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Emerging roles of alkali cation/proton exchangers in organellar homeostasis

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Abstract

The regulated movement of monovalent cations such as H^+, Li^+, Na^+ and K^+ across biological membranes influences a myriad of cellular processes and is fundamental to all living organisms. This is accomplished by a multiplicity of ion channels, pumps and transporters. Our insight into their molecular, cellular and physiological diversity has increased greatly in the past few years with the advent of genome sequencing, genetic manipulation and sophisticated imaging techniques. One of the revelations from these studies is the emergence of novel alkali cation/protons exchangers that are present in endomembranes, where they function to regulate not only intraorganellar pH but also vesicular biogenesis, trafficking and other aspects of cellular homeostasis.

Introduction

Ion transport across the cell surface and organellar membranes is essential for cellular survival and proliferation. Inorganic ions play a defining role in cytoplasmic and organellar pH and volume homeostasis, in the provision of energy, thetranslocation of organic solutesandin cellular excitability and contractility. Much is known about transporters on the plasma membrane, which have been studied extensively by isotopic or electrophysiological means, or by employing ion-specific fluorescent probes that can be selectively targeted to the cytoplasm. By contrast, endomembrane compartments are highly dynamic structures that are not readily accessible and therefore much less amenable to study. As a consequence, our understanding of organellar ion transport is at present rudimentary. In some cases, the same or related transporters operate at both the surface and internal membranes. In these instances, lessons learned from studying plasmalemmal function can be extrapolated to infer the function of the organellar transporters. Alkali cation/proton exchangers (or antiporters), commonly referred to as Na^+/H^+ exchangers (NHEs), are one such example.

Mammalian NHEs are integral membrane proteins with a proposed secondary structure of 12 transmembrane segments at their N-terminus, followed by a hydrophilic C-terminus that is oriented towards the cytoplasm and the target for various regulatory molecules [1–3]

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(illustrated in Figure 1). Though their tertiary and quaternary structures have yet to be elucidated, biochemical analyses of plasmalemmal NHEs suggest that they assemble and function as homodimers [4,5•], a structure that compares favourably with the recent crystal structure of the more distantly related bacterial *Escherichia coli* Na^+/H^+ antiporter NhaA [6•]. However, unlike its bacterial homologues that operate as electrogenic transporters, the mammalian NHEs are generally thought to catalyze the electroneutral exchange of alkali cations (Li^+, Na^+ or K^+) for protons, though their cation selectivity can differ amongst isoforms. The basic transport kinetic properties are known with certainty for the isoforms resident on the plasmalemma [4] but are not fully established for the endomembrane isoforms, which have remained refractory to direct measurements for technical and topological reasons. The objective of this review is to summarize our current understanding of the distribution and function of members of the mammalian NHE family, with particular emphasis on those present in internal membrane compartments.

Genetic and subcellular diversity

Mammalian NHEs constitute a family of nine related gene products (NHE1–9) that share ~25–70% amino acid identity overall (Figure 2a) [2,7••]. In addition to the NHE gene family, mammals also possess another novel cluster of distant NHE-related genes, termed NHA1 and NHA2 on the basis of their closer homology to the fungal/plant NHA1 and bacterial NhaA Na^+/H^+ antiporters [7••]. Their functional and physiological properties, however, are presently unknown.

The recognized members of the NHE family can be broadly classified into two major subgroups on the basis of their primary structure similarity and principal subcellular location, that is plasma-membrane-type or endomembrane-type transporters (Figure 2a). NHE1 through NHE5 (~40–60% identity) are all targeted to the plasma membrane, but isoforms such as NHE3 and NHE5 can also enter a recycling endosomal pool (Figure 2b). NHE6 to NHE9 are thought to reside predominantly, though not exclusively, in endomembrane compartments. This latter group can be further subdivided into the endosomal/*trans*-Golgi network (TGN) cluster (encompassing NHE6, 7 and 9, which share ~60–70% identity) and NHE8 that exhibits the least similarity (25% identity) to other NHEs [2,7••]. Orthologues for the mammalian endosomal/TGN NHE cluster can be found in all eukaryotes, the best studied being the yeast *Saccharomyces cerevisiae* Nhx1 that resides in the late endosomal/prevacuolar compartment [7••]. By contrast, NHE8 is seemingly restricted to animal cells, suggesting a more recent emergence in evolution to satisfy a particular physiological need.

