BIOCHEMISTRY
including biophysical chemistry & molecular biology
Current Topic

Biochemistry **2011,** *50,* 443–450 443
DOI: 10.1021/bi101082e

Organellar Na^+/H^+ Exchangers: Novel Players in Organelle pH Regulation and Their Emerging Functions

Ryuichi Ohgaki,[‡,∥] Sven C. D. van IJzendoorn,[§] Masafumi Matsushita,[‡] Dick Hoekstra,[§] and Hiroshi Kanazawa*[,‡]

[‡]*Department of Biological Sciences, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043, Japan, and* [§]*Department of Cell Biology, Section Membrane Cell Biology, University Medical Center Groningen, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.* [∥]*Present address: Division of Bio-system Pharmacology, Department of Pharmacology, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan.*

Received July 6, 2010; Revised Manuscript Received December 17, 2010

ABSTRACT: Mammalian Na^+/H^+ exchangers (NHEs) play a fundamental role in cellular ion homeostasis. NHEs exhibit an appreciable variation in expression, regulation, and physiological function, dictated by their dynamics in subcellular localization and/or interaction with regulatory proteins. In recent years, a subgroup of NHEs consisting of four isoforms has been identified, and its members predominantly localize to the membranes of the Golgi apparatus and endosomes. These organellar NHEs constitute a family of transporters with an emerging function in the regulation of luminal pH and in intracellular membrane trafficking as expressed, for example, in cell polarity development. Moreover, specific roles of a variety of cofactors, regulating the intracellular dynamics of these transporters, are also becoming apparent, thereby providing further insight into their mechanism of action and overall functioning. Interestingly, organellar NHEs have been related to mental disorders, implying a potential role in the brain, thus expanding the physiological significance of these transporters.

Regulation of ion homeostasis by ion-transporting proteins, including pumps, transporters, and channels, is a crucial task for living organisms. Mammalian Na^+/H^+ exchangers (NHEs),[1] which play such a fundamental role in the regulation of the cellular ionic environment (*1*−*4*), are integral membrane ion transporters that mediate the electroneutral exchange of H^+ with Na^+ or K^+. All mammalian NHEs are proposed to possess 12 transmembrane helices in their N-termini that constitute the ion translocation domain, while the cytoplasmic C-terminus plays an important role in the binding of various regulatory proteins. Nine mammalian NHE isoforms (NHE1−9) have been identified thus far, and they appear to display considerable variation in tissue expression, subcellular localization, ion transport characteristics, pharmacological properties, and regulatory mechanisms (*3*, *4*). Two novel members of the family (*SLC9C1* and *SLC9C2*) have recently been identified, but phylogenic analysis demonstrates that they are only distantly related to the other family members and, hence, appear to be derived from a distinct evolutionary origin (*5*). On the basis of subcellular localization and phylogenic analysis, the NHEs can be categorized into two types: plasma membrane NHEs (NHE1−5) and organellar NHEs (NHE6−9). However, the criteria for this distinction into two categories are occasionally somewhat ambivalent. Thus, although NHE8 is categorized as an organellar NHE, the transporter may largely localize to the apical plasma membrane domain in polarized epithelial cells, while on the basis of phylogenic criteria and its localization when ectopically expressed in several cell lines, NHE8 can be classified as an organellar NHE. However, for the sake of consistency, we will adopt in this review the phylogenic analysis-dependent categorization, as indicated above.

Plasma membrane NHEs constitute an electroneutral exchange of cytoplasmic H^+ with extracellular Na^+ and are involved in pH regulation, osmolarity control, cell volume regulation, and absorption of salt and water in epithelia (*2*−*4*). They attract widespread clinical attention as they appear to be related to a variety of disorders such as epilepsy, essential hypertension, ischemia/reperfusion injury, and congenital secretory diarrhea (*2*−*4*). In contrast, our knowledge of organellar NHEs, which mediate both Na^+/H^+ and K^+/H^+ exchange, is rather limited. Organellar NHEs localize to various intracellular compartments along the secretory and endocytic pathways. Accordingly, it has been proposed that by facilitating proton efflux, organellar NHEs, next to the well-known V-ATPases, contribute to the establishment and/or fine-tuning of the luminal pH. Although likely of obvious importance, a direct link between this activity and an ensuing physiologically relevant effect remains to be determined.

In this review, the expression, localization, and ion selectivity of organellar NHEs will be highlighted first, and then recent advances in the knowledge of their role as a determinant of organellar pH by mediating proton efflux will be discussed. Emerging functions in intracellular trafficking as well as molecular interactions that regulate intracellular dynamics will also be

*To whom correspondence should be addressed. Phone: +81 (0)6 68505812. Fax: +81 (0)6 68505812. E-mail: kanazawa@bio.sci.osaka-u.ac.jp.

