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Review

Tethering, recycling and activation of the epithelial sodium–proton exchanger, NHE3

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Summary

NHE3 is a sodium–proton exchanger expressed predominantly in the apical membrane of renal and intestinal epithelia, where it plays a key role in salt and fluid absorption and pH homeostasis. It performs these functions through the exchange of luminal sodium for cytosolic protons. Acute regulation of NHE3 function is mediated by altering the total number of exchangers in the plasma membrane as well as their individual activity. Traffic between endomembrane and plasmalemmal pools of NHE3 dictates the density of exchangers available at the cell surface. The activity of the plasmalemmal pool, however, is not fixed and can be altered by the association with modifier proteins, by post-translational alterations (such as cAMP-mediated phosphorylation) and possibly also *via* interaction with specific plasmalemmal phospholipids. Interestingly, association with cytoskeletal components affects both levels of regulation, tethering NHE3 molecules at the surface and altering their intrinsic activity. This paper reviews the role of proteins and lipids in the modulation of NHE3 function.

Introduction

Acid–base balance is regulated both at the cellular and whole organism levels. Specialized systems for the elimination of CO_2/HCO_3^- and other H^+ equivalents control systemic pH. At the cellular and subcellular levels, homeostasis is maintained by a fine balance between net acid generation and extrusion. In mammalian cells, H^+ (equivalents) generated metabolically are extruded by two main pathways: (1) through the efflux of organic acids (e.g. lactate) *via* H^+ symporters, and (2) in exchange for extracellular Na^+ by an antiport mechanism. The entities responsible for the latter process are known as Na^+/H^+ exchangers (NHEs). Na^+/H^+ exchange provides a robust, electroneutral pathway for net acid extrusion by utilizing the energy inherent to the inward Na^+ concentration gradient generated by the sodium/potassium-ATPase (Na^+/K^+-ATPase) (Bobulescu and Moe, 2006; Brett et al., 2005; Donowitz and Li, 2007; Fliegel, 2008; Malo and Fliegel, 2006; Orlowski and Grinstein, 2007; Slepkov et al., 2007).

In addition to their key role in pH homeostasis, it has recently become apparent that NHEs serve many other functions: they are instrumental to Na^+ (re)absorption in the renal and gastrointestinal tracts; they play an important role in cell volume regulation, particularly in guarding cells against shrinkage; they seem to be stringently required for macropinocytosis; and they have been proposed to be involved in cell migration. This functional versatility stems in part from the evolutionary divergence of a primordial NHE into multiple isoforms with unique properties and specialized functions (Brett et al., 2005; Orlowski and Grinstein, 2004).

NHE isoforms

There are nine known NHE isoforms in the mammalian genome (Brett et al., 2005; Orlowski and Grinstein, 2004). These can be grossly subdivided into two groups: those that reside and function predominantly in the plasma membrane (NHE1–5) and those that are found largely or exclusively in endomembrane organelles (NHE6–9). The latter can in turn be subdivided into two separate phylogenetic clades: NHE6, 7 and 9, which are related to the yeast NHX1 (sodium–proton exchanger; non-mammalian) and reside in the endosomal/TGN (trans-Golgi network) pathway; and NHE8 that has orthologues in *Dictyostelium*, worms and flies, and in mammals is found both in endomembranes and on the apical membrane of certain epithelia (Brett et al., 2005; Orlowski and Grinstein, 2004; Slepkov et al., 2007). In addition, a distinct type of NHEs related to the bacterial NhaA and yeast NHA1 (sodium–proton antiporter) was recently identified also in the mammalian genome (Brett et al., 2005; Xiang et al., 2007). Like the transporters of lower organisms, the mammalian paralogues NHA1 and NHA2 are likely to be electrogenic (see below) and may have unique functions, although remarkably little is known about them at present.

