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Review

# Common themes and problems of bioenergetics and voltage-gated proton channels

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**Abstract**

The existence of a proton-selective pathway through a protein is a common feature of voltage-gated proton channels and a number of molecules that play pivotal roles in bioenergetics. Although the functions and structures of these molecules are quite diverse, the proton conducting pathways share a number of fundamental properties. Conceptual parallels include the translocation by hydrogen-bonded chain mechanisms, problems of supply and demand, equivalence of chemical and electrical proton gradients, proton wells, alternating access sites, p$K_a$ changes induced by protein conformational change, and heavy metal participation in proton transfer processes. An archetypal mechanism involves input and output proton pathways (hydrogen-bonded chains) joined by a regulatory site that switches the accessibility of the bound proton from one 'channel' to the other, by means of a p$K_a$ change, molecular movement, or both. Although little is known about the structure of voltage-gated proton channels, they appear to share many of these features. Evidently, nature has devised a limited number of mechanisms to accomplish various design strategies, and these fundamental mechanisms are repeated with minor variation in many superficially disparate molecules. 

*Keywords:* Proton; Proton channel; Proton transport; pH regulation; Hydrogen ion; Proton permeability

## 1. Introduction

It may not be obvious that there is much in common between molecules important in bioenergetics and voltage-gated proton-selective ion channels in cell membranes. Channels by definition are passive entities that tend to dissipate electrochemical gradients, whereas bioenergetics is concerned with systems that generate and transfer biological energy. The goal of this review is to discuss what we know about the properties of voltage-gated proton-selective channels in the context of exploring the areas in which proton channels and bioenergetics overlap. A fundamental common feature is that many important bioenergetic molecules have pathways or 'channels' that translocate protons from one place to another, in some cases across cell membranes. The *intent* of the proton transfer may be quite different in bacteriorhodopsin than in a proton channel in a human neutrophil membrane, but in both cases, protons are moved selectively through a protein. The hope is that the voltage-gated proton channel will provide a relatively simple model of proton translocation, which is amenable to certain kinds of measurements that would be more difficult to achieve in

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a pathway that, for example, does not span the membrane, or whose function requires the presence of an entourage of subunits that obfuscate the workings of the proton conduction pathway. Our ability to interpret some of the properties of voltage-gated proton channels has certainly benefited from the existence of a large body of knowledge about many other molecules that share the property of conducting protons. What at this time appears to be a fundamental difference is that voltage-gated $H^+$ channels are more like ion channels than pumps or carriers [1]. When a voltage-gated $H^+$ channel is open it presumably conducts $H^+$ current without any further conformational change, in contrast with carriers or pumps that require a conformational change during each transport cycle.

## 2. What are voltage-gated proton channels?

Ion channels are relatively simple mechanisms for the transport of ions across cell membranes. Unlike pumps, they do not require an energy source like ATP, but instead they allow passive flow of an ion down its electrochemical gradient. With no applied voltage, ionic current flows through open ion channels from high to low concentration. Carriers differ from ion channels in two respects. Many carriers, symporters or antiporters, transport two different molecular species across cell membranes, either in the same direction or in opposite directions, with fixed stoichiometry. For example, the $Na^+/H^+$-antiport is electroneutral, exchanging one $Na^+$ for one $H^+$ with each cycle. The $Na^+/Ca^{2+}$ exchanger is electrogenic, exchanging three $Na^+$ for one $Ca^{2+}$. Ion channels simply allow ions to permeate with relatively little formal interaction between the ions. Another major difference is that carriers undergo a conformational change with each transport cycle, which switches the accessibility of the site from one side of the membrane to the other. An open ion channel is considered to be available to any ion entering from either side of the membrane. No explicit conformational change of the ion channel protein is required for permeation to occur.

Voltage-gated proton channels were discovered in 1982 in snail neurons, by Thomas and Meech [2]. They simultaneously recorded membrane current and intracellular pH, $pH_i$, in snail neurons under voltage-clamp conditions. Upon injection of HCl there were $pH_i$ changes consistent with conductive $H^+$ efflux, which were independent of $Na^+$ or $HCO_3^-$ and occurred only at depolarized voltages. They concluded that an ion channel permeable to $H^+$ was opened by depolarization.

### 2.1. Voltage-gating

The voltage-clamp technique has proven invaluable in elucidating the properties of ion channels [3], because many important ion channels are voltage-gated. 'Gating' in ion channels refers to the switching between two distinct conformational states of the channel molecule, open and closed. An open channel conducts ionic current at a constant rate, typically $10^6$–$10^7$ ions/s, until the channel closes, in which state it does not conduct measurable current. 'Voltage-gating' simply means that the likelihood that a channel opens or closes depends on the transmembrane potential. Strongly voltage-dependent ion channels have an open probability, $P_{open}$, which varies from near 0 to near 1.0 within a 10–20 mV range. By controlling the membrane potential using voltage-clamp technique, the response of the channel to this critically important parameter is readily determined. Well-established voltage-clamp protocols exist that allow measurement of the rate of activation (channel opening) and deactivation (channel closing) at a given voltage [4]. Some ion channels inactivate during long voltage pulses, meaning that they open, but then inactivate or close into a state in which they are refractory to opening again with the same stimulus. Voltage-gated proton channels do not inactivate, but stay open as long as an appropriate voltage is sustained. When $H^+$ currents decay with time, this indicates that the unidirectional $H^+$ efflux was sufficient to increase $pH_i$ and thus decrease the driving force [5].

### 2.2. Ion selectivity

Many ion channels are selective, which means that they allow only certain ions to pass through. The relevant concentration gradient is that of the permeant species of ion. With applied voltage, current will flow in either direction through ion channels, gener-

ally in a simple, nearly Ohmic manner, reversing direction at the reversal potential, $V_{rev}$. The selectivity of an ion channel is determined by measuring $V_{rev}$, which will be near the Nernst potential, $E_X$, of the permeable ionic species X [6]:

$$E_X = \frac{RT}{zF}\log\frac{[X]_o}{[X]_i} \quad (1)$$

where $R$ is the gas constant, $T$ is the absolute temperature, F is Faraday's constant, $z$ is the charge of the ion, and $[X]_o$ and $[X]_i$ indicate the ionic concentrations outside and inside the cell, respectively. If more than one permeable ion species is present, then $V_{rev}$ will fall between the Nernst potentials of the two species, according to their relative permeability. A convenient method for estimating relative permeability is to measure $V_{rev}$ first with one ionic species in the bath and then after complete replacement with another ion. The relative permeability can then be estimated from the change in $V_{rev}$:

$$\Delta V_{rev} = \frac{RT}{zF}\ln\frac{P_X[X]_o}{P_Y[Y]_i} \quad (2)$$

where $[X]$ and $[Y]$ are the concentrations of X and Y respectively, and $P_X/P_Y$ is the relative permeability of X compared with Y. From measurements of $V_{rev}$ in a variety of ionic solutions, it has been determined that the voltage-gated $H^+$ channel is highly selective for $H^+$ over other ions. In particular, there is no measurable change in $V_{rev}$ when the bathing cation is changed among $Na^+$, $K^+$, $TMA^+$, $TEA^+$, $NMG^+$, $Li^+$, and $Cs^+$ or when the anion is changed among $Cl^-$, aspartate$^-$, $MeSO_3^-$, glutamate$^-$, or isethionate$^-$ [7–12]. The only exception occurs when substrates for the $Na^+/H^+$-antiporter are present and antiport alters local pH; this effect can be inhibited by blockers of the antiporter [7,11]. Because changing the concentration or species of any other cation or anion present has no measurable effect on $V_{rev}$, no other ion has detectable permeability thorough these channels. As defined by Eq. 2, the relative permeability of $H^+$ compared with all other ions has been estimated to be $>10^6$–$10^8$ [5,9–11,13,14].

### 2.3. Four types of $H^+$ channels

Every year voltage-gated $H^+$ channels are reported for the first time in several new cell types. Based on descriptions in the existing literature it is possible to distinguish at least four varieties of $H^+$ channels [15]. Named for the cells in which they are expressed, these are '***n***' in neurons, '***o***' in oocytes, '***e***' in epithelial cells, and '***p***' in phagocytes. The $H^+$ channel seems to be ubiquitous in phagocytes and other white blood cells, being present in neutrophils, eosinophils, macrophages, basophils, osteoclasts, microglia, and mast cells [10–12,16–21] as well as numerous cell lines related to or derived from these cells. The main properties that distinguish these channels are gating kinetics. Type ***n*** channels open and close rapidly, within milliseconds, whereas type ***p*** channels are quite slow, and may require several minutes after a voltage change to fully reach a new steady-state $P_{open}$ [16]. Presumably the differences in behavior reflect the existence of several isoforms of the $H^+$ channel. The molecular identity of the voltage-gated $H^+$ channel remains unknown, however. One reason that $H^+$ channels have not been isolated is that there are no high affinity markers for the channel. The most potent inhibitors are divalent cations, especially $ZnCl_2$, which bind to many proteins and thus are not useful for this purpose.

