Review

Structure and function of the human Na$^+$/H$^+$ exchanger isoform 1

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Sodium proton exchangers (NHEs) constitute a large family of polytopic membrane protein transporters found in organisms across all domains of life. They are responsible for the exchange of protons for sodium ions. In archaea, bacteria, yeast and plants they provide increased salt tolerance by removing sodium in exchanger for extracellular protons. In humans they have a host of physiological functions, the most prominent of which is removal of intracellular protons in exchange for extracellular sodium. Human NHE is also involved in heart disease, cell growth and in cell differentiation. NHE's physiological roles and the intriguing pathological consequences of their actions, make them a very important target of structural and functional studies. There are nine isoforms identified to date in humans. This review provides a brief overview of the human NHE's physiological and pathological roles and cellular/tissue distribution, with special attention to the exemplar member NHE1. A summary of our knowledge to date of the structure and function of NHE1 is included focusing on a discussion of the recent discrepancies reported on the topology of NHE1. Finally we discuss a newly discovered relative of the NHE1 isoform, the Na$^+$/Li$^+$ exchanger, focusing on its predicted topology and its potential roles in disease.

Introduction

Sodium proton exchangers (NHEs) are ubiquitous transmembrane (TM) proteins found universally in all organisms. They work by exchanging extracellular sodium or lithium ions for intracellular protons. In lower organisms from archaea to plants, NHEs confer increased salt tolerance allowing organisms to live in environments containing relatively high sodium concentrations. In animal cells, they are linked to a variety of physiological roles with the most important being regulation of intracellular pH and cell volume.

The Na$^+$/H$^+$ exchange system was first identified by Aickin and Thomas in 1977 as a system of intracellular pH (pH$_i$) regulation.[1] Since the first NHE gene, dubbed NHE1, was cloned in 1989

allowing the amino acid sequence to be predicted,[2] many studies of its physiological roles, tissue distribution, cellular localization, expression, molecular mechanisms and molecular structure have been undertaken. To date nine isoforms, hNHE1-9, have been identified in the human NHE (hNHE1) family—hNHE1 being the ubiquitous member known as the 'housekeeping enzyme'. However, despite its uncomplimentary designation, NHE1 has a multitude of important and specific tasks[3-6] and its basic role of maintaining pH$_i$ and cell volume affect cell growth, proliferation, migration and apoptosis. While the diverse physiological roles of NHE1 make it interesting to study, perhaps even more intriguing are NHE1's pathological roles. NHE1 plays important roles in two of the most devastating diseases facing society today, heart disease and cancer. Ischemic heart disease causes damage to the myocardium by a temporary blockage of blood flow to the heart. Tissue oxygen deprivation causes the cell to respire anaerobically resulting in a decrease in pH$_i$ due to build up of metabolic intermediates such as lactic acid and protons. Since pH$_i$ must be kept in a very tight range for cell function and survival, NHE1 corrects the imbalance by removal of intracellular protons. However, this causes an increase in the cellular sodium levels which in turn are corrected by the Na$^+$/Ca^{2+} exchanger acting in reverse transport mode. This causes the intracellular calcium concentration to increase to a critical level triggering a number of detrimental calcium dependent pathways including apoptosis, which result in cell death and tissue damage.[7] Studies in mice and other species have shown that inhibition of NHE1 improves heart tissue recovery after ischemic/reperfusion injury.[7]

NHE1 also has an important role to play in cancer. In some types of cancer, NHE1 is critically involved in pH regulation in tumor cells and is important for the formation of invadopodia, structures allowing tumor cell migration and invasion.[8]

Clinical trials on the use of NHE1 inhibitors in heart disease have been disappointing[9,10] but hopefully detailed structural and functional information will enable the design of improved compounds that will prove beneficial for many patients suffering from cancer or heart disease.

NHE Physiology

The other eight isoforms of human NHE identified to date have different tissue distributions, subcellular localization and expression. NHE2, first discovered in rats,[11] is highly expressed throughout most

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of the intestine,[12] parts of the kidney,[13] and gastric parietal cells, where it is involved in Na⁺ reabsorption, cell longevity, but it is not essential for acidification of the stomach.[14] Additionally, NHE2 has been found in skeletal muscle and testis.[15] NHE3 and NHE4 were discovered simultaneously[16] and although they both are present in the gastrointestinal tract and kidney, NHE3 is more prominent in the intestine whereas hNHE4 is more prominent in the stomach.[16] In the kidney NHE3 is expressed in the apical membrane of the proximal tubule and the thick ascending limb,[17] whereas NHE4 is specifically localized to the basolateral epithelia membrane of the medulla.[12] NHE5 is predominately expressed in the brain[18,19] where the concentration of protons is critical for neuronal signaling. It is thought to have unique regulatory mechanisms from other NHE isoforms; recent studies have shown regulation by endocytosis[20] and binding to the intracellular adapter protein RACK1.[21] NHE5 is also expressed in sperm,[22] where it is important for sperm motility, and in an osteosarcoma cell line.[23] Although less is known about NHE6-9, they are distinct in that they are expressed intracellularly unlike the other isoforms, which are all expressed on the plasma membrane. These isoforms are thought to be involved in maintaining the pH of their respective intracellular compartments.[24]

