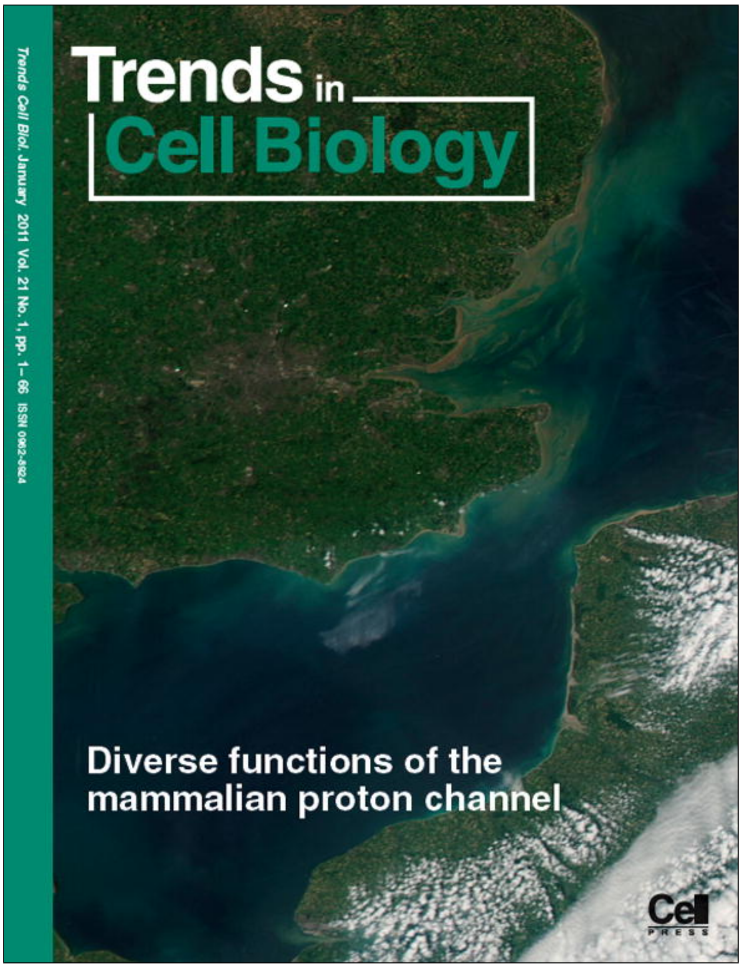
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Diverse functions of the
mammalian proton channel
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Review

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pH regulation and beyond: unanticipated functions for the voltage-gated proton channel, HVCN1

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Electrophysiological studies have implicated voltage-gated proton channels in several specific cellular contexts. In neutrophils, they mediate charge compensation that is associated with the oxidative burst of phagocytosis. Molecular characterization of the hydrogen voltage-gated channel 1 (HVCN1) has enabled identification of unanticipated and diverse functions: HVCN1 not only modulates signaling from the B-cell receptor following B-cell activation and histamine release from basophils, but also mediates pH-dependent activation of spermatozoa, as well as acid secretion by tracheal epithelium. The importance of HVCN1 in pH regulation during phagocytosis was established by surprising evidence that indicated its first-responder role. In this review, we discuss recent findings from a functional perspective, and the potential of HVCN1 as a therapeutic target for autoimmune and other diseases.

Introduction

The hydrogen voltage-gated channel 1 (HVCN1, also called H_V1 and VSOP) was a mysterious protein for several years. Proton currents were identified in snail neurons and amphibian oocytes in the 1980s [1–3], in mammalian cells in 1991 [4], and in human cells in 1993 [5–7]. However, the gene that encodes the channel was not discovered until 2006 when two groups cloned human and murine HVCN1 genes [8,9], which prompted a wave of new studies on voltage-gated proton channel function and regulation. Historically, proton currents have been associated with the NADPH oxidase in phagocytic cells, where they mediate rebalancing of charges across the plasma membrane to allow optimal NADPH-oxidase-mediated reactive oxygen species (ROS) production [10–13]. Availability of an HVCN1-deficient mouse line has facilitated further functional characterization of HVCN1, and has opened new avenues for the investigation of its roles in other cell types. We now know that the proton channel is expressed in such diverse cell types as neutrophils, basophils, B cells, spermatozoa and airway epithelial cells, and that it performs specific functions in each of them. This review highlights the significance of such functions and the emerging potential to exploit HVCN1 as a therapeutic target in applications such as allergy, autoimmune diseases and contraception.

Key electrophysiological properties of the proton channel

Consideration of the electrophysiological properties of voltage-gated proton channels has led to the conclusion that their fundamental function is acid extrusion from cells [14]. Proton channels are perfectly selective for protons. Furthermore, they are ion channels, not carriers or exchangers, and acid extrusion occurs independently of other ionic concentrations [1,2,14]. Voltage-gated proton channels open upon membrane depolarization, with a sigmoid time course. However, their open probability depends strongly on pH on both sides of the membrane. The threshold voltage at which proton channels begin to open has been determined empirically to be $40(\mathrm{pH_o{-}pH_i})$ +20 mV [15]. In practical terms, this means that at symmetrical pH, the channel opens 20 mV positive to the Nernst potential for protons, E_H, that increasing $\mathrm{pH_o}$ or decreasing $\mathrm{pH_i}$ by one unit shifts the entire g_H-V (proton conductance–voltage) relationship by −40 mV, and that at all physiologically achievable pH values, the channel opens only when the electrochemical proton gradient is outwards. Thus, proton channels are designed to open only when this will result in acid extrusion from cells.

Proton channels have been found in a multitude of cell types within two dozen species, based on both direct voltage-clamp evidence and gene homology [16]. In electrophysiological studies, which have been widely used to provide unequivocal evidence of expression, proton channel function is characterized in the plasma membrane. However, indirect evidence supports the presence of proton channels in phagosomes [17] and the Golgi complex [18]. When overexpressed in HeLa cells, HVCN1 protein appears mainly in intracellular compartments [19]; whether this reflects a catabolic pathway or a function in organelles is unclear.

The current that flows through a single proton channel is small, compared to that in other ion channels. For a moderate driving force, a Na^+ or K^+ channel might conduct a few picoamperes of current (~10^7 ions/s), but a proton

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channel conducts only a few femtoamperes of current ($\sim10^4$ H^+/s) [20]. The smaller conductance reflects the much lower permeant ion concentration; the K^+ concentration in cells is 10^6 times larger than the H^+ concentration. Nevertheless, macroscopic (collective) proton currents in many cells are as large or larger than K^+ currents [21], because nature is clever enough to express large numbers of H^+ channels. The presence of a tiny conductance is useful for proton channels in small organelles such as phagosomes, because this enables a finely controlled response.