Although there are distinct functional differences, the main properties of voltage-gated proton channels are similar in all tissues where they have been studied: they open with depolarization, their voltage-dependence is strongly sensitive to $pH_o$ and $pH_i$ such that they only carry outward current under physiological conditions, they are inhibited by polyvalent cations, such as $Zn^{2+}$, $Cd^{2+}$, and $La^{3+}$, and they are extremely selective for $H^+$ over other ions. No direct measurements of single channel currents have been reported. The unitary $H^+$ current amplitude can be estimated from the stochastic fluctuations in $H^+$ currents. In snail neurons [22] and human skeletal myotubes [8] no excess noise was detected, but in human neutrophils, current fluctuations were detected, and a rough estimate of 10 fS was reported [16]. This conductance indicates currents on the order of 1 fA ($10^{-15}$ A) for a 100 mV driving voltage, which corresponds with 6250 $H^+$ $s^{-1}$ channel$^{-1}$. The single $H^+$ channel conductance is three to four orders of magnitude smaller than that of most ion channels, and is not clearly larger than the transport rate of some carriers.

## 3. Functions of voltage-gated proton channels

The key to understanding the function of voltage-gated proton channels is to recognize their exquisite regulation by $pH_o$ and $pH_i$. Although $H^+$ channels are voltage-gated and open upon depolarization of the membrane potential, the voltage range at which the channels open varies dramatically. Lower $pH_o$ or higher $pH_i$ shifts the voltage–activation relationship to more positive voltages, by 40 mV/unit change in the pH gradient, $\Delta pH$ ($pH_o - pH_i$) [14]. The result of this regulation is that $H^+$ channels open only at voltages positive to $E_H$ and therefore conduct only outward current. These properties seem ideally suited for the $H^+$ channel to function as an acid extrusion mechanism. The maximal rate of $H^+$ extrusion by $H^+$ channels in small cells is two orders of magnitude greater than for other acid extruding membrane transporters, such as $Na^+/H^+$-antiport, $Na^+$-$HCO_3^-$ symport or the $H^+$-ATPase [5]. $H^+$ efflux through channels is passive and driven by outward electrochemical gradient, and thus no metabolic energy must be expended. In contrast the $H^+$-ATPase consumes ATP directly and the $Na^+/H^+$-antiporter, for example, extrudes $H^+$ at the expense of dissipating the $Na^+$ gradient, which subsequently must be restored by the $Na^+$,$K^+$-ATPase. The voltage-gated proton channel is thus a cleverly designed and efficient mechanism for extruding acid from cells.

Proton channels perform a number of specific functions in different cells. There is strong evidence that $H^+$ channels are activated during the respiratory burst in phagocytes [23]. These white blood cells (neutrophils, macrophages, and eosinophils) engulf and kill bacteria by secreting reactive oxygen species such as the superoxide anion, $O_2^-$. The enzyme responsible, NADPH oxidase, is electrogenic and releases a proton into the cell for each $O_2^-$ produced [24,25]. The $H^+$ conductance is activated during the respiratory burst and serves to eliminate excess positive charge from the cell. Inhibiting $H^+$ currents during the respiratory burst results in depolarization of the membrane potential, a decrease in $pH_i$ [24,26], and reduced release of $O_2^-$ [27]. The $H^+$ conductance is also activated during spreading of human neutrophils [28] and recovery from an acute acid load in osteoclasts [20].

In snail neurons where $H^+$ channels were discovered, $H^+$ currents may be activated during nerve impulses, or action potentials. Part of the action potential reflects influx of $Ca^{2+}$ through $Ca^{2+}$ channels. This $Ca^{2+}$ is rapidly extruded by the $Ca^{2+}/H^+$ antiporter, resulting in locally high levels of protons just inside the cell membrane. This drop in local $pH_i$ combined with the depolarization inherent in the action potential combine to activate $H^+$ currents, thus eliminating metabolic acid [29–31].

Activation of the $H^+$ conductance appears to facilitate inositol triphosphate dependent $Ca^{2+}$ oscillations in frog oocytes [32], and may contribute to alkalinization following fertilization in newt oocytes [7].

$H^+$ channels are expressed at a high density in alveolar epithelial cells [33]. It was recently hypothesized that these $H^+$ channels might contribute to the elimination of $CO_2$ by the lung. The diffusion of $CO_2$ across the blood–gas barrier is facilitated by carbonic anhydrase, which allows diffusion of $CO_2$ also in the form of $HCO_3^-$ and $H^+$ (buffered). $H^+$ extruded through $H^+$ channels across the apical membrane of alveolar epithelial cells may combine with $HCO_3^-$ transported by the $Cl^-/HCO_3^-$ exchanger to form $CO_2$ in the alveolar subphase [34].

## 4. How do protons get from one place to another inside proteins? Water wires and hydrogen-bonded chains (HBC)

It is well known that protons diffuse in aqueous solutions by a mechanism very different from that of other ions. The mobility (or conductivity, or diffusion coefficient) of $H^+$ is about five times larger than that of other monovalent cations of a size similar to the hydronium ion, $H_3O^+$ [35], which is the predominant form in which protons exist in solution, rather than as unhydrated free protons, $H^+$ [36]. Since de Grotthuss [37], it has been recognized that protons can travel through water by hopping from one water molecule to another, in contrast with other cations that must diffuse around and between the water molecules. A refinement of this mechanism came about when it was realized that sustained proton conduction requires an additional process, the reorientation of water molecules, in order to facilitate subsequent proton hopping events [38,39]. Variations on this

theme have been described as 'structural diffusion' [40]. Further refinement came with the recognition of the importance of hydrogen bonding in the special conduction mechanism of $H^+$ [36,39–43].

The importance of the two separate processes required for proton conduction becomes more obvious and easier to understand when one considers a proton channel. In general terms, a proton channel can be defined as a linear hydrogen-bonded chain (HBC) that a proton can hop across. If the entire HBC is composed of water molecules, as in the pore of the pentadecapeptide antibiotic gramicidin channel, it is called a 'water-wire' [44]. In a homogeneous HBC, such as a water wire, cooperative proton transfers are favored energetically [45]. In most bioenergetic proton channels, protonatable side groups of amino acids are believed to form part of the HBC, with water molecules bridging any gaps in the chain. Ion channels are generally thought of as water-filled pores that form a hydrophilic pathway for ions to cross the membrane. Selective ion channels, those that allow only certain ionic species to permeate, are believed to have at least one narrow region in which ions or water molecules are constrained to move in single file [46]. The narrow region serves as a 'selectivity filter' where steric, chemical, and electrostatic constraints are employed to discriminate among ions [47]. This is our general conceptual view, and although broader regions also likely exist, the minimum requirement of a proton channel must be a continuous hydrogen-bonded chain that provides a pathway across a low dielectric barrier (the cell membrane, or through the interior of a protein). John Nagle and colleagues [44,48,49] proposed and developed the idea that HBC conduction mechanisms provide a realistic model for proton conduction, and stressed the importance of the two-step nature of this mechanism. After a proton hops across the HBC, the chain is oriented such that another proton cannot enter from the same side. The groups comprising the HBC must first reorient. Key properties and predictions of the 'hop-turn' mechanism listed below.

(1) The hopping step is faster than the turning (reorientation) step by roughly an order of magnitude [44,49,50]. This prediction has been supported by molecular dynamics calculations [51]; proton transfer between waters occurs in $\sim$1 fs [52]. Its generality, however, may depend on the precise nature of the HBC. Recent evidence suggests that D defects have much higher mobility in ice than hydronium ions [53]. One implication of hopping being faster than reorientation is that although quantum effects, such as tunneling, may occur, they are unlikely to be rate determining. Thus, the deuterium isotope effect on $H^+$ conduction through gramicidin (1.2–1.35) [54], chloroplast $F_0$ proton channels (1.7) [55], or voltage-gated $H^+$ channels ($\sim$1.9) [13] is similar to or greater than the isotope effect for bulk conductance in aqueous solution (1.41) [56], but likely would be much greater (e.g. 6 or 7) if tunneling were rate-limiting [36,57].

(2) Both hopping and turning carry a partial charge across the membrane. For proton conduction through ice, Scheiner and Nagle [58] calculated that the proton-hopping step carries 64% and the reorientation step 36% of the total charge. Depending on the nature of the HBC, the fractions can vary substantially [49]. The importance of this property is that both $H^+$ flux and its requisite companion, the rearrangement of hydrogen bonds to permit a subsequent proton transport event, can be driven by transmembrane voltage. It is evident from molecular dynamics simulations that applied voltage must drive defects across the gramicidin water-wire. In simulations without applied voltage, the simulated proton hops around within a small cluster of favorably oriented water molecules with little net movement over 400 ps [59]. In real data, the average interval between complete $H^+$ conduction events (including all requisite hopping and turning) is only 500 ps at 7 M HCl and 300 mV (data of Cukierman et al. [60]). Furthermore, because it carries a partial charge, the bonding defect that obligatorily must 'permeate' the channel after each proton hopping event may be induced by membrane potential. This voltage-activated reorientation is analogous to the induced rotation of water molecules in the vicinity of a hydronium ion that is required to account for the observed anomalously high conductivity of $H^+$ in water [36].

(3) Because the turning step is slower than hopping, its initiation is crucial in limiting $H^+$ conductance. In general, the turning step should be independent of pH if it is initiated by breaking hydrogen bonds between neutral waters [61]. This feature has been useful in attempts to explain the

apparent pH independence of the unitary conductance of $F_0$ and voltage-gated $H^+$ channels [5,55]. There has been recent interest in the precise nature of the rate determining step in $H^+$ conductance in gramicidin, which in one proposal is defect entry into the distal end of the pore [62]. The difficulty is in explaining why the conductance of gramicidin is proportional to the proton concentration, $[H^+]$, over a range spanning five orders of magnitude [1]. Initiation of the defect at the proximal end of the channel could occur by proton-induced orientation of a water molecule in the pore, possibly enhanced by applied voltage as just discussed. The electric field inside the 25 Å gramicidin channel at 125 mV is the same as the field 6 Å away from a univalent ion in bulk water, $5\times10^5$ V/cm [35].