The evolutionary story of NHEs is intriguing since in addition to the mammalian family of NHEs, archaea, bacteria, yeast, plants and simpler animals all express NHEs. However not all of these NHEs have the same roles or function in the same manner. In lower organisms as well as plants they confer salt tolerance by allowing the organism to flourish in high salt containing environments. Consequently, while the mammalian NHEs are activated in low pH, the NHEs from simpler organisms are activated by high pH. It is also interesting that the stoichiometry of exchange is not conserved: bacteria have an electrogenic 1Na⁺/2H⁺ exchange;[25] yeast, mammals and plants have an electroneutral 1Na⁺/1H⁺ exchange; and an invertebrate NHE has been identified that exchanges 2Na⁺/1H⁺ making it suitable for some divalent cation transport.[26] The specificity of transport is also not equivalent: yeast exchangers can transport Na⁺, K⁺ or Li⁺[27] whereas most others are selective for only Na⁺ or Li⁺. These many differences make the exploration of structure-function relationships increasingly interesting as it is thought that all NHEs share quite similar three dimensional structure or cation coordination sites[28] while possessing a diverse range of functional roles. The differences in cation selectivity cannot be explained solely by different regulatory domains since many of the exchangers lack complex regulatory sites but still have varying properties. Additionally, amino acid sequences of NHEs vary considerably. Within the hNHE family the sequence identity ranges between about 25% and 65%.

NHE Structure, First Insights

The many diverse physiological roles, the curious variability in functional specificity of NHEs in different organisms, as well as the obvious clinical and agricultural applications stemming from being able to manipulate NHE1 activity, have driven structural research. Initial studies tried to determine the membrane topology of NHE1 which is important in the prediction of mechanisms of activation and inhibition, drug binding and in identifying potential pore lining TM helices. The first insight into human NHE1 structure was done with hydropathy analysis using the Kyte-Doolittle algorithm[29]

(Fig. 1). This is a simple yet informative approach. However, NHE1's membrane domain is not simply a hydrophobic anchor that holds it in the membrane; it is a transporter that moves charged hydrophilic molecules across a lipophilic barrier. For this reason hydropathy analysis, which relies on individual amino acid hydrophobicities and a 'window' of a fixed size accounting simply for the neighboring amino acids, is not able to accurately predict polar and charged regions that make up the translocation pore through the membrane. In 2000 the Wakabayashi group tested the hydropathy model further by employing cysteine scanning mutagenesis.[30] This resulted in the generally accepted topology model of NHE1 referred to here as model 1, (Fig. 2) which was widely accepted until very recently when Landau et al., published a computational model based on bioinformatics and protein fold-alignment[31] (model 2, Figs. 3 and 4). Here we compare both models in light of the current functional and biophysical data, and we contrast the advantages and pitfalls of the applied methods.

Before comparing the two NHE1 models it should be noted that in 2005 the full length three dimensional structure of the *Escherichia coli* NHE, ecNhaA was elucidated.[32] This was the first NHE to have its structure solved and has provided some functional clues for NHE1 as well as a 'structural guide' for looking at family members in other organisms. Additionally, there have been nuclear magnetic resonance (NMR) studies on individual putative TM helices of NHE1 taken from the Wakabayashi model. The solution NMR structures have been elucidated for the putative TM segments IV, VII and IX[33-35] while that of TM XI has also been determined (unpublished observation). This detailed structural information allows more informed functional assays as well as the prediction of the molecular mechanism of NHE1 function.

Model 1,[30] (Fig. 2, TM Segments, I–XII)

Initial observations on the topology of the membrane domain were derived from hydropathy analysis such as the Kyte-Doolittle hydropathy algorithm[29] (Fig. 1) and gave varying results. Most of these analyses showed 12 TM segments through the membrane with the amino and carboxyl termini of the protein both being located on the cytoplasmic side. However, varying the parameters within different algorithms led to ambiguities in the prediction of TM segments (not shown). The general model of 12 TM segments was a rough consensus, anchored by the by evidence suggesting that the cytosolic regulatory tail must be internal. However, there was initially no direct evidence for the computer predictions and some who believed that the first TM segment was removed as a signal peptide disputed the 12 TM segment model. Of the NHEs, NHE3,[36] and NHE6,[37] have been shown to have signal peptides. One report[38] suggests NHE1 has a signal peptide though this has not been confirmed and is not widely accepted to be correct. Limited chymotryptic digestion of NHE1 revealed that TMI and TMII are not required for the function of NHE1, while other putative extracellular loops were protected from cleavage.[39] N-linked glycosylation was shown in the first extracellular loop between the putative TMI and TMII supporting an extracellular localization of this region.[40]

