacuolar H+-ATPase activity, effects of chronic AT_1 blockade also have been reported. In in vivo microperfused distal tubules from remaining nephrons in two-thirds nephrectomized rats, chronic AT_1 blockade reduced bicarbonate reabsorption in close association with the reduction of synthesis and insertion of apical H+-ATPase.[118] AT_1 receptors couple intracellularly mainly to phospholipase C, Ca^{2+}, and the protein kinase C (PKC) pathway.[119] The signaling pathway involved in activation of vacuolar H+-ATPase activity by angiotensin II, however, needs to be elucidated. Moreover, in contrast to the studies of H+-ATPase activation by angiotensin II, a study using permeabilized CCD segments showed a specific dose-dependent inhibitory effect of angiotensin II on the vacuolar H+-ATPase.[120]

Regulation of H+-ATPase activity includes mechanisms such as trafficking from submembraneous pools, regulation by cytosolic activator and inhibitor proteins, or gene expression and protein expression.[121] Colchicine, an agent that disrupts the microtubular network, prevents the angiotensin II–induced stimulation of vacuolar H+-ATPase in intercalated cells of isolated mouse cortical collecting ducts.[117] This

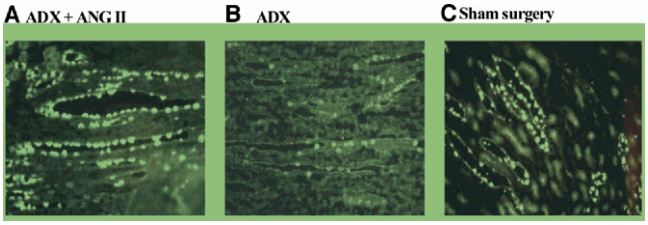

Figure 3 Immunofluorescence staining using antibodies against the 56-kDa H^+-ATPase B1 subunit in sections of rat kidney medulla. Magnification 400x. (A) In collecting ducts from an ADX rat administered angiotensin II (ANG II), cells with intense apical immunoreactivity corresponding to α-intercalated cells were frequently seen. (B) In collecting ducts from ADX rat, cells had a marked attenuation of staining. (C) Cells from medullary collecting ducts from sham surgery rat showed intense staining on the apical membrane similar to that seen in tubules from ANG IIδinfused rat, although staining does not appear to be as widespread within the tubules. (Color version of figure is available online.)

suggests that vacuolar H^+-ATPases, some of its subunits, or other regulatory proteins must traffic to the membrane. Studies to date indicate that H^+-ATPase is regulated to only a minor extent by transcriptional and translational mechanisms, but further work in this area is needed.

Little is known about the in vivo regulation of the various subunits of the H^+-ATPase. Recently, we examined the expression of H^+-ATPase subunits B1 and a4 in an animal model of selective aldosterone deficiency (adrenalectomized [ADX] rats maintained on glucocorticoid replacement). The effect of exogenous angiotensin II on the in vivo expression of the H^+-ATPase B1 subunit also was studied.[122] Our data showed an increase in the relative protein abundance of B1 subunit isoform of the H^+-ATPase after chronic infusion of angiotensin II in the renal medulla of ADX rats. This increase in H^+-ATPase expression by angiotensin II was limited to intercalated cells in the medullary collecting ducts (Fig 3). The expression of the a4 subunit, by contrast, was not altered by either ADX or exogenous angiotensin II.[122]

Aldosterone has both indirect and direct effects on proton secretion. In the connecting segment and cortical collecting duct, aldosterone stimulates the electrogenic vacuolar H^+-ATPase through increased reabsorption of Na^+ in principal cells. This renders the tubule lumen more negative and thus facilitates proton diffusion into the lumen.[123] In the OMCD, aldosterone also stimulates proton secretion, but the effect persists in the absence of Na^+ reabsorption, suggesting direct activation of vacuolar H^+-ATPase activity.[124] Although aldosterone stimulates H^+-ATPase–dependent bicarbonate reabsorption in all collecting duct segments, different sensitivities of vacuolar H^+-ATPase enzymatic activity in distinct collecting duct segments have been described.[125] Aldosterone, similar to other steroid hormones, works mainly through increasing gene transcription. Thus, activation of the vacuolar H^+-ATPase likely is owing to increased transcription of one or more subunits. The regulation of other ion-transport processes by aldosterone involves genomic changes in transcription and synthesis of transport proteins, such as subunits of the epithelial Na^+ channel ENaC or the Na^+/K^+-ATPase.[126]

The findings in our study using ADX rats suggest that the removal of aldosterone results in a decrease in the relative abundance and expression of B1 H^+-ATPase in the medullary, but not the cortical, collecting duct.[122] We also have found that aldosterone increases a4 H^+-ATPase protein synthesis in a cell line of the collecting duct.[127,128] Immunofluorescence with confocal microscopy further revealed that aldosterone promotes trafficking of the a4 subunit toward plasma membrane in this cell line.[128] This dual effect of aldosterone (ie, to promote trafficking of the a4 subunit to the cell membrane and to increase a4 protein synthesis) likely is required to facilitate assembly of the H^+-ATPase proton pump and thus increased hydrogen ion secretion.