The way we have presented the concept of proton conduction across an HBC may be an oversimplification for some real channels. A key question is whether a single continuous HBC exists that *simultaneously* spans the entire channel. In the case of gramicidin, the HBC is a simple water wire [46,54,63,64]. Early molecular dynamics simulations concluded that the waters in the pore were aligned, and that a cation in the pore aligned the waters on either side across the entire length of the pore [65,66]. The concept of a permion, a chain of oriented waters that accompany an ion during permeation through a channel, emphasizes the coupling between ion and water movement through gramicidin [67]. More recent simulations including polarizability indicate that an ion may orient only two waters on either side, and that longer-range interactions disappear [68,69]. Orientational faults between water molecules inside gramicidin are predicted to occur due to interaction between water and the channel wall, so that a proton in the channel rapidly jumps among a small cluster of waters, but cannot cross the entire channel until the misaligned waters reorient [59]. In a sense, the rate of proton permeation through gramicidin is more accurately described as the rate of defect permeation. It must be borne in mind that: (a) both protons and defects can migrate through the channel without displacing the water molecules inside the pore; (b) this property results in the $H^+$ conductance in gramicidin being 14–25 times greater than that of any other cation [70–72]; and (c) consequently, both processes must occur at least an order of magnitude faster than ionic diffusion (by cations other than $H^+$) through the channel.

Proton transport across a channel that is not water-filled could also occur by hopping across an HBC that need not span the entire membrane simultaneously. It has been suggested that a proton permeating such a channel, by virtue of its altering local charge distribution, might induce a 'travelling wave' of transient conformational change that would facilitate $H^+$ jumping [73].

## 5. Voltage-gated $H^+$ channels are not water-filled pores

The voltage-gated proton channel appears to conduct protons across an HBC that is formed at least in part by side groups of amino acids, rather than a simple water-filled channel. The evidence for this conclusion has been discussed at greater length elsewhere [1,5] and will simply be summarized here. Many of the arguments are based on comparison with proton permeation through gramicidin and other channels.

(1) The voltage-gated channel is extremely selective for protons (and deuterons) over any other ion. A relative permeability $>10^6$ higher for $H^+$ than any other ion [5,9–11,13,14] reflects a degree of selectivity unheard of for 'normal' water-filled ion channels, but could be explained by an HBC conduction mechanism. The $H^+$ selectivity of the influenza A viral proton channel has been proposed to result from a constriction in the pore formed by histidine residues that interrupts the water wire at one point [74].

(2) The $H^+$ conductance is not proportional to $H^+$ concentration, $[H^+]$. The $H^+$ conductance of water-filled channels like gramicidin is nearly directly proportional to $[H^+]$ over a wide range spanning pH 5 to 0 (Fig. 1). In contrast, $H^+$ conductance of the voltage-gated $H^+$ channel and the $F_0$ proton channel of the chloroplast proton pump is nearly pH independent over a range pH 8 to 4 [5,14,55,75,76]. If one views permeation as a simple binding site in the channel that saturates with permeant ion concentration, then voltage-gated or $F_0$ channels apparently saturate at $\sim$8 orders of magnitude lower $[H^+]$ than gramicidin. Considering that $H^+$ conduction through an HBC occurs in two steps, hopping and

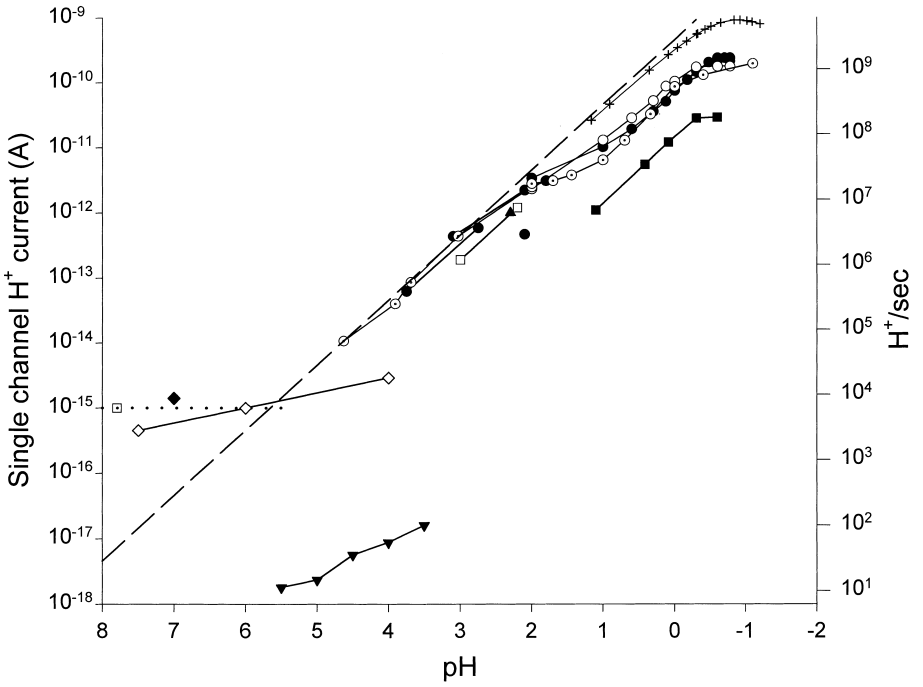


Fig. 1. Compilation of data in the literature for single-channel $H^+$ currents through various types of proton conducting channels. Data from each source are connected by lines. All values have been scaled linearly with voltage to 100 mV. The dashed line shows the maximum $H^+$ conductance if diffusion of $H^+$ to the channel were rate-limiting (details in [1]), assuming a capture radius of 0.87 Å as found for gramicidin [94]. Note that the $H^+$ conductance is proportional to $[H^+]$ over a wide range, for several channels that are believed to be water-filled pores. The product of the conductivity of concentrated HCl at 25°C [112] and its concentration is plotted for comparison (+), after arbitrary scaling. The parallel behavior of this value and the conductance of ion channels studied at high [HCl] suggests that the apparent saturation of single-channel $H^+$ current may simply reflect bulk properties of HCl. If so, then the apparent saturation of $H^+$ conductance of the gramicidin water wire reflects diffusion limitation external to the channel, and the maximum $H^+$ flux that a water wire can sustain has not been reached. Possible explanations of the 'shoulder' in the gramicidin data between pH 2 and 1 are discussed in [1]. Sources of data for the channels are: (●) 'gA' gramicidin A [54,60,70,71,94]; (○) 'gA dimer' covalently linked gramicidin dimer in GMO membranes [60,113]; (⊙) gramicidin A [114]; (▲) M2 proton channel of the influenza A virus [115]; (▼) '5-HT' serotonin receptor $H^+$ selective 'leakage' current [116]; (♦) 'MotA' flagellar motor torque generator [117]; (dotted line, □) $F_0$ the proton channel component of $H^+$-ATPase [55,118,119]; and (■) LSLLLSL a synthetic channel believed to comprise a trimer of the given sequence [120]. The voltage-gated $H^+$ channel estimates (◇) are based on noise measurements at pH 6 [16] and the $pH_i$ dependence of the conductance measured in inside-out patches [75,77]. The value given for MotA is based on $H^+$ flux/torque generator (1200 $H^+$/revolution, eight torque generators/motor) during flagellar rotation at 100 Hz [117], with the protonmotive force scaled linearly with voltage [121] to 100 mV; the passive $H^+$ flux through Mot A incorporated into bilayers is substantially less [122,123]. The 5-HT receptor current appears to be an acid-induced 'leak' mode of the serotonin receptor, induced by 5-HT addition, but not part of the normal transport cycle [116]. Reproduced from [1], with permission of the *Israel Journal of Chemistry*.

turning, and that we do not know which is rate determining in voltage-gated $H^+$ channels, the binding site analogy should not be carried too far. The point is that voltage-gated $H^+$ channels behave very differently from water-filled pores.

(3) The single channel conductance is very small. Unitary $H^+$ channel currents are too small to have been observed directly, but estimates from $H^+$ current fluctuations suggest a conductance of 10–100 fS [8,16,22], which is $\sim 10^3$ smaller than most other ion channels. This comparison should be tempered by consideration of the quite small permeant ion concentration at neutral pH. The highest $[H^+]$ at which voltage-gated $H^+$ currents have been successfully measured is pH 4.0 [77], and the weak increase in conductance as pH is lowered from 7.5 to 4.0 provides no indication that the conductance would increase more rapidly at lower pH (◇, Fig. 1). We thus tentatively conclude that the channel is close to saturation, although additional measurements would be

preferable to deduction by extrapolation. The small conductance can be explained if the HBC mechanism includes one or more 'inefficient' proton transfer steps between amino acid side groups. In contrast, in the classical water-filled ion channel gramicidin the mechanism of proton conduction is the water-wire variant of HBC [63,64] and its conductance increases with $[H^+]$, reaching 300 pA ($2\times10^9$ $H^+$/s) at very low pH [60].