The first detailed testing of the topology of NHE1 was by Wakabayashi and colleagues.[30] They constructed a functional 'cysteine-less' NHE1 by mutating all the native cysteines to alanines, then mutating individual cysteines at strategic positions. The
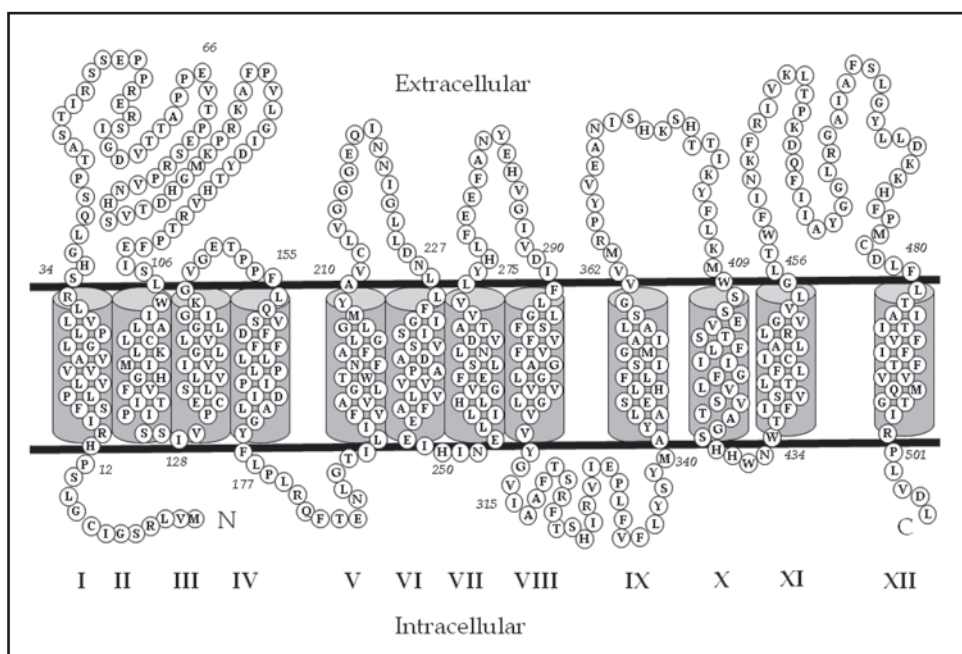
Figure 1. Hydropathy model of NHE1 topology based on the Kyte-Doolittle algorithm.[29,30] The two black bars represent the span of the membrane bilayer; the amino and carboxyl termini are denoted by the large N and C respectively; the Roman numerals denote the putative TM segment number and the small italic numbers indicate amino acid residue numbers. Residues past amino acid number 505 are omitted for clarity.
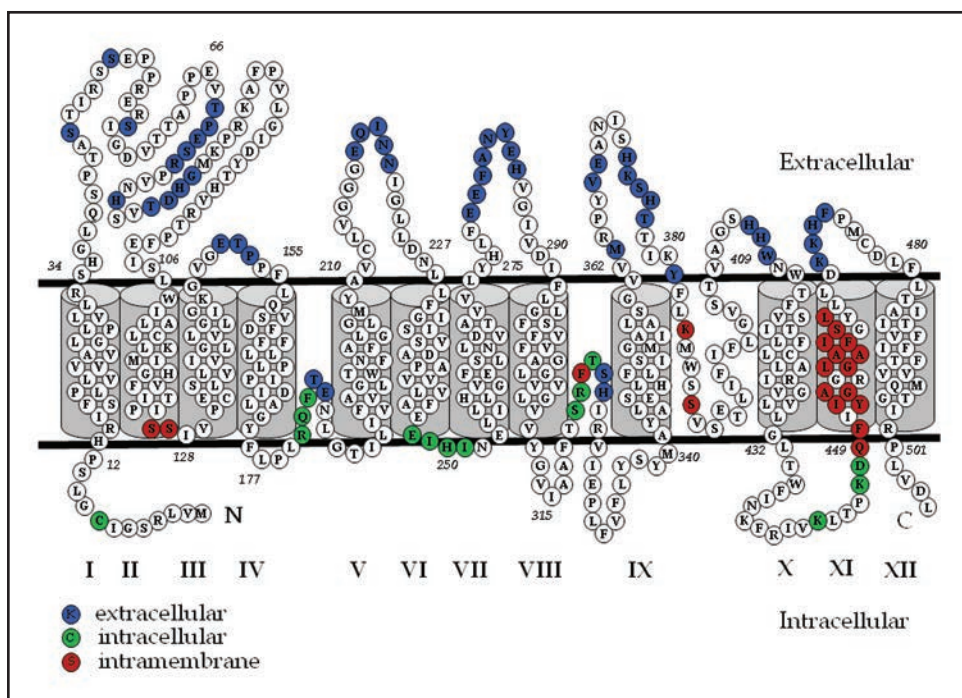