[1]Abbreviations: NHE, Na^+/H^+ exchanger; TGN, trans-Golgi network; ER, endoplasmic reticulum; CLC, chloride channel; V-ATPase, vacuolar ATPase; GPHR, Golgi pH regulator; siRNA, small interfering RNA; RACK1, receptor for activated protein kinase C-1; PKC, protein kinase C; ABCB4, ATP-binding cassette subfamily B member 4; MVB, multivesicular body; SCAMP, secretory carrier membrane protein; XLMR, X-linked mental retardation; AEE, apical early endosome; ARE, apical recycling endosome; SAC, subapical compartment.

Published on Web 12/20/2010

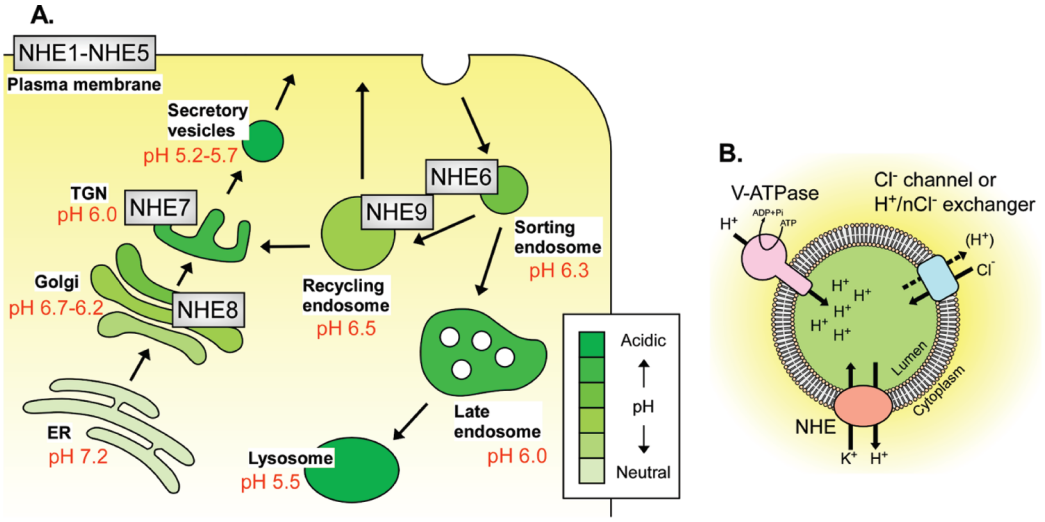

FIGURE 1: Organellar NHEs and luminal pH. (A) Localization of organellar NHEs and a luminal pH gradient along the secretory and endocytic pathway. Of the nine NHE isoforms, NHE1−5 are mainly localized to the plasma membrane, while NHE6−9 are defined as organellar NHEs. NHE6 and NHE9 colocalize to some extent at sorting/recycling endosomes. NHE7 and NHE8 are localized at TGN and mid/trans-Golgi stacks, respectively. These organellar NHEs are fairly "dynamic", implying that they are subjected to intracellular trafficking and thus continuously shuttle between organelles and the plasma membrane. Each intracellular compartment is maintained at an acidic pH, which is important for various biological processes, including membrane trafficking. Organellar NHEs are thought to be involved in the establishment and/or fine-tuning of a specific, compartment-dependent luminal pH. (B) Schematic illustration of pH regulation in organelles. The maintenance of a defined luminal acidic pH is proposed to be accomplished by the carefully balanced activities of inward proton pumping and proton efflux. Vacuolar H^+-ATPase, V-ATPase, primarily facilitates the acidification of the organellar lumen by pumping H^+ into the lumen. This acidification step is promoted by a counteranion flux through either Cl^- channels or Cl^-/H^+ exchangers, which shunt inside positive membrane potential across the organellar membrane. Organellar NHEs mediate the efflux of H^+ from the lumen in an exchange with K^+, which is abundantly present in the cytoplasm, and/or Na^+.

addressed. Intriguingly, several findings suggest a link between NHEs and neuronal disorders, and we will consider possible underlying mechanisms of these pathological conditions.

EXPRESSION, SUBCELLULAR LOCALIZATION, AND ION SELECTIVITY

The four organellar NHEs (NHE6−9) identified thus far show a relatively broad tissue distribution at the transcription level (*6*−*10*), although they are particularly expressed in skeletal muscle (NHE6, -7, and -8), brain (NHE6 and -7), and kidney (NHE8). NHE9 exhibits a fairly ubiquitous expression. Although the subcellular localization and intracellular dynamics of organellar NHEs have been only partially characterized, they primarily localize to distinct intracellular compartments, when expressed in several cell lines. Thus, NHE6 and NHE9 show a comparable localization in sorting/recycling endosomes (*8*, *11*). NHE7 and NHE8 are mainly localized at the trans-Golgi network (TGN) (*7*) and mid/trans-Golgi stacks (*8*), respectively (Figure 1A).

