Perspectives

Proton Transport and Cell Function

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The past five years have witnessed an explosion of information on the many and varied roles of H^+ transport in cell function. H^+ transport is involved in three broad areas of cell function: (*a*) maintenance and alteration of intracellular pH for initiation of specific cellular events, (*b*) generation of pH gradients in localized regions of the cell, including gradients involved in energy transduction, and (*c*) transepithelial ion transport. These processes each involve one or more of several H^+ translocating mechanisms. The first section of this review will discuss these H^+ translocating mechanisms and the second part will deal with the cellular functions controlled by H^+ transport.

Mechanisms of H^+/OH^- Transport

Primary active H^+ transport

Primary active H^+ transport mechanisms consume energy directly for the translocation of H^+. This energy can be derived from redox reactions, light, or ATP. Since most of the primary active H^+ translocating processes are electrogenic, their action will produce only a membrane potential difference unless there is some provision in the membrane for counter ion movement. This is usually accomplished by a simple conductive pathway through which Cl^- can move in the same direction as, or cations (e.g., K^+ or Na^+) can move in the opposite direction to H^+ transport. As will be seen below, some of the active H^+ transport mechanisms do generate primarily a membrane potential difference, while others move significant amounts of acid across membranes. To date, there are no known primary active OH^- or HCO_3^- transport mechanisms.

H^+ translocation by the electron transport chain. In the mitochondrion, three electron transport complexes (reduced nicotinamide-adenine dinucleotide dehydrogenase, the b-c_1 complex, and cytochrome oxidase) translocate H^+ into the cytoplasm to generate a membrane potential of 150–180 mV and a pH gradient of 0.5–1.0 unit. In chloroplasts, a single electron transport complex (b_6-f) pumps H^+ into the thylakoid space to produce a pH gradient of 3.0–3.5 units with only a minimal potential difference. Although there is considerable data on the structure and membrane location of these complexes, only little is known about the precise coupling of H^+ translocation to redox reactions by the electron transport complexes (1).

Photochemical H^+ translocation. Bacteriorhodopsin, a 26,000-D retinal-containing protein which accomplishes light-driven H^+ translocation in *Halobacterium halobium,* is perhaps the best studied H^+ translocating molecule (2). Because of its crystalline arrangement in vivo, it has provided a unique opportunity to study the three-dimensional structure of a membrane transport protein and correlate it with its amino acid sequence. This protein translocates H^+ by cycling through a series of six photochemical reactions, one of which takes up and another of which releases H^+ (2).

H^+ translocating ATPases. H^+ translocating ATPases are used by cells either to generate large pH gradients (e.g., >6 pH units across the gastric epithelium) or to transduce the energy in pH gradients to ATP. Properties of the various types of H^+ ATPase are summarized in Table I. There are currently two types which have been extensively studied and several others which are newly discovered.

The first type is called F_0F_1 ATPase, named for the coupling factors F_0 and F_1 found in early research on oxidative phosphorylation (3). This ATPase is found in bacterial cells, mitochondria, and chloroplasts. The F_0 subunit is an intramembranous proton "well" or H^+ conductance pathway, which consists of three integral membrane proteins. The F_1 subunit consists of five peripheral membrane proteins which together confer ATPase activity to the F_0F_1 complex. This type of H^+ ATPase typically is inhibited by <1 μM *NN'*-dicyclohexylcarbodiimide (DCCD).[1] The F_0F_1 H^+ ATPases in mitochondria and in a few bacteria are also inhibited by oligomycin.

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1. *Abbreviations used in this paper:* DCCD, *NN'*-dicyclohexylcarbodiimide; pH_i, intracellular pH.

Table I. Types of H^+ ATPase

Type	Inhibitors			References
	Oligomycin	Vanadate	DCCD I_{50}	
			μM	
F_0F_1	+	−	0.25	3
Phosphorylated				
Gastric	−	+	>10	4
Neurospora plasma membrane	−	+	8	5
Turtle bladder	−	−	40	66
Chromaffin granule	−	−	150	47, 48
Lysosome	−	−	100	63
Clathrin-coated	−	−	100	61, 62
Neurospora vacuole	−	−	0.65	6

The second type of H^+ translocating ATPase is a variant of the Na^+/K^+ ATPase (4, 5). It consists of two to three integral membrane subunits, one of which is an $\simeq$100,000-D protein similar to the α-subunit of the Na^+/K^+ ATPase. Like the Na^+/K^+ ATPase, these enzymes undergo a reaction cycle involving a phosphorylated intermediate and are inhibited by vanadate. Examples are the gastric H^+/K^+ ATPase (4) and the plasma membrane H^+ pump of *Neurospora crassa* (5).

