Perspective

NOX Family NADPH Oxidases: Do They Have Built-in Proton Channels?

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Proton channels allow cells to extrude excess acid with a high efficiency and therefore are one of the important homeostatic mechanisms that maintain the intracellular pH in a range compatible with life. Proton currents are highly expressed in phagocytes where they are thought to contribute to charge and pH compensation during NADPH oxidase activation. By analogy with the cytochrome C oxidase of mitochondria, it therefore has been suggested that the phagocyte NADPH oxidase possesses a built-in proton channel. This concept is supported by a variety of arguments: (a) electrophysiologically and pharmacologically distinct proton currents can be observed in phagocytes upon NADPH oxidase activation and these currents are absent in phagocytes from patients lacking the NADPH oxidase); (b) expression of full-length or COOH-terminal truncated NOX proteins (the gp91^{phox} subunit of the phagocyte NADPH oxidase, or several of its recently cloned homologues) leads in many instances to the appearance of proton currents; and (c) a series of histidines within the third transmembrane domain of NOX proteins could act as "proton wire" and, indeed, mutation of these histidines leads to a loss of H^+ channel function. Importantly, these arguments do not imply that NOX proteins are the ubiquitously expressed H^+ channels, but rather that they contain a built-in, specialized form of H^+ channel. An alternative explanation, not favored, but also not formally excluded by our results, would be that NOX proteins act as channel modulators. Our current working hypothesis is as follows: histidines within the third transmembrane domain of NOX proteins act as a hydrogen-bounded chain (HBC), which slowly reorients within the membrane to form a functional proton channel. Under resting conditions the channel is not functional, because critical histidine residues are bound to heme molecules. Upon heme reduction, the histidines become available to conduct protons, thereby linking electron and proton transfer. To unambiguously establish the proposed channel function of NOX proteins, their isolation and functional reconstitution will be necessary.

Proton Currents, Conductances, and Channels

20 yr ago, Thomas and Meech (1982) described the first voltage-activated proton currents in the plasma membrane of eukaryotic cells, the currents that are the focus of this perspective. These first recordings were performed in neurons from the snail Helix Stagnalis. Since then, similar currents have been observed in a variety of cells from different phyla, including mammalian and human cells (for review see Eder and DeCoursey, 2001). The unique features of the currents led to numerous speculations as to the nature of the underlying transport protein. For years, it was prudently referred to as "proton conductance," before the term "channel" became generally accepted. Like typical ion channels, proton channels do not require ATP or coupling to other ions and exhibit complex gating properties. Unlike typical ion channels however, protons channels are markedly temperature dependent, (Q10 = 10 in the range 24–36°C) (Kuno et al., 1997), nearly perfectly selective for H^+ over other ions ($p_H/p_x > 10^6$), and their unitary conductance, estimated from noise analysis, is in the fS range (Bernheim et al., 1993). These unusual properties, as well as the unique way by which protons can move in water and across membrane proteins, suggest that the underlying transport system is not a water-filled pore, and thus not a channel according to its classical definition. The H^+ transport protein is therefore most likely structurally unrelated to classical ion channels. For this reason, attempts to clone the channel by homology have proven unsuccessful, and the lack of high-affinity pharmacological tools has further hampered their molecular identification. Based on the electrophysiological and pharmacological characteristics, it is likely that there are different types of H^+ channels, not only in different cell types (DeCoursey, 1998), but even within a given cell type (Banfi et al., 1999).