Of interest, several lines of evidence support the notion that organellar NHEs continuously cycle between intracellular compartments and the plasma membrane by means of vesicular transport mechanisms (*11*−*14*). Their distribution is thus likely dictated by an equilibrium between the pools at two or more compartments. However, in certain types of cells such as polarized epithelial cells, NHEs defined as organellar NHEs are mainly expressed at the plasma membrane. For instance, NHE6 and NHE9 are largely expressed at the plasma membrane of auditory hair cells of *Xenopus laevis* (*15*). Furthermore, NHE8 appears to be exclusively localized at the apical surface of epithelial cells in the intestine (*10*) and kidney (*9*, *14*) of rodents, suggesting its roles in absorption of Na^+ and water. Such biaxial localization is reported also for plasma membrane isoforms, NHE3 and NHE5, which are located in endosomal compartments besides the plasma membrane (*5*, *16*). These data thus indicate that the assigned categorization as either plasma membrane or organellar NHE is not necessarily mutually exclusive in terms of localization. However, the exact intracellular dynamics of organellar NHEs in these cases remains to be determined, which may contrast with that of bona fide plasma membrane NHEs.

A prominent distinction between organellar and plasma membrane NHEs, other than their subcellular localization and phylogenic relationship, is their selectivity for alkaline cations. Thus, whereas organellar NHEs operate as both K^+/H^+ and Na^+/H^+ exchangers (refs *7*, *8*, and *15* and unpublished data of H. Kanazawa et al.), plasma membrane NHEs solely mediate Na^+/H^+ exchange. Nevertheless, as a common feature, NHEs are polytopic transmembrane proteins that are composed of 12 putative membrane-spanning helices in the N-terminus and a cytoplasmic hydrophilic C-terminus. Compared to the C-terminal cytoplasmic tail, the amino acid sequences of the N-terminal transmembrane segments, which directly mediate the transmembrane crossing of ions, are highly conserved in plasma membrane and organellar NHEs. Accordingly, this would suggest that the actual mechanism of ion translocation per se in both NHE families is likely very similar. The molecular basis of the broader ion selectivity for organellar membrane NHEs (exchanging both Na^+ and K^+ for H^+) is unclear so far, yet NHE6, NHE7, and NHE9 possess a unique insertion at the putative second extracellular loop that contains many charged residues. This region has been suggested to be important for ion selectivity and inhibitor binding (*5*) and may therefore account for their ability to transport both Na^+ and K^+. Meanwhile, NHE8, which is only distantly related to the three other isoforms, belongs to a different clade in the phylogenic analysis (*5*) and lacks the insertion in the corresponding region. Therefore, the observed K^+/H^+ exchange in this case may have a unique molecular basis. At any rate, it is of

note that organellar NHEs exhibit similar K_M and V_{max} values for Na^+ and K^+ (ref *8* and unpublished data of H. Kanazawa et al.) and may thus mainly operate as K^+/H^+ exchangers under physiological conditions where the cytoplasmic K^+ concentration is relatively high (~140 mM) and that of Na^+ low (~10 mM).

PH REGULATION OF THE ORGANELLE LUMEN

The luminal pH values of organelles along the secretory and endocytic pathways exhibit a gradient, ranging from pH ~5.0 to ~7.0 (*17*, *18*). In the secretory pathway, the organellar pH progressively declines in the outbound direction, i.e., from the endoplasmic reticulum (ER) to Golgi, TGN, and secretory vesicles. By contrast, in the endocytic pathway, organellar acidity shows the opposite trend and gradually decreases in an inbound direction, the lysosomes being most acidic. Moreover, steep pH gradients exist within the recycling endosomal system of polarized epithelial cells (*19*−*23*). Clearly, it has been well established that the acidic pH is important for maintaining proper intracellular trafficking (sorting and vesiculation) and membrane fusion (e.g., in the late endolysosomal pathway), post-translational modification, dissociation of the receptor−ligand complex, and uptake of neurotransmitters (*17*−*24*). However, despite the well-recognized overall importance in cell biology, the molecular mechanism(s) underlying the fine-tuning of the pH in each organelle is still poorly understood.