The plasmalemmal NHEs have varied tissue distribution and function. Some, like the ubiquitously expressed NHE1, are restricted to the plasma membrane and serve housekeeping functions such as pH homeostasis and cell volume regulation (Bobulescu and Moe, 2006; Fliegel, 2008; Orlowski and Grinstein, 2007; Slepkov et al., 2007). Others, like the apical epithelial isoform NHE3, can cycle between the surface membrane and intracellular compartments and have highly specialized functions including trans-epithelial Na^+ transport and systemic acid–base homeostasis (Bobulescu and Moe, 2006; Donowitz and Li, 2007). The unique tissue distribution of NHE3 (detailed below) implicates it in critical physiological processes like the control of systemic volume and pH. In addition, its specific subcellular localization confers complexity to its regulation, facilitating dynamic alterations in function. Because of its distinguishing features and its functional importance we have chosen to make NHE3 the focus of this review.

Tissue distribution

NHE3 is found almost exclusively in the epithelia of renal and gastrointestinal tissue (Brant et al., 1995; Orlowski et al., 1992; Tse et al., 1992), although there is some suggestion that a low level of expression also occurs in human thymus, prostate, testis and ovary (Brant et al., 1995). Renal expression of NHE3 occurs in distinct segments of the nephron and is exclusive to the luminal side of tubular epithelial cells (Bobulescu et al., 2005a). Specifically, NHE3 is located in the proximal tubule and the thick descending limb of the loop of Henle (Biemesderfer et al., 1997). Within the gastrointestinal tract, NHE3 is more widely distributed. It can be found in epithelia from stomach, small intestine and large intestine. It is found throughout all segments of the colon but its expression is limited to the jejunum of the small intestine and the corpus and antrum of the stomach (Orlowski et al., 1992; Tse et al., 1992).

NHE3 structure

NHE3 shares with the other NHE isoforms a bipartite structure: an N-terminal, 12 pass transmembrane domain (residues 1–454 in the case of NHE3), followed by a relatively unstructured cytosolic C-terminal domain (residues 455–831) (Fig. 1). The transmembrane domain mediates ion exchange while the cytosolic C-terminus regulates activity and interacts with the cytoskeleton and other ancillary molecules. In contrast to most other isoforms, significant empirical evidence exists to support this structure. The addition of epitopes to NHE3 confirmed that the first loop, linking transmembrane helices 1 and 2, is extracellular (Kurashima et al., 1998). A similar approach confirmed that the cytosolic C-terminus is intracellular (Kurashima et al., 1998). Although this proposed structure for NHE3 is widely accepted (Donowitz et al., 2005; Orlowski and Grinstein, 2004; Weinman et al., 2005; Zachos et al., 2005), there is some conflicting evidence suggesting that epitopes within the cytosolic C-terminus may be exposed to the extracellular milieu (Biemesderfer et al., 1998), and a single *in vitro* analysis suggests that NHE3 may contain a N-terminal signal sequence which is cleaved, leaving only 11 transmembrane domains (Zizak et al., 2000).

Mutational analysis of, and comparisons to, NHE1 have implicated transmembrane (TM) domains IV and VII of NHE3 in ion transport and TM domain IX as the site of binding to amiloride analogues (Orlowski and Kandasamy, 1996; Slepkov et al., 2005). Experiments utilizing chimeric and truncation mutants enabled the localization of different regulatory domains within the C-terminus. These include sites responsible for cAMP-mediated inhibition, a calcineurin homologous protein (CHP)-binding site and regions responsible for phorbol ester and okadaic acid-mediated inhibition (Cabado et al., 1996; Levine et al., 1995; Wakabayashi et al., 1995). Similarly, by mutating specific residues in the C-terminus it was shown that both ezrin and NHERF-1/2 (sodium–hydrogen exchanger regulatory factor) bind to this section of NHE3 (Cha et al., 2006; Weinman et al., 2003b).

In contrast to NHE1 (Counillon et al., 1994), NHE3 is generally thought not to be glycosylated in most systems (Orlowski and Grinstein, 2004). Rabbit renal brush border membranes and porcine LLCPK cells appear to be exceptions, because experiments utilizing inhibitors of glycosylation in combination with SDS-PAGE analysis suggested that, in these systems, NHE3 is glycosylated. Moreover, in these studies inhibition of glycosylation prevented the apical expression of NHE3 (Bizal et al., 1996; Soleimani et al., 1996). Canine, rat, human and opossum NHE3, which are seemingly not glycosylated, must possess other apical targeting motifs (Bizal et al., 1996; Durkan et al., 2007). Finally, as with NHE1 (Fuster et al., 2008; Hisamitsu et al., 2006; Hisamitsu et al., 2004), the functional unit of NHE3 in the apical plasma membrane is likely to be a homodimer or possibly a higher order oligomer (Fafournoux et al., 1994).