Engineering Proton Channels by the Insertion of Transmembrane Histidines

A simple mechanism of voltage-dependent proton transport has been suggested by histidine-scanning mutagenesis of the *Shaker* K^+ channel. While probing for the accessibility of charged residues within the voltage sensor of the *Shaker* K^+ channel, Starace et al. (1997) have mutated arginine residues within the *Shaker* fourth transmembrane domain (S4) into histidines

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(Starace and Bezanilla, 2001). Histidine was chosen because its protonation can be detected as pH-dependent changes in the gating currents, allowing to test the successive accessibility of the residues to the intracellular and extracellular solvent. Remarkably, voltage-dependent translocation of the histidines produced protons currents that recapitulated several features of voltage-gated proton channels: the currents were voltage activated, pH dependent, highly temperature sensitive, and extremely selective for H^+ ions. The unitary events were below resolution, the models predicting maximal transport rates of 178 ions/s per protein. In the mutated *Shaker* K^+ channel, voltage-gated proton currents could be generated by mutations of any of three residues that are aligned along the axis of an α-helix: R365H, R368H, and R371H. However, the current properties of the three histidine mutants were different. Proton transport by the R365H and R368H mutants required protonation of the residues on one side of the membrane, followed by voltage-dependent translocation and subsequent deprotonation on the other side. These histidine residues were thus able to transport protons across the membrane only in the presence of a pH gradient. In contrast, depolarization of the R371H mutant placed the histidine residue in a position accessible both from the internal or the external side, thereby generating a proton-conducting pore that allowed the continuous flow of protons. The channel then behaved in a similar way to the viral proton channel M2, whose pore contains a histidine residue that provides the strong proton selectivity of the channel (Pinto et al., 1997). The fact that a single histidine mutation can transform the voltage sensor of a K^+ channel into a voltage-gated proton channel suggests that a similar mechanism might be found in naturally occurring proton channels.

Proton Channels and the Phagocyte NADPH Oxidase

The phagocyte NADPH oxidase is a multisubunit enzyme that participates in the killing of microorganisms by phagocytes through the generation of toxic oxygen radicals (Henderson and Chappel, 1996; Babior, 1999). This enzyme is crucial for host defense against microbial pathogens, and patients with chronic granulomatous disease (CGD), who lack a functional oxidase, suffer from severe recurrent infections (Henderson and Chappel, 1996; Babior, 1999). The large membrane-bound subunit of the phagocytic NADPH oxidase, $gp91^{phox}$, allows the flow of electron currents from intracellular NADPH to external oxygen (Schrenzel et al., 1998). Based on the analogy with the mitochondrial NADPH oxidase and on thermodynamic considerations, $gp91^{phox}$ was proposed long ago to act as a proton channel to allow acid extrusion and charge compensation (Henderson et al., 1987). Since the original channel postulate, several lines of evidence have supported the view that $gp91^{phox}$ is an H^+ channel. pH measurements indicated that H^+ channels are closely linked to the oxidase (Kapus et al., 1992), and that their activation requires the assembly of a functional oxidase, but not its redox function (Nanda et al., 1994a). Patch-clamp recordings confirmed the presence of voltage-activated H^+ currents in phagocytic cells (Demaurex et al., 1993a), and revealed that H^+ channel activity develops in parallel with $gp91^{phox}$ during granulocytic differentiation (Qu et al., 1994).

Proton current recordings quickly indicated that $gp91^{phox}$ was not the only proton channel, even in phagocytes. First, proton currents were observed in several cell types that do not express the phagocytic NADPH oxidase, including the original preparation in which proton currents were first described (Thomas and Meech, 1982; for review see DeCoursey, 1998). Second, proton currents were observed in cells from CGD patients lacking the $gp91^{phox}$ subunit (Nanda et al., 1994b). Third, the observed proton currents did not match the properties of the $gp91^{phox}$ channel inferred from pH measurements. At that time, only outward proton currents could be recorded by the patch-clamp technique, whereas pH data consistently showed that $gp91^{phox}$ catalyzed proton influx, in phagocytes as well as in CHO cells expressing full-length or N-truncated $gp91^{phox}$ (Henderson et al., 1995, 1997; Henderson, 1998). For these reasons, most electrophysiologists, including ourselves, thought at the time that voltage-gated proton channels were functionally coupled to, but physically distinct from $gp91^{phox}$ (Demaurex et al., 1993b).