Plasma-membrane-type NHEs

The plasma-membrane-type NHEs (NHE1–5) preferentially catalyze the electroneutral exchange of one extracellular Na^+ for one cytosolic H^+. Li^+ is also a transportable substrate, but its rate of translocation is generally slower than that of Na^+. NHE1 is by far the best studied prototypical isoform. It has a ubiquitous tissue distribution, resides only at the plasma membrane (basolateral membrane in polarized epithelia) and is thought to be a ‘housekeeping’ enzyme responsible for maintaining cytoplasmic pH and controlling cellular

volume. In fibroblasts and possibly other cell types, the carboxy-terminal cytoplasmic tail of NHE1 also serves as a scaffold for the assemblage of various actin-binding proteins (ezrin, radixin and moesin) and signalling complexes, transmitting signals from activated growth factor and integrin receptors to pathways regulating cell proliferation, shape, adhesion and migration (summarized in Table 1) [3,8,9].

The other members of this subgroup, NHE2–5, have a more limited tissue distribution and, in some polarized cell types, are confined to discrete regions of the plasma membrane, suggestive of more specialized functions. For instance, both NHE2 and NHE3 are localized at the apical surface of renal and gastrointestinal epithelial cells as well as other epithelia and play important roles in salt, bicarbonate and fluid (re)absorption. Unlike NHE2, however, NHE3 (and the neuronal NHE5) is further sorted to the recycling endosomal pathway.

Roles in recycling endosomes

Accumulation of NHE3 in recycling endosomes may serve as an important regulated reservoir of functional transporters. It constitutes a sizeable source of spare exchangers when their abundance at the surface needs to be increased acutely. Conversely, internalization into endomembrane storage sites serves to downregulate the number of active surface transporters in a rapid and reversible manner. Delivery to the internal pool seems to follow clathrin-dependent endocytosis. NHE3 has been detected in clathrin-coated pits at the base of microvilli [10] and expression of a dominant-negative allele of dynamin interfered with its internalization [11]. It is noteworthy that dynamin is required not only for clathrin-mediated endocytosis but also for caveolar uptake and that extraction of cholesterol with cyclodextrin, which disrupts rafts and caveolae, also impaired NHE3 endocytosis [12]. However, cyclodextrin non-selectively also affects clathrin-mediated internalization, which is the predominant pathway for apical endocytosis and the likely mechanism utilized by NHE3.

There is evidence that NHE3 is functional in endocytic and endosomal membranes. If operating in the same direction as it does at the surface, NHE3 would move H^+ into the vesicular lumen while delivering Na^+ to the cytosol (illustrated in Figure 3). This would result in a luminal acidification, which has been detected not only after heterologous overexpression in fibroblastic cells [13] but also in native systems [14,15]. Indeed, there is compelling evidence that the presence and activity of NHE3 in endosomes affect some aspects of epithelial physiology. Specifically, Gekle *et al.* [14,16,17,18•,19] have demonstrated that a functional NHE3 is required for optimal albumin uptake and subsequent delivery to lysosomes in renal cells (Figure 3). Importantly, the activity of the exchangers in endocytic vesicles, and not at the surface membrane, was found to be the crucial parameter. Inhibition of NHE3 activity significantly attenuated the rate of fusion of plasma-membrane-derived albumin-laden vesicles with early (sorting) endosomes, thereby blocking subsequent trafficking and processing of albumin [16]. Acidification of the endosomal lumen is known to be required for proper routing of internalized cargo [20], but it is not clear whether this is the mechanism whereby NHE3 contributes to albumin uptake and traffic. Indeed, the contribution of ‘forward’ Na^+/H^+ exchange to endosomal acidification can only be small and

transient (i.e. during the formation of the primary endocytic vesicle), as Na^+ is predicted to be depleted rapidly from the lumen, unless continuously restored by active transporters like the Na^+/K^+-ATPase. The latter, however, is not expected to be found in apical endosomes, as it is restricted to the basolateral membrane. Moreover, it is well established that vacuolar-type H^+-pumps (V-ATPases), in conjunction with electrogenic chloride transporters of the CLC family, are the primary means of endosomal acidification [21,22•]. Accordingly, apical endosomes of epithelial cells were found to be only marginally more acidic than cells devoid of NHE3 (6.20 ± 0.02 in wild type versus 6.30 ± 0.03 in NHE3-null cells) [17]. The fact that ATP-dependent H^+-pumping is predominant raises the possibility that the acidification generated by the V-ATPases, together with the depletion of luminal Na^+, may eventually reverse the direction of the combined Na^+/H^+ gradient that drives NHE activity. In this event, NHE3 or other endosomal NHEs may in fact function to dissipate the acidification generated by the H^+-pumps and serve to regulate the pH setpoint of these compartments.