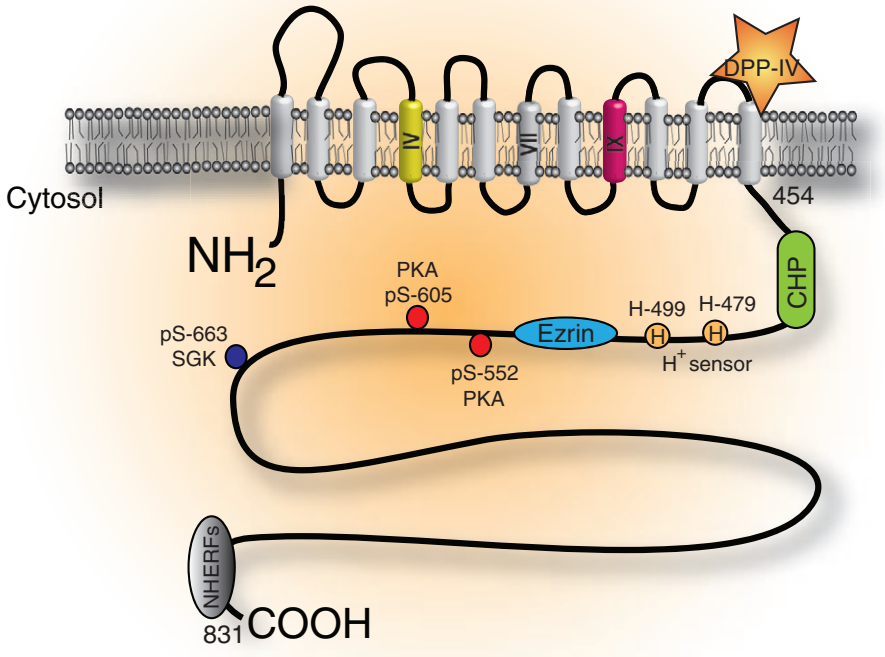

Fig. 1. The structure of NHE3 is divided into 12 transmembrane domains (residues 1–454) and a large cytosolic C-terminal domain (residues 455–831). The transmembrane domains implicated in ion transport are depicted in yellow and the domain responsible for inhibition by amiloride is depicted in pink (transmembrane domain IX). The diagram also indicates the putative binding sites for CHP (calcineurin homologous protein), ezrin and the NHERFs (sodium–hydrogen exchanger regulatory factor), the proton modifier sites (H^+ sensor) and the sites that are phosphorylated by either protein kinase A (PKA) and serum and glucocorticoid kinase (SGK). DPP, dipeptidyl peptidase.

Function

NHE3 mediates the exchange of extracellular Na^+ for intracellular H^+ with a stoichiometry of 1:1. Sodium–proton exchange through NHE3 is therefore electroneutral. The driving force of this cross-membrane exchange is the large inward gradient of Na^+ generated by the Na^+/K^+-ATPase. Consequently the exchanger is secondarily active. NHE3 is capable of functioning in reverse (exchanging intracellular Na^+ for extracellular H^+) if the chemical gradients are inverted (Alexander et al., 2007; Wakabayashi et al., 2003). Studies where the extracellular concentration of Na^+ was varied revealed a Hill coefficient $\approx$1, suggesting a single extracellular Na^+-binding site (Levine et al., 1993). NHE3 preferentially exchanges Na^+ for H^+, although extracellular lithium and H^+ will compete for the Na^+-binding site at comparatively high concentrations. Kinetic analysis of cytosolic H^+ binding revealed a more complex picture (Levine et al., 1993). The Hill coefficient with respect to internal H^+ is approximately 2, indicating cooperativity in the activation of NHE3 by H^+. Based on this and other observations, the existence of two intracellular H^+-binding sites has been postulated: one is presumably the substrate or H^+-transport site whereas the other is an allosteric modifier site that dictates the 'set point' at which the exchanger becomes acutely activated. Above the set point Na^+/H^+ exchange is downregulated, preventing excessive alkalinization of the cytosol. Mutational analysis suggested that the allosteric modifier activity depends on two histidine residues in the juxta-membrane region of the cytosolic C-terminus of the exchanger (Cha et al., 2003).