With the discovery of H^+ ATPases in numerous intracellular organelles, new types of H^+ ATPase are emerging. Many of these ATPases are inhibited only by >10 μM DCCD and are resistant to vanadate and oligomycin. For example, *Neurospora* (6) and *Saccharomyces* (7) both have vanadate-inhibitable H^+ ATPases on their plasma membranes and F_0F_1 ATPases in their mitochondria, but the intracellular storage vacuole contains a vanadate- and oligomycin-resistant but DCCD-sensitive H^+ ATPase. Thus, as many as three different kinds of H^+ ATPase have been found in a single organism.

Secondary active H^+ transport

These transport mechanisms do not consume energy directly, but rather use preformed ion gradients (typically Na^+, K^+, or Cl^-) to transport H^+, OH^-, or HCO_3^-. These transporters move H^+, OH^-, or HCO_3^- electroneutrally in the opposite direction to the movement of Na^+, K^+, or Cl^- and have been termed antiporters. Antiporters can generate pH gradients of only one or two units in cells because the ion gradients that drive them are only one or two log units in magnitude.

Cation/H^+ antiporters. Nigericin and monensin, cation/H^+ antiporters which are ionophores produced by *Streptomyces* species have been purified and their structure is well established (8). Little is known about the structure of Na^+/H^+ or K^+/H^+ antiporters from higher organisms, but two lines of evidence suggest that they are much more complex. First, simple ionophores, like monensin, rapidly distribute themselves in all the membranes of a cell, making them highly toxic (9). In contrast, the Na^+/H^+ antiporter of the mammalian renal proximal tubule is localized to the apical plasma membrane (10), and the K^+/H^+ antiporter appears to be localized to mitochondria (11). Thus, antiporters in higher organisms must have recognition mechanisms for specific membranes. Second, there is now evidence that mammalian cell Na^+/H^+ antiporters have several cation binding sites which may differ on the two sides of the membrane (12–15). Na^+/H^+ antiporters from higher organisms are, thus far, all inhibited by 10^{-4}–10^{-3} M amiloride.

Anion/OH^- antiporters. The best studied of this class of transporters is the erythrocyte Band 3 protein which is responsible for Cl^-/HCO_3^- exchange. This 97-kD molecule has been purified and its tertiary structure has been partially solved (16). Anion/OH^- antiporters have also been identified in epithelia (17, 18), but little is known about the structure of these molecules. Transporters of this class all appear to be inhibited by the stilbene disulfonates.

Passive H^+/OH^- conductances

All membranes are permeable to H^+/OH^- to a certain extent, and this property will tend to collapse gradients formed by H^+ translocating mechanisms. The true H^+/OH^- permeability of artificial and biological membranes is controversial, with published measurements of, on one hand, 10^{-9} cm/s (19, 20) or, on the other hand, 10^{-4} cm/s (21) for pure phospholipid vesicles and from 10^{-4} to 10^{-3} cm/s or higher for biological membranes (22, 23). Progress in this area has been slow because of the limited number of methods available for making these measurements (electron paramagnetic resonance and fluorescent probes being the main ones) and because vanishingly small quantities of certain biological contaminants and synthetic compounds appear to greatly alter the native H^+ permeability of membranes (20). No specific passive H^+ conductance channels have been isolated from biological membranes. Postulated mechanisms explaining the high H^+/OH^- permeability in biological membranes (compared with that of other small cations) include linear water aggregates (24) and/or lipophilic weak acids (25) in membranes.