The organelle pH is controlled by a complex balance of proton pumping and proton efflux activities across organellar membranes (Figure 1B). The primary catalyst responsible for acidification is the V-ATPase, a proton-pumping vacuolar ATPase (*25*, *26*). It is also well-known that organellar membranes exhibit Cl^- conductance that assists acidification by compensating for the negative effect of the membrane potential, generated by H^+ pumping. The best-characterized Cl^- transport proteins are the members of the CLC family, consisting of Cl^- channels and electrogenic Cl^-/H^+ exchangers (*27*) on endosomal membranes and the GPHR (Golgi pH regulator) on the Golgi membrane (*28*). The observation that pharmacological inhibition of V-ATPase results in an immediate dissipation of the proton gradient across organellar membranes suggests the existence of a proton efflux pathway. The CLC-mediated electrogenic Cl^-/H^+ exchange is not likely responsible for this proton efflux because the immediate loss of protons from Golgi and secretory granules occurs under even cytoplasmic Cl^--free conditions. Accordingly, it is therefore likely that organellar NHEs play a role in this process, as may be inferred from studies with Nhx1p, a yeast homologue of organellar NHEs. Thus, in a strain lacking Nhx1p, a significant decrease is seen in the luminal pH of the endosomal compartment, presumably because of the lack of proton efflux from the lumen (*29*). Some more recent studies provided further insight into the consequences of an altered expression of organellar NHEs on organellar pH in mammalian cells. Thus, overexpression of NHE6, NHE8, and NHE9 alkalizes the lumen of the specific organelles in which they are located, strongly indicating an enhanced proton extrusion activity (*8*, *30*). Alternatively, when the expression of endosomal NHEs was suppressed by siRNA, i.e., NHE6 alone in HepG2 cells, or in case of a double knockdown of both NHE6 and NHE9 in HeLa cells (*30*, *31*), the luminal pH of the endosomes shifted to a more acidic pH, consistent with a decreased rate of proton release. These data demonstrate that organellar NHEs can control the organellar pH at steady state by effectively mediating the efflux of protons from their lumen, resulting in a significant net change in the luminal pH.

At present, the mechanism that determines fixation of an organellar lumen pH within a narrow and specific range is poorly understood. Several potential mechanisms, participating in the regulation of V-ATPase proton pumping, have been proposed and include the assembly and/or disassembly of V_1-V_o complexes (*32*, *33*), the regulation of the amount of pumps present on the organelles (*34*), the efficiency of pumping and/or ATP-hydrolysis coupling, and the existence of organelle-specific isoforms (*26*, *35*). However, the relative contribution of each of these mechanisms in setting an organelle-specific pH remains to be elucidated. Given their specific localization, in conjunction with the evidence discussed in the previous paragraph, it is tempting to speculate that the distinct localization of organellar NHEs contributes to the establishment of an organelle-specific pH (*8*, *36*). To demonstrate this ability at the level of ion transport activity, it is necessary to establish a reliable in vitro assay system that allows comparative biochemical analysis of each isoform. Furthermore, the level of expression of a given NHE on a given organelle should be revealed as a determinant of proton efflux activity on the organellar membrane. Needless to say, the possibility of other regulatory events should also be addressed in the future, including a potential regulation via modifications such as phosphorylation, regulatory binding partners, and other molecular mechanisms, analogous to what has been reported for plasma membrane NHEs. Interestingly, the activity of the plasma membrane-residing NHE1 displays a clear acidic pH dependence. The requirement for proton binding as an allosteric modifier of transport as such rather than as a substrate has been postulated (*37*). Although significantly interesting, the versatility of analogous mechanisms of organellar NHEs in assisting the control of luminal pH remains to be established.

EMERGING FUNCTIONS OF ORGANELLAR NHES IN INTRACELLULAR MEMBRANE TRAFFICKING

What is the importance of pH regulation by organellar NHEs, and what are the specific physiological consequences in terms of organelle functioning? Studies of the yeast homologue Nhx1p have provided convincing support for its role in various aspects of intracellular membrane trafficking (*38*). Thus, in a yeast mutant strain lacking Nhx1p, the prevacuolar endosomes display aberrant structures, suggesting a role of the transporter in maintaining organellar morphology and integrity. Moreover, missorting occurs with vacuolar enzymes, destined for secretion to the extracellular space. Also, endocytosed proteins and lipids, normally destined for the vacuole, remain trapped in aberrant endosomal compartments, further supporting the notion that vesicular transport from endosome to vacuole is impaired in the mutant strain. Whether these alterations also underlie the cause of missorting due to lateral membrane perturbations that may deter the system's sorting machinery and/or the requirement of membrane vesiculation events for assembly of transport vehicles remains to be determined.