The situation changed when Schrenzel et al. (1998) demonstrated that the electron currents generated by the oxidase could be recorded using the patch-clamp technique. For the first time, activation of the oxidase could be induced in patch-clamped cells during proton current recordings. The effect on the proton currents was dramatic. In the presence of an active oxidase, the threshold of voltage activation was shifted by −50 mV, the current activated faster and deactivated much more slowly, and was blocked by the histidine-reactive agent DEPC (Banfi et al., 1999). The oxidase-associated proton currents were not strictly coupled to electron transport, as they persisted in the presence of the inhibitor DPI or in the absence of the electron acceptor oxygen. However, they were absent in cells from CGD patients lacking either the $gp91^{phox}$ or the $p47^{phox}$ subunit, indicating that channel activation required not only $gp91^{phox}$, but also the assembly of a fully functional oxidase. Cells from CGD patients displayed normal outward proton currents that could be up-regulated by physiological stimuli, confirming that a proton channel distinct from $gp91^{phox}$ was present in phagocytes (Banfi

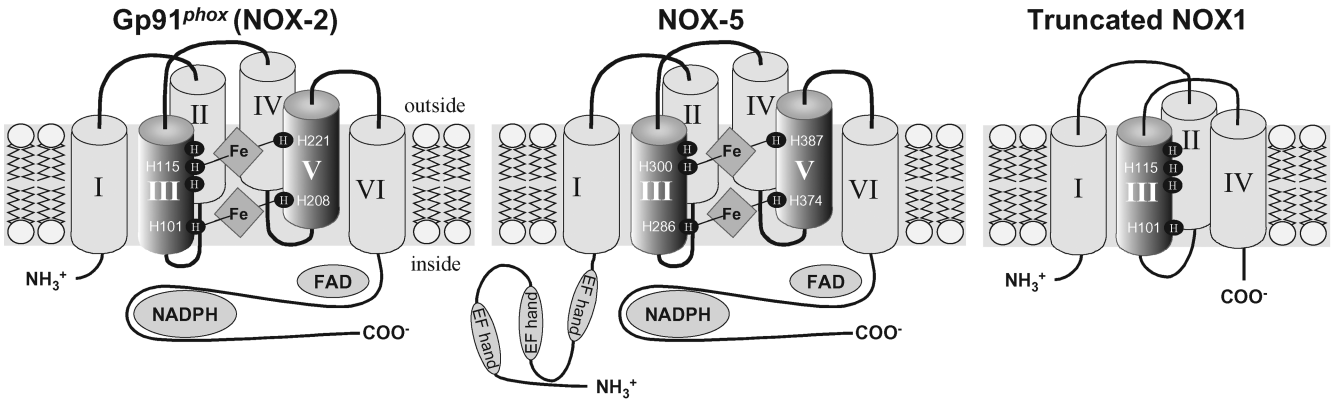

FIGURE 1. Predicted membrane topology and functional domains of NOX proteins associated with proton currents. All proteins contain a string of histidines in the third transmembrane domain and generate H^+ currents when expressed in HEK-293 cells. Currents associated with the truncated NOX-1 have a high threshold of voltage activation. Currents associated with NOX-2/gp91^{phox} isoform have a low threshold of voltage activation, but only when the two hemes are removed from the cytochrome. Currents associated with NOX-5, which contains three EF hands domains, are observed only at high cytosolic calcium concentrations.

et al., 1999). Based on these observations, we concluded that gp91^{phox} was probably the low threshold channel associated with an active oxidase. Accordingly, transfection of full-length or truncated gp91^{phox} was associated with H^+ currents in CHO cells and in HEK-293 cells (Henderson, 1998; Henderson and Meech, 1999; Maturana et al., 2001). The gp91^{phox}-associated currents activated at low voltages and were blocked by histidine reagents, as observed in activated phagocytes, but only when the heme molecules contained in the cytochrome were removed (Maturana et al., 2001). These heme groups are essential for electron transport and are noncovalently coordinated by histidine residues in the third and fifth transmembrane domains (Fig. 1). Mutation of the heme-ligating His 115 residue abolished the H^+ currents, whereas mutations in neighboring His residues strongly reduced the currents (Henderson and Meech, 1999; Maturana et al., 2001). These histidine are aligned along the axis of an α helix (Fig. 1), suggesting that they might function as a "proton wire" as in the mutated *Shaker* K^+ channel (Starace et al., 1997; Starace and Bezanilla, 2001).