Regulation

The apical membrane and subapical vesicular pools of NHE3 appear to be in dynamic equilibrium, regulated by a bewildering assortment of agents and conditions. These include aldosterone, glucocorticoids, lysophosphatidic acid, albumin, extracellular acidity and glucose-induced cell swelling, all of which upregulate the number of apical surface transporters, as well as cholinergic agonists, dopamine, TNF and elevated blood pressure, which reduce transporter density (see reference [23] for review). The number and variety of signalling pathways that have been invoked in this regulation – either directly or indirectly – is perplexing, including an array of serine/threonine kinases [10,24–26], non-receptor tyrosine kinases [26,27], phospholipases [28] and other lipid-metabolizing enzymes [29,30]. Of particular interest is the observation that a large fraction of the apical NHE3 is firmly anchored along the sides and near the tip of microvilli, which restricts the mobility of the exchangers and presumably retains them on the apical membrane. NHE3 is thought to attach to actin filaments that are formed and/or stabilized by Rho-family GTPases [31•]. Bridging between the integral membrane and cytoskeletal proteins is thought to be mediated by PDZ-based adaptor proteins (NHERF1, NHERF2, PDZK1, MAST205, Shank2) and/or ezrin (Table 1) [32–36]. Substantive internalization may require detachment of NHE3 from such anchorage sites, making the latter prime targets for the regulation of traffic between surface and endomembrane compartments. Despite the mechanistic uncertainty of this step, recent analyses of the later phases of endocytosis of NHE3 induced in response to elevated levels of cAMP and Ca^{2+} have identified a crucial role for synaptotagmin I in the binding of NHE3 and subsequent recruitment of the adaptor protein complex AP2 and clathrin [37].

Of all the NHE isoforms, NHE5 bears the highest similarity to NHE3. It was therefore not unexpected to find NHE5 not only on the plasmalemma but also in an endomembrane compartment. This observation was first made following ectopic expression in fibroblasts, but was subsequently validated in PC12 neuroendocrine cells and in primary hippocampal neurons. In the heterologous expression system, NHE5 was also internalized via clathrin-coated structures in a dynamin-dependent fashion [38]. The adaptor/scaffolding protein, β-arrestin, probably mediates the association between NHE5 and clathrin [39•]. The two proteins interact directly both *in vitro* and *in vivo*, and overexpression of β-arrestin

redirected NHE5 from the surface to endomembranes. The linkage with β-arrestin raises the possibility that both NHE5 and neurotransmitter-activated G-protein-coupled receptors, which are also internalized upon association with β-arrestin, may co-localize within the same clathrin-coated pits. Such an assembly has the potential to promote more rapid vesicle acidification, ligand dissociation and recycling of the receptors.

Endomembrane-type NHEs

Compared with the mammalian plasma-membrane-type NHEs, considerably less is known about the function, regulation and trafficking of the endomembrane transporters NHE6–9. These isoforms are widely expressed and appear to share distinct but overlapping compartments along the secretory and endocytic pathways (see Figure 2).

Subcellular distribution

NHE7 is located largely in the *trans*-Golgi network (TGN) and perinuclear recycling vesicles when expressed stably in CHO and MCF-7 cells [40,41]. Like other TGN-resident proteins such as TGN38, a minor fraction of NHE7 also appears to transiently shuttle to the cell surface, at least in non-polarized cells [41]. NHE8 accumulates within the *mid*-cisternae to *trans*-cisternae of the Golgi complex when heterologously expressed in COS7 cells [42]. However, in renal proximal tubule and intestinal epithelial cells, native NHE8 localizes predominantly at the microvillar membrane surface and to a lesser extent in intermicrovillar-coated pits and/or subapical vesicles [43], suggesting that it may undergo recycling to and from the apical surface. By comparison, the distributions of NHE6 and NHE9 overlap considerably with markers of the recycling endosomal pathway, though they tend to partition differentially to early and recycling endosomes, respectively [42]. Again, their relative distribution may also be influenced by the cell type in which they are expressed. A striking example is found in the sensory hair cells of the vestibular system, where NHE6 and NHE9 are concentrated not only in intracellular vesicles of the cell bodies but also apically at the tips of stereocilia—the sensory organelles that mediate mechano-electrical transduction [44]. It thus appears that the membrane sorting of these isoforms is dependent on the cell context (i.e. cell-specific adaptors, scaffolding or anchoring proteins, cytoskeletal elements), forming a mosaic wherein individual isoforms address specialized needs both within vesicles and at the cell surface.

Kinetic features

A distinguishing kinetic feature of the endomembrane class of NHEs is their ability to mediate not only Na^+ (or Li^+) but also K^+ in exchange for H^+, which contrasts with the high selectivity for Na^+ displayed by their plasma-membrane-type counterparts. This was shown initially for NHE7 [40] and has since been demonstrated directly or inferred indirectly for other mammalian endomembrane isoforms [42,44], as well as for their orthologues in yeast (i.e. Nhx1) [45•] and plants [46]. Since K^+ is the major cytoplasmic alkali cation, and most endomembrane compartments are acidic, the prevailing view is that these transporters use the combined electrochemical ion gradients to direct the inward movement of K^+ in exchange for luminal H^+, thereby serving as an electroneutral alkalinizing mechanism.