Analogous observations concerning a potential role of NHE6 in the maintenance of cell polarity, regulating the retention of resident apical plasma membrane lipids and proteins (*30*), were recently reported (Figure 2). In a polarized human hepatoma cell line, HepG2, NHE6 localizes in the recycling endosomal system, including the subapical compartment, the equivalent of

the common recycling endosome, which is considered to be a major sorting station for the segregation and polarized delivery of proteins and lipids to the apical or basolateral membrane surface domains (*39*, *40*). Both knockdown and overexpression of NHE6, which shifted the luminal endosomal pH of 6.6 to 5.9 and 7.3, respectively, cause a defect in the maintenance of the apical domain, as reflected by a defect in apical protein recycling. Thus, these data suggest that tuning of pH (range) in the apical recycling compartments by NHE6 is a prerequisite for efficient apical recycling. The molecular mechanism underlying the defect in recycling has not yet been determined but may conceivably be related to a failure of recruiting trafficking machinery components to the surface of recycling endosomes in a pH-dependent manner, as previously reported (*41*−*44*). Specifically, it has been shown that RACK1 (receptor for activated protein kinase C-1) is involved in the apical localization of the bile canalicular ABC transporter ABCB4 in HepG2 cells (*45*). Because RACK1 is an NHE6 binding protein (see also below), it is tempting to speculate that the loss of ABCB4 at the canalicular domain reflects a defect in apical recycling due to the loss of interaction between NHE6 and RACK1.

Recent evidence suggests a role for NHE8 in late endosomal morphology and membrane trafficking from late endosomal multivesicular bodies (MVBs) to lysosomes (*46*). Depletion of NHE8 resulted in an enlargement and clustering of late endosomes into the perinuclear region, accompanied by an increased level of degradation of epidermal growth factor. Still, a pH-related role remains enigmatic, because the luminal pH of the late endosomes was not altered. However, in this case, the possibility that an altered pH in the Golgi apparatus, where the majority of NHE8 is located, may indirectly affect the endosomal function as a result of a malfunctioning of trafficking between Golgi and late endosomes cannot be excluded.

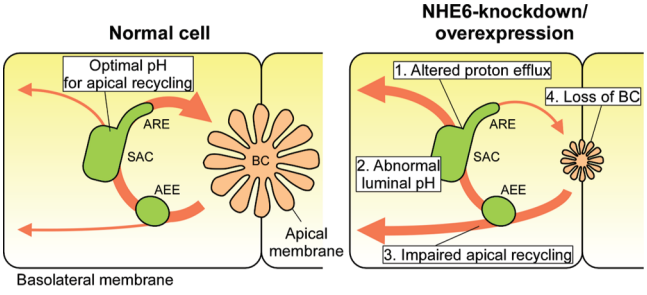

FIGURE 2: Functional role of NHE6 in maintaining the cell polarity of HepG2 cells. Overexpression or knockdown of NHE6 induces an increased or decreased rate of efflux of H^+ from apical endosomal compartments, respectively. The perturbed luminal pH results in an impaired apical recycling and/or retention of lipids and proteins, and the subsequent loss of bile canalicular structures, suggesting the existence of an optimal pH (range) for mechanisms underlying the maintenance of the bile canaliculi. Rather than an effective apical recycling, in these cells, transcytosis of apical components from the apical to the basolateral membrane occurs via AEE and ARE/SAC, triggered by a malfunctioning of the luminal pH.

REGULATORY BINDING PARTNERS FOR ORGANELLAR NHES

Valuable insight into the mechanisms of action of plasma membrane NHEs has been obtained by revealing a variety of regulatory cofactors. In particular, the C-termini of NHE1 and NHE3 have been shown to be susceptible to such regulatory interactions (*3*, *4*, *16*, *47*). The molecular and functional nature of these binding molecules is rather diverse and includes signaling molecules, metabolic enzymes, scaffolds, cytoskeleton elements, and lipids that belong to the phosphoinositide species. An additional level of regulation is provided by the presence of phosphorylation sites. The significance of such interactions and modifications ranges from affecting the stability of the protein and its subcellular localization to the regulation of the ion transport activity per se (*3*, *4*, *16*, *47*). Clearly, thus far, much less is known about such regulatory molecular interactions in the case of organellar NHEs. Nevertheless, an increasing number of studies identify proteins that interact with these transporters (Table 1).