NADPH Oxidase Homologues: More Proton Channels?

The recognition that a histidine motif was critical for proton channel activity led us to the cloning of gp91^{phox} homologues. Using this motif as template for a search in EST databases, we found two clones coding for gp91^{phox} homologues (Banfi et al., 2000), which are now members of the growing family of NADPH oxidases (NOX). So far, five NOX isoforms have been identified in different tissues that share similar features as gp91^{phox} (Lambeth, 2002). They contain NADPH and FAD binding regions, and two heme groups anchored by four histidine residues (Fig. 1). NOX1 and NOX4 generate superoxide constitutively, whereas NOX5, which contains three EF-hand motifs, produces superoxide in a Ca^{2+}-dependent manner (Banfi et al., 2001). Consistent with their H^+ channel function, all the NOX isoforms expressed in HEK-293 cells were able to generate voltage-activated H^+ currents (Banfi et al., 2000, 2001; Maturana et al., 2001). Importantly, NOX1S, a truncated form of NOX1 unable to catalyze electron transport but retaining the proton channel histidine motif, was still able to conduct protons. Moreover, NOX5, which bears three EF hand motifs, generated H^+ currents in a Ca^{2+}-dependent manner. Thus, distinct patterns of current activation were observed in NOX transfectants depending on the isoform expressed, whereas the ability to conduct protons correlated with the presence of the histidine motif. These observations strongly suggest that the NOX histidine motif can form a "proton wire" in cell membranes, as in the case of the mutated *Shaker* voltage channel.

Do NOX Proteins Really Have a Built-in H^+ Channel or Do They Rather Modulate H^+ Channels?

While the arguments outlined above strongly suggest that NOX proteins possess a built-in H^+ channel, they do not provide conclusive evidence and several studies from the group of Tom DeCoursey suggest instead that the phagocyte NADPH oxidase is a channel modulator, rather than a channel itself. However, although the results presented in these studies should be taken seriously, the conclusions are not convincing for the following reasons.

(a) Studies showing the presence of normal H^+ currents in a gp91^{phox} deficient cell line (DeCoursey et al., 2001) are not conclusive, because the existence of such gp91^{phox}-independent currents had been known for a long time from studies in CGD patients (Nanda et al., 1994b; Banfi et al., 1999). Compared with blood phago-