(4) There are no pure blockers of $H^+$ channels. All known inhibitors appear to shift the voltage dependence of gating as though their effects were indirect. Divalent metals cations may bind to the channel and alter the transmembrane voltage sensed by the channel molecule. Organic inhibitors may reduce $H^+$ current indirectly, by altering local pH [5]. Many 'normal' ion channels have large, funnel-shaped vestibules, which also serve as effective sites for high-affinity binding by antibodies or peptide inhibitors derived from venoms and toxins. If the voltage-gated $H^+$ channel has no pore per se, but only an HBC, then there may be little opportunity for occlusion.

(5) There is a large deuterium isotope effect. The ratio of $H^+$ to $D^+$ current is $\sim$1.9 in voltage-gated $H^+$ channels [13]. This isotope effect is comparable with that in chloroplast $F_0$ proton channels [55], but larger than that for conduction in bulk solution [56] or in gramicidin channels [54]. The large isotope effect can be explained if proton transfer reactions (or hydrogen bond rearrangement) inside the channel are rate determining.

(6) The temperature dependence of the $H^+$ conductance is extraordinarily large, with activation energy, $E_a$ 18–27 kcal/mol [78]. This $E_a$ is larger than that for any of several proton-related processes that occur in bulk solution (e.g. hydrolysis, buffer deprotonation, dielectric relaxation, etc.) and thus suggests that events within the channel HBC are rate determining. By comparison with the small $E_a$ of 4.8 kcal/mol in the water-filled gramicidin channel [54], the voltage-gated proton channel employs a far less efficient proton translocation mechanism.

Although we do not know the primary structure of the voltage-gated proton channel, we have deduced the general nature of the conduction pathway. Several distinctive properties point to the conclusion that the conduction pathway is not a simple water-filled pore, but includes side groups of amino acids. Thus there is a general resemblance with other proton channels in bioenergetic proteins, most of which are believed to include amino acid side groups and some intercalated water molecules.

## 6. Is there a special mechanism of rapid proton conduction at the surface of the membrane?

This question typically arises when it appears that the supply of protons provided by diffusion to or from a sink or source (e.g. a $H^+$ channel) is inadequate to account for the magnitude of $H^+$ flux measured. The supply of protons to a $H^+$-selective channel (i.e. one with molecular dimensions) provided by simple diffusion of $H^+$ is $\sim$0.1 fA ($10^{-16}$ A or $\sim$600 $H^+$/s) at pH 7 [1]. If measured single-channel currents exceed this limit, then additional mechanisms must be proposed. One such mechanism is the idea that protons may be conducted in the plane of the surface of the membrane, perhaps by HBC's composed of protonatable phospholipid headgroups or proteins [79], at a rate that exceeds diffusion in bulk solution. A related idea is that there may be a barrier to proton movement into and out of the membrane plane, although such a barrier would exacerbate the problem if the demand for protons exceeds the supply. There has been extensive discussion of the question whether protons generated by the electron transport powered proton pumps of mitochondria, chloroplasts, and bacteria, simply diffuse through the aqueous solution in 'delocalized coupling' [80,81] or travel preferentially across the surface of the membrane to nearby ATPases in 'localized coupling' [82,83].

Several other situations have presented the problem of accounting for the supply of protons. Kasianowicz et al. [84] recorded astoundingly large $H^+$ flux across membrane doped with protonophores. They concluded that hydrolysis was a significant source of protons. The high turnover of carbonic anhydrase presented a problem because the catalytic group releases $10^6$ $H^+$ $s^{-1}$; which is $10^3$ faster than the predicted limit for a diffusion controlled reaction given a p$K_a$ near 7 [85,86]. The solution is that buffer rather than water is the main proton acceptor [87]. Proton uptake by the bacterial reaction center occurs

at high pH with an apparent rate constant nearly $10^3$ faster than the limit for a diffusion-controlled protonation reaction, apparently due to surface charge effects as well as conformational changes in site accessibility [88]. The unitary conductance of the $F_0$ proton channel in bilayers is independent of pH between pH 5.6 and 8.0 and has been estimated to be $>10$ fS and as high as 1 pS, at least transiently [55]. This result seems problematic because the $F_0$ is a highly $H^+$ selective channel, and could not be selective if it had a large diameter. If one calculates [89,90] the diffusion-limited supply of protons to a channel of reasonable dimensions, one obtains $\sim$1 fA at pH 6 and 0.01 fA at pH 8 [1], values $10^2$–$10^4$ times smaller than the 1 pS value (=100 fA at 100 mV). To explain the problematic supply of protons, Althoff et al. [55] suggested favorable channel geometry, negative charges near the channel mouth [91], and rapid surface diffusion [92]. The single-channel current through voltage-gated $H^+$ channels is too small to have been detected directly [8,16,22]. However, using current fluctuation analysis, the unitary conductance has been estimated to be $\sim$10 fS at pH 6.0 [16], a value on the verge of exceeding the apparent diffusion limit (Fig. 1). At pH 7.5, the estimated conductance is 4.5 fS [1], well above the simple diffusion limit.

Without speculating whether $H^+$ conduction across HBC's in the plane of the membrane might occur more rapidly than $H^+$ diffusion in bulk solution, or whether there may be a barrier to proton movement into or out of the plane of the membrane, it might be more constructive simply to attempt to determine the extent to which the bulk proton concentration can be scaled by this type of mechanism. The upper limit can be set by postulating that the existence of surface conduction pathways potentially enables the entire membrane to serve as a proton collector. The surface of proteins has been proposed to play an analogous role as a proton collecting antenna [93]. The question can then be reduced to determining the surface area available to supply each $H^+$ channel. In cells with a high density of $H^+$ channels, such as several leukocytes [5,11,17–19], each channel has $\sim$450 nm$^2$ membrane area that could act as a proton collector [1]. If one assumes an effective 'capture distance' of 0.87 Å, which is the capture radius determined for $H^+$ conduction through gramicidin channels [94] – that is the distance from which a proton can jump to the membrane from $H_3O^+$ or buffer – then the theoretical limiting current is roughly equal to the best estimate of actual measured $H^+$ current at pH 7.5 [1]. Thus we find that each $H^+$ channel apparently uses most of the available membrane are in feeding its insatiable proton habit.

## 7. Equivalence of chemical and electrical proton gradients

The basic mechanism of energy transduction in many biological systems is the generation of a transmembrane proton gradient, which is used to drive the production of ATP. The total driving force on protons in such a system, the protonmotive force,

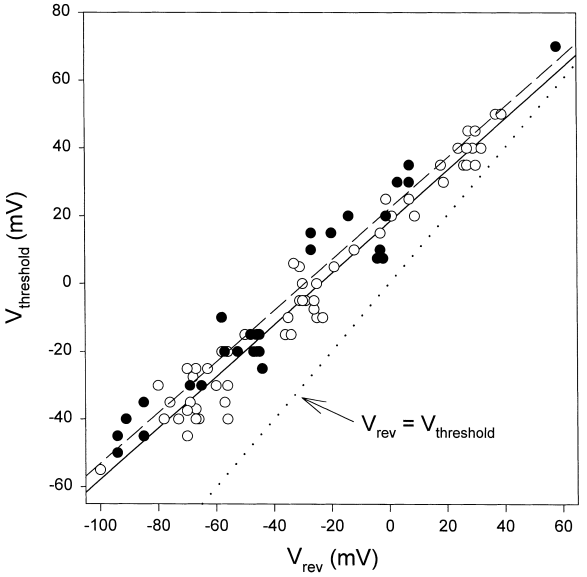


Fig. 2. The potential at which clearly time-dependent outward current was detected, $V_{threshold}$, is plotted as a function of $V_{rev}$ measured in the same cell and the same solution. $V_{threshold}$ was examined using voltage increments of 5 mV or less in rat alveolar epithelial cells in primary culture. Open symbols indicate measurements in $H_2O$, filled symbols in $D_2O$. The data encompass $pH_o$ 6.5–10.0 and $pD_o$ 7–10, and $pH_i$ 5.5–7.5 and $pD_i$ 7.0–9.0. The lines show the results of linear regression on the $H_2O$ data: $V_{threshold}=0.76\ V_{rev}+18$ mV (solid line), and the $D_2O$ data: $V_{threshold}=0.75\ V_{rev}+22$ mV (dashed line). The dotted line shows equality between $V_{threshold}$ and $V_{rev}$. Because all the data fall above this line, $H^+$ channels open only positive to $E_H$ and thus only when they will conduct outward current. Reproduced from [13], by copyright permission of The Rockefeller University Press.