Importantly, others reported that in microdissected cortical and medullary collecting duct segments from ADX rats, a pharmacologic dose of aldosterone increased enzyme H^+-ATPase activity irrespective of potassium levels.[129] Another recent study showed nongenomic targeting of the a4 subunit of vacuolar H^+-ATPase to the apical membrane by aldosterone.[130] This nongenomic stimulatory effect of aldosterone required protein kinase C and was associated with a rapid transient increase of intracellular Ca^{2+}.

A level of regulation involving changes in protein levels, however, may be important as well. Our results in medullary collecting tubule segments suggest that both aldosterone and angiotensin II provide an additional level of regulation by altering in vivo subunit protein abundances.[122] This may occur either by changing protein synthesis or by altering protein degradation. Taken together, these studies suggest that angiotensin II and aldosterone affect vacuolar H^+-ATPase activity through different mechanisms that may be specific to both the segment and the cell type.

Vacuolar H^+-ATPase in Disease

RTA

In children, dRTA almost always is observed as a primary entity. Prominent clinical features include impairment of growth, polyuria, hypercalciuria, nephrocalcinosis, lithiasis, and K^+ depletion. Progression of nephrocalcinosis may lead to the development of chronic renal failure. If detected early in life, therapeutic correction of the acidosis by continuous alkali administration may induce resumption of normal growth, arrest of nephrocalcinosis, and preservation of renal function.[131] Primary dRTA may be observed sporadically or with autosomal-dominant or autosomal-recessive transmission.[132] Patients with recessive dRTA typically are affected severely, presenting either with acute illness or with growth failure in the early years of life. Two types of recessive dRTA have been differentiated by the presence or absence of sensorineural hearing loss, but appear otherwise clinically similar. Karet et al[133] recently showed that most patients with dRTA and nerve deafness present mutations in the *ATP6V1B1* gene encoding the B1-subunit of H^+-ATPase. A genome-wide linkage screen of a set of 31 kindreds with sporadic or

autosomal-recessive dRTA without sensorineural deafness resulted in the identification of *ATP6V1B1* as the first gene associated with this disorder.[26] This gene is encoded in 14 exons, yielding the B1 subunit of 513 amino acids. The B1 subunit is expressed by interdental cells and endolymphatic sac epithelia, accounting for the associated hearing impairment.

Sporadic or autosomal-recessive dRTA without sensorineural deafness is the most frequently encountered primary form. Karet et al[134] also showed that this form may be caused by mutations in the gene *ATP6V0A4* encoding the 116-kd subunit of vacuolar H^+-ATPase. Characterization of the *ATP6V0A4* genomic locus revealed that it comprises 23 exons; 20 of these exons encode the 840 amino acid a4-subunit of the vacuolar H^+-ATPase.[26] However, several patients with *ATP6V0A4* mutations have developed hearing loss, usually in young adulthood.[135] It is now known that *ATP6V0A4* also is expressed within the human inner ear. These findings provide further evidence for genetic heterogeneity in renal distal RTA, and extend the spectrum of disease causing mutations in *ATP6V1B1* and *ATP6V0A4*.

In contrast, autosomal-dominant distal RTA is typically a milder disease. Autosomal-dominant dRTA was found to be associated in several kindred with mutations in the gene encoding the Cl^--HCO_3^- exchanger AE1 or band 3 protein,[136] affecting the ability of α-intercalated cells to transport protons into the lumen of the collecting duct, and thus reducing the net acid secretion.

The recent generation of mice deficient in *Atp6v1b1*, the murine vacuolar H^+-ATPase B1-subunit homolog (*Atp6v1b1-/-*)[137] may provide a useful tool for dissecting mechanisms of distal urinary acidification.[138] Although *Atp6v1b1-/-* mice fed a standard laboratory diet produce urine that is significantly more alkaline than that of wild-type littermates, *Atpv16b1-/-* mice are not acidotic. After oral acid challenge, however, *Atp6v1b1-/-* mice develop a metabolic acidosis that is more severe than that seen in wild-type controls while still maintaining inappropriately alkaline urine.[138] *Atp6v1b1-/-* mice may prove a useful tool in future studies, elucidating the mechanisms of mammalian vacuolar H^+-ATPase assembly and/or trafficking.

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