However, because of the crudeness of the ion transport assays used to date – usually involving intact or permeabilized cells – the precise cation selectivity and energetics of these transporters are ill defined. A precise characterization awaits more refined measurements in purified reconstituted *in vitro* systems.

Roles and regulation

The physiological importance of NHEs to endosomal function is not well understood, but insights from the study of the *S. cerevisiae* Nhx1 suggest that alkali cation/ proton exchange may influence not only luminal ion homeostasis but also vesicle biogenesis and protein trafficking. Initially, Nhx1 was found to protect yeast cells from the cytotoxic effects of high saline environments by sequestering excess cytoplasmic Na^+ into endosomes [47]. However, subsequent analyses revealed an important role of Nhx1 in the trafficking of proteins from the late endosome to the vacuole. Null (*nhx1Δ*) or functionally inactive mutants of Nhx1 displayed an atypical distension of the prevacuolar compartment that was accompanied by aberrant accumulation and processing of vacuolar-targeted proteins originating either from the late Golgi (e.g. carboxypeptidase Y) or internalized from the cell surface (e.g. the G protein-coupled receptor Ste3p) [48]. This effect was relatively specific, as sorting of other vacuolar-targeted proteins (e.g. alkaline phosphatase) that did not pass through the prevacuolar compartment was unaffected.

The molecular basis for these observed defects is unclear but is probably linked to disruption in K^+-dependent and/ or pH-dependent endosomal processes. Though not examined in the context of Nhx1 function, K^+ is known to act as a regulatory co-factor of the Kex2/furin family of endoproteases required for maturation of newly synthesized proteins in the secretory pathway of yeast as well as animal cells [49]. Hence, alterations in intraorganellar K^+ concentrations could conceivably compromise proper protein processing. Disturbances of intravesicular K^+ concentration are also likely to have an impact on organellar volume.

By contrast, acidification of endomembrane compartments is well recognized as a crucial determinant of protein processing and trafficking along the biosynthetic and endocytic pathways [50–52]. Indeed, recent analyses of *nhx1Δ* mutant cells revealed that the endosomal (and, unexpectedly, also the cytoplasmic) compartments were more acidic than those of wild type cells and that treatment with weak bases could alleviate the severe defect in vacuolar protein trafficking [45]. These data support the notion that endomembrane Nhx/ NHEs serve as a crucial alkalinizing mechanism to finely control intraorganellar pH that, by an ill-defined mechanism, modulates endosome biogenesis and trafficking.

While the available evidence indicates that endomembrane Nhx/NHEs influence organellar function through ionic/pH changes, they may also exert other effects by interacting with proteins required for normal membrane traffic. In this regard, Gyp6, a specific GTPase-activating protein (GAP) for Ypt6, the orthologue for the mammalian Rab6-GTPase, was found to bind to the hydrophilic C-terminal segment of Nhx1 and to co-localize with the transporter at the prevacuolar compartment [53•]. This is an intriguing observation since Ypt6 is implicated in endosome to Golgi membrane trafficking. Additional genetic manipulations supported a functional interrelationship between Gyp6, Nhx1 and Ypt6, with

Gyp6 acting as a negative regulator of both Nhx1 and Ypt6 both to control intraendosomal pH and retrograde traffic from the endosome to the late Golgi. However, interpretation of these data is complicated by membrane topological studies of Nhx1 showing that its C-terminus (or a portion thereof) lies within the endosomal lumen [54], whereas Gyp6 is cytoplasmic or tethered to the outer leaflet of the endosomal membrane. Hence, the precise nature of these molecular interactions, linking Nhx1-regulated endosomal ion homeostasis to membrane traffic, remains obscure.

The factors that govern the trafficking and regulation of mammalian endomembrane NHEs are also largely unknown. Recently, a yeast two-hybrid screen identified members of the secretory carrier membrane protein family (i.e. SCAMP 1, 2 and 5) as interacting partners of NHE7 [55•]. SCAMPs are transmembrane proteins localized in the Golgi and post-Golgi compartments and thought to be involved in membrane traffic, though their precise roles are not well defined [56]. Consistent with this view, a dominant-negative deletion mutant of SCAMP2 caused NHE7 (as well as wild type SCAMP2) to redistribute to recycling endosomes, suggesting that it plays a role in the retrieval of NHE7 from recycling endosomes to the TGN [55•]. Recently, SCAMP2 was found to bind to the small GTPase ADP-ribosylation factor 6 (Arf6) and to phospholipase D1 in PC12 cells [57], indicating that it may facilitate recruitment of vital elements of the trafficking machinery involved in vesicle formation and fusion [58]. This raises the prospect that NHE7 may form a macromolecular complex that includes not only SCAMP2 but also Arf6. Such an assembly could provide a potential link between endomembrane NHEs and vesicle trafficking along the TGN/endosome pathway. Intriguingly, in a recent study Hurtado-Lorenza *et al.* [59••] showed that Arf6 and its associated guanine-nucleotide-exchange factor ARNO also interacted with the integral membrane c-subunit and a2-subunit, respectively, of the V-ATPase in early endosomes. Significantly, the association of ARNO with the a2-subunit was dependent on endosomal acidification and necessary for subsequent vesicle traffic between early and late endosomes. Thus, the V-ATPase, in addition to pumping H^+, appears capable of sensing the optimal intraorganellar pH and transmitting that signal across the membrane, possibly by a pH-dependent conformational change in the a2-subunit. In turn, the ‘activated’ a2-subunit becomes competent to recruit cytoplasmic proteins required for vesicle maturation along the degradative pathway. It would be interesting to determine whether this molecular paradigm also applies to NHE7 as well as other endomembrane NHEs, whereby intraorganellar acidification to a ‘defined’ pH setpoint – regulated partly by the NHEs themselves – triggers a conformational change in the transporters that allows for the scaffolding of other proteins important for membrane traffic.