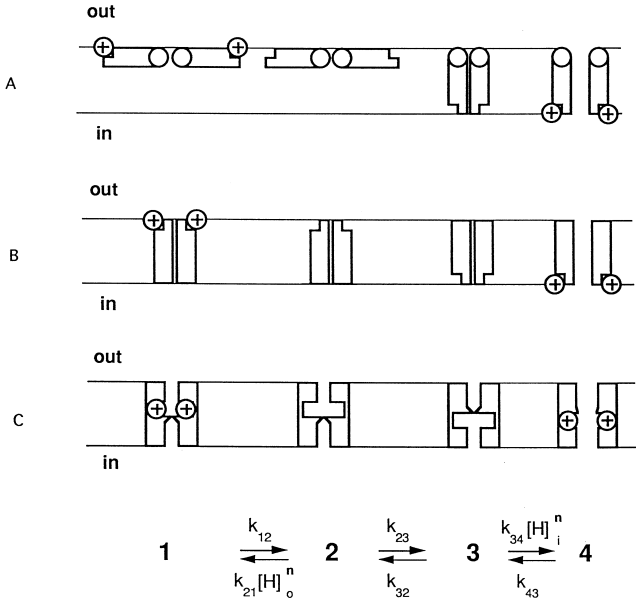


Fig. 3. A model showing how the opening of voltage-gated $H^+$ channels could be regulated by voltage and ΔpH. The state diagram and rate constants that define the model are at the bottom, above which are three possible physical representations. The mechanism can be envisioned as: (A) a 'butterfly' in which the protonation site on each channel protomer or 'wing' moves across the membrane; (B) distinct external and internal sites which when protonated allosterically prevent protonation at the opposite site; (C) a protonation site in a proton well whose accessibility depends on a small conformational change, or other variants not illustrated. The diagram in C would be a proton carrier [89] if the conformational change occurred in the protonated state, i.e. if there were direct transitions between states 1 and 4 and if no conducting pore was formed. In each case, the formation of a conducting $H^+$ channel requires a conformational change in each channel protomer which can occur only when the regulatory site is deprotonated. Thus the closed channel conformation is stabilized by external protons, and the open configuration is stabilized by internal protonation of the same site, which is possible only after the conformational change exposes the protonation site to the internal solution. The probability of channel opening is therefore increased by high $pH_o$ or low $pH_i$. The voltage dependence of $H^+$ channel gating could arise either from voltage-dependent binding/unbinding of protons to the regulatory protonation site, or from a voltage-dependent conformational change, or some combination of the two. In the version of the model used for the calculations, we assigned all of the voltage-dependence to proton binding, so that the regulatory sites behave like 'proton wells' as postulated for the proton channel of $H^+$-ATPases [80,95]. Reproduced from [14], with copyright permission of The Rockefeller University Press.

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$\Delta\mu_H^+$ is the sum of electrical and concentration differences across the membrane:

$$\Delta\mu_H^+ = V - E_H \quad (3)$$

where $V$ is the transmembrane potential in mV and $E_H$ (also in mV) is the Nernst potential for $H^+$, the voltage at which the electrical and chemical terms are equal and opposite:

$$E_H = \frac{RT}{F}\log\frac{[H^+]_o}{[H^+]_i} = 58.2\Delta pH \quad (4)$$

where ΔpH is the pH gradient defined as $pH_o - pH_i$ and the temperature is 20°C (because that is the temperature at which we usually do experiments). Passive $H^+$ currents in a non-voltage-clamped cell are driven by $V - E_H$ and bring the membrane potential toward $E_H$. Although ΔpH and the electrical driving force $V - E_H$ are thermodynamically equivalent, it is important to note that the kinetics of transport driven by these forces may differ markedly [48,95].

The voltage dependence of $H^+$ current gating is strongly affected by both $pH_o$ and $pH_i$ [29] and to a rough approximation appeared to be fixed in a definite relation with $\Delta\mu_H^+$ [96]. More quantitative studies revealed that although there is a linear relationship between $E_H$ and $V_{threshold}$, which is defined as the minimum depolarizing voltage required to activate detectable $H^+$ current, there is not a direct correspondence [13,97]. Fig. 2 shows that the rate of change of $V_{threshold}$ is only 75% of the change in $V_{rev}$. This is equivalent to a shift of 40 mV for a 1 unit change in ΔpH [14]:

$$V_{threshold} = 20\ mV - 40\ \Delta pH. \quad (5)$$

For comparison, the dashed line in Fig. 2 shows strict equivalence of $\Delta\mu_H^+$ and $V_{threshold}$. The biological significance of the observed relationship is that over a wide range of $pH_o$ and $pH_i$, voltage-gated $H^+$ channels open only when there is an outward electrochemical driving force, and hence carry only outward current. This property is essential for a channel that is expressed at high levels in the plasma membranes of many cells, because cellular metabolism produces acid that must be extruded continually. Proton entry is something that cells under normal circumstances try to avoid.

## 8. Proton wells

Mitchell proposed the idea of a '*proton well*' for $H^+$-ATPases [80,81,98]. A proton well is a pathway through which protons travel part way across the membrane potential field. The 'concentration' of protons in such a well will be increased or decreased from the bulk concentration according to the fraction of the membrane potential that they sense. It may be more accurate to speak of the likelihood of protonation of a site in a proton well, rather than 'proton concentration' per se, because at physiological pH, the concentration of water is $10^9$ higher than that of free protons, and thus a channel or vestibule that encloses a volume of 100 water molecules will have a nominal probability of containing a proton of $10^{-7}$ (in the absence of local charges or membrane potential). The proton exit pathway of cytochrome *c* oxidase is a proton well, because reversing the membrane potential can drive proton influx [99].

In modeling the regulation of voltage gating of $H^+$ channels by pH [14], it is necessary to incorporate mechanisms for both voltage dependent and pH dependent regulation of gating. The model is illustrated and described in Fig. 3. There are proton-binding steps at both sides of the membrane, and at least one required conformational change (in addition to channel opening). The model behaved similarly in most respects when the voltage dependence was assigned to the proton binding steps as when it was assigned to the conformational change. However, some aspects of the data were modeled better by assuming that proton binding was the main source of voltage dependence. Hence we proposed the existence of 'proton wells' that sense the pH on either side of the membrane and use this information to set the voltage dependence of gating [14].

## 9. Alternating access

Another conceptual parallel is the idea of *alternating access* to a site within the protein. The idea of alternating access is key to the function of ion pumps [100] and carriers [95] in cell membranes. The main reason pumps and carriers are so much slower than ion channels in terms of their rate of ion transport is that each ion translocation event (which moves one or several ions depending on stoichiometry) requires a conformational change in the protein, whose effect is to switch the accessibility of the bound ion from the solution on one side of the membrane to the solution on the other side. Bacteriorhodopsin can pump protons across membranes because the key proton-binding site, the retinal Schiff base, first is accessible to the cytoplasmic solution and then switches to the extracellular solution [101]. Respiratory oxidases, such as cytochrome *c*, employ a switch in the accessibility of proton binding groups to regulate proton translocation [102]. The key assumption of the model in Fig. 3 that imparts $\Delta$pH dependence to $H^+$ channel gating is that of alternating access. The protonation sites can be accessible to only one side of the membrane or the other, but not both simultaneously. In a sense, proton conduction across an HBC could be considered a form of alternating access, in that HBC orientation determines whether a proton can enter from one side or the other.

## 10. Conformational changes can induce p$K_a$ shifts

The fundamental (in a sense miraculous) mechanism responsible for the ability of bacteriorhodopsin to transmute light into chemical energy is a dramatic lowering of the p$K_a$ of the retinal Schiff base during the conformational change (i.e. the accessibility change discussed above) induced by photon absorption [103]. The high p$K_a$ before the light reaction makes the base a proton acceptor when it is accessible to the cytoplasmic solution, and the lower p$K_a$ (a change on the order of 5 Units [104]) leads to release of the proton to the extracellular solution. Coupling of proton flux to ATP synthesis is accomplished in ATP synthase by electrostatic reduction of the p$K_a$ of the site facing the basic side of the membrane resulting in deprotonation when it approaches a positively charged Arg [105,106]. Proton release during translocation by respiratory cytochromes results from a large reduction of the p$K_a$ of a histidine imidazole group [102]. Nicotinamide nucleotide transhydrogenase pumps protons across the membrane during reduction of $NADP^+$ to NADPH, by a mechanism that may also involve a p$K_a$ shift [107]. The key element of the proton shuttle of carbonic anhydrase is a histidine that exists in two conformations. That the preferred conformation depends

strongly on pH [108] suggests that the effective p$K_a$ of this group is altered.

Our model for proton channel gating does not include explicit p$K_a$ shifts, but such a mechanism could be invoked to provide the requisite alternating access. The conformational change that we postulate switches the accessibility of the regulatory protonation sites from one side of the membrane to the other [14] could be effected by simultaneously lowering the p$K_a$ of one site and raising the p$K_a$ of the other. Other possibilities include steric alterations or gross molecular movement. Furthermore, given the small unitary conductance, large isotope effect, and strong temperature dependence, we cannot rule out the possibility that $H^+$ conduction through voltage-gated $H^+$ channels may involve participation of the channel, conceivably in the form of a p$K_a$ shift, during each transfer event. If the 'channel' behaved thus like a carrier with respect to permeation, there would still have to be an additional gating mechanism to account for the strongly voltage-dependent gating.

## 11. Metal binding can control proton reactions

Heavy metals play a central role in proton transfer reactions in several molecules. Complex interactions between copper and iron atoms in cytochrome *c* oxidase are central to the electron transfers that result in proton pumping [102,109]. A zinc atom coordinated between three histidine residues plays an key role in carbonic anhydrase by lowering the p$K_a$ of a bound water molecule to form the $OH^-$ that combines with $CO_2$ to form $HCO_3^-$ [110]. The proton shuttle in carbonic anhydrase is inhibited by $Cu^{2+}$ and $Hg^{2+}$ which are believed to bind to a histidine residue involved in proton shuttling [111].

Voltage-gated proton channels are unusual in having few known inhibitors. The best known are metals, particularly $Cd^{2+}$ and $Zn^{2+}$ [2,5,15,22,30]. $Zn^{2+}$ binds proton channels with higher affinity than many other ion channels [77]. There is no direct evidence that metals are a normal component of the channel, although this possibility is intriguing. More intriguing, the effects of externally applied $Zn^{2+}$ resemble qualitatively the effects of external protons (i.e., lowering $pH_o$), raising the possibility that $Zn^{2+}$ may bind to the regulatory external protonation sites (Fig. 3). There is striking competition between $Zn^{2+}$ and $H^+$, with stoichiometry suggesting that at least two protonation sites act in concert to coordinate the $Zn^{2+}$ at its binding site [77].