Interestingly, some binding factors have been reported to bind to both types of NHEs, in spite of the fact that the amino acid sequences in the regions responsible for binding are not necessarily conserved. Most of interaction partners thus far identified appear to participate in the regulation of the intracellular location of organellar NHEs, in particular in controlling the shuttling of NHEs between two compartments (see also below). Unfortunately, because of methodological limitations, biochemical investigations that aimed to assess the potential importance of these interacting proteins in regulating ion transport activity have been frustrated. Some specific interaction partners for NHE6 and NHE7 will be briefly reviewed in the following sections. However, so far, spatiotemporal regulation of their interaction with

Table 1: Binding Partners of Organellar NHEs

binding partner	NHE	description	ref
RACK1[a]	NHE6, -7, -9	regulation of surface levels of NHE (NHE6)	*12*
angiotensin-II receptor AT2	NHE6	ligand-dependent formation of a complex with NHE6	*67*
SCAMP1, -2, -5[a]	NHE7	trafficking of NHE7 between TGN and recycling vesicles (SCAMP2)	*48*
caveolin-1	NHE7	association of NHE7 with caveolae and/or lipid rafts	*56*
β-actin, vimentin	NHE7	interaction in focal adhesion sites	*56*
CD44	NHE7	association with NHE7 in lipid rafts	*56*
calmodulin[a]	NHE7	regulation of ion transport activitiy	*56*
GLUT1	NHE7	identified in NHE7 interactome	*56*
junction plakoglobin, desmoplakin			
myosin heavy chain 9			
myosin light polypeptide 6			
tropomyosin-4			
Na^+/K^+-ATPase			

[a]These proteins are also identified as binding partners of plasma membrane NHEs.

binding partners is still poorly understood and merits further investigation.

Binding Partners for NHE7. In terms of its molecular interactions, the most extensively studied organellar NHE is NHE7, which is primarily localized to the TGN. Among others, the transporter has been shown to interact with secretory carrier membrane proteins (SCAMPs) (*48*), representing integral membrane proteins with four transmembrane segments. Five SCAMP isoforms known to date localize to Golgi and post-Golgi membranes (*49*) and have been implicated in vesicular trafficking in the secretory pathway (*50*−*52*). Moreover, these proteins (especially SCAMP2) have also been reported to be involved in the regulation of the plasma membrane localization of NHE5 (*53*), and the serotonin transporter (*54*). The interaction between NHE7 and SCAMP2 plays a role in the retrieval of NHE7 to the TGN from endosomal compartments. Thus, disruption of the interaction causes NHE7 to accumulate in TfR-positive endosomes. Interestingly, SCAMPs specifically interact with NHE7 despite the fact that the amino acid sequence of the SCAMP-binding region is also highly conserved in NHE6 and NHE9. Conceivably, other elements in NHE7 (co)determine the binding specificity with SCAMP2. Indeed, recently, a short stretch of amino acids was identified in the C-terminus of NHE7, proximal to the membrane-spanning region, acting as a determinant for the transporter's partitioning between TGN and endosomes (*55*). Deletion of this sequence in NHE7, which is not present in NHE6, resulted in an NHE6-resembling endosomal distribution of NHE7. However, the exclusiveness of this region for the potential binding to SCAMP2 has not been demonstrated in a direct manner and merits further work. The same holds true for other SCAMPs (SCAMP1 and SCAMP5), which have also been demonstrated to associate with NHE7, but the functional relevance and specificity of these interactions remain to be determined.

In addition to SCAMPs, several binding partners for NHE7 have been reported. Caveolin-1 interacts with the C-terminus of NHE7 and colocalizes with NHE7 in detergent-insoluble microdomains (*13*), implying the existence of two distinct pools of NHE7 (i.e., caveolae- and non-caveolae-associated) with distinct dynamics on the membrane such as the kinetics of internalization. Vimentin and β-actin colocalize with NHE7 at focal adhesions in migrating cells and have been postulated to interact with NHE7 (*56*), possibly to control cell adhesion and migration. Of all known binding partners, only the Ca^{2+}−calmodulin (CaM) complex has been suggested to be directly involved in the regulation of the ion transport activity of organellar NHEs (*56*). Indeed, treatment with CaM inhibitors abolishes the uptake of $^{86}Rb^+$, a radioactive K^+ analogue, into intracellular compartments, implying that in vivo K^+ uptake may be similarly abolished, thereby impeding the transporter's H^+ exchange capability. Interestingly, the Ca^{2+}−CaM complex is also known to associate with the C-terminus of NHE1 and stimulate ion transport activity (*57*, *58*). Thus, there might be a common regulatory mechanism that involves the Ca^{2+}−CaM complex for both plasma membrane and organellar NHEs, even though the CaM-binding motifs are not conserved. Interestingly, upon activation of protein kinase C (PKC) with phorbol esters, NHE7 translocates to the cell surface where it interacts with CD44 in detergent-resistant microdomains (*56*), raising the possibility that phosphorylation by PKC may alter the localization and/or ion transport activity of NHE7.