Figure 2. Schematic diagram of the two dimensional topology of the NHE1 isoform of the Na⁺/H⁺ exchanger after Wakabayashi et al.[30] (Model 1). I–XII indicate TM segments, amino and carboxyl termini are denoted by the large N and C respectively and residue numbers are denoted by the small italic numbers. The legend in bottom left corner denotes the accessibility of various residues based on the results of cysteine mutagenesis and chemical modification experiments.[30]

intact and permeabilized cells. In intact cells, residues located on the extracellular surface of the membrane would be labeled; in permeabilized cells residues accessible to the cytosol would also be labeled; residues that remained unlabelled in both intact cells and permeabilized cells were totally inaccessible and therefore 'hidden' within the membrane. Figure 2 illustrates their topology model, referred to here as model 1. It suggests a 12 TM segment model with an internal cytosolic tail. There were two unusual regions. Previously, hydropathy analysis suggested that amino acids 433–477 were extracellular loop number VI. However there were many externally inaccessible amino acids between 449–465 so this segment was proposed to be TM XI. TM X of the hydropathy model (amino acids 386–407) had several amino acids that were inaccessible from the cell exterior and these amino acids were assigned as an intramembrane segment—entering and leaving the membrane from the extracellular side. This solved the otherwise impossible 13 TM topology that would result if amino acids 382–403 were simply turned into a TM segment. The 300 amino acid carboxyl-terminus of NHE1 was located in the cytosol since a large amount of functional data has proven that these amino acids are the site of cellular regulation including studies showing in vivo phosphorylation[41,42] and association with intracellular proteins.[43] Other evidence for the intracellular location of the carboxyl terminus included experiments in which carboxyl-terminal tags required permeabilization of cells for immunoreactivity.[44] Thus a variety of studies supported an intracellular location for the tail.

In model 1 other features include the glycosylation of extracellular loop 1 (amino acids 37–105), which was assigned an extracellular location, and a cysteine close to the amino-terminus (amino acid number 8), which was only labeled in permeabilized cells placing the N-terminus in the cytosol and linking TMI to the rest of the protein. Additionally, since this model was published, extracellular loop 1 has been reported to be involved in differential volume sensing[45] further supporting an extracellular location. Confusingly, two intracellular loops,

mutated cysteines were then tested for either extracellular, intracellular or TM location by labeling with biotin maleimide or MTSET ([2-(Trimethylammonium)Ethyl]Methanethiosulfonate) in both amino acids 176–189 and amino acids 320–330, contained residues that were accessible from outside the cell. To explain this discrepancy they proposed that these loops dipped into the membrane, possibly
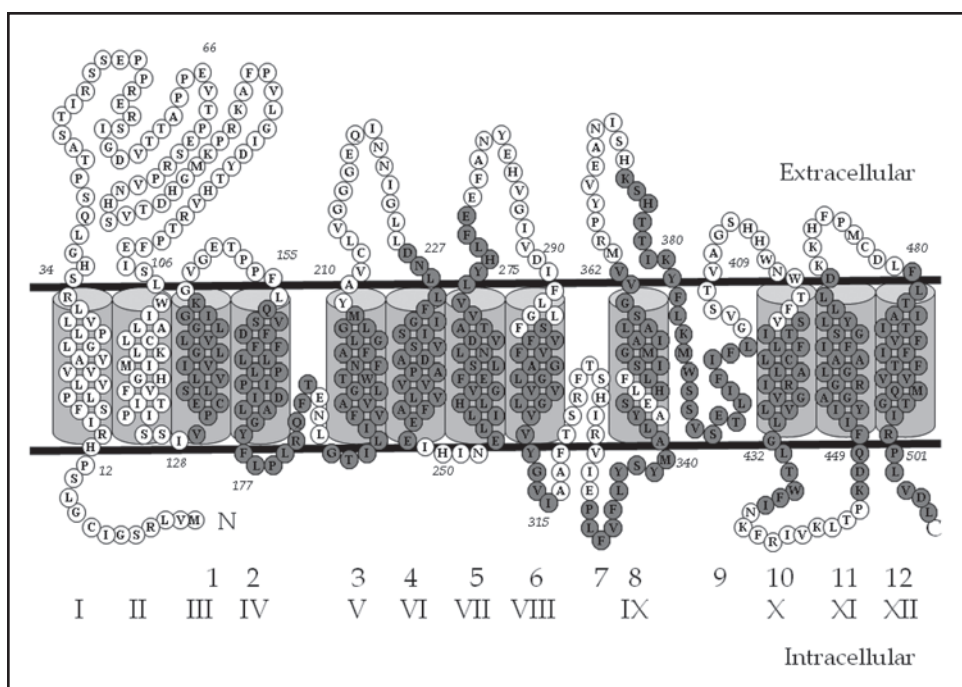
Figure 3. Two dimensional topology of NHE1 isoform of the Na⁺/H⁺ exchanger after Wakabayashi et al.[30] (see Fig. 2) with the residues of the TM segments predicted by Landau et al.[31] shaded gray. TM I–XII segments as described in Figure 2. The TM segments predicted by Landau et al. are labeled 1–12. The amino and carboxyl termini are denoted by the large N and C respectively and residue numbers are denoted by the small italic numbers.
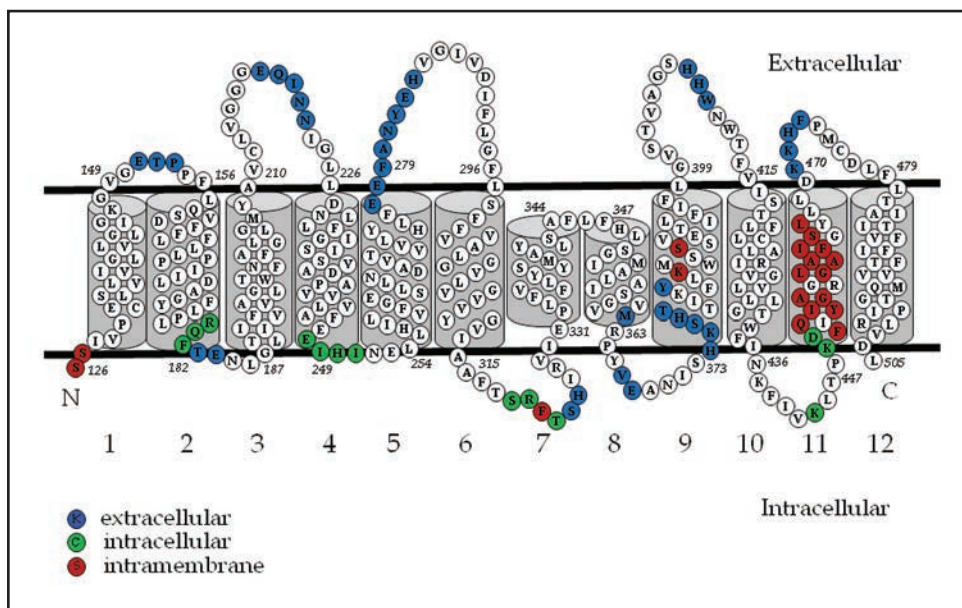