Cell Functions Controlled by H^+ Transport

Maintenance of intracellular pH (pH_i)

Most cells maintain pH_i at 6.9–7.5 (26). This is well above the electrochemical equilibrium value of 6.0–6.5 that is predicted by the Nernst equation from the interior negative potential difference of 60–90 mV. Because of passive H^+/OH^- conductances, pH_i would rapidly fall to equilibrium values were it not for specific H^+ transport mechanisms which extrude protons to raise pH_i. In sea urchin eggs, crayfish neurons, *Necturus* proximal tubule, *Escherichia coli,* and mouse soleus muscle, the ionic mechanism appears to be Na^+/H^+ exchange (26). In snail neu-

rons, squid giant axons, and barnacle muscle, it appears to be Na^+ and HCO_3^- exchange for H^+ and Cl^- (26). Since the Na^+ gradient ($Na_{outside}^+$:Na_{inside}^+) across most cell membranes is $\simeq$10:1, these transporters could theoretically raise the cell pH to one unit above the medium pH. The measured pH_i of 6.9–7.5, therefore, represents a steady state balance between passive H^+ entry and secondary active exit. By altering either of these processes, the cell can change pH_i toward one of the two extremes.

In cells that contain H^+ ATPases on the plasma membrane, it would seem likely that these ATPases also participate in pH_i regulation. Cell types which have H^+ translocating ATPases on the plasma membrane include all bacteria (27), unicellular eukaryotes such as *Neurospora* (5), and certain proton-transporting epithelial cells, such as the gastric oxyntic cell (4) and the turtle bladder, mitochondria-rich cell (28). As an example, the bacterial F_0F_1 ATPase (running in reverse) becomes responsible for maintaining pH_i above electrochemical equilibrium when electron transport (or photochemical H^+ ejection in *Halobacterium halobium*) is inhibited (29). Under normal respiratory conditions, but only in alkaline medium, the Na^+/H^+ antiporter becomes involved in pH_i regulation (30). Thus, in bacteria, at least three mechanisms (electron transport, F_0F_1 ATPase, and Na^+/H^+ antiporter) are involved in pH_i regulation, depending upon the growth conditions.

pH_i and transport. The maintenance of pH_i above electrochemical equilibrium in bacteria and unicellular eukaryotes appears to be responsible for multiple transport functions. Small molecules such as glucose, lactose, and amino acids are carried into the cell by cotransport with H^+, and thus derive their energy from the H^+ electrochemical gradient (31). Other nutrients, such as glutamate and melibiose, are cotransported with Na^+. Since bacterial cells do not have Na^+/K^+ ATPases, the low intracellular Na^+ is maintained by a Na^+/H^+ antiporter, which pumps Na^+ out of the cell by utilizing the cell's H^+ gradient (32).

pH_i and insulin action. There is growing evidence that the effect of insulin to increase the rate of glycolysis in muscle is mediated by changes in pH_i, which in turn is regulated by the Na^+/H^+ antiporter (33). It has been shown in frog sartorius muscle that insulin stimulates an amiloride-sensitive increase in both intracellular Na^+ and pH. This effect is converted to acidification by removal of extracellular Na^+, which strongly implicates the role of a reversible Na^+/H^+ exchanger in the process. Furthermore, it has now been shown that the effect of insulin on the rate of glycolysis is dependent upon extracellular Na^+ and is inhibited by amiloride (33). Phosphofructokinase, an extremely pH-sensitive enzyme and the rate-limiting enzyme of glycolysis, appears to be the locus where insulin-induced pH changes exert their effect.

pH_i and cell division. Numerous cell types undergo a rapid rise in pH_i and an uptake of Na^+ after stimulation by mitogens and growth factors. Fertilization causes a similar phenomenon in the sea urchin egg (34). In foreskin fibroblasts, this alkalinization is converted to acidification by Na^+ removal (35). In 3T3 cells (36, 37) and human foreskin fibroblasts (35, 38), this process is blocked by amiloride, suggesting that it is mediated by a Na^+/H^+ antiporter. In fibroblasts (36) and neuroblastoma cells (39), amiloride also blocks the increase in DNA synthesis caused by growth factors. When taken together, these data provide strong evidence that Na^+/H^+ exchange is one important link between growth factor or mitogen binding and cell division.

pH_i and cell volume regulation. Most cells have mechanisms on their plasma membranes for the regulation of cell volume. In response to volume changes which arise from alterations in extracellular osmolality, cells move NaCl or KCl across their membranes to return cell volume towards normal. These ion movements appear to be coupled to H^+ or OH^- transport, and are probably triggered by changes in cell pH. In the *Amphiuma* erythrocyte (40), volume regulatory increase involves NaCl uptake via coupled Na^+/H^+ and Cl^-/HCO_3^- exchangers, while volume regulatory decrease involves KCl loss via coupled K^+/H^+ and Cl^-/HCO_3^- exchangers. In the *Necturus* gallbladder (41), volume regulatory increase also occurs via coupled Na^+/H^+ and Cl^-/HCO_3^- exchangers.