## 12. Conclusions

The conceptual framework describing our knowledge of a number of radically different molecules shares a surprising number of parallels: proton translocation by special HBC mechanisms, problems of proton supply and demand, equivalence of chemical and electrical proton gradients, proton wells, alternating access sites, p$K_a$ changes induced by protein conformational change, and heavy metal participation in proton transfer processes. An archetypal mechanism involves input and output proton pathways (HBC's) that are joined by a regulatory site that switches the accessibility of the bound proton from one 'channel' to the other, by means of a p$K_a$ change, molecular movement, or both. The only requirement for inclusion in this review is that the molecule translocates protons through a hydrophobic environment, such as a protein interior or a cell membrane. Some of the parallels drawn may seem strained or contrived, but we simply have attempted to assemble information that we hope will stimulate cross-fertilization between fields. It seems evident that nature has devised a limited number of fundamental mechanisms to accomplish various design problems, and that these clever and subtle mechanisms are repeated with minor variation in many superficially disparate molecules. Our understanding of our particular favorite molecule, the voltage-gated proton channel, has benefited substantially from work done on other molecules. We hope that we will eventually learn enough to be able to return some small insights from the types of measurements that we can make on this channel. If not, then we will continue unabashedly to enjoy the intellectual benefits of progress in other areas.

## 13. Note added in proof

Although the molecular identity of the voltage-gated proton channel remains elusive, there is evidence that the $gp91^{phox}$ component of NADPH oxidase plays some role in proton extrusion during the

respiratory burst in phagocytes [L.M. Henderson, S. Thomas, G. Banting, J.B. Chappell, The arachidonate-activatable, NADPH oxidase-associated $H^+$ channel is contained within the multi-membrane-spanning N-terminal region of gp91-*phox*, Biochem. J. 325 (1997) 701–705; L.M. Henderson, R.W. Meech, Evidence that the product of the human X-linked CGD gene, pg91-*phox*, is a voltage-gated $H^+$ pathway, J. Gen. Physiol. 114 (1999) 771–785]. It was reported recently that expression of NOH-1, a molecule with some structural similarity to $gp91^{phox}$, enhances the proton conductance of HEK cells [B. Bánfi, A. Maturana, S. Jaconi, S. Arnaudeau, T. Laforge, B. Sinha, E. Ligeti, N. Demaurex, K.-H. Krause, A mammalian $H^+$ channel generated through alternative splicing of the NADPH oxidase homolog NOH-1, Science 287 (2000) 138–142].

Although it remains true that voltage-gated proton currents in unstimulated cells open only when there is an outward electrochemical $H^+$ gradient, when NADPH oxidase is active in phagocytes, inward $H^+$ currents can occur [B. Bánfi, J. Schrenzel, O. Nüsse, D.P. Lew, E. Ligeti, K.-H. Krause, N. Demaurex, A novel $H^+$ conductance in eosinophils: unique characteristics and absence in chronic granulomatous disease, J. Exp. Med. 190 (1999) 183–194]. This 'abnormal' behavior is the result of dramatic alterations in the properties of proton channels in phagocytes during the respiratory burst [T.E. DeCoursey, V.V. Cherny, W. Zhou, L.L. Thomas, PMA enhances proton currents during the respiratory burst in human neutrophils, Biophys. J. 78 (2000) 131A].

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## References

[1] T.E. DeCoursey, V.V. Cherny, An electrophysiological comparison of voltage-gated proton channels, other ion channels, and other proton channels, Israel J. Chem., in press.
[2] R.C. Thomas, R.W. Meech, Hydrogen ion currents and intracellular pH in depolarized voltage-clamped snail neurones, Nature 299 (1982) 826–828.
[3] A.L. Hodgkin, A.F. Huxley, A quantitative description of membrane current and its application to conduction and excitation in nerve, J. Physiol. 117 (1952) 500–544.
[4] A.L. Hodgkin, A.F. Huxley, The components of membrane conductance in the giant axon of Loligo, J. Physiol. 116 (1952) 473–496.
[5] T.E. DeCoursey, V.V. Cherny, Voltage-activated hydrogen ion currents, J. Membr. Biol. 141 (1994) 203–223.
[6] W. Nernst, Zur Kinetik der in Lösung befindlichen Körper. Theorie der Diffusion, Z. Phys. Chem. 2 (1888) 613–637.
[7] M.E. Barish, C. Baud, A voltage-gated hydrogen ion current in the oocyte membrane of the axolotl, Ambystoma, J. Physiol. 352 (1984) 243–263.
[8] L. Bernheim, R.M. Krause, A. Baroffio, M. Hamann, A. Kaelin, C.-R. Bader, A voltage-dependent proton current in cultured human skeletal muscle myotubes, J. Physiol. 470 (1993) 313–333.
[9] N. Demaurex, S. Grinstein, M. Jaconi, W. Schlegel, D.P. Lew, K.-H. Krause, Proton currents in human granulocytes: regulation by membrane potential and intracellular pH, J. Physiol. 466 (1993) 329–344.
[10] A. Kapus, R. Romanek, A.Y. Qu, O.D. Rotstein, S. Grinstein, A pH-sensitive and voltage-dependent proton conductance in the plasma membrane of macrophages, J. Gen. Physiol. 102 (1993) 729–760.
[11] T.E. DeCoursey, V.V. Cherny, $Na^+$-$H^+$ antiport detected through hydrogen ion currents in rat alveolar epithelial cells and human neutrophils, J. Gen. Physiol. 103 (1994) 755–785.
[12] M. Kuno, J. Kawawaki, F. Nakamura, A highly temperature-sensitive proton conductance in mouse bone marrow-derived mast cells, J. Gen. Physiol. 109 (1997) 731–740.
[13] T.E. DeCoursey, V.V. Cherny, Deuterium isotope effects on permeation and gating of proton channels in rat alveolar epithelium, J. Gen. Physiol. 109 (1997) 415–434.
[14] V.V. Cherny, V.S. Markin, T.E. DeCoursey, The voltage-activated hydrogen ion conductance in rat alveolar epithelial cells is determined by the pH gradient, J. Gen. Physiol. 105 (1995) 861–896.
[15] T.E. DeCoursey, Four varieties of voltage-gated proton channels, Frontiers Biosci. 3 (1998) d477–d482.
[16] T.E. DeCoursey, V.V. Cherny, Potential, pH, and arachidonate gate hydrogen ion currents in human neutrophils, Biophys. J. 65 (1993) 1590–1598.
[17] D.V. Gordienko, M. Tare, S. Parveen, C.J. Fenech, C. Robinson, T.B. Bolton, Voltage-activated proton current in eosinophils from human blood, J. Physiol. 496 (1996) 299–316.
[18] J. Schrenzel, D.P. Lew, K.-H. Krause, Proton currents in human eosinophils, Am. J. Physiol. 271 (1996) C1861–C1871.
[19] V.V. Cherny, W. Zhou, L.L. Thomas, T.E. DeCoursey, Proton currents in human basophils, Biophys. J. 76 (1999) A349 (abstract).