Despite its predominance in endomembranes, a minor fraction of NHE7 also shuttles to and from the plasma membrane, analogous to the plasma membrane/recycling-type NHEs (i.e. NHE3 and 5). In MCF-7 cells, surface NHE7 concentrates in caveolae/lipid-enriched rafts [41]. This association is mediated through direct binding of the cytoplasmically oriented C-terminal tail of NHE7 with caveolin-1, perhaps stabilizing the transporter at the cell surface [41]. However, internalization of NHE7 appears to occur exclusively through the clathrin-mediated pathway, suggesting that NHE7 may exist in dynamic equilibrium between caveolae/lipid rafts and non-caveolae/lipid rafts. Curiously, disruption of the interaction with dominant-negative mutants of caveolin-1, despite shifting NHE7 to non-caveolae/lipid raft

fractions, did not appreciably affect its subcellular distribution. Hence, the functional significance of this association remains obscure.

The presence of endomembrane NHEs at the cell surface raises the possibility that they may also fulfil more specialized roles in regulating cytoplasmic ion homeostasis. For instance, in vestibular hair cells that express NHE6 and NHE9 in their stereocilia, the apical endolymph fluid lacks extracellular Na^+ and instead is highly enriched in K^+. Hill *et al.* [44] provided intriguing evidence that these NHEs use the high extracellular K^+ concentration to expel intracellular H^+ generated by the Ca^{2+}/H^+ exchange activity of the apical plasma membrane Ca^{2+}-ATPase isoform 2 (PMCA2). PMCA2 is required to extrude sufficient Ca^{2+} into the surrounding endolymph to generate otoconia, the calcium carbonate crystals embedded in the otolithic membrane that shifts in response to gravity or linear acceleration, thereby stimulating the hair cells. These data suggest that NHE6 and NHE9 are functionally coupled with PMCA2 to maintain the pH of hair bundles while supporting robust extrusion of Ca^{2+}.

Conclusions

While the contributions of plasma-membrane-type NHEs to cellular and systemic physiology are well documented, the observation that some of these isoforms (i.e. NHE3 and NHE5) can be internalized into recycling endosomes and the discovery of novel isoforms (NHE6–9) localized to discrete compartments along the exocytic and endocytic pathways highlights broader roles of these transporters in cell function. The endomembrane-type transporters appear to operate physiologically as K^+/H^+ exchangers, but there is a need to better define their kinetic properties (cation selectivity, affinities, energetics) under more defined experimental conditions. Two-hybrid screening has begun to identify interacting proteins that underlie their membrane sorting and regulation. The combination of RNAi methodologies, powerful imaging techniques and deployment of existing as well as novel organelle-specific, cation-sensitive, fluorescent probes should help to distinguish their roles in intraorganellar ion homeostasis and as scaffolds for recruitment of proteins involved in carrier vesicle coat formation and traffic. This could be particularly challenging, as the endomembrane isoforms appear to share overlapping compartments; thus, selection of suitable cell model systems will be crucial. A key question is whether their abilities to modulate organellar pH is functionally coupled with the recruitment of trafficking machinery, or whether these processes are regulated independent of each other. These approaches should help to provide a more complete understanding of the roles of alkali cation/ proton exchangers in cellular/organellar homeostasis.