Generation of H^+ gradients in localized regions of the cell

Energy transduction in mitochondria, chloroplasts, and bacteria. Energy transduction is the best studied example of a localized pH gradient. The Mitchell hypothesis, which was first proposed in 1961 (42), suggested that the link between the mitochondrial electron transport chain and the phosphorylative generation of ATP was a H^+ gradient formed across the mitochondrial inner membrane. H^+, which is ejected from the mitochondrial matrix by the electron transport chain, was hypothesized to return to the mitochondrial interior through a specialized ATP-forming molecule, the F_0F_1 ATPase. Considerable data have been amassed to support this now accepted hypothesis (43). A similar process also occurs in chloroplasts.

The large electrical gradient across the mitochondrial inner membrane would result in conductive cation entry and mitochondrial swelling unless a mechanism existed to remove cations. Mitchell (42) proposed that cation/H^+ antiporters could perform this function. Garlid (11) has shown that electroneutral K^+/H^+ exchange does take place in isolated mitochondria. This process appears to be tightly regulated by the mitochondrial volume itself, since large variations in K^+/H^+ exchange activity can be observed (11) under conditions where the K^+ or H^+ gradients change only slightly.

In *Halobacterium halobium,* patches of membrane called purple membrane appear during O_2 starvation. These membranes consist of crystalline arrays of bacteriorhodopsin, a protein which contains the visual pigment chromophore, retinal. On exposure to light, bacteriorhodopsin ejects H^+ outwardly, taking the place of the electron transport chain. In a classic experiment, Racker and Stoeckenius (44) reconstituted bacteriorhodopsin and mitochondrial F_0F_1 ATPase into a single artificial membrane and produced light-sensitive ATP generation.

H^+ transport in storage granules. The adrenal chromaffin granule is responsible for the uptake and storage of catechol-

amines before their secretion. The uptake process is driven by a proton gradient, which is in turn generated by a H^+ ATPase (45). While it was originally believed that this enzyme resembled mitochondrial F_0F_1 ATPase (46), it has more recently been shown (47, 48) that it is not inhibited by oligomycin and that its subunits correspond neither in molecular weight nor in immunoreactivity to mitochondrial subunits.

An acidic intravesicular pH has also been found in secretory granules of other cells, including platelets (49), mast cells (50), pancreatic cells (51), and pituitary cells (52). Less is known about the H^+ transport mechanisms in these cells although presumably they also involve H^+ pumps.

H^+ transport in endocytosis and intracellular digestion. Clathrin-coated vesicles are responsible for receptor-mediated endocytosis, the process whereby hormones, viruses, certain toxins, lipoproteins, asialoglycoproteins, and their respective receptors are taken up by a variety of cells (53). Recently, these vesicles have been shown to have acidic interiors (54), and agents which raise the intravesicular pH, such as protonophores or weak bases (55), appear to inhibit receptor-mediated endocytosis. Several possible functions for an interior acid environment in these vesicles have been proposed. Receptors and ligands become dissociated at low pH, enabling the membrane receptors to be recycled and the ligand to be passed along either to lysosomes for degradation or to other parts of the cell. A similar mechanism may exist for nutrient substances, such as iron, which is brought into the cell by receptor-mediated endocytosis. At low pH, iron dissociates from its plasma-binding protein, transferrin, but transferrin remains bound to its receptor on the membrane (56). Thus, intravesicular acidity allows iron to dissociate but leaves transferrin bound to the receptor, so that both transferrin and its membrane receptor can be recycled to the surface, where transferrin would be released. In addition, intravesicular acidity is required for the transmembrane transport and intracellular release of viral particles (57) and certain proteins, such as diphtheria toxin (58).

Some of the hormones, viral particles, and proteins taken up by endocytosis are degraded in lysosomes by an energy-requiring process (59, 60) with enzymes having pH optima between 4.0 and 5.0. This digestive process is dependent upon acidification of the intralysosomal space by a membrane-bound H^+ ATPase. The H^+ ATPases from lysosomes and clathrin-coated vesicles appear to be similar and are inhibited by DCCD but not by oligomycin or vanadate (61–63).