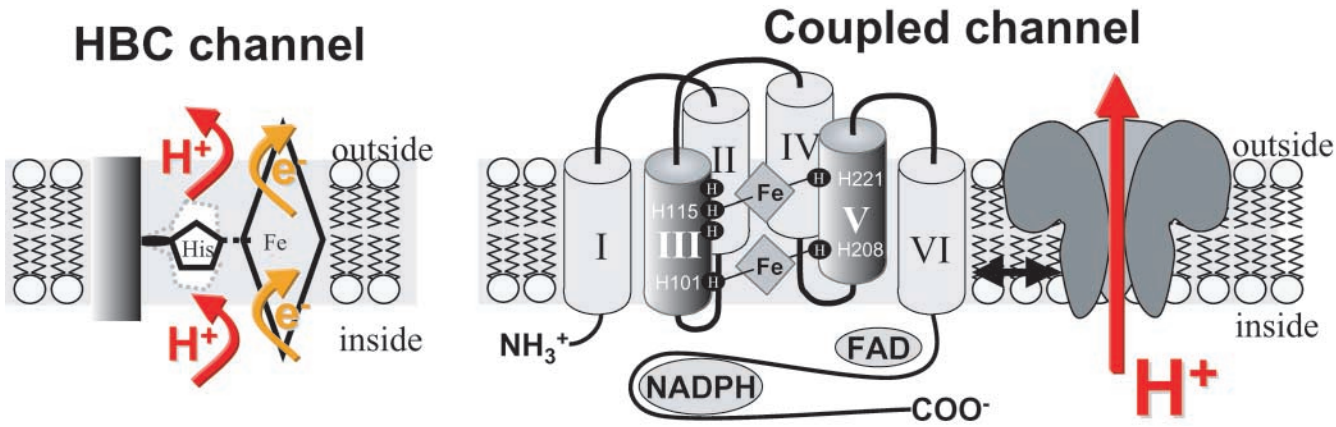

FIGURE 2. Proton conduction by NOX isoforms. We propose that NOX are channels that carry protons through a hydrogen-bonded chain (HBC). The protons flow is limited by the mobility of histidine residues, which act as heme ligands and couple proton and electron transport. DeCoursey et al. postulate that NOX modulates a separate, but closely associated voltage-gated proton channel.

cytes, these cell lines have quite low gp91^{phox} levels and the contribution of gp91^{phox} to the total current is difficult to assess. The authors briefly mention some current measurements in CGD granulocytes, but show only derived data that cannot be interpreted. Interestingly, DeCoursey et al. also observed significant differences in the PMA-activated currents between control and gp91^{phox} -deficient cells, confirming a connection between the NADPH oxidase and proton channels.

(b) Another line of argument against a role of gp91^{phox} as H^+ channel comes from a comparison of the size of electron currents (which reflects NADPH oxidase activity; Schrenzel et al., 1998) and the size of H^+ currents. De Coursey et al. (2001) observed a poor correlation and concluded therefore that oxidase activation modulates a preexisting channel distinct from gp91^{phox}. However, the lack of correlation is difficult to interpret, because the oxidase-associated currents do not require concomitant electron fluxes (Banfi et al., 1999), and only limited tools are available to distinguish the gp91^{phox}-associated H^+ currents from other types of H^+ currents. In our hands, the two most distinguishing features of the gp91^{phox}-associated H^+ currents are their capability to support long-lasting inward currents and the block by DEPC. None of these had been tested in the study of DeCoursey et al. (2001)

(c) Finally, in a recent paper, Morgan et al. (2002) failed to detect voltage-gated proton currents in COS cells stably expressing a functional phagocytic oxidase (COS*phox*; see also Price et al., 2002). A problem in this study is, however, that the authors also failed to detect electron currents in the patched cells. This is surprising, since the arachidonic acid-stimulated superoxide generation of these cells was in the same order of magnitude as that of neutrophils (35 nmol/10^7 cells/min in COS*phox* cells, as compared with 41 nmol/10^7 cells/min in human neutrophils; Price et al., 2002). The authors did also not use any other single cell techniques (e.g., NBT, DCF) to document NADPH oxidase activation under their experimental conditions. Thus, it appears that there was a problem of activation of the NADPH oxidase under patch-clamp conditions, which logically would have prevented detection of gp91^{phox}-associated H^+ currents. Indeed, our studies suggest that under physiological conditions, the assembled NADPH oxidase conducts protons only upon activation (Banfi et al., 1999).

The lack of current in COS-7 cells might also reflect a cell-specific defect that disturbs gp91^{phox} proton channel function without affecting electron transport. In this respect it is interesting to note that upon expression of gp91^{phox} in Cos-7 cells, the predominant species detected in immunoblots was the 65 kD precursor (Yu et al., 1998). In contrast, in HEK-293 cells the size of the expressed gp91^{phox} protein was similar to the one observed in neutrophils (Maturana et al., 2001), indicating that the gp91^{phox} protein is able to mature to its fully glycosylated state in HEK-293, but not in COS-7 cells. Presently, nothing is known about the role of glycosylation in the H^+ channel function of gp91^{phox}. However, our model predicts that NOX proteins conduct protons only when adopting a specific configuration (see below). Interestingly, COS-7 cells are the only cells so far devoid of proton channel activity. Although one might argue that this makes them a clean model for functional expression, this property might also reflect their inability to assemble a functional channel or to provide the appropriate membrane environment to sustain proton transfer.