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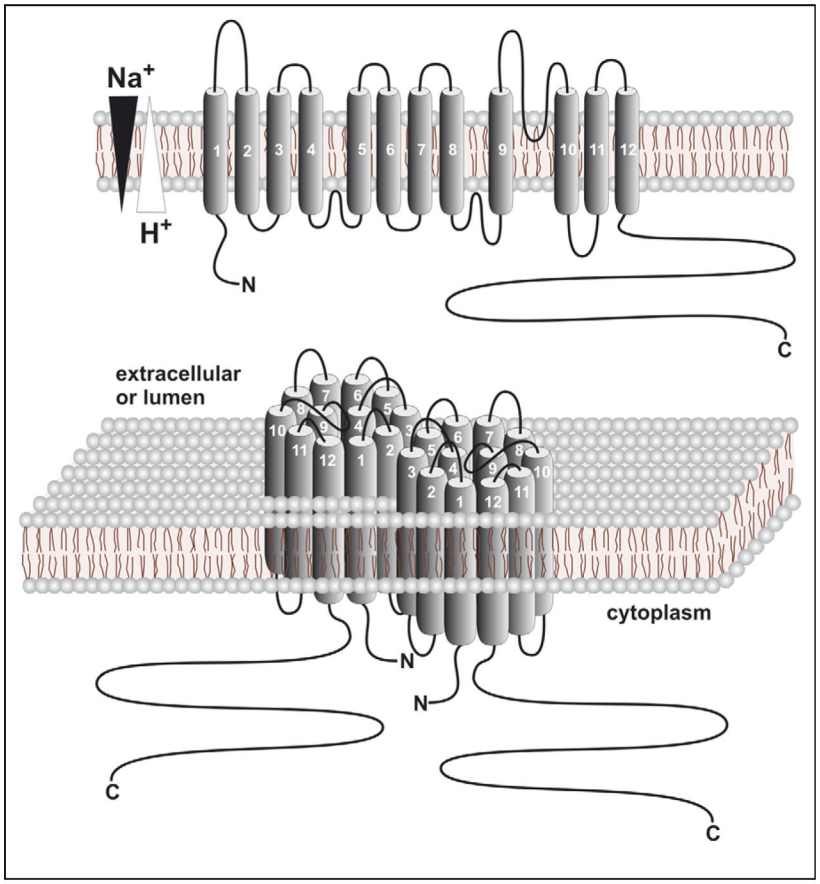

Figure 1.

Predicted membrane topology and higher ordered structure of mammalian alkali cation/proton exchangers.

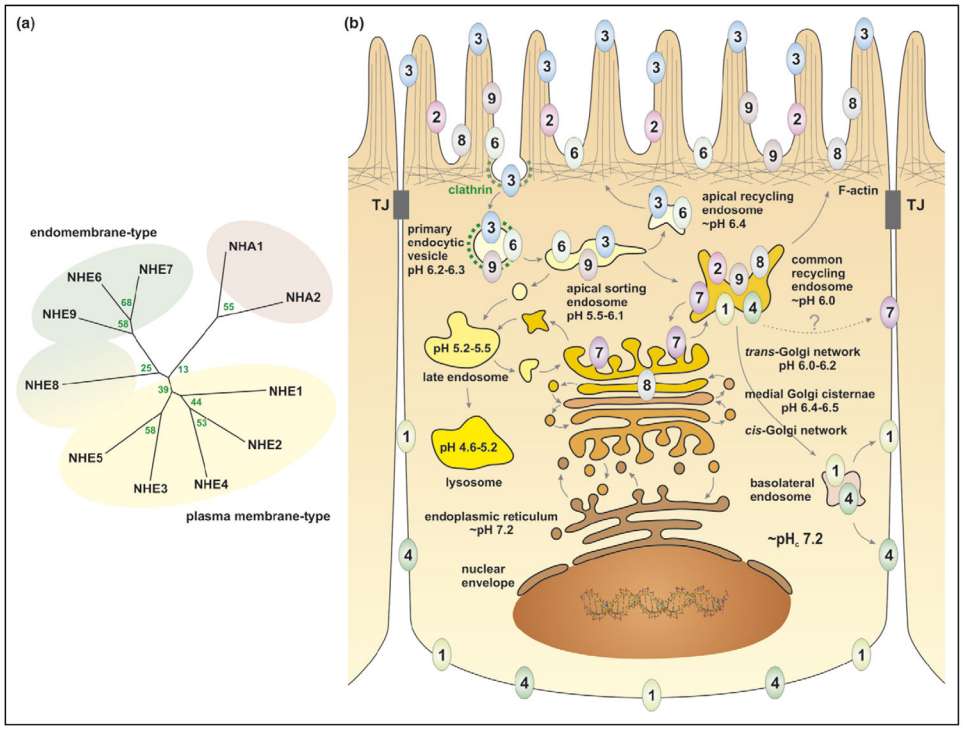

Figure 2.