Discovery of the gene that encodes the proton channel was hampered by a lack of selective inhibitors. The most potent inhibitor is Zn^{2+}, which although rather promiscuous, inhibits H^+ currents more effectively than Ca^{2+} currents, for example [22]. Unlike traditional ion channel blockers, Zn^{2+} does not occlude the channel, but instead binds to the external surface of the molecule where it slows channel opening and shifts the voltage dependence positively [23]. The effects of Zn^{2+} are strongly attenuated at low pH_o, which reflects competition between protons and Zn^{2+} for binding sites. Modeling of this competition has suggested that Zn^{2+} binding is coordinated between 2–3 histidine residues [23]. Subsequently, mutation of the human proton channel has confirmed that His^{140} and His^{193} (Figure 1) both contribute to Zn^{2+} effects [8]. Zn^{2+} appears to bind at the interface between monomers in the HVCN1 dimer (see below), where it prevents channel opening [24,25].

HVCN1 protein and structure–function relationships

In 2006, human HVCN1 was cloned [5], as were homologous genes in mice and *Ciona intestinalis* [9]. The human HVCN1 (Figure 1) is expressed in two isoforms, the full-length protein (273 amino acids), and a short form, which lacks the first 20 amino acids; thus far, documented only in B cells [26]. The latter protein isoform derives from translation from an alternative initiation site. One naturally occurring mutation has been identified, M91T, which reduces the probability of channel opening [27].

The protein encoded by the HVCN1 gene has two remarkable features. First, no explicit aqueous pore that might provide a conduction pathway similar to other ion channels is evident [8,9]. This feature was not altogether unexpected, because several properties of proton channels have suggested the lack of a conventional aqueous pathway. Deuterium conductance is only 50% that of H^+ [28], conduction is strongly temperature-sensitive [29,30] and the channel is perfectly selective [31,32], which suggests that protons permeate proton channels by a hydrogen-bonded chain mechanism [33] that does not require a continuous aqueous pathway. A recent study has concluded that protons cross frozen or non-exchangeable waters without displacing them [34]. Larger ions, in contrast, permeate ordinary ion channels by moving in single file along with water molecules. The second remarkable feature of HVCN1 is its similarity to the voltage-sensing domain (VSD) of voltage-gated K^+, Na^+ and Ca^{2+} channels

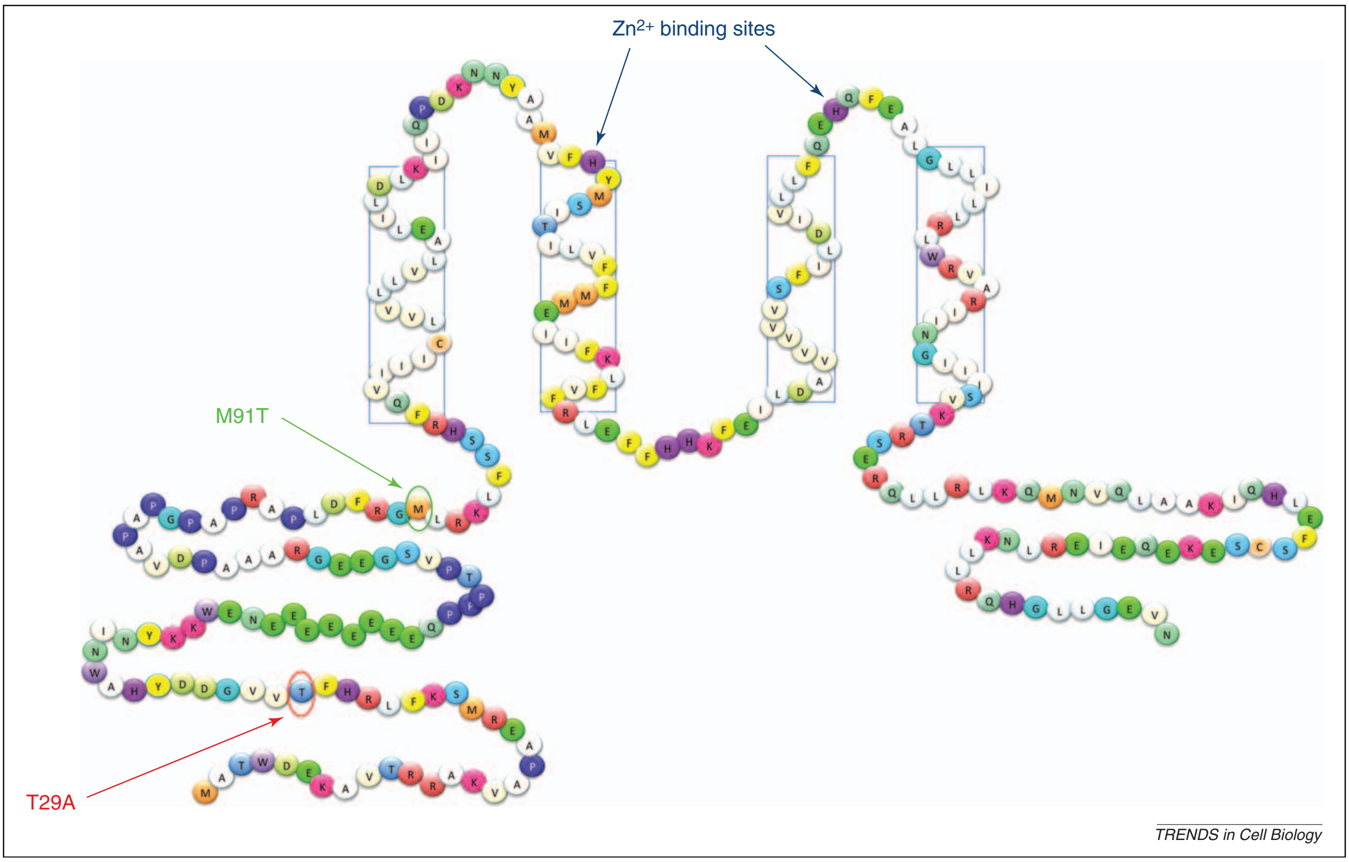

Figure 1. Sequence of human HVCN1 showing its arrangement in the membrane. Two sites on the intracellular N terminus have been shown to influence channel opening: phosphorylation of Thr^{29} strongly enhances opening in leukocytes [45], whereas the missense mutation M91T reduces it in airway epithelial cells [27]. Histidines that constitute Zn^{2+} binding sites [8,24,25] are indicated, together with transmembrane domains (four rectangular boxes).