Whereas we do not favor the model proposed by DeCoursey and colleagues, i.e., activation of a structurally unrelated H^+ channel through NOX proteins, the currently available results do not exclude this possibility. How could such a scenario work? The electrogenic and

acid-generating activity of the NADPH oxidase would be the most obvious explanation for a functional coupling to an unrelated H^+ channel. Indeed, such functional coupling between proton channels and other membrane transporters has been demonstrated previously (Demaurex et al., 1995). However, this possibility can be excluded because truncated forms of gp91^{phox} and NOX1, which are not capable of transporting electrons and generating intracellular acid, have an H^+ channel–inducing activity as potent as full length NADPH oxidases. Thus, protein–protein interaction between the NH_2-terminal and/or transmembrane region of NOX proteins with either the H^+ channel or an intermediate protein would have to be postulated.

A Model of Proton Conduction by NOX Proteins

We thus favor the following model of proton conduction, based on the structures of NOX proteins and the effects of histidine mutations, heme depletion, and oxidase activation (Fig. 2). This model is derived from the mechanism of proton transfer within the viral M2 channel (Tang et al., 2002) and the proton-conductive path in His-mutated *Shaker* (Starace and Bezanilla, 2001). We propose that NOX are H^+ channels that possess a hydrogen-bounded chain (HBC), made up in part by the transmembrane histidine residues that ligate the heme moieties embedded within the cytochrome. During oxidase activation, the heme iron changes from a high-spin hexacoordinated state to a low-spin pentacoordinated state (Doussiere et al., 1996), a transition that increases the affinity of the heme for O2. Two of the heme ligands are histidine residues, one (His 115 in gp91^{phox}) being the central residue in the putative HBC chain (Maturana et al., 2001). Heme release from the His 115 residue both increases the flexibility of the imidazole ring and its accessibility to the solvent pH, thereby favoring proton transfer through the HBC. In this heme-free conformation, the central histidine residue might be accessible to both the internal and external solvent pH, forming a very efficient proton pore as in the artificial *Shaker* proton channel (Starace and Bezanilla, 2001). This is suggested by the effects of the histidine-reagent DEPC, which selectively blocks the gp91^{phox}-associated inward currents in cells lacking heme or with an active oxidase (Banfi et al., 1999; Maturana et al., 2001). The binding of heme thus determines the conductive properties of the NOX channels, and underlies the transitions between currents of resting and activated phagocytes. This model might account for the diverging results obtained in different cellular contexts. Because heme ligation prevents protonation of the critical His 115 residue, the presence of heme might prevent gp91^{phox} channel function in COS-7 cells. Removal of the heme or activation of the oxidase might be required to rescue the channel function in this cell type. Alternatively, the lack of proton currents might reflect structural or conformational defects within the gp91^{phox} HBC chain. To generate voltage-dependent proton currents, the HBC must be properly oriented within the membrane and accessible to the solvent pH. Abnormal maturation or glycosylation of the gp91^{phox} protein might render the channel dysfunctional, as even minor alterations in the protein structure can affect the mobility of the HBC within the membrane or its accessibility to protons.

Concluding Remarks

Because of the redundancy of NOX isoforms and the lack of a clean expression system for functional reconstitution, the question whether NOX are channels or channel modulators remains a point of discussion. What steps should we take now to clarify the channel status of NOX proteins? History has shown that assessing gp91^{phox} channel function can be deceiving, and that patch-clamp recordings, albeit considered the gold standard, can miss clear channel phenotypes. Heterologous expression of putative channel proteins in alien cell lines is also prone to uncertainties, because the specific post-translational modifications required to reconstitute functional channels cannot be guaranteed. The current controversy will undoubtedly stimulate the use of more creative and innovative approaches to investigate the relationship between NOX proteins and proton channels.