Genetic diversity and membrane distribution of mammalian alkali cation/proton exchangers. **(a)** Phylogenetic relationships of human alkali cation/proton exchangers were determined by multiple sequence alignments using the CLUSTAL W algorithm [68] and the radial tree was drawn using TreeView [69]. The GenBank™ accession numbers for the various NHEs are as follows: NHE1 to NHE9: NM_003047, NM_003048, NM_004174, NM_001011552, NM_004594, NM_006359, NM_032591, NM_015266, NM_173653, respectively; NHA1, NM_139173 and NHA2, NM_178833. **(b)** The diagram displays a composite subcellular distribution of the mammalian $Na^+(K^+)/H^+$ exchangers (NHE1–4, 6–9) in a prototypical epithelial cell. In native epithelia, these isoforms exhibit cell-specific expression. NHE5 is not depicted since it is found predominantly in neuronal cells but exhibits a subcellular distribution analogous to that of NHE3. The various biosynthetic and endocytic routes are shown, along with approximate values for the luminal pH of the various endomembrane compartments, as extrapolated from published data of different cell types.

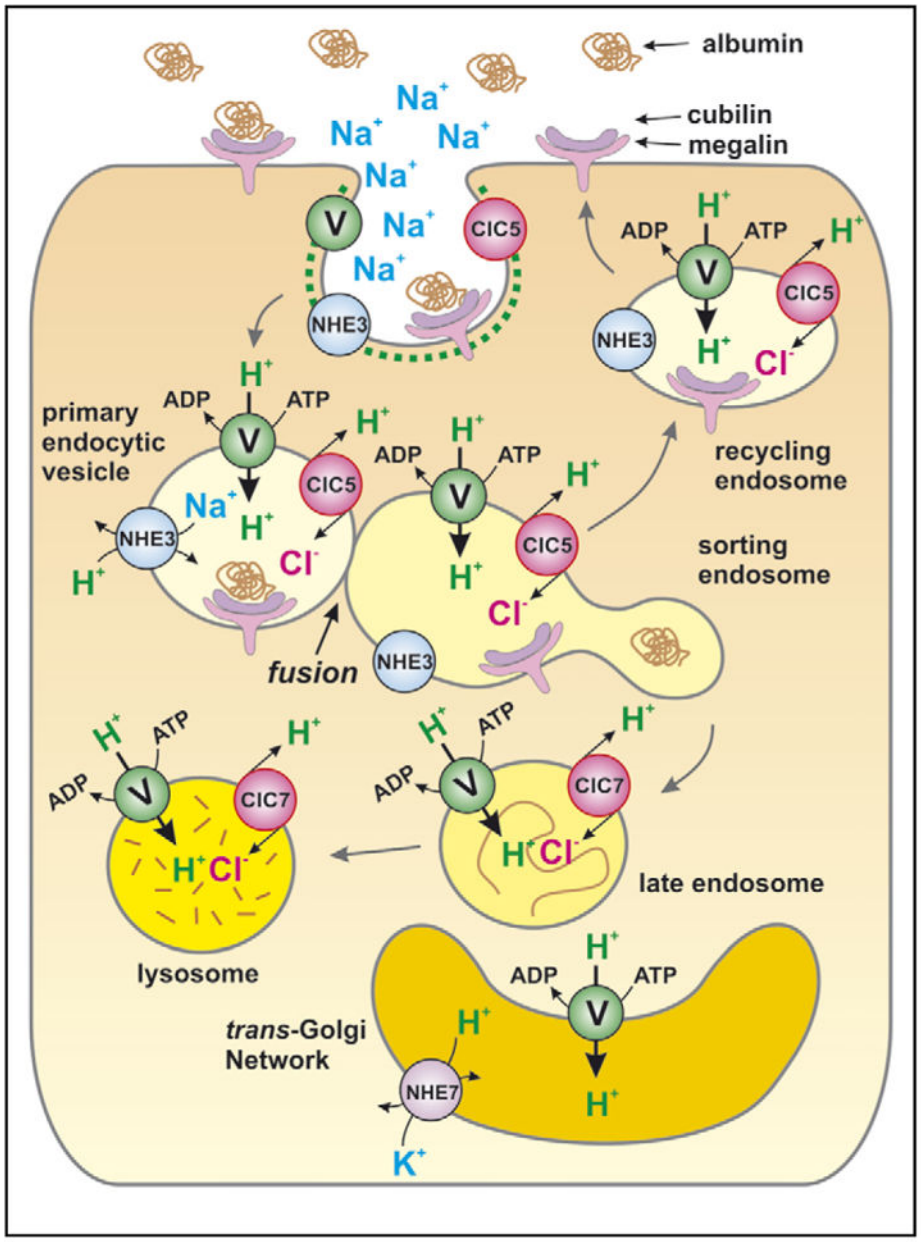

Figure 3.