[20] T. Nordström, O.D. Rotstein, R. Romanek, S. Asotra, J.N.M. Heersche, M.F. Manolson, G.F. Brisseau, S. Grinstein, Regulation of cytoplasmic pH in osteoclasts: contribution of proton pumps and a proton-selective conductance, J. Biol. Chem. 270 (1995) 2203–2212.
[21] C. Eder, H.-G. Fischer, U. Hadding, U. Heinemann, Properties of voltage-gated currents of microglia developed using macrophage colony-stimulating factor, Pflügers Archiv. 430 (1995) 526–533.
[22] L. Byerly, Y. Suen, Characterization of proton currents in neurones of the snail, Lymnaea stagnalis, J. Physiol. 413 (1989) 75–89.
[23] T.E. DeCoursey, S. Grinstein, Ion channels and carriers in leukocytes, in: J.I. Gallin, R. Snyderman (Eds.), Inflammation: Basic Principles and Clinical Correlates, 3rd edn., Lippincott, Williams and Wilkins, Philadelphia, PA, 1999, pp. 639–659.
[24] L.M. Henderson, J.B. Chappell, O.T.G. Jones, The superoxide-generating NADPH oxidase of human neutrophils is electrogenic and associated with an $H^+$ channel, Biochem. J. 246 (1987) 325–329.
[25] J. Schrenzel, L. Serrander, B. Bánfi, O. Nüsse, R. Fouyouzi, D.P. Lew, N. Demaurex, K.-H. Krause, Electron currents generated by the human phagocyte NADPH oxidase, Nature 392 (1998) 734–737.
[26] L.M. Henderson, J.B. Chappell, O.T.G. Jones, Internal pH changes associated with the activity of NADPH oxidase of human neutrophils: further evidence for the presence of an $H^+$ conducting channel, Biochem. J. 251 (1988) 563–567.
[27] L.M. Henderson, J.B. Chappell, O.T.G. Jones, Superoxide generation by the electrogenic NADPH oxidase of human neutrophils is limited by the movement of a compensating charge, Biochem. J. 255 (1988) 285–290.
[28] N. Demaurex, G.P. Downey, T.K. Waddell, S. Grinstein, Intracellular pH regulation during spreading of human neutrophils, J. Cell Biol. 133 (1996) 1391–1402.
[29] L. Byerly, R. Meech, W. Moody, Rapidly activating hydrogen ion currents in perfused neurones of the snail, Lymnaea stagnalis, J. Physiol. 351 (1984) 199–216.
[30] R.W. Meech, R.C. Thomas, Voltage-dependent intracellular pH in Helix aspersa neurones, J. Physiol. 390 (1987) 433–452.
[31] C.J. Schwiening, H.J. Kennedy, R.C. Thomas, Calcium-hydrogen exchange by the plasma membrane Ca-ATPase of voltage-clamped snail neurons, Proc. R. Soc. Lond. Ser. B. 253 (1993) 285–289.
[32] S. Humez, T. Collin, F. Matifat, P. Guilbault, F. Fournier, $InsP_3$-dependent $Ca^{2+}$ oscillations linked to activation of voltage-dependent $H^+$ conductance in Rana esculenta oocytes, Cell. Signal. 8 (1996) 375–379.
[33] T.E. DeCoursey, Hydrogen ion currents in rat alveolar epithelial cells, Biophys. J. 60 (1991) 1243–1253.
[34] T.E. DeCoursey, Do voltage-gated proton channels in alveolar epithelial cells contribute to $CO_2$ elimination by the lung? Am. J. Physiol. Cell Physiol. 278 (2000) C1–C10.
[35] R.A. Robinson, R.H. Stokes, Electrolyte Solutions, Butterworths, London, 1959, 571 pp.
[36] B.E. Conway, J.O'M. Bockris, H. Linton, Proton conductance and the existence of the $H_3O\bullet$ ion, J. Chem. Phys. 24 (1956) 834–850.
[37] C.J.T. deGrotthuss, Sur la décomposition de l'eau et des corps qu'elle tient en dissolution à l'aide de l'électricité galvanique, Ann. Chimie LVIII (1806) 54–74.
[38] H. Danneel, Notiz über Ionengeschwindigkeiten, Zeitschrift für Elektrochemie und angewandte physikalische Chemie 11 (1905) 249–252.
[39] J.D. Bernal, R.H. Fowler, A theory of water and ionic solution, with particular reference to hydrogen and hydroxyl ions, J. Chem. Phys. 1 (1933) 515–548.
[40] M. Eigen, L. De Maeyer, Self-dissociation and protonic charge transport in water and ice, Proc. R. Soc. Lond. Ser. A. 247 (1958) 505–533.
[41] M.L. Huggins, The role of hydrogen bonds in conduction by hydrogen and hydroxyl ions, J. Am. Chem. Soc. 53 (1931) 3190–3191.
[42] N. Agmon, Hydrogen bonds, water rotation and proton mobility, J. Chem. Phys. 93 (1996) 1714–1736.
[43] G. Zundel, Proton polarizability of hydrogen bonds and proton transfer processes, their role in electrochemistry and biology, Institut für Physikalische Chemie der Universität München, 1997, 250 pp.
[44] J.F. Nagle, H.J. Morowitz, Molecular mechanisms for proton transport in membranes, Proc. Natl. Acad. Sci. USA 75 (1978) 298–302.
[45] S. Scheiner, Proton transfers in hydrogen-bonded systems: cationic oligomers of water, J. Am. Chem. Soc. 103 (1981) 315–320.
[46] A. Finkelstein, O.S. Andersen, The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport, J. Membr. Biol. 59 (1981) 155–171.
[47] B. Hille, Ionic selectivity of Na and K channels of nerve membranes, in: G. Eisenman. (Ed.), Membranes: A Series of Advances, Dekker, New York, 1975, pp. 255–323.
[48] J.F. Nagle, M. Mille, H.J. Morowitz, Theory of hydrogen bonded chains in bioenergetics, J. Chem. Phys. 72 (1980) 3959–3971.
[49] J.F. Nagle, S. Tristram-Nagle, Hydrogen bonded chain mechanisms for proton conduction and proton pumping, J. Membr. Biol. 74 (1983) 1–14.
[50] M.-S. Chen, L. Onsager, J. Bonner, J. Nagle, Hopping of ions in ice, J. Chem. Phys. 60 (1974) 405–419.
[51] R. Pomès, B. Roux, Free energy profiles for $H^+$ conduction along hydrogen-bonded chains of water molecules, Biophys. J. 75 (1998) 33–40.
[52] K. Drukker, S.W. de Leeuw, S. Hammes-Schiffer, Proton transport along water chains in an electric field, J. Chem. Phys. 108 (1998) 6799–6808.
[53] J.P. Cowin, A.A. Tsekouras, M.J. Iedema, K. Wu, G.B. Ellison, Immobility of protons in ice from 30 to 190 K, Nature 398 (1999) 405–407.
[54] M. Akeson, D.W. Deamer, Proton conductance by the gramicidin water wire: model for proton conductance in the $F_0F_1$ ATPases?, Biophys. J. 60 (1991) 101–109.

[55] G. Althoff, H. Lill, W. Junge, Proton channel of the chloroplast ATP synthase, $CF_0$: its time-averaged single-channel conductance as a function of pH, temperature, isotopic and ionic medium composition, J. Membr. Biol. 108 (1989) 263–271.
[56] G.N. Lewis, T.C. Doody, The mobility of ions in $H^2H^2O$, J. Am. Chem. Soc. 55 (1933) 3504–3506.
[57] J.E. Crooks, Proton transfer to and from atoms other than carbon, in: C.H. Bamford, C.F.H. Tipper (Eds.), Chemical Kinetics, Vol. 8, Proton Transfer, Elsevier, New York, 1977, pp. 248–250.
[58] S. Scheiner, J.F. Nagle, Ab initio molecular orbital estimates of charge partitioning between Bjerrum and ionic defects in ice, J. Phys. Chem. 87 (1983) 4267–4272.
[59] R. Pomès, B. Roux, Structure and dynamics of a proton wire: a theoretical study of $H^+$ translocation along the single-file water chain in the gramicidin A channel, Biophys. J. 71 (1996) 19–39.
[60] S. Cukierman, E.P. Quigley, D.S. Crumrine, Proton conduction in gramicidin A and in its dioxolane-linked dimer in different lipid bilayers, Biophys. J. 73 (1997) 2489–2502.
[61] J.F. Nagle, Theory of passive proton conductance in lipid bilayers, J. Bioenerg. Biomembr. 19 (1987) 413–426.
[62] L.R. Phillips, C.D. Cole, R.J. Hendershot, M. Cotten, T.A. Cross, D.D. Busath, In gramicidin hydrogen transport the rate limiting water reorientation must be initiated at the channel exit, Biophys. J. 76 (1999) A25.
[63] V.B. Myers, D.A. Haydon, Ion transfer across lipid membranes in the presence of gramicidin A: the ion selectivity, Biochim. Biophys. Acta 274 (1972) 313–322.
[64] D.G. Levitt, S.R. Elias, J.M. Hautman, Number of water molecules coupled to the transport of sodium, potassium and hydrogen ions via gramicidin, nonactin or valinomycin, Biochim. Biophys. Acta 512 (1978) 436–451.
[65] S.-W. Chiu, S. Subramaniam, E. Jakobsson, J.A. McCammon, Water and polypeptide conformations in the gramicidin channel: a molecular dynamics study, Biophys. J. 56 (1989) 253–261.
[66] D.H.J. Mackay, P.H. Berens, K.R. Wilson, A.T. Hagler, Structure and dynamics of ion transport through gramicidin A, Biophys. J. 46 (1984) 229–248.
[67] R. Elber, D.P. Chen, D. Rojewska, R. Eisenberg, Sodium in gramicidin: an example of a permion, Biophys. J. 68 (1995) 906–924.
[68] P.C. Jordan, Ion–water and ion–polypeptide correlations in a gramicidin-like channel: a molecular dynamics study, Biophys. J. 58 (1990) 1133–1156.
[69] K.A. Duca, P.C. Jordan, Ion–water and water–water interactions in a gramicidinlike channel: effects due to group polarizability and backbone flexibility, Biophys. Chem. 65 (1997) 123–141.
[70] S.B. Hladky, D.A. Haydon, Ion transfer across lipid membranes in the presence of gramicidin A: I. studies of the unit conductance channel, Biochim. Biophys. Acta 274 (1972) 294–312.
[71] E. Neher, J. Sandblom, G. Eisenman, Ionic selectivity, saturation, and block in gramicidin A channels. II. Saturation behavior of single channel conductances and evidence for the existence of multiple binding sites in the channel, J. Membr. Biol. 40 (1978) 97–116.
[72] G.A. Woolley, V. Zunic, J. Karanicolas, A.S.I. Jaikaran, A.V. Starostin, Voltage-dependent behavior of a 'ball-and-chain' gramicidin channel, Biophys. J. 73 (1997) 2465–2475.
[73] A.K. Dunker, D.A. Marvin, A model for membrane transport through α-helical protein pores, J. Theor. Biol. 72 (1978) 9–16.
[74] L.H. Pinto, G.R. Dieckmann, C.S. Gandhi, C.G. Papworth, J. Braman, M.A. Shaughnessy, J.D. Lear, R.A. Lamb, W.F. DeGrado, A functionally defined model for the $M_2$ proton channel of influenza A virus suggests a mechanism for its ion selectivity, Proc. Natl. Acad. Sci. USA 94 (1997) 11301–11306.
[75] T.E. DeCoursey, V.V. Cherny, Voltage-activated proton currents in membrane patches of rat alveolar epithelial cells, J. Physiol. 489 (1995) 299–307.
[76] T.E. DeCoursey, V.V. Cherny, Voltage-activated proton currents in human THP-1 monocytes, J. Membr. Biol. 152 (1996) 131–140.
[77] V.V. Cherny, T.E. DeCoursey, pH-dependent inhibition of voltage-gated $H^+$ currents in rat alveolar epithelial cells by $Zn^{2+}$ and other divalent cations, J. Gen. Physiol. 114 (1999) 819–838.
[78] T.E. DeCoursey, V.V. Cherny, Temperature dependence of voltage-gated $H^+$ currents in human neutrophils, rat alveolar epithelial cells, and mammalian phagocytes, J. Gen. Physiol. 112 (1998) 503–522.
[79] J.F. Nagle, R.A. Dilley, Models of localized energy coupling, J. Bioenerg. Biomembr. 18 (1986) 55–64.
[80] P. Mitchell, Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism, Nature 191 (1961) 144–148.
[81] P. Mitchell, A commentary on alternative hypotheses of protonic coupling in the membrane systems catalysing oxidative and photosynthetic phosphorylation, FEBS Lett. 78 (1977) 1–20.
[82] D.B. Kell, On the functional proton current pathway of electron transport phosphorylation: an electrodic view, Biochim. Biophys. Acta 549 (1979) 55–99.
[83] R.J.P. Williams, The history and the hypotheses concerning ATP-formation by energised protons, FEBS Lett. 85 (1978) 9–19.
[84] J. Kasianowicz, R. Benz, S. McLaughlin, How do protons cross the membrane–solution interface? Kinetic studies on bilayer membranes exposed to the protonophore S-13 (5-chloro-3-tert-butyl-2′-chloro-4′nitrosalicylanilide), J. Membr. Biol. 95 (1987) 73–89.
[85] M. Eigen, Proton transfer, acid-base catalysis, and enzymatic hydrolysis. Part I: elementary processes, Angewandte Chemie, International Edition 3 (1964) 1–19.
[86] D.N. Silverman, S.H. Vincent, Proton transfer in the catalytic mechanism of carbonic anhydrase, CRC Crit. Rev. Biochem. 14 (1983) 207–235.
[87] B.-H. Jonsson, H. Steiner, S. Lindskog, Participation of