REFERENCES

Babior, B.M. 1999. NADPH oxidase: an update. *Blood.* 93:1464–1476.

Banfi, B., A. Maturana, S. Jaconi, S. Arnaudeau, T. Laforge, B. Sinha, E. Ligeti, N. Demaurex, and K.H. Krause. 2000. A mammalian H+ channel generated through alternative splicing of the NADPH oxidase homolog NOH-1. *Science.* 287:138–142.

Banfi, B., G. Molnar, A. Maturana, K. Steger, B. Hegedus, N. Demaurex, and K.H. Krause. 2001. A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes. *J. Biol. Chem.* 276:37594–37601.

Banfi, B., J. Schrenzel, O. Nusse, D.P. Lew, E. Ligeti, K.H. Krause, and N. Demaurex. 1999. A novel H(+) conductance in eosinophils: unique characteristics and absence in chronic granulomatous disease. *J. Exp. Med.* 190:183–194.

Bernheim, L., R.M. Krause, A. Baroffio, M. Hamann, A. Kaelin, and C.R. Bader. 1993. A voltage-dependent proton current in cultured human skeletal muscle myotubes. *J. Physiol.* 470:313–333.

DeCoursey, T.E. 1998. Four varieties of voltage-gated proton channels. *Front. Biosci.* 3:d477–d482.

DeCoursey, T.E., V.V. Cherny, D. Morgan, B.Z. Katz, and M.C. Dinauer. 2001. The gp91phox component of NADPH oxidase is not the voltage-gated proton channel in phagocytes, but it helps. *J. Biol. Chem.* 276:36063–36066.

Demaurex, N., S. Grinstein, M. Jaconi, W. Schlegel, D.P. Lew, and K.H. Krause. 1993a. Proton currents in human granulocytes: regulation by membrane potential and intracellular pH. *J. Physiol.* 466:329–344.

Demaurex, N., J. Orlowski, G. Brisseau, M. Woodside, and S. Grinstein. 1995. The mammalian Na+/H+ antiporters NHE-1, NHE-2,

and NHE-3 are electroneutral and voltage independent, but can couple to an H+ conductance. *J. Gen. Physiol.* 106:85–111.

Demaurex, N., J. Schrenzel, M.E. Jaconi, D.P. Lew, and K.H. Krause. 1993b. Proton channels, plasma membrane potential, and respiratory burst in human neutrophils. *Eur. J. Haematol.* 51: 309–312.

Doussiere, J., J. Gaillard, and P.V. Vignais. 1996. Electron transfer across the O2- generating flavocytochrome b of neutrophils. Evidence for a transition from a low-spin state to a high-spin state of the heme iron component. *Biochemistry.* 35:13400–13410.

Eder, C., and T.E. DeCoursey. 2001. Voltage-gated proton channels in microglia. *Prog. Neurobiol.* 64:277–305.

Henderson, L.M. 1998. Role of histidines identified by mutagenesis in the NADPH oxidase-associated H+ channel. *J. Biol. Chem.* 273: 33216–33223.

Henderson, L.M., G. Banting, and J.B. Chappell. 1995. The arachidonate-activable, NADPH oxidase-associated H+ channel. Evidence that gp91-phox functions as an essential part of the channel. *J. Biol. Chem.* 270:5909–5916.

Henderson, L.M., and J.B. Chappel. 1996. NADPH oxidase of neutrophils. *Biochim. Biophys. Acta.* 1273:87–107.

Henderson, L.M., J.B. Chappell, and O.T. Jones. 1987. The superoxide-generating NADPH oxidase of human neutrophils is electrogenic and associated with an H+ channel. *Biochem. J.* 246:325–329.

Henderson, L.M., and R.W. Meech. 1999. Evidence that the product of the human X-linked CGD gene, gp91-phox, is a voltage-gated H(+) pathway. *J. Gen. Physiol.* 114:771–786.

Henderson, L.M., S. Thomas, G. Banting, and J.B. Chappell. 1997. The arachidonate-activatable, NADPH oxidase-associated H+ channel is contained within the multi-membrane-spanning N-terminal region of gp91-phox. *Biochem. J.* 325:701–705.