Binding Partners for NHE6. Receptor for activated protein kinase C-1 (RACK1), a scaffold protein, was demonstrated to interact with NHE6 (but in vitro interactions with NHE7 and NHE9 have also been reported) through the highly conserved region in the C-terminus (*12*). RACK1 dictates the distribution of NHE6 between the plasma membrane and endosomes and, in this way, presumably contributes to the establishment of the luminal pH of sorting/recycling endosomes. Moreover, it was shown, although indirectly, that NHE6 is involved in endocytosis via modulating endosomal pH (*12*). Presumably, RACK1, by an as yet unknown mechanism, causes the retention of NHE6 at the plasma membrane, like that reported for a RACK1 regulation of the plasma membrane localization of other transporters and receptors (*45*, *59*, *60*). Furthermore, RACK1, originally identified as an adaptor protein for activated PKC (*61*), is known as a scaffold protein that interacts with metabolic enzymes, kinases, receptors, ion channels, and other scaffolds (*62*, *63*). Thus, RACK1 may link NHEs to other proteins and alter the ion transport activity in a manner similar to that reported for other ion-transporting proteins (*64*−*66*).

Binding of NHE6 with angiotensin II receptor subtype AT2 is regulated by its ligand, angiotensin II (*67*). Although angiotensin II is known to regulate the function of Na^+/H^+ exchange activity in several cell types such as vascular smooth muscle cells (*68*), cardiac myocytes (*69*), and lymphocytes (*70*) in a manner partially dependent on PKC, the NHE isoform engaged in these phenomena has not been specified so far. It is tempting to speculate that NHE6 is responsible for the Na^+/H^+ exchange activity, observed to be upregulated by angiotensin II in these cells. It is also worthwhile to analyze the potential involvement of PKC, mediated by binding of RACK1 to NHE6.

LINK TO MENTAL DISEASE: IMPLICATION FOR ORGANELLAR NHES IN BRAIN FUNCTION

An increasing number of reports reveal the involvement of organellar NHEs, in particular NHE6 and NHE9, in neurological disorders. Four different types of mutated *NHE6* alleles were identified in families with Angelman syndrome-like X-linked mental retardation (XLMR), characterized by clinical symptoms that include microcephaly, seizure, ataxia, and absence of speech (*71*). Alterations in the *UBE3A* locus encoding a ubiquitin ligase have been identified as a cause of Angelman syndrome (OMIM 105830) (*72*). However, a considerable number (10−15%) of patients are unrelated to aberrant *UBE3A* (*72*), and several other diseases that present symptoms resembling those of Angelman syndrome exist (*73*, *74*). Thus, alternative causative genes have been postulated. One of the mutant NHE6 proteins identified in these patients carries an internal deletion of two amino acids at residues Glu255 and Ser256 in a putative transmembrane segment (*71*). The corresponding residues for Glu255 are highly conserved among all NHEs and have been demonstrated to be critically important for the ion transport activity in NHE1 (*75*, *76*), NHE6 (*30*), and NHE8 (*8*). Moreover, this mutant was shown to be abnormally ubiquitinated when ectopically expressed in HeLa cells. As a consequence, degradation of mutant NHE6 was accelerated, compared to that of wild-type NHE6, via lysosome- and proteasome-dependent degradation pathways (*31*), suggesting that patients are challenged by a severe decrease in the amount and activity of NHE6. Interestingly, NHE9, which is also located in sorting/recycling endosomes, has been genetically associated with neurological disorders such as attention deficient hyperactivity disorder (*77*) and family-based autisms (*78*). However, it remains to be elucidated how

mutations in the *SLC9A9* locus encoding NHE9 contribute to the etiology and pathogenesis of the disease.