Figure 4. Two dimensional topology of NHE1 isoform of the Na⁺/H⁺ exchanger after Landau et al.[31] (Model 2). TM segments are indicated by 1–12, amino and carboxyl termini are denoted by the large N and C respectively and residue numbers are denoted by the small italic numbers. The legend in bottom left corner denotes the accessibility of various residues based on the results of cysteine mutagenesis and chemical modification experiments.[30]

externally applied modifying reagents. The model was an important step forward in the understanding of the protein's topology and represented a large undertaking with mutation and analysis of many residues of the protein. The assignment of intracellular locations to several residues accessible from the outside was somewhat subjective, but was necessary to bring the model to a logical conclusion.

Model 2,[31] (Figs. 3 and 4 TM Segments 1–12)

A new model recently challenged model 1. Landau et al.,[31] used the crystal structure of *E. coli* NhaA (herein called NhaA) as the starting model for bioinformatics and computational analysis to propose a potential three-dimensional structure and therefore a new topology, referred to as model 2. They used fold alignment algorithms and bioinformatics to determine TM segments of NHE1. It should be noted that NhaA and NHE1 are not very similar, sharing only 10% sequence identity in the membrane domain and have different functional characteristics such as differing stoichiometry. Nonetheless, with the premise that structurally NhaA and NHE1 are similar, NhaA structure was a template on which the NHE1 model structure was constructed.

Figure 3 illustrates their predicted TM segments superimposed on the earlier model and Figure 4 illustrates their model. The two models share many similarities but also have important differences. Firstly, the initial two TM segments are missing in model 2. This region of NHE1 has been the subject of much debate. There is evidence that TM I is actually a signal peptide,[38] TM I and II are not functionally important based on chymotryptic digestions,[39] there is high sequence variability in this region between NHEs, and the first two TM segments of NhaA are important for activity.[32] Thus, Landau et al. suggested that TM I and II are not part of the canonical 12 TM segments, though they may exist as an additional two TM segments. Also the first intracellular, intramembrane segment (amino acids 177–188) were changed to be part of TM 2.

It is of interest to note that quite a few of the residues that were shown to be extracellular by cysteine accessibility studies, are now predicted to be intracellular or intramembrane in model 2 (Fig. 4).