Hormonal regulation

Several hormones modulate the activity of NHE3. The best-described mechanism involves parathyroid hormone (PTH) (Azarani et al., 1995; Azarani et al., 1996). Engagement of the PTH receptor raises intracellular cAMP levels thereby activating protein kinase A (PKA), which phosphorylates NHE3 at serines 552 and 605, a mechanism dependent on both ezrin and the NHERF (see below). This ultimately leads to inhibition of NHE3 activity (Collazo et al., 2000). Dopamine also inhibits NHE3 activity. Engagement of the dopaminergic receptor in the proximal tubule activates PKA- and protein kinase C (PKC)-dependent pathways, both of which are thought to be necessary for the endocytosis of NHE3 and the consequent decrease in its activity (Bacic et al., 2003; Gomes and Soares-da-Silva, 2004; Hu et al., 2001; Pedrosa et al., 2004; Wiederkehr et al., 2001).

By contrast, NHE3 activity is increased by angiotensin II (Dixit et al., 2004; Xu et al., 2006). This occurs through a redistribution of NHE3 to the plasma membrane; a process that is dependent on c-Src (Tsuganezawa et al., 1998), phosphatidylinositol 3-kinase (du Cheyron et al., 2003) and an increased synthesis of the exchanger (Xu et al., 2006). Glucocorticoids also increase NHE3 activity. Acutely, a direct phosphorylation of the cytosolic C-terminus of NHE3 by serum and glucocorticoid kinase (SGK) at serine 663 causes the redistribution of NHE3 from endomembranes to the apical membrane (Wang et al., 2005; Yun, 2003; Yun et al., 2002). More chronic exposure to glucocorticoid leads to an increased overall expression of the exchanger (Ambuhl et al., 1999; Kandasamy and Orlowski, 1996). Insulin increases NHE3 activity by similar means. Chronic exposure to insulin increases surface expression of NHE3 *via* SGK-catalyzed phosphorylation (Fuster et al., 2006). Finally, beta adrenergic stimulation (*via* epinephrine or norepinephrine) also activates NHE3 (Hall et al., 1998).

Role in mammalian physiology

NHE3 plays a critical role in salt (re)absorption, in the maintenance of intravascular volume and in pH homeostasis (Alexander and Grinstein, 2006; Bobulescu and Moe, 2006). Accordingly, NHE3-null mice display a decreased blood pressure, diarrhea and an acidic plasma pH (Schultheis et al., 1998). These combined effects result in volume contraction, as evinced by the severely elevated plasma rennin and angiotensin levels found in these animals (Schultheis et al., 1998). The diarrhea is secondary to an increased osmotic load in the lumen of the intestine and provides direct evidence for the important role that NHE3 plays in Na^+ absorption. Interestingly, decreased NHE3 expression caused by inflammatory cytokines appears to induce diarrhea in inflammatory bowel diseases (Clayburgh et al., 2006; Sullivan et al., 2008). The importance of NHE3 to renal Na^+ and bicarbonate absorption was similarly demonstrated. Both microperfusion and micropuncture studies on NHE3-null mice revealed a dramatic decrease ($\approx$50%) in proximal tubular Na^+ and bicarbonate absorption (Lorenz et al., 1999; Wang et al., 1999). If not for a significant reduction in their glomerular filtration rate and increased distal Na^+ absorption, these mice would not be viable (Lorenz et al., 1999; Schnermann, 1999).

The movement of Na^+ across the apical membrane creates a slight but significant osmotic gradient that is responsible for the osmotically driven flux of water (approximately 1201day^{-1}) (Lorenz et al., 1999). NHE3 should therefore also contribute to the passive paracellular flux of other ions – such as calcium and magnesium – from the lumen of the proximal tubule. NHE3 plays a further role in acid–base homeostasis by indirectly facilitating the proximal tubular absorption of citrate as well as the luminal retention of ammonium (Bobulescu and Moe, 2008; Brennan et al., 1988; Nagami, 1988). Finally, the exchanger has also been implicated in the absorption of filtered proteins from the proximal tubular lumen (Gekle et al., 2004), probably through a physical interaction with megalin (Biemesderfer et al., 1999).