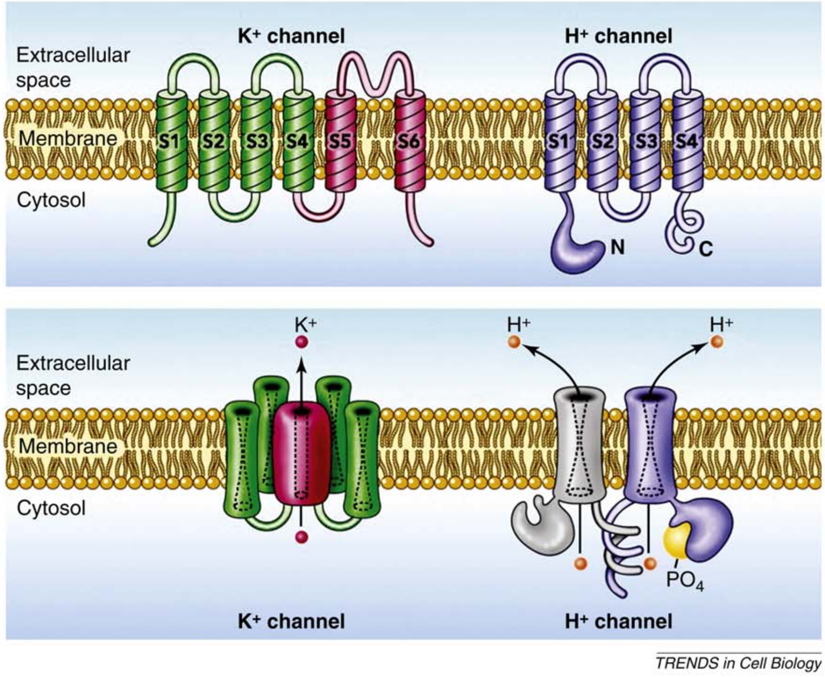

Figure 2. Architectural distinction between K^+ and proton channels. Voltage-gated K^+ channels are tetramers of the subunit shown in the top left panel. The four S5–S6 domains combine to create a single central pore in the assembled channel (lower panel). The voltage-gated H^+ channel (right, upper and lower panel) is a dimer, but each protomer contains its own conduction pathway and can function as a monomer. Proton channel activity is greatly enhanced by phosphorylation at Thr^{29}. Figure modified from [21] with permission of the American Physiological Society.

[8,9]. Most voltage-gated ion channels are tetramers of subunits that span the membrane six times (Figure 2). The first four domains (S1–S4) comprise the voltage-sensing apparatus (VSD), and the remaining two domains (S5–S6) from each of the four monomers collectively form a single aqueous pathway across the membrane. Although using 24 membrane-spanning domains to form a single pore might seem needlessly elaborate, the result is a fourfold amplification of the voltage sensitivity of a single VSD. For ion channels that mediate nerve impulses or trigger contraction of muscle fibers, extreme sensitivity to small changes in membrane potential is essential.

The discovery of the HVCN1 gene, and its homology with the VSDs of other, more widely studied ion channels, has greatly increased interest in this molecule, with the result that new discoveries are being made rapidly. Diverse evidence indicates that the proton channel exists as a dimer, with a separate conduction pathway in each protomer [35–37]. Although most voltage-gated ion channels are tetramers with a single conduction pathway (Figure 2), the ClC family of Cl^- channels and Cl^-/H^+ antiporters also are dimers with permeation pathways in each subunit [38–40]. Truncation of the HVCN1 C terminus results in monomeric channels that retain most functions of the dimer [24,36,37]. The two protomers do not function independently in the dimer, however, but interact during gating [24,25,41,42]. One consequence of this cooperativity is that the dimer has twice the voltage sensitivity of the monomer [41], despite channel opening being slower [24,25,37]. Teleologically, strong voltage sensitivity might be more important to the cell than rapid opening kinetics [24]. For example, in phagocytes, proton channels counterbalance the effects of NADPH oxidase, which remains active for several to many minutes (Box 1). Strong voltage sensitivity activates proton channels before excessive depolarization can occur, which precludes self-inhibition of this electrogenic enzyme [10] that both produces and is inhibited by membrane depolarization [13].

Proton channels in phagocytes regulate NADPH oxidase activity

The best-established function of proton channels is in leukocytes that phagocytose and kill bacteria (Box 1). Proton channel properties change dramatically in activated leukocytes [43,44]. In the "enhanced gating mode", they open faster and at less positive voltages, and close more slowly, which substantially increases the proton current. Enhanced gating results primarily from phosphorylation of HVCN1 by protein kinase C (PKC) at Thr^{29} of the intracellular N terminus [45], and is prevented or reversed by PKC inhibitors [46,47]. Part of this response might involve arachidonic acid (AA) [6,48], which is produced locally by activated phagocytes, although it is unclear whether the effect is direct or via AA-mediated stimulation of PKC [47]. Without enhanced gating, proton channels can still open during NADPH oxidase activity; they would be activated by membrane depolarization, decreased intracellular pH (pH_i), and increased pH in the extracellular milieu or phagosome. However, in the enhanced gating mode, more proton channels open with smaller depolarization. This, in turn, improves the efficiency of NADPH oxidase by 15–20% by minimizing depolarization-induced self-inhibition [49].

At the same time that proton flux compensates charge, it also prevents large changes in both cytoplasmic and phagosomal pH. Regulation of pH_i is essential, because NADPH oxidase activity is optimal at pH_i 7.5 and

Box 1. NADPH oxidase and ROS

A schematic representation of NADPH oxidase and HVCN1 function in phagocytes (Figure I). The NADPH oxidase enzymatic complex assembles on the plasma (or phagosome) membrane of granulocytes, macrophages and B cells upon stimulation. The membrane-bound components, gp91^{phox} (*phox* refers to phagocyte oxidase) and p22^{phox}, bind the cytosolic components (p47^{phox}, p67^{phox}, p40^{phox} and Rac) that are recruited to the membrane, which in phagocytic cells, invaginate to produce a phagosome. NADPH oxidase produces superoxide anion ($O_2\bullet^-$), which is a precursor to other ROS. Most $O_2\bullet^-$ dismutates to H_2O_2, which is converted to HOCl by myeloperoxidase. Gp91^{phox}, also called Nox2, contains an electron transport chain that comprises NADPH, FAD and two heme groups that pass electrons sequentially to O_2 at an external binding site, to produce $O_2\bullet^-$. The enzyme is electrogenic, because electrons extracted from cytoplasmic NADPH are translocated to extracellular or intraphagosomal O_2 that is thereby reduced to $O_2\bullet^-$ [10]. Without charge compensation, the membrane would depolarize to extreme positive voltages at which NADPH oxidase would cease to function [13]. Proton currents provide most of this charge compensation [49], and alleviate the cytosolic acidification that results from NADPH utilization and reconstitution by the hexose monophosphate shunt, which also inhibits NADPH oxidase [51]. Other transporters that might contribute to pH regulation include ClC-3 (a Cl^-/H^+ antiporter), H-ATPase, and the Na^+/H^+ antiporter. ROS enable clearance of engulfed bacteria, as shown by impaired immune responses in chronic granulomatous disease (CGD) patients [81]. CGD occurs when mutations to components of NADPH oxidase prevent its function. The disease affects phagocytes and, to a lesser extent, B cells [82]. The primary goal of the high levels of ROS produced by phagocytes is clearance of bacteria. In B cells, where ROS production is about 10 times smaller than in phagocytes, ROS have been implicated in BCR signaling [66]. ROS temporarily inhibit protein tyrosine phosphatases to allow initiation and amplification of BCR downstream signaling pathways. ROS oxidize the cysteine residue in the catalytic domain of tyrosine phosphatases to sulfenic acid (–SOH). The reaction is reversible and the active thiolate anion ($-S^-$) can be quickly reformed by reduction, which is mediated by the many reducing agents present in the cytosol. In recent years, the number of signaling pathways and phosphatases shown to be regulated by oxidation has increased considerably [83,84]. Figure reprinted from [21] with permission of the American Physiological Society.