TM III–VIII of model 1, and TM 1–6 of model 2 are essentially equivalent in topology (Fig. 3). Important differences in the models

composing part of the pore structure. Therefore, these regions could be placed on the inside of the membrane and yet were accessible outside the cell. This paper provided convincing arguments to explain some of the disputes about the amino-terminal region—all mutated residues in the first extracellular loop were modified only by

are downstream of TM VIII (TM 6, model 2). TM IX of model 1 (amino acids 338–360) is modeled as part of two smaller segments TM 7 (amino acids 331–344) and TM 8 (amino acids 349–362, Figs. 3 and 4). Model 1 earlier proposed an intramembrane segment from amino acids 380–405 to account for lack of external accessibility of some amino acids within this segment. Part of this segment (amino acids 374–398) now becomes TM 9 in model 2. Model 2, like model 1, suggests that some extracellular accessible amino acids have intracellular location. This includes amino acids, 183, 184, 324, 325, 367 and 368. The reasoning is again that they were near or in the pore. Amino acids 373–377 were accessible to extracellular reagents when mutated to cysteine[30] and were placed in an extracellular location in model 1, but in model 2 they are placed intracellular or within TM 9.

An examination of the NMR structures of some of the TM segments of human NHE1 provides some support for either model. TM IV of NHE1 (amino acids 155–180) was shown to have an extended region in the middle of the segment and only residues 169–176 were α-helical.[33] Despite a lack of significant amino acid identity with TM IV of NhaA, there was a lot of structural similarity between these TM segments.[46] However, in model 2 amino acids 226–248 are proposed to form TM4 which contradicts the structural alignment based on the determined NMR structure of amino acids 155–180. Therefore, the structural similarity between TM IV of NhaA and TM IV of model 1 provides support for the topological predictions of model 1.

Other available structural data seems to support model 2. The structure of amino acids 339–363, TM IX of model 1, was that of a kinked peptide with helices at either end.[35] These amino acids were predicted to be TM IX in model 1, but were predicted to be part of TM segments 7 and 8 of model 2. Their structure is more similar to that of part of TM 7 and 8 with a link in between. In addition, the same study[35] showed that Ser[351] is a pore lining residue which is in agreement with model 2's predictions.

Landau et al.[31] presented other bioinformatics and biochemical evidence for their model. Most relevant for comparison to model 1, the cumulative mutagenesis data has been superimposed on model 2. This showed that many residues implicated in ion translocation are located close together and in the pore, residues shown to affect drug binding are clustered together, though they are not close in the primary structure. Additionally, residues shown to have no effect on NHE activity are located on the fringes of the structure. This provided compelling data to support model 2. Also, titratable residues involved in ion translocation are located in the pore of the model, where they are located in NhaA, giving further suggestion that the TM segments are accurately arranged.

Opposing model 2 is a variety of direct biochemical evidence, which is especially strong in the amino terminal half of NHE1. In their initial experiments, Wakabayashi et al.,[30] demonstrated that the first extracellular loop from amino acids 40–82 had a large number of externally accessible residues. Additionally, N-linked glycosylation has been demonstrated to occur on mature NHE1,[47,48] and is localized to amino acid 75 of this region. Thus it seems inconceivable that this domain is not found in an extracellular location in the mature protein.

Equipped with these two models and all their supporting and refuting evidence, an interesting picture of NHE1 emerges. Model 1

comes from a historical point of view supported by direct and indirect experimental evidence, but also carries some assumptions from the hydropathy model about the number of TM segments and is limited by the fact that not all of the residues in NHE1 were tested. Model 2 uses a new approach that relies on the most up-to-date three-dimensional structure information available and bioinformatics to compose a three-dimensional model of NHE1, yet it still assumes 12 TM segments and structural similarity to NhaA, which may or may not be the case. Since model 1 is only two-dimensional it cannot be completely evaluated and scrutinized like model 2. Based solely on the biochemical data, there are regions of both topology models that have some validity. In a three dimensional view, model 2 does not explain all the current NHE1 data but it does explain a significant amount. The NMR data evaluating the structure of NHE1 TM segments cannot alone refute either model but suggests that model 2 offers a better view of the latter portion of the TM segments (7–12) whereas model 1 may be more accurate in the first TM segments, especially in the region of TM IV and the first extracellular loop. More NMR data of other TM segments, and/or the three dimensional structure of the full length NHE1 membrane domain will be required to resolve the ambiguities. Nonetheless, these two models provide researchers with fodder for future studies in order to better understand structure-function relationships in NHE1.

Model 3, The Na⁺/Li⁺ Exchanger, A Partially Inverted NHE1? (Fig. 5)

It has long been known that Na⁺/Li⁺ exchange is a property of erythrocytes and other cell types. It occurs with a stoichiometry of 1:1 similar to NHE1.[49] This activity has been shown to be elevated in essential hypertension and in diabetic nephropathy.[50-54] Na⁺/Li⁺ exchange has similar characteristics to Na⁺/H⁺ exchange though it has notable differences in that it is insensitive to inhibitors of NHE1.[55,56] Genetic analysis suggests that NHE1 is not a direct candidate for essential hypertension,[57] though this does not preclude an involvement of regulators involved in hypertension that affect Na⁺/H⁺ exchange function.