Regulation of the distribution of NHE3 at the apical membrane

Endosomal recycling

NHE3 displays a characteristic subcellular distribution (Fig. 2), which facilitates its acute regulation. It is found not only in the apical plasma membrane (Kaunisto and Rajaniemi, 2002; Noel et al., 1996; Pushkin et al., 2000) but also in a sub-apical endomembrane compartment (Biemesderfer et al., 1997; D'Souza et al., 1998). Little is known about the identity of this endomembrane compartment, although NHE3 in this locale is known to exchange with the plasma membrane population (Kurashima et al., 1998). The movement of exchangers between compartments serves as a means of altering their number at the plasma membrane, in turn, altering apical NHE3 activity. Carbachol (Li et al., 2004), albumin (Klisic et al., 2003), glucocorticoids (Bobulescu et al., 2005b) and sodium–glucose co-transport (Zhao et al., 2004) mediate their effects on NHE3 activity through this mechanism.

Intracellular NHE3 is known to be active (D'Souza et al., 1998) and has been proposed to contribute to the acidification of the endomembrane compartment (through the exchange of luminal Na^+ for cytosolic H^+). This process may facilitate the degradation and/or release of cargo having undergone endocytosis through megalin/cubilin; two scavenging proteins known to co-localize with NHE3 at the base of microvilli and in endosomes of the proximal tubule (Biemesderfer et al., 1999; Hryciw et al., 2004). Finally, there is emerging evidence that NHE3 exists in more than one type of intracellular compartment. Our cell culture work

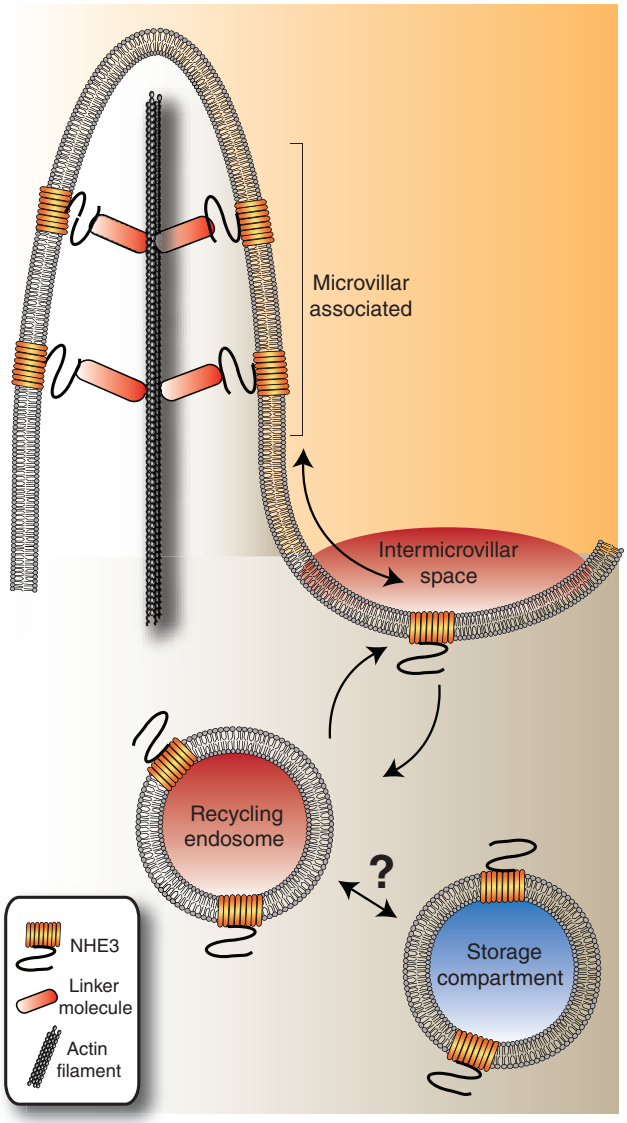

Fig. 2. A model depicting the proposed subcellular distribution of NHE3 in epithelial cells. Note that NHE3 is located in two distinct locations on the apical membrane: most exchangers are tethered to the actin skeleton on microvilli whereas a smaller sub-population is found in the intramicrovillar space. The latter is more likely to internalize and exchange with endomembrane compartments. Endomembrane exchangers can recycle to the apical membrane but with complex kinetics that suggest the existence of fast- and slow-exchanging sub-compartments.

suggests that one population of exchangers is in rapid exchange with the cell surface, while another intracellular population undergoes little constitutive recycling (Alexander et al., 2005) (Fig. 2). It is possible that this later population is potentially waiting to be recruited to the cell surface when increased activity of the exchanger is required acutely.