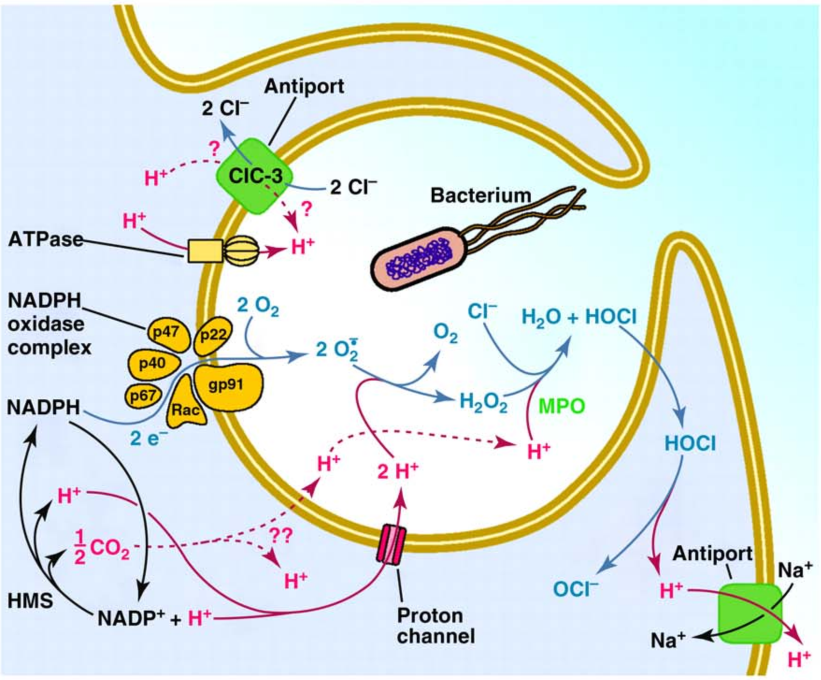

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Figure I. Schematic representation of NADPH oxidase and HVCN1 function in phagocytes.

decreases drastically at more acidic and more basic pH [50]. The importance of proton channels in minimizing pH_i changes during phagocytosis has been demonstrated recently in a study using the pH-sensitive dye seminaphtorhodafluor [51]. When neutrophils first engulf opsonized zymosan particles, there is a precipitous drop in pH_i, followed by an almost equally rapid recovery. This acidification reflects H^+ generation as a consequence of NADPH oxidase activity, since it is abolished by the oxidase inhibitor, diphenylene iodonium. Inhibition of Na^+/H^+ antiport or proton channel activity prevents pH_i recovery. Acidification is more rapid in the presence of Zn^{2+} (but not inhibitors of other transporters) and in HVCN1-deficient cells [51], which indicates that the proton channel is the first transporter to respond during phagocytosis. When proton channels are inhibited or absent, pH_i drops to levels that are known to inhibit NADPH oxidase [50]. This raises the philosophical question of whether charge compensation or pH_i regulation is more important to the cell. Fortunately, proton channels perform both functions simultaneously and inseparably.

Using proton channels to compensate charge in phagocytes has two additional benefits [49]. Proton efflux minimizes osmotic effects that could occur if other ions performed this function. If all charge were compensated by K^+, the phagosome would swell to ~20 times its original volume [49,52]. Finally, H^+ is required inside the phagosome as a substrate to form H_2O_2 and HOCl, the main products of

NADPH oxidase, both of which are generated at a high rate [53].

The downstream effects of HVCN1 deficiency in neutrophils have been investigated both in terms of ROS production and neutrophil function [17,51,54,55]. All studies have supported the concept that HVCN1 is required for optimal ROS production. Furthermore, NADPH-oxidase-dependent bacterial killing is significantly reduced in HVCN1-deficient neutrophils *in vitro* [54]. Bacterial clearance in HVCN1-deficient mice *in vivo* after peritoneal injection of *Staphylococcus aureus* is slightly but not significantly impaired. Clearance of *Pseudomonas aeurginosa* and *Burkholderia cepacia* is not impaired. The residual ROS production in HVCN1-deficient cells might be sufficient to ensure bacterial clearance to a point at which the infection can be resolved. Patients with variant forms of chronic granulomatous disease (CGD, Box 1), whose NADPH oxidase activity is reduced to 3–30% of normal, generally have milder symptoms than CGD patients with complete absence of NADPH oxidase activity [56–60]. However, defects in HVCN1 activity might become more relevant in different settings of inflammation and/or infection. Further work could clarify this point. HVCN1-deficient neutrophils also have diminished chemotactic responses to the bacterial peptide *N*-formyl-Met-Ile-Val-Ile-Leu (fMIVIL) *in vitro* [55]. Ca^{2+} entry is reduced, which in turn impairs migration and actin depolymerization. It would be interesting to establish whether *in vivo* defects in HVCN1-deficient mice are more apparent in neutrophil responses in which migration is crucial.

HVCN1 in basophils facilitates histamine release

Another cell with abundant HVCN1 expression is the basophil [61], which is not surprising given its lineage relationship with eosinophils and neutrophils. By contrast, basophils lack NADPH oxidase, which seems to be the *raison d'être* for proton channels in phagocytes. Agents that stimulate histamine release by basophils, including anti-IgE, phorbol 12-myristate 13-acetate, and the chemotactic peptide fMLF (formyl-Met-Leu-Phe) all enhance the gating of proton channels in human basophils [62]. Inhibition of proton channels with Zn^{2+} abolishes histamine release, which suggests that proton channels in basophils compensate charge or regulate pH. Measurement of pH_i during anti-IgE stimulation reveals acidification, which is exacerbated by Zn^{2+}. Thus, stabilization of pH_i is a probable function of HVCN1 in basophils. Histamine release is a clear example of a cellular process that is unrelated to NADPH oxidase activity, but is mediated by proton channels.