buffer in the catalytic mechanism of carbonic anhydrase, FEBS Lett. 64 (1976) 310–314.
[88] P. Maróti, C.A. Wraight, Kinetics of $H^+$ binding by the $P^+Q_{A-}$ state of bacterial photosynthetic reaction centers: rate limitation within the protein, Biophys. J. 73 (1997) 367–381.
[89] P. Läuger, Diffusion-limited ion flow through pores, Biochim. Biophys. Acta 455 (1976) 493–509.
[90] B. Hille, Ionic Channels of Excitable Membranes, 2nd edn., Sinauer Associates, Sunderland, MA, 1992, 607 pp.
[91] P.C. Jordan, How pore mouth charge distributions alter the permeability of transmembrane ionic channels, Biophys. J. 51 (1987) 297–311.
[92] M. Prats, J.F. Tocanne, J. Teissie, Lateral proton conduction at a lipid/water interface. Effect of lipid nature and ionic content of the aqueous phase, Eur. J. Biochem. 162 (1987) 379–385.
[93] V. Sacks, Y. Marantz, A. Aagaard, S. Checover, E. Nachliel, M. Gutman, The dynamic feature of the proton collecting antenna of a protein surface, Biochim. Biophys. Acta 1365 (1998) 232–240.
[94] E.R. Decker, D.G. Levitt, Use of weak acids to determine the bulk diffusion limitation of $H^+$ ion conductance through the gramicidin channel, Biophys. J. 53 (1988) 25–32.
[95] P. Läuger, Electrogenic Ion Pumps, Sinauer Associates, Sunderland, MA, 1991, 313 pp.
[96] G.L. Lukacs, A. Kapus, A. Nanda, R. Romanek, S. Grinstein, Proton conductance of the plasma membrane: properties, regulation, and functional role, Am. J. Physiol. 265 (1993) C3–C14.
[97] R.C. Thomas, Changes in the surface pH of voltage-clamped snail neurones apparently caused by $H^+$ fluxes through a channel, J. Physiol. 398 (1988) 313–327.
[98] P. Mitchell, Proton translocation mechanisms and energy transduction by adenosine triphosphatases: an answer to criticisms, FEBS Lett. 50 (1975) 95–97.
[99] D.A. Mills, S. Ferguson-Miller, Proton uptake and release in cytochrome *c* oxidase: separate pathways in time and space?, Biochim. Biophys. Acta 1365 (1998) 46–52.
[100] O. Jardetzky, Simple allosteric model for membrane pumps, Nature 211 (1966) 969–970.
[101] J.K. Lanyi, Bacteriorhodopsin as a model for proton pumps, Nature 375 (1995) 461–463.
[102] M. Wikström, A. Bogachev, M. Finel, J.E. Morgan, A. Puustinen, M. Raitio, M. Verkhovskaya, M.I. Verkhovsky, Mechanism of proton translocation by the respiratory oxidases: the histidine cycle, Biochim. Biophys. Acta 1187 (1994) 106–111.
[103] W. Stoeckenius, R.H. Lozier, R.A. Bogomolni, Bacteriorhodopsin and the purple membrane of halobacteria, Biochim. Biophys. Acta 505 (1979) 215–278.
[104] J.K. Lanyi, Proton translocation mechanism and energetics in the light-driven pump bacteriorhodopsin, Biochim. Biophys. Acta 1183 (1993) 241–261.
[105] R.H. Fillingame, Coupling $H^+$ transport and ATP synthesis in $F_1F_0$-ATP synthases: glimpses of interacting parts in a dynamic molecular machine, J. Exp. Biol. 200 (1997) 217–224.
[106] T. Elston, H. Wang, G. Oster, Energy transduction in ATP synthase, Nature 391 (1998) 510–513.
[107] J. Rydström, X. Hu, O. Fjellström, J. Meuller, J. Zhang, C. Johansson, T. Bizouarn, Domains, specific residues and conformational states involved in hydride ion transfer and proton pumping by nicotinamide nucleotide transhydrogenase from Escherichia coli, Biochim. Biophys. Acta 1365 (1998) 10–16.
[108] S.K. Nair, D.W. Christianson, Unexpected pH-dependent conformation of His-64, the proton shuttle of carbonic anhydrase II, J. Am. Chem. Soc. 113 (1991) 9455–9458.
[109] R.B. Gennis, Multiple proton-conducting pathways in cytochrome oxidase and a proposed role for the active-site tyrosine, Biochim. Biophys. Acta 1365 (1998) 241–248.
[110] D.W. Christianson, C.A. Fierke, Carbonic anhydrase: evolution of the zinc binding site by nature and by design, Acc. Chem. Res. 29 (1996) 331–339.
[111] C.K. Tu, G.C. Wynns, D.N. Silverman, Inhibition by cupric ions of $^{18}O$ exchange catalyzed by human carbonic anhydrase II, J. Biol. Chem. 256 (1981) 9466–9470.
[112] B.B. Owen, F.H. Sweeton, The conductance of hydrochloric acid in aqueous solutions from 5 to 65°, J. Am. Chem. Soc. 63 (1941) 2811–2817.
[113] S. Cukierman, Flying protons in linked gramicidin monomers, Israel J. Chem., in press.
[114] G. Eisenman, B. Enos, J. Hägglund, J. Sandblom, Gramicidin as an example of a single-filing ionic channel, Ann. New York Acad. Sci. 329 (1980) 8–20.
[115] K.C. Duff, R.H. Ashley, The transmembrane domain of influenza A M2 protein forms amantadine-sensitive proton channels in planar lipid bilayers, Virology 190 (1992) 485–489.
[116] Y. Cao, S. Mager, H.A. Lester, $H^+$ permeation and pH regulation at a mammalian serotonin receptor, J. Neurosci. 17 (1997) 2257–2266.
[117] M. Meister, G. Lowe, H.C. Berg, The proton flux through the bacterial flagellar motor, Cell 49 (1987) 643–650.
[118] G. Schoenknecht, W. Junge, H. Lill, S. Engelbrecht, Complete tracking of proton flow in thylakoids – the unit conductance of $CF_0$ is greater than 10 fS, FEBS Lett. 203 (1986) 289–294.
[119] H. Schindler, N. Nelson, Proteolipid of adenosinetriphosphatase from yeast mitochondria forms proton-selective channels in planar lipid bilayers, Biochemistry 21 (1982) 5787–5794.
[120] W.F. DeGrado, J.D. Lear, Conformationally constrained α-helical peptide models for protein ion channels, Biopolymers 29 (1990) 205–213.
[121] D.C. Fung, H.C. Berg, Powering the flagellar motor of Escherichia coli with an external voltage source, Nature 375 (1995) 809–812.
[122] D.F. Blair, H.C. Berg, The MotA protein of *E. coli* is a proton-conducting component of the flagellar motor, Cell 60 (1990) 439–449.
[123] B. Stolz, H.C. Berg, Evidence for interactions between MotA and MotB, torque-generating elements of the flagellar motor of *Escherichia coli*, J. Bacteriol. 173 (1991) 7033–7037.