Recently, it was shown that the NHE1 transcript can be alternatively spliced to yield a protein with Na⁺/Li⁺ exchange capability and which was incapable of Na⁺/H⁺ exchange.[56] The splicing event results in removal of nucleotides 809–1105, corresponding to amino acids Val[78]-Phe[176], which includes TM segments II–IV of model 1 or TM segments 1 and 2 of model 2. TM IV is known to be involved in mediating sensitivity to inhibition by amiloride analogues and other compounds.[46,56] Its absence may account for the resistance of the Na⁺/Li⁺ exchanger to inhibition. How the NHE1 protein mediates a Na⁺/Li⁺ exchange as opposed to Na⁺/H⁺ exchange remains unknown and would be of keen interest in investigations as to mechanisms of cation coordination and transport. It is not yet clear whether this protein is the sole mediator of Na⁺/Li⁺ exchange in cells and in fact, recently[58] another candidate Na⁺/H⁺ exchanger protein has been discovered that can mediate Na⁺/Li⁺ exchange.

The topology of the Na⁺/Li⁺ exchanger splice variant of NHE1 has not been addressed in detail. As mentioned above, three TM segments, amino acids Val[78]-Phe[176] are deleted in the splice variant. The original model presented by Zerbini et al.[56] implied that the balance of the transmembrane segments folded, such that the cytosolic tail remained intracellular. However, details were not
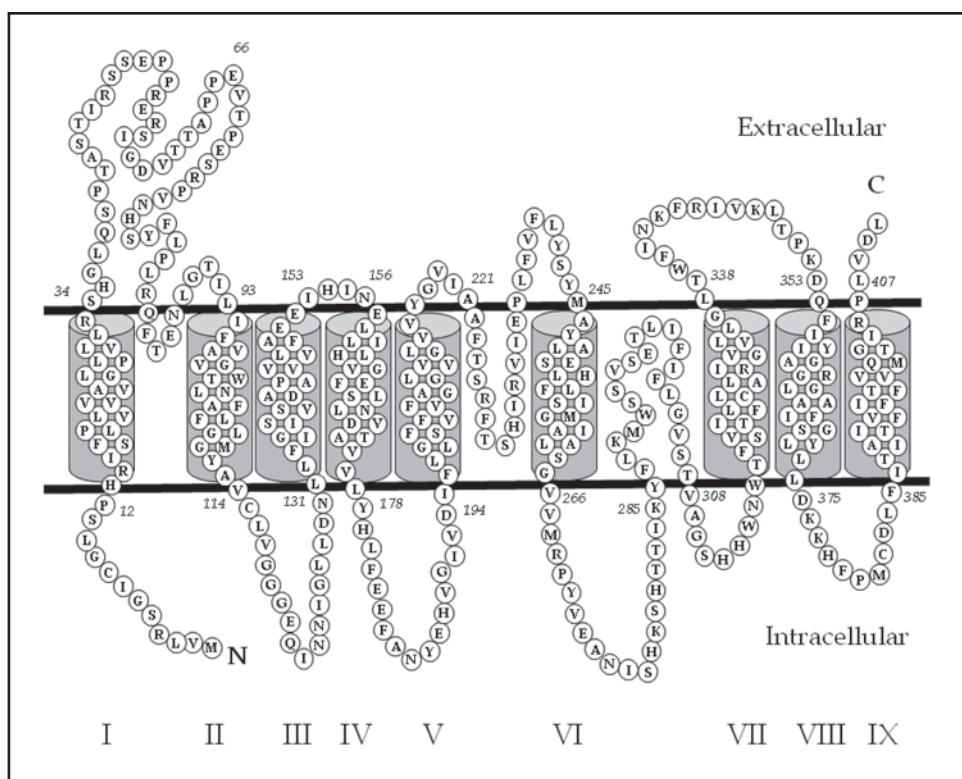
Figure 5. Hypothetical model of two dimensional topology of splice variant of the NHE1 isoform of the Na⁺/H⁺ exchanger after Wakabayashi et al.[30] with amino acids Val[78]-Phe[176] removed as indicated by Zerbini et al.[56] TM segments are indicated by I–IX, amino and carboxyl termini are denoted by the large N and C respectively and residue numbers are denoted by the small italic numbers.
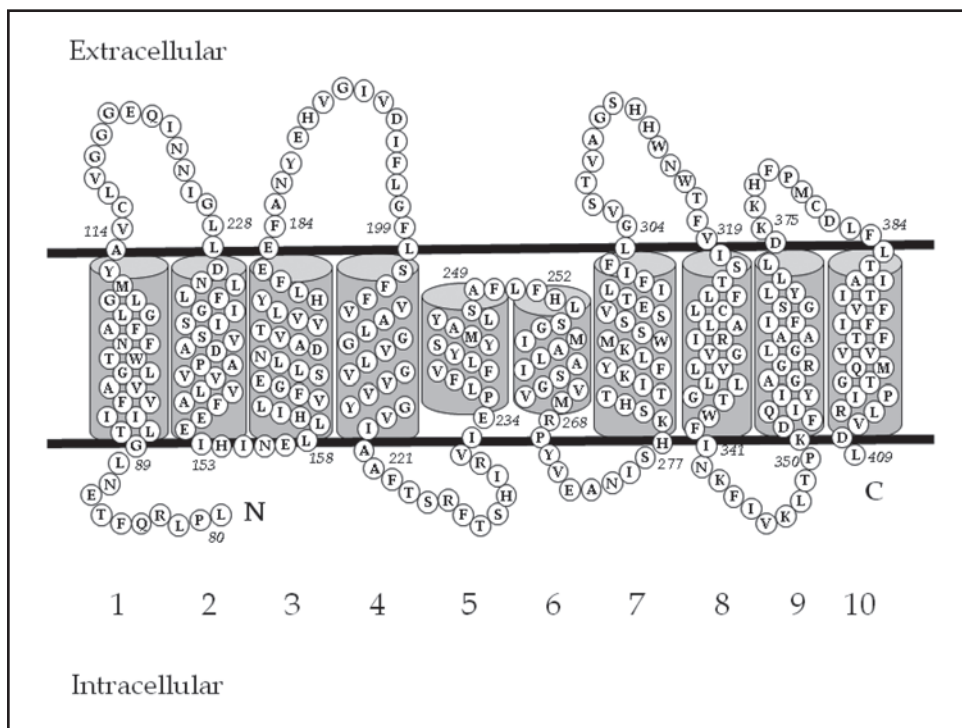