Cytoskeletal tethering

Ultimately, the balance between exocytosis and endocytosis dictates the apical expression level of NHE3. The rate of endocytosis, in turn, depends on the number of exchangers that are available for internalization at the membrane. In this regard, it is important to appreciate that not all the plasmalemmal exchangers are equally susceptible to endocytosis. Only those that can diffuse in the membrane to enter clathrin-coated pits can be internalized whereas those that are immobile cannot. Indeed, a sizable sub-population of the apical NHE3 appear to be tethered to the cytoskeleton, which results in restricted motion and the inability to be engaged by the endocytic machinery. As a result, the cytoskeleton can regulate NHE3 availability and activity. In support of this notion, perturbations of actin (either through pharmacological inhibitors or genetic manipulation of the small Rho-GTPases) were found to inhibit NHE3 activity (Kurashima et al., 1999; Szaszi et al., 2000). More recent studies confirmed that an intact actin cytoskeleton is necessary for optimal NHE3 activity. Manipulation of microvillar actin architecture through the use of *Clostridium difficile* toxin B, inhibited NHE3 activity and redistributed the apical exchangers to an endomembrane compartment (Alexander et al., 2005; Hayashi et al., 2004). The best evidence that the actin cytoskeleton retains NHE3 in the apical membrane was provided by measuring the lateral mobility of the exchangers in the plane of the membrane. Determinations of fluorescence recovery after photobleaching (FRAP) demonstrated that NHE3 is largely immobile in the apical plasma membrane of epithelia (Alexander et al., 2005; Cha et al., 2004). The first study by Cha and colleagues used an NHE3 construct tagged with green fluorescent protein (GFP) at its extreme cytosolic C-terminus and found that greater than 50% of the exchangers were immobile in the plane of the membrane (Cha et al., 2004). These studies were limited by the fact that fluorescence from GFP-tagged NHE3 in sub-apical endosomes could not be resolved from the plasmalemmal exchangers. Furthermore, the attachment of a GFP moiety to the C-terminus of the exchanger, the domain thought to be responsible for tethering NHE3 to the cytoskeleton, may have altered its behavior. Instead of GFP, the second study utilized NHE3 that was tagged by an extracellular epitope, three tandem HA motifs (Alexander et al., 2005). By reacting the exofacial epitopes with antibodies added extracellularly, we were able to detect exclusively those exchangers located on the apical plasma membrane. Fab fragments were used for immunolabeling to preclude cross-linking of the exchangers, which might have altered their mobility. Using this approach, we found that 70% of apically expressed NHE3 was immobile. Further investigation concluded that this immobility was not the result of association of the exchangers with lipid rafts. Instead, the reduced rate of recovery after photobleaching was attributed to association with the cytoskeleton, because the mobile fraction of the exchanger increased markedly when the actin filaments were perturbed. Taken together, these results support the concept that NHE3 is localized to the apical plasma membrane, at least in part, through a physical retention mechanism mediated by the microvillar actin cytoskeleton.

Is NHE3 attached to the actin cytoskeleton by NHERF and ezrin?