A highly relevant question to be addressed concerns the issue of how these NHEs are involved in neural functions and how the defects of these NHEs would cause disorders. It is of note that both spontaneous null mutation (identified in *swe* mice) and targeted disruption of the *Nhe1* locus in mice result in epileptic-like seizures, ataxia, and growth retardation, which overlap with symptoms observed in the patients of Angelman syndrome and related diseases (*79*, *80*). Although a causal relationship is still unclear, it was shown that in *Nhe1* knockout mice the expression of the Na^+ channel, $Na_v1.2$, is upregulated in hippocampal and cortical regions, which could thus account for the observed neuronal overexcitability (*81*). It is therefore worthwhile to investigate whether similar mechanisms could account for defects in patients that suffer from organellar NHE-related neuronal disorders. The possibility that the etiology of the disease may be related to basic changes in processes as fundamental as cell polarity development is not to be excluded, as discussed above. After all, the brain is particularly enriched in polarized cells, such as neurons and glial cells. The development and maintenance of dendrites and spines rely heavily on trafficking through recycling endosomes (*82*, *83*). The latter also underlies long-term potentiation, a cellular mechanism responsible for learning and memory, which is disturbed in *Ube3a* knockout mice (*84*) and is thought to be perturbed in patients with Angelman syndrome (*85*). Given the role of NHE6 in polarized trafficking and hepatocyte polarity, one could speculate that NHE6, and probably NHE9, may play a role in the brain by regulating polarized membrane trafficking and polarization of neuronal cells through the regulation of endosomal pH. Magnetic resonance spectroscopy analysis of a patient with an Angelman syndrome-like disease revealed an accumulation of the glutamate−glutamine complex in basal ganglia (*71*), which was proposed to account, at least in part, for the clinical symptoms. As excess glutamate is well-known to exert excitoxic and neurodegenerative effects (*86*), it will be of interest to investigate the role of NHE6 in the trafficking of glutamate receptors or transporters involved in the response or clearance of glutamate at synaptic clefts. Recently, a study in patients displaying a mental retardation distinct from Angelman syndrome also revealed mutations in the *NHE6* locus. These patients were pathohistologically characterized with neuronal loss and tau deposition (*87*). As tau, a microtubule binding protein, plays a pivotal role in the growth and integrity of axons and dendrites in neurons (*88*), this finding further strengthens the possible linkage between NHE6 and the polarity of neuronal cells. Consistently, NHE6, NHE7, and NHE9 exhibit relatively elevated transcript expression levels in brain, even though they are rather widely expressed in other organs (*6*−*8*). Also, expression analysis by immunoblotting in mouse tissue revealed large amounts of NHE6 in the brain (H. Kanazawa et al., unpublished data). Further detailed histological and cell biological studies are needed to elucidate the function of NHE6 in the brain and, directly correlated therewith, the pathogenesis in the mental disorders mentioned above. *Nhe6* knockout mice partially mimic phenotypical features of patients suffering from mental disorders, including motor hyperactivity and a lowered threshold for pharmacologically induced seizures (Deltagen KO mouse and Phenotypic Data). Hence, this knockout system may prove to be a promising animal model for studying the molecular basis of these diseases.

CONCLUDING REMARKS

Different lines of evidence demonstrate that organellar NHEs act as a novel family of key players in the regulation of organellar pH. Depletion or overexpression of organellar NHEs results in an alteration of the luminal pH of specific organelles. The physiological consequences of such an alteration of pH were recently revealed in the case of NHE6, demonstrating its involvement in maintaining a limited pH range in the lumen of recycling endosomes as a requirement for proper apical membrane recycling and maintenance of cell polarity in HepG2. Recent studies also show that mutations in organellar NHEs are genetically linked to several neurological diseases. Given that neuronal cells, like epithelial cells, are polarized cells, it is tempting to speculate that the malfunctioning of organellar NHEs and subsequently defects in intracellular trafficking contribute to the pathogenesis of these diseases. Further studies are needed to identify interacting proteins that regulate organellar NHE function and dynamics and to unravel the molecular basis by which luminal pH regulates (polarized) membrane trafficking. In this context, it will also be particularly interesting to unravel the identity and mechanisms underlying the actions of distinct protein affectors of NHEs, including the specificity and dynamics of these interactions as well as the potential relevance of recruitment of these interacting platforms into distinct lateral membrane microdomains. Whether all such interactions serve the exclusive purpose of maintaining the organellar pH within strict limits or whether a local H^+ extrusion may lead to a transient but local pH change, thus creating a specific microenvironment at the organellar surface for specific catalytic reactions, is similarly a challenge for future research.

REFERENCES

1. Casey, J. R., Grinstein, S., and Orlowski, J. (2010) Sensors and regulators of intracellular pH. *Nat. Rev. Mol. Cell Biol. 11*, 50–61.
2. Slepkov, E. R., Rainey, J. K., Sykes, B. D., and Fliegel, L. (2007) Structural and functional analysis of the Na^+/H^+ exchanger. *Biochem. J. 401*, 623–633.
3. Orlowski, J., and Grinstein, S. (2004) Diversity of the mammalian sodium/proton exchanger SLC9 gene family. *Pfluegers Arch. 447*, 549–565.
4. Malo, M. E., and Fliegel, L. (2006) Physiological role and regulation of the Na^+/H^+ exchanger. *Can. J. Physiol. Pharmacol. 84*, 1081–1095.
5. Brett, C. L., Donowitz, M., and Rao, R. (2005) Evolutionary origins of eukaryotic sodium/proton exchangers. *Am. J. Physiol. 288*, C223–C239.
6. Numata, M., Petrecca, K., Lake, N., and Orlowski, J. (1998) Identification of a mitochondrial Na^+/H^+ exchanger. *J. Biol. Chem. 273*, 6951–6959.
7. Numata, M., and Orlowski, J. (2001) Molecular cloning and characterization of a novel $(Na^+,K^+)/H^+$ exchanger localized to the trans-Golgi network. *J. Biol. Chem. 276*, 17387–17394.
8. Nakamura, N., Tanaka, S., Teko, Y