Involvement of the apical Na^+/H^+ exchanger NHE3 in endosomal pH and reabsorption of albumin in renal proximal tubules. Acidification of intracellular compartments is driven primarily by the vacuolar-type (V) H^+-ATPase pumps (V-ATPases) [21]. In addition, vesicle acidification is facilitated by counterion Cl^- influx pathways that minimize the generation of a large inside-positive voltage that would otherwise impede V-ATPase function. In proximal tubular epithelia, the Cl^- conductance in apical early and recycling endosomes is mediated by ClC-5, an electrogenic $2Cl^-/H^+$ exchanger of the CLC family of channels/transporters [22]. Acidification of the late endosomal and lysosomal compartments is aided by another CLC member, ClC-7; depicted as an exchanger though its precise mode of operation (exchanger or channel) is uncertain. The apical Na^+/H^+ exchanger isoform 3 (NHE3) also

contributes to acute acidification of early/recycling endosomes. The glomerular filtrate in the proximal tubule, which contains plasma-levels of Na^+, is internalized into primary endocytic vesicles upon their formation. This establishes an outwardly directed Na^+ gradient that is used by NHE3 to propel the inward movement of H^+; a process that terminates upon dissipation of the Na^+ gradient. Disruption of vesicle acidification by pharmacological inhibition or gene knockout of either the V-ATPase, ClC-5 or NHE3 impedes endocytosis of albumin by the megalin-cubilin scavenger receptor complex and its subsequent degradation in lysosomes (reviewed in reference [19]). The molecular mechanisms underlying this pH-associated effect are ill defined, but may result from impaired dissociation of the albumin-receptor complex and/or biogenesis of recycling vesicles.

Table 1

Characteristics of mammalian alkali cation/proton exchangers

Isoform	Tissue distribution	Membrane location	Main functions	Interacting partners	References
NHE1	Ubiquitous	• Plasma membrane • Basolateral membrane of epithelia	• Na^+:H^+ (1:1) • Cytosolic pH • Cell volume • Cell proliferation • Cell shape, adhesion and migration • Fluid secretion	• Ca^{2+}/calmodulin • CHP1, 2 • Tescalcin (CHP3) • 14-3-3 • Carbonic anhydrase II • Ezrin/radixin/moesin • ERK1/2 • p38 MAPK • $p90^{rsk}$ • p160ROCK (ROCK1) • NIK • PIP_2	[2,3,8,9,60–63]; and references therein
NHE2	Gastrointestinal tract > skeletal muscle ⋙ kidney, brain, uterus, testis ≫ heart	• Plasma membrane • Apical membrane of epithelia	• Na^+:H^+ (1:1) • Fluid secretion	• CHP1, 2	[60,61]
NHE3	Kidney, intestines (other epithelia)	• Apical membrane of epithelia • Early/recycling endosomes	• Na^+:H^+ (1:1) • Na^+ and HCO_3^- (re)absorption • Early endosome acidification • Renal tubule albumin reabsorption	• PKA • PKC • CHP1, 2 • Megalin • DPP-IV • NHERF1, 2 • PDZK1 • MAST205 • Shank2 • Ezrin • Synaptotagmin I	[2,10,23–25,32–37,60,61, 64–66]

Isoform	Tissue distribution	Membrane location	Main functions	Interacting partners	References
NHE4	Stomach ≫ ≫ kidney	• Basolateral membrane of epithelia	• Na^+:H^+ (1:1) • Cytosolic pH	• CHP1	[60]
NHE5	Brain (neurons)	• Plasma membrane • Recycling endosomes	• Na^+:H^+ (1:1)	• β-arrestin1, 2 • RACK1	[39•,67]
NHE6	Ubiquitous	• Early/recycling endosomes • Apical membrane in hair cell stereocilia	• K^+ (Na^+):H^+ • Organellar pH?	?	
NHE7	Ubiquitous	• TGN • Endosomes • Plasma membrane	• K^+ (Na^+):H^+ • Organellar pH?	• SCAMP1, 2, 5 • Caveolin-1	[41,55•]
NHE8	Ubiquitous	• Golgi complex • Endosomes • Apical membrane in renal epithelia	• K^+ (Na^+):H^+ • Organellar pH?	?	
NHE9	Ubiquitous	• Recycling endosomes • Apical membrane in hair cell stereocilia	• K^+ (Na^+):H^+ • Organellar pH?	?	

CHP, calcineurin B-homologous protein; ERK, extracellular signal-regulated protein kinase; MAPK, mitogen-activated protein kinase; $p90^{rsk}$, p90 ribosomal S6 kinase, p160ROCK/ROCK1, Rho-associated, coiled-coil containing protein kinase 1; NIK, Nck-interacting kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; DPP-IV, dipeptidyl peptidase IV; NHERF, Na^+/H^+ exchanger regulatory factor; PDZK1, PDZ domain-containing protein highly homologous to rat Diphor-1; MAST205, microtubule-associated serine/threonine kinase-205 kDa that contains a Ser/Thr kinase domain and a PDZ domain; Shank2, SH3 and multiple ankyrin repeat domains 2; RACK1, receptor for activated protein kinase C; SCAMP, secretory carrier membrane protein.

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