Because attachment to the actin cytoskeleton contributes to the apical retention of NHE3, potentially altering its activity, understanding the nature of this interaction has become a research priority. Unexpected insights were gained from studies designed to isolate the factor responsible for the cAMP-mediated inhibition of NHE3, which led to the purification of the NHERF (Weinman et al., 1993). Subsequent co-immunoprecipitation experiments revealed that NHERF and NHE3 interact directly *in vivo*. A PDZ domain in the C-terminus of NHERF-1 binds to the cytosolic tail of NHE3 (Weinman et al., 2003b). As NHE3 lacks a canonical PDZ-binding domain, the precise binding location is not known, although the four carboxyl terminal amino acid residues, STHM,

have been implicated (Weinman et al., 2003b). Concurrently, NHERF was independently identified in a screen for ezrin-binding partners and named ezrin-binding protein 50 (EBP50) (Reczek et al., 1997). A third independent study demonstrated ezrin to bind to the regulatory subunit II of protein kinase A (PKA R_{II}) (Dransfield et al., 1997). The fact that NHE3 needs to be associated with NHERF and ezrin to enable cAMP-mediated inhibition was then confirmed by reconstituting the system (Weinman et al., 2000b). These findings led to the generation of the current model of cAMP-mediated inhibition of NHE3, which postulates that the exchanger associates with PKA indirectly *via* NHERF and ezrin (Donowitz et al., 2005; Minkoff et al., 1999; Weinman et al., 2005; Weinman et al., 2000a; Weinman et al., 2001). When activated, PKA phosphorylates NHE3 at serine residues 552 and 605 (Kurashima et al., 1997; Zhao et al., 1999) mediating, or at least facilitating, the inhibition of the exchanger (Weinman et al., 2000b).

Identification of a second NHERF isoform, NHE3 kinase A-regulatory protein (E3KARP/NHERF-2), has provided further complexity to the proposed model. The second isoform shares some of the functions described for NHERF-1, as it can similarly mediate inhibition of NHE3 by cAMP (Lamprecht et al., 1998; Yun et al., 1998). It also appears to be necessary for the Ca^{2+}-mediated inhibition of NHE3 activity (Choi et al., 2004; Kim et al., 2002; Lee-Kwon et al., 2003). A third member of the NHERF family, NHERF-3/PDZK1, also physically interacts with NHE3 *in vitro* and augments cAMP-mediated inhibition (Cinar et al., 2007; Thomson et al., 2005).

Ezrin not only binds to NHERF and PKA but is also known to associate with actin *via* its C-terminal domain (Niggli and Rossy, 2008). It has therefore been tacitly assumed that the same protein complex that controls NHE3 phosphorylation also serves to anchor the exchangers to the cytoskeleton. However, the hypothesis that NHE3 is attached to the apical actin skeleton through NHERF and ezrin has never been directly tested and recent findings call into question the validity of this model. First, the ezrin knockout mouse does not display any of the phenotypic features that would be anticipated from altered NHE3 activity caused by changes in its retention or regulation (Saotome et al., 2004; Tamura et al., 2005). Second, NHE3 localization studies in these animals failed to show any redistribution of the exchangers (Saotome et al., 2004). Of note, the phenotype of the NHERF-1 knockout animal is also inconsistent with major relocalization of NHE3. These mice are characterized by renal phosphate wasting and this phenotype appears also in humans with NHERF-1 mutations (Karim et al., 2008). Similarly, while NHE3 lost susceptibility to inhibition by cAMP in NHERF-1-null mice (Cunningham et al., 2004; Murtazina et al., 2007; Weinman et al., 2003a), the localization of the exchangers was not noticeably altered in these animals (Broere et al., 2008; Shenolikar et al., 2002). Third, recent studies reveal that ezrin can bind to NHE3 directly (Cha and Donowitz, 2008; Cha et al., 2006), calling into question the need for NHERF-1 in an association between the actin cytoskeleton and NHE3. Lastly, another PDZ adaptor protein, Shank2, appears to alter NHE3 activity at least in part by regulating the cell surface expression (Han et al., 2006). Therefore, the possibility that it may tether NHE3 to the actin cytoskeleton must also be considered.

The preceding brief overview of the literature makes it clear that while the role of ezrin and NHERF isoforms in regulating the phosphorylation of NHE3 is well established, their participation in targeting and tethering the exchangers is less clear and requires additional study.