Figure 6. Hypothetical model of two dimensional topology of splice variant of the NHE1 isoform of the Na⁺/H⁺ exchanger after Landau et al.[31] with amino acids Val[78]-Phe[176] removed as indicated by Zerbini et al.[56] TM segments are indicated by 1–10, amino and carboxyl termini are denoted by the large N and C respectively and residue numbers are denoted by the small italic numbers.

provided. In model 1 of NHE1 topology (Fig. 2), amino acids Val[78]-Phe[176] traverse the membrane three times, while in model 2, (Figs. 3 and 4) deletion of this segment removes 2 TM segments. Removal of 3 TM segments leaves the protein with an uneven number of TM segments and the balance of the topology of the protein following amino acid Leu[177] (or Leu[78] of the Na⁺/Li⁺ exchanger) should be reversed in its topology. This results in a hypothetical topology with a Na⁺/H⁺ exchanger with a regulatory, and previously cytosolic tail, that is now external to the cell. Figure 5 illustrates this hypothetical topology, which is a new presentation of this putative structure of the protein.

Is there any evidence that could support the existence of a NHE1 gene transcript with a tail external to the cell surface? Surprisingly the answer is yes. An earlier report showed that the carboxyl terminal of NHE1 had an extracellular epitope.[59] Interestingly, this was found in red blood cells that contain significantly high Na⁺/Li⁺ exchanger activity. Another group also reported that the NHE3 isoform of the Na⁺/H⁺ exchanger also has an exoplasmic tail, though it is not known if it also has a similar splice variant of the NHE3 message. We hypothesize that the splicing of NHE1 could explain the presence of an extracellular C-terminal epitope. The deletion of an odd number of TM segments results in a protein with a topology that is mostly inverted when compared with the unsliced protein. This unusual arrangement would mean that in the splice variant form of the protein, a single early TM segment associates with other TM segments with an inverted orientation. While this arrangement seems unusual, it should be remembered that Na⁺/H⁺ exchanger activity is reversible, so that with alterations of cation coordination sites, a reversal of direction by the balance of the protein is not unreasonable. In addition, the first TM segment of NHE1 is usually regarded as more of a targeting signal, rather than a mediator of cation transport.[38] This leaves the balance of the protein intact for cation transport.

While model 1 predicts an interesting phenomenon, if model 2 turns out to be closer to the correct structure of the NHE1 protein, the splicing event removes TM segments 3 and 4 and leaves a 10 TM segment protein with an intracellular tail

(Fig. 6). It will be interesting to see which of these turns out to be correct.

Because of the lack of a clear physiological role for Na⁺-Li⁺ exchange, there has been less impetus for study of this protein compared to that for the intact NHE1 protein. Nevertheless, it provides an interesting model of cation binding and transport, and possibly, an unusual model of splicing that leads to an inverted functional protein with an altered topology compared with the unsliced protein.

Concluding Remarks

The NHE1 isoform of the Na⁺/H⁺ exchanger plays a key role in the regulation of intracellular pH and is important in cell growth and differentiation. It is important in the etiology of several diseases including cancer, and in various types of heart disease. There is recently some controversy on the fundamental structure of the protein. Two differing topological models of NHE1 have emerged. It is surprising that at this late stage in the study of the protein, even such a basic understanding of the protein's topology is in doubt. The controversy underscores the need for further studies elucidating the structure of the protein. Adding to the mystique of NHE physiology, the NHE1 transcript appears to be spliced to form an unusual cation transporter with distinct specificity and characteristics from the normal protein. We present topological models of this protein, which could suggest an interesting phenomenon in membrane protein transport.

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