HVCN1 sustains optimal B-cell receptor (BCR) signaling

Proton currents were discovered in human B cells in 2002 [63], and the HVCN1 protein was identified in a proteomic screen of plasma membrane proteins expressed by mantle cell lymphoma cells in the peripheral blood [64]. Protein expression levels in resting naïve and memory B cells are comparable to those in granulocytes; however, HVCN1 is downregulated in proliferating B cells, such as cells in the germinal center or primary cells stimulated *in vitro* via CD40 (a tumor necrosis factor receptor family member) in the presence of interleukin-4 [26]. Downregulation might be mediated by BCL6 (B-cell lymphoma 6), because HVCN1 appears to be a direct target for BCL6-mediated transcriptional repression [65]. This pattern of expression suggests HVCN1 involvement in the initial phase of B-cell activation, which is impaired during *in vivo* and *in vitro* stimulation of HVCN1-deficient B cells. The impairment is mediated by diminished ROS production following BCR stimulation. ROS have been postulated to ensure correct BCR signal propagation by inhibition of protein phosphatases, such as SH2-containing protein tyrosine phosphatase-1, SHP-1 (Figure 3 and Box 1). This has been somewhat controversial [66] because of the possibility that reducing conditions in the cytosol scavenge ROS as soon as they are formed. Recent evidence, however, suggests that there are mechanisms to block temporarily the reducing action of cytosolic scavenging proteins such as peroxiredoxin [67]. The HVCN1-deficient model therefore provides a new way to illustrate how attenuation of ROS production in B cells has a detrimental effect on BCR downstream signaling. Diminished ROS correlate with diminished oxidation of the protein tyrosine phosphatase SHP-1, which dephosphorylates and therefore inhibits a crucial kinase in B-cell activation, spleen tyrosine kinase (Syk) [68]. Syk controls many downstream pathways such as mitogen-activated protein kinase activation, Ca^{2+} mobilization (from endoplasmic reticulum stores as well as entry from the extracellular milieu) and phosphoinositide 3-kinase (PI3K) activation [69,70]. Syk activation is indeed impaired in BCR-stimulated HVCN1-deficient cells, but not in the initial phases of activation; only at later time-points. The defect can be rescued by treating the cells with low doses of the SHP-1 inhibitor sodium stibogluconate [71]. Surprisingly, not all Syk downstream pathways are affected equally, because neither extracellular signal-regulated kinase activation nor Ca^{2+} mobilization is impaired. This suggests that these events are controlled by the very early activation of Syk and are not affected by the absence of sustained Syk activation. By contrast, the protein kinase Akt, which is downstream of PI3K [72], is impaired in HVCN1-deficient cells and this results in decreased cell metabolism, as both mitochondrial respiration and glycolysis are diminished following BCR stimulation (Figure 3). The defects in HVCN1-deficient B cells are specific for BCR stimulation, since downstream signaling from Toll-like receptor 4 and CD40 is unimpaired.

Consistent with the BCR-specific defect in signaling, HVCN1 has been found to be associated with the BCR complex and to colocalize with the receptor upon stimulation. This raises the possibility that close proximity of H^+ transport to the BCR might be important. Whether proximity is necessary to support NADPH oxidase activity or for other reasons is unknown and requires further investigation. Intriguingly, Ca^{2+} entry is impaired in the absence of HVCN1 in neutrophils [55] but not in B cells [26], which might reflect different signal regulation of Ca^{2+} mobilization in neutrophils and B cells, or differences in membrane depolarization. Ca^{2+} entry is impaired by the large depolarization attained by HVCN1-deficient neutrophils, which attenuates entry of positive ions [55]. In

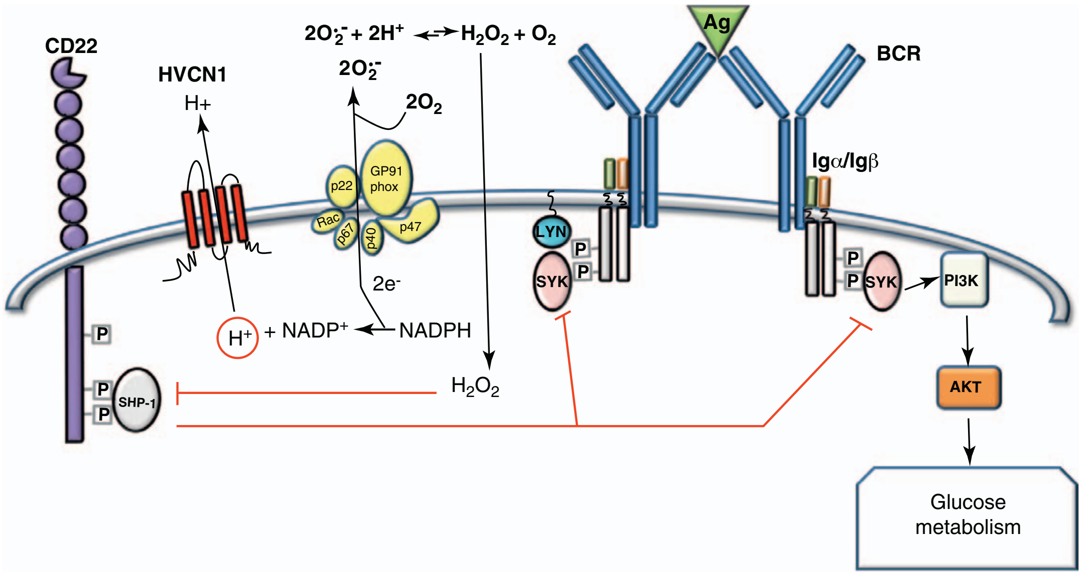

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Figure 3. Schematic representation of HVCN1 in the context of BCR signaling. Antigen binding to the BCR results in phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the Igα/β heterodimer by Lyn, a src-family tyrosine kinase, creating docking sites for Syk [85]. This serves to amplify BCR signaling by further recruitment and activation of Syk, which leads to PI3K activation, activation of Akt and increased glucose uptake and metabolism. Amplification of signaling is negatively regulated by CD22, which is also phosphorylated by Lyn, providing a docking site for protein tyrosine phosphatase SHP-1 [86]. SHP-1 dephosphorylates Syk, counterbalancing ITAM/Syk-mediated signal amplification. SHP-1 is inhibited by ROS, which oxidize a cysteine residue in the catalytic site of the enzyme. BCR stimulation results in ROS generated by the NADPH oxidase enzymatic complex, which transfers electrons across the plasma/endosome membrane to molecules of oxygen. The transfer of one electron results in the production of $O_2\bullet^-$ that combines with protons to form H_2O_2 and O_2, which freely diffuse through the membrane ($2O_2\bullet^- + 2H^+ \rightarrow H_2O_2 + O_2$). ROS generate a localized oxidizing environment that leads to inhibition of SHP-1, which results in amplification of BCR signals. HVCN1 sustains NADPH oxidase activity through charge compensation and intracellular pH regulation; therefore, in the absence of HVCN1, the oxidizing environment cannot be maintained and this results in SHP-1 remaining more active, which diminishes BCR signal strength. Figure adapted from [26].

contrast with neutrophils, B cells have substantial K^+ conductances [73] that would abrogate any depolarization due to NADPH oxidase activity. Consequently, the driving force for Ca^{2+} entry should be preserved.

HVCN1 regulates human spermatozoa activation

Gene expression analysis (http://biogps.gnf.org/#goto=genereport&id=84329 and [9]) has revealed HVCN1 expression in human testis, and recently, a role for HVCN1 in human spermatozoa has been proposed [74]. Spermatozoa reside in the testes in a quiescent state that is maintained by low cytosolic pH, below 6.5. The abundant proton channels are thought to be inhibited by a high concentration of Zn^{2+} present in seminal fluid. Once the sperm enter the female reproductive tract, Zn^{2+} might be buffered by albumin and other proteins, which relieves the inhibition. The resultant efflux of acid through proton channels increases pH_i, which triggers capacitation (maturation) that is necessary for egg fertilization.

Other species might lack this mechanism. Zn^{2+} enhances sperm motility in the Japanese eel [75]. Mouse spermatozoa lack proton currents, which suggests a different mechanism of alkalinization [74], although the murine cells investigated were less mature than their human counterparts [76]. Notably, HVCN1-deficient mice do not exhibit obvious fertility defects.

HVCN1 regulates pH in airway mucosa

A recent study by Iovannisci *et al.* [27] has demonstrated HVCN1 involvement in establishing the pH in airway epithelia. Low pH is associated with diseases such as asthma and cystic fibrosis, whereas alkaline pH is associated with rhinitis [77,78], which emphasizes that pH regulation of airway surface liquid (ASL) needs to be finely tuned. The pH in ASL is controlled by acid secretion from airway epithelial cells. As in most cells, multiple mechanisms contribute to acid secretion. In human nasal mucosa, proton channels secrete over half the acid measured in ASL; this fraction changes in chronic rhinosinusitis [78]. Iovannisci *et al.* [27] have found that HVCN1 opens when extracellular pH exceeds 7. They have emphasized that, in airway epithelia, proton channel gating is modulated by the difference in pH across the membrane rather than depolarization, because the epithelial membrane potential is stable.

This group identified a human subject with a missense mutation that produced a substitution of Met → Thr at position 91. Mutation M91T inhibited channel opening, with the mutant requiring +20 mV additional depolarization or ~0.5 units more alkaline mucosal pH to open the same number of channels. The mutation is likely to be heterozygous, which implies two possible scenarios: (i) the mutant has dominant expression, in which case the observed effect is mediated by M91T/M91T dimers; or (ii) dimers with the altered phenotype are composed of one mutant and one wild-type copy, which suggests that M91T/M91T dimers have an even stronger phenotype. Surprisingly, Met^{91} is not well conserved among species. *Macaca mulatta, Canis familiaris* and *Bos taurus* have Thr at position 91 and mice have Arg. It is not clear if this

Box 2. Major questions for future research

- What is the crystal structure?
- What interactions take place between monomers during gating of the dimeric proton channel complex?
- Where is the permeation pathway? How is selectivity achieved? What is the difference between the open and closed states?
- Which cells express proton channels, and for what purposes?
- Are there other naturally occurring mutations in the HVCN1 sequence that have consequences for its function?
- Can selective inhibitors of HVCN1 be identified? Can inhibitors be targeted to specific tissues? Will these inhibitors ameliorate diseases that might benefit from HVCN1 inhibition?

produces different characteristics for HVCN1 in different species.

Therapeutic implications of targeting HVCN1

As descriptions of HVCN1 involvement in diverse cellular processes proliferate, the potential of HVCN1 as a therapeutic target expands. Beyond the archetypal role of HVCN1 in supporting bactericidal ROS production by phagocytes, recent studies have described a role for HVCN1 in regulating production of ROS intended for signaling. Significantly, in basophils, spermatozoa and airway epithelia, HVCN1 serves functions altogether unrelated to ROS production. Its regulation of histamine release by basophils and the threshold of activation of autoreactive B cells make HVCN1 a desirable therapeutic target in allergic reactions and autoimmune diseases that are characterized by hyperreactive B cells, such as rheumatoid arthritis and systemic lupus erythematosus. For B cells in particular, the potential for HVCN1 inhibitors to impair optimal B-cell activation without complete abrogation of B-cell responses should provide a useful therapeutic window, which avoids the increased susceptibility to infections risked by strong immunosuppression. Some B-cell malignancies have been shown to rely on BCR signaling [79,80]; thus, inhibition of HVCN1 in HVCN1-expressing tumors could help reduce the survival signal that cells derive via engagement of their BCR. An HVCN1 inhibitor introduced into the female reproductive system might block capacitation (activation) of spermatozoa and thus act as a contraceptive. In asthma and cystic fibrosis, inhibition of HVCN1 should ameliorate mucosal acidification and prevent epithelial injury that might contribute to asthma pathogenesis. However, can HVCN1 inhibition be achieved? Its structural similarities to other voltage-gated cation channels raise the possibility that some side-targeting might occur. Even if side-targeting could be avoided, the lack of a crystal structure makes it difficult to predict which residues mediate proton transport and how the conformation could be altered to achieve proton transport inhibition. Identification of an effective inhibitor might be a difficult task, but surely the therapeutic possibilities will be abundant.

Concluding remarks

Interest in voltage-gated proton channels increased greatly after identification of the HVCN1 gene in 2006. The molecule has its own unique features, with perfect selectivity, low conductance, strong modulation by pH and unusual dimeric architecture. Its homology to the VSD of other voltage-gated ion channels makes it a unique model system in which voltage-gating mechanisms can be studied. Many questions remain unanswered about the structure of the channel (Box 2); surely a great boost to HVCN1 research would come from unveiling the crystal structure. In addition to helping to unravel the molecular mechanisms that underlie proton conductance, structural information would probably prove invaluable in the design of HVCN1 inhibitors. These inhibitors, in turn, would help us to understand further HVCN1 functions in cells.

To date, HVCN1 has been shown to regulate neutrophil, basophil and B-cell activation, human sperm capacitation and pH in mucosal airway epithelial cells. As expression in more cells is discovered, new functions will undoubtedly be identified. Furthermore, additional mutations or polymorphisms that alter channel properties might emerge. Exploration of what are now only putative proton channels in other species will provide clues to understanding differences in HVCN1 properties in diverse species. Beyond its intrinsic interest to evolutionary biologists, comparison of a wide range of proteins will illuminate our understanding of how these extraordinary molecules work.

References

1 Thomas, R.C. and Meech, R.W. (1982) Hydrogen ion currents and intracellular pH in depolarized voltage-clamped snail neurones. *Nature* 299, 826–828

2 Byerly, L. *et al.* (1984) Rapidly activating hydrogen ion currents in perfused neurones of the snail, *Lymnaea stagnalis*. *J. Physiol.* 351, 199–216

3 Barish, M.E. and Baud, C. (1984) A voltage-gated hydrogen ion current in the oocyte membrane of the axolotl, *Ambystoma*. *J. Physiol.* 352, 243–263

4 DeCoursey, T.E. (1991) Hydrogen ion currents in rat alveolar epithelial cells. *Biophys. J.* 60, 1243–1253

5 Bernheim, L. *et al.* (1993) A voltage-dependent proton current in cultured human skeletal muscle myotubes. *J. Physiol.* 470, 313–333

6 DeCoursey, T.E. and Cherny, V.V. (1993) Potential, pH, and arachidonate gate hydrogen ion currents in human neutrophils. *Biophys. J.* 65, 1590–1598

7 Demaurex, N. *et al.* (1993) Proton currents in human granulocytes: regulation by membrane potential and intracellular pH. *J. Physiol.* 466, 329–344

8 Ramsey, I.S. *et al.* (2006) A voltage-gated proton-selective channel lacking the pore domain. *Nature* 440, 1213–1216

9 Sasaki, M. *et al.* (2006) A voltage sensor-domain protein is a voltage-gated proton channel. *Science* 312, 589–592

10 Henderson, L.M. *et al.* (1987) The superoxide-generating NADPH oxidase of human neutrophils is electrogenic and associated with an H^+ channel. *Biochem. J.* 246, 325–329

11 Henderson, L.M. *et al.* (1988) Superoxide generation by the electrogenic NADPH oxidase of human neutrophils is limited by the movement of a compensating charge. *Biochem. J.* 255, 285–290

12 Henderson, L.M. *et al.* (1988) 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, 563–567

13 DeCoursey, T.E. *et al.* (2003) The voltage dependence of NADPH oxidase reveals why phagocytes need proton channels. *Nature* 422, 531–534

14 DeCoursey, T.E. (2003) Voltage-gated proton channels and other proton transfer pathways. *Physiol. Rev.* 83, 475–579

15 Cherny, V.V. *et al.* (1995) The voltage-activated hydrogen ion conductance in rat alveolar epithelial cells is determined by the pH gradient. *J. Gen. Physiol.* 105, 861–896

16 DeCoursey, T.E. (2008) Voltage-gated proton channels. *Cell Mol. Life Sci.* 65, 2554–2573

17 Okochi, Y. *et al.* (2009) Voltage-gated proton channel is expressed on phagosomes. *Biochem. Biophys. Res. Commun.* 382, 274–279

18 Schapiro, F.B. and Grinstein, S. (2000) Determinants of the pH of the Golgi complex. *J. Biol. Chem.* 275, 21025–21032

19 Li, S.J. *et al.* (2010) The role and structure of the carboxyl-terminal domain of the human voltage-gated proton channel Hv1. *J. Biol. Chem.* 285, 12047–12054

20 Cherny, V.V. *et al.* (2003) Properties of single voltage-gated proton channels in human eosinophils estimated by noise analysis and by direct measurement. *J. Gen. Physiol.* 121, 615–628

21 DeCoursey, T.E. (2010) Voltage-gated proton channels find their dream job managing the respiratory burst in phagocytes. *Physiology (Bethesda)* 25, 27–40

22 Mahaut-Smith, M.P. (1989) The effect of zinc on calcium and hydrogen ion currents in intact snail neurones *J. Exp. Biol.* 145, 455–464

23 Cherny, V.V. and DeCoursey, T.E. (1999) pH-dependent inhibition of voltage-gated H^+ currents in rat alveolar epithelial cells by Zn^{2+} and other divalent cations. *J. Gen. Physiol.* 114, 819–838

24 Musset, B. *et al.* (2010) Zinc inhibition of monomeric and dimeric proton channels suggests cooperative gating. *J. Physiol.* 588, 1435–1449

25 Musset, B. *et al.* (2010) Oligomerization of the voltage gated proton channel. *Channels (Austin)* 4, 260–265

26 Capasso, M. *et al.* (2010) HVCN1 modulates BCR signal strength via regulation of BCR-dependent generation of reactive oxygen species. *Nat. Immunol.* 11, 265–272

27 Iovannisci, D. *et al.* (2010) Function of the HVCN1 proton channel in airway epithelia and a naturally occurring mutation, M91T. *J. Gen. Physiol.* 136, 35–46

28 DeCoursey, T.E. and Cherny, V.V. (1997) Deuterium isotope effects on permeation and gating of proton channels in rat alveolar epithelium. *J. Gen. Physiol.* 109, 415–434

29 DeCoursey, T.E. and Cherny, V.V. (1998) Temperature dependence of voltage-gated H^+ currents in human neutrophils, rat alveolar epithelial cells, and mammalian phagocytes. *J. Gen. Physiol.* 112, 503–522

30 Kuno, M. *et al.* (2009) Temperature dependence of proton permeation through a voltage-gated proton channel. *J. Gen. Physiol.* 134, 191–205

31 DeCoursey, T.E. and Cherny, V.V. (1994) Voltage-activated hydrogen ion currents. *J. Membr. Biol.* 141, 203–223

32 Lukacs, G.L. *et al.* (1993) Proton conductance of the plasma membrane: properties, regulation, and functional role. *Am. J. Physiol.* 265, C3–14

33 Nagle, J.F. and Morowitz, H.J. (1978) Molecular mechanisms for proton transport in membranes. *Proc. Natl. Acad. Sci. U. S. A.* 75, 298–302

34 Ramsey, I.S. *et al.* (2010) An aqueous H^+ permeation pathway in the voltage-gated proton channel Hv1. *Nat. Struct. Mol. Biol.* 17, 869–875

35 Lee, S.Y. *et al.* (2008) Dimeric subunit stoichiometry of the human voltage-dependent proton channel Hv1. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7692–7695

36 Tombola, F. *et al.* (2008) The voltage-gated proton channel Hv1 has two pores, each controlled by one voltage sensor. *Neuron* 58, 546–556

37 Koch, H.P. *et al.* (2008) Multimeric nature of voltage-gated proton channels. *Proc. Natl. Acad. Sci. U. S. A.* 105, 9111–9116

38 Miller, C. and White, M.M. (1980) A voltage-dependent chloride conductance channel from *Torpedo* electroplax membrane. *Ann. N. Y. Acad. Sci.* 341, 534–551

39 Middleton, R.E. *et al.* (1996) Homodimeric architecture of a ClC-type chloride ion channel. *Nature* 383, 337–340

40 Ludewig, U. *et al.* (1996) Two physically distinct pores in the dimeric ClC-0 chloride channel. *Nature* 383, 340–343

41 Gonzalez, C. *et al.* (2010) Strong cooperativity between subunits in voltage-gated proton channels. *Nat. Struct. Mol. Biol.* 17, 51–56

42 Tombola, F. *et al.* (2010) The opening of the two pores of the Hv1 voltage-gated proton channel is tuned by cooperativity. *Nat. Struct. Mol. Biol.* 17, 44–50

43 Bánfi, B. *et al.* (1999) A novel H^+ conductance in eosinophils: unique characteristics and absence in chronic granulomatous disease. *J. Exp. Med.* 190, 183–194

44 DeCoursey, T.E. *et al.* (2000) Simultaneous activation of NADPH oxidase-related proton and electron currents in human neutrophils. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6885–6889

45 Musset, B. *et al.* (2010) Identification of Thr^{29} as a critical phosphorylation site that activates the human proton channel Hvcn1 in leukocytes. *J. Biol. Chem.* 285, 5117–5121

46 Bankers-Fulbright, J.L. *et al.* (2001) Regulation of human eosinophil NADPH oxidase activity: a central role for PKCδ. *J. Cell Physiol.* 189, 306–315

47 Morgan, D. *et al.* (2007) Sustained activation of proton channels and NADPH oxidase in human eosinophils and murine granulocytes requires PKC but not $cPLA_{2\alpha}$ activity. *J. Physiol.* 579, 327–344

48 Henderson, L.M. and Chappell, J.B. (1992) The NADPH-oxidase-associated H^+ channel is opened by arachidonate. *Biochem. J.* 283, 171–175

49 Murphy, R. and DeCoursey, T.E. (2006) Charge compensation during the phagocyte respiratory burst. *Biochim. Biophys. Acta* 1757, 996–1011

50 Morgan, D. *et al.* (2005) The pH dependence of NADPH oxidase in human eosinophils. *J. Physiol.* 569, 419–431

51 Morgan, D. *et al.* (2009) Voltage-gated proton channels maintain pH in human neutrophils during phagocytosis. *Proc. Natl. Acad. Sci. U. S. A.* 106, 18022–18027

52 Reeves, E.P. *et al.* (2002) Killing activity of neutrophils is mediated through activation of proteases by K^+ flux. *Nature* 416, 291–297

53 Winterbourn, C.C. *et al.* (2006) Modeling the reactions of superoxide and myeloperoxidase in the neutrophil phagosome: implications for microbial killing. *J. Biol. Chem.* 281, 39860–39869

54 Ramsey, I.S. *et al.* (2009) Hv1 proton channels are required for high-level NADPH oxidase-dependent superoxide production during the phagocyte respiratory burst. *Proc. Natl. Acad. Sci. U. S. A.* 106, 7642–7647

55 El Chemaly, A. *et al.* (2010) VSOP/Hv1 proton channels sustain calcium entry, neutrophil migration, and superoxide production by limiting cell depolarization and acidification. *J. Exp. Med.* 207, 129–139

56 Lew, P.D. *et al.* (1981) A variant of chronic granulomatous disease: deficient oxidative metabolism due to a low-affinity NADPH oxidase. *N. Engl. J. Med.* 305, 1329–1333

57 Shurin, S.B. *et al.* (1983) Impaired granulocyte superoxide production and prolongation of the respiratory burst due to a low-affinity NADPH-dependent oxidase. *Blood* 62, 564–571

58 Roos, D. *et al.* (1992) Chronic granulomatous disease with partial deficiency of cytochrome b_{558} and incomplete respiratory burst: variants of the X-linked, cytochrome b_{558}-negative form of the disease. *J. Leukoc. Biol.* 51, 164–171

59 Curnutte, J.T. (1993) Chronic granulomatous disease: the solving of a clinical riddle at the molecular level. *Clin. Immunol. Immunopathol.* 67, S2–15

60 Bu-Ghanim, H.N. *et al.* (1995) Molecular analysis in three cases of $X91^-$ variant chronic granulomatous disease. *Blood* 86, 3575–3582

61 Cherny, V.V. *et al.* (2001) Voltage-gated proton currents in human basophils. *Biologicheskie Membrany* 18, 458–465

62 Musset, B. *et al.* (2008) A pH-stabilizing role of voltage-gated proton channels in IgE-mediated activation of human basophils. *Proc. Natl. Acad. Sci. U. S. A.* 105, 11020–11025

63 Schilling, T. *et al.* (2002) Voltage-activated proton currents in human lymphocytes. *J. Physiol.* 545, 93–105

64 Boyd, R.S. *et al.* (2009) Protein profiling of plasma membranes defines aberrant signaling pathways in mantle cell lymphoma. *Mol. Cell Proteomics* 8, 1501–1515

65 Basso, K. *et al.* (2010) Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. *Blood* 115, 975–984

66 Reth, M. (2002) Hydrogen peroxide as second messenger in lymphocyte activation. *Nat. Immunol.* 3, 1129–1134

67 Woo, H.A. *et al.* (2010) Inactivation of peroxiredoxin I by phosphorylation allows localized H_2O_2 accumulation for cell signaling. *Cell* 140, 517–528

68 Dustin, L.B. *et al.* (1999) Expression of dominant-negative src-homology domain 2-containing protein tyrosine phosphatase-1 results in increased Syk tyrosine kinase activity and B cell activation. *J. Immunol.* 162, 2717–2724

69 Kurosaki, T. (1997) Molecular mechanisms in B cell antigen receptor signaling. *Curr. Opin. Immunol.* 9, 309–318

70 Beitz, L.O. *et al.* (1999) SYK is upstream of phosphoinositide 3-kinase in B cell receptor signaling. *J. Biol. Chem.* 274, 32662–32666

71 Pathak, M.K. and Yi, T. (2001) Sodium stibogluconate is a potent inhibitor of protein tyrosine phosphatases and augments cytokine responses in hemopoietic cell lines. *J. Immunol.* 167, 3391–3397

72 Franke, T.F. *et al.* (1997) PI3K: downstream AKTion blocks apoptosis. *Cell* 88, 435–437

73 Wulff, H. *et al.* (2004) K^+ channel expression during B cell differentiation: implications for immunomodulation and autoimmunity. *J. Immunol.* 173, 776–786

74 Lishko, P.V. *et al.* (2010) Acid extrusion from human spermatozoa is mediated by flagellar voltage-gated proton channel. *Cell* 140, 327–337

75 Yamaguchi, S. *et al.* (2009) Zinc is an essential trace element for spermatogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 106, 10859–10864

76 Florman, H.M. *et al.* (2010) Shedding light on sperm pHertility. *Cell* 140, 310–312

77 Fischer, H. and Widdicombe, J.H. (2006) Mechanisms of acid and base secretion by the airway epithelium. *J. Membr. Biol.* 211, 139–150

78 Cho, D.Y. *et al.* (2009) Proton secretion in freshly excised sinonasal mucosa from asthma and sinusitis patients. *Am. J. Rhinol. Allergy* 23, e10–13

79 Chen, L. *et al.* (2008) SYK-dependent tonic B-cell receptor signaling is a rational treatment target in diffuse large B-cell lymphoma. *Blood* 111, 2230–2237

80 Davis, R.E. *et al.* (2010) Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature* 463, 88–92

81 Holland, S.M. (2010) Chronic granulomatous disease. *Clin. Rev. Allergy Immunol.* 38, 3–10

82 Volkman, D.J. *et al.* (1984) B cell lines as models for inherited phagocytic diseases: abnormal superoxide generation in chronic granulomatous disease and giant granules in Chediak–Higashi syndrome. *J. Immunol.* 133, 3006–3009

83 Meng, T.C. *et al.* (2002) Reversible oxidation and inactivation of protein tyrosine phosphatases *in vivo*. *Mol. Cell* 9, 387–399

84 Finkel, T. (2003) Oxidant signals and oxidative stress. *Curr. Opin. Cell Biol.* 15, 247–254

85 Reth, M. and Brummer, T. (2004) Feedback regulation of lymphocyte signalling. *Nat. Rev. Immunol.* 4, 269–277

86 Cornall, R.J. *et al.* (1998) Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity* 8, 497–508