Localization to different plasma membrane domains

Not only are the exchangers segregated into surface and endomembrane populations but evidence also exists to support the co-existence of at least two different apical sub-populations of exchangers. Using a variety of techniques including transmission electron microscopy, immunofluorescence microscopy and gradient ultra-centrifugation, Biemesderfer and colleagues have shown that NHE3 exists in at least two distinct sites within the apical plasma membrane of the renal proximal tubule (Biemesderfer et al., 1999). The first is an active population or more active population that resides along the microvilli of the brush border and co-immunoprecipitates with dipeptidyl peptidase IV (Girardi et al., 2001; Girardi et al., 2004). The second is a less active or possibly inactive population that co-immunoprecipitates with megalin and is found in the regions between microvilli, i.e. the inter-microvillar spaces (Biemesderfer et al., 2001). Utilizing similar techniques, McDonough and colleagues provided evidence that physiologically relevant stimuli such as a high salt diet or PTH administration decrease proximal tubular sodium absorption and concomitantly induce the redistribution of NHE3 from microvilli to the inter-microvillar space (Yang et al., 2008; Zhang et al., 1999).

Analysis of fluorescence recovery after photobleaching are consistent with the idea that two separate populations of NHE3 co-exist on the apical membrane. These studies provided evidence for a rapidly mobile fraction of exchangers and a second, poorly mobile fraction (Alexander et al., 2005; Cha et al., 2004). Scanning electron microscopy revealed that about 70% of the exchangers are associated with microvilli whereas the remainder are scattered in the inter-microvillar spaces. Remarkably, the fraction of microvilli-associated NHE3 is similar to the fraction of immobile exchangers detected by photobleaching. These findings suggest that the exchangers on the microvilli are tethered to the cytoskeleton whereas those in the inter-microvillar space are free to diffuse (Alexander et al., 2005).

Another means of partitioning populations of NHE3 within the plasma membrane may involve the association with lipid microdomains, often called rafts. Using the criteria of detergent solubility and sedimentation pattern on a sucrose gradient, Donowitz and coworkers inferred that NHE3 localizes to lipid rafts (Li et al., 2001). In an epithelial cell culture model, they were able to show that disruption of lipid rafts inhibited NHE3 activity, suggesting that the raft-associated population was active (Murtazina et al., 2006). However, our fluorescence recovery studies failed to detect changes in NHE3 mobility following disruption of rafts, implying that partition into lipid microdomains is not the primary mechanism whereby NHE3 is immobilized and retained in apical membranes (Alexander et al., 2005). Instead it is possible that an interaction between NHE3 and membrane lipids may control the activity of the exchangers. Accordingly, Fuster and colleagues were able to demonstrate that polyphosphoinositides alter NHE3 activity, although in their experiments, depletion of cholesterol failed to alter NHE3 activity (Fuster et al., 2004). Similarly, we were able to increase NHE3 activity by adding a cone-shaped lipid, lysophosphatidyl choline, to the lipid bilayer (Alexander et al., 2007). Jointly, these experiments point to a role of lipids in the control of NHE3 activity but whether lipids segregate the exchangers into sub-populations or alter their lateral mobility is not obvious.

Conclusions

In order to regulate intravascular volume, pH and blood pressure, second-to-second modulation of the amount of Na^+ and water

absorbed from the renal tubule and the intestine must be achieved. One of the primary sites of regulation is the apical entry step in the proximal tubule, a process mediated by NHE3. As discussed above, acute regulation of the net activity of the exchangers is accomplished, in part, by altering their number in the functional domain through redistribution between the plasma membrane and endomembrane populations. Further regulatory complexity is conferred by altering the state of activation of individual exchangers within the plasma membrane. This can be accomplished through interactions with regulatory proteins, such as megalin, or by post-translational modifications, notably phosphorylation by cAMP-dependent kinase. In addition, there are other regulatory elements, such as the cytoskeleton and selected lipids that in all likelihood affect both the activity and the distribution of NHE3. Their mode of action at the molecular level should be the subject of more detailed future studies.

List of abbreviations

CHP	calcineurin homologous protein
DDP	dipeptidyl peptidase
GFP	green fluorescent protein
LLCPK	porcine kidney epithelial cells
NHA	sodium–proton antiporter
NHE	Na^+/H^+ exchanger
NHERF	sodium–hydrogen exchanger regulatory factor
NHX	sodium–proton exchanger
PKA	protein kinase A
PKC	protein kinase C
PTH	parathyroid hormone
SGK	serum and glucocorticoid kinase
TGN	trans-Golgi network
TM	transmembrane

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