Transepithelial ion transport

Epithelial H^+ transport via H^+ ATPases. Numerous epithelia secrete acid. Of these, many (e.g., the gastric gland, the turtle urinary bladder, and the renal distal tubule) do so by means of a H^+ translocating ATPase located at the apical pole of the epithelial cell. Regulation of these ATPases has been a focus of particular interest. For example, H^+ secretion in the stomach is regulated by such agents as histamine, acetylcholine, and pentagastrin. The precise mechanism of increased H^+ secretion is not known, but it appears that there is an increase in the number of H^+/K^+ ATPases in the apical plasma membrane brought about by fusion of intracellular vesicles with this membrane (64).

The mitochondria-rich cell of the turtle bladder also appears to increase its H^+ secretory capability by fusion of membrane vesicles with the plasma membrane (65). This H^+ ATPase has properties (66) which make it more similar to the lysosomal or clathrin-coated vesicle pumps (DCCD-inhibitable, oligomycin- and vanadate-resistant) than to the gastric pump.

Epithelial H^+ transport via Na^+/H^+ exchange. Experiments in the intact mammalian renal proximal tubule for many years suggested that H^+ secretion was the result of Na^+/H^+ exchange at the luminal membrane (67). An electroneutral Na^+/H^+ antiporter was found in isolated brush border membranes (68), and recently, the antiporter was shown (10) to be absent in basolateral membranes, making vectorial transport via this mechanism possible. However, several groups have found that luminal Na^+ removal does not eliminate all acidification (67). At least one group also finds a H^+ translocating ATPase in brush border membranes (69). This ATPase activity may be due to contamination from intracellular vesicles, many of which contain H^+ ATPases. Alternatively, the clathrin-coated vesicle ATPase may actually inhabit the plasma membrane, since regions of the brush border appear to be coated with clathrin (70). The weight of evidence still favors Na^+/H^+ exchange as the ion transport mechanism responsible for acidification in the proximal tubule, but the evidence for alternative H^+ transport mechanisms cannot entirely be discounted.

Epithelial NaCl transport. Several epithelia which do not generate a net H^+ flux still utilize H^+ transport mechanisms in the transport of Na^+ and Cl^-. For example, the rat small intestine appears to move Na^+ and Cl^- across the brush border by means of coupled Na^+/H^+ and Cl^-/OH^- antiporters rather than by NaCl cotransport (18). In the *Necturus* gallbladder, both NaCl cotransport and the antiporters are present (71), but it is not clear which is responsible for net NaCl movement. In the mammalian renal proximal tubule, NaCl is the major salt transported after the HCO_3^- in tubular fluid has been maximally reduced by the action of the Na^+/H^+ antiporter. Coupled Na^+/H^+ and Cl^-/HCO_3^- exchangers have been proposed (17) to effect this NaCl transport. At present, there is evidence both for (17) and against (72) this hypothesis.

Conclusion and Future Goals

The biological roles for H^+ transport go well beyond the maintenance of pH_i within certain limits for proper functioning of enzymes and other chemical reactions in the cell. pH_i is involved in the transport of small molecules in bacteria, the process of fertilization, cell division, and cell volume regulation. Localized regions of acidity are responsible for energy transduction and appear critical for the endocytic and digestive functions of the cell. Lastly, transcellular H^+ movement plays an important transport role in many epithelial tissues. One challenge for the future will be understanding how the varied H^+ transport proteins

are synthesized, regulated, and degraded. It will also be important to establish the mechanisms whereby H^+ transporters are compartmentalized (i.e., inserted into their specific membrane loci) so as to enable the cell to utilize a single mechanism (H^+ transport) for such a large array of different processes without the disastrous complication of significant "cross-talk" between the systems. The lack of precise pH_i measurements in intact cells has been a major obstacle to progress in this area. Advances in the use of intracellular fluorescent dyes, ^{31}P nuclear magnetic resonance, and pH_i electrodes may permit further understanding of these control mechanisms in the intact cell. In addition, the isolation of molecules responsible for H^+ transport will potentially allow precise determination of the factors which regulate their activity and their locations within the cell.

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