Kapus, A., K. Szaszi, and E. Ligeti. 1992. Phorbol 12-myristate 13-acetate activates an electrogenic H(+)-conducting pathway in the membrane of neutrophils. *Biochem. J.* 281:697–701.

Kuno, M., J. Kawawaki, and F. Nakamura. 1997. A highly temperature-sensitive proton current in mouse bone marrow-derived mast cells. *J. Gen. Physiol.* 109:731–740.

Lambeth, J.D. 2002. Nox/Duox family of nicotinamide adenine dinucleotide (phosphate) oxidases. *Curr. Opin. Hematol.* 9:11–17.

Maturana, A., S. Arnaudeau, S. Ryser, B. Banfi, J.P. Hossle, W. Schlegel, K.H. Krause, and N. Demaurex. 2001. Heme histidine ligands within gp91(phox) modulate proton conduction by the phagocyte NADPH oxidase. *J. Biol. Chem.* 276:30277–30284.

Morgan, D., V.V. Cherny, M.O. Price, M.C. Dinauer, and T.E. DeCoursey. 2002. Absence of proton channels in COS-7 cells expressing functional NADPH oxidase components. *J. Gen. Physiol.* 119:571–580.

Nanda, A., J.T. Curnutte, and S. Grinstein. 1994a. Activation of H+ conductance in neutrophils requires assembly of components of the respiratory burst oxidase but not its redox function. *J. Clin. Invest.* 93:1770–1775.

Nanda, A., R. Romanek, J.T. Curnutte, and S. Grinstein. 1994b. Assessment of the contribution of the cytochrome b moiety of the NADPH oxidase to the transmembrane H+ conductance of leukocytes. *J. Biol. Chem.* 269:27280–27285.

Pinto, L.H., G.R. Dieckmann, C.S. Gandhi, C.G. Papworth, J. Braman, M.A. Shaughnessy, J.D. Lear, R.A. Lamb, and W.F. DeGrado. 1997. A functionally defined model for the M2 proton channel of influenza A virus suggests a mechanism for its ion selectivity. *Proc. Natl. Acad. Sci. USA.* 94:11301–11306.

Price, M.O., L.C. McPhail, J.D. Lambeth, C.H. Han, U.G. Knaus, and M.C. Dinauer. 2002. Creation of a genetic system for analysis of the phagocyte respiratory burst: high-level reconstitution of the NADPH oxidase in a nonhematopoietic system. *Blood.* 99: 2653–2661.

Qu, A.Y., A. Nanda, J.T. Curnutte, and S. Grinstein. 1994. Development of a H(+)-selective conductance during granulocytic differentiation of HL-60 cells. *Am. J. Physiol.* 266:C1263–C1270.

Schrenzel, J., L. Serrander, B. Banfi, O. Nusse, R. Fouyouzi, D.P. Lew, N. Demaurex, and K.H. Krause. 1998. Electron currents generated by the human phagocyte NADPH oxidase. *Nature.* 392: 734–737.

Starace, D.M., and F. Bezanilla. 2001. Histidine scanning mutagenesis of basic residues of the S4 segment of the *Shaker* k+ channel. *J. Gen. Physiol.* 117:469–490.

Starace, D.M., E. Stefani, and F. Bezanilla. 1997. Voltage-dependent proton transport by the voltage sensor of the *Shaker* K+ channel. *Neuron.* 19:1319–1327.

Tang, Y., F. Zaitseva, R.A. Lamb, and L.H. Pinto. 2002. The gate of the influenza virus M2 proton channel is formed by a single tryptophan residue. *J. Biol. Chem.* 14:14.

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

Yu, L., M.T. Quinn, A.R. Cross, and M.C. Dinauer. 1998. Gp91(phox) is the heme binding subunit of the superoxide-generating NADPH oxidase. *Proc. Natl. Acad. Sci. USA.* 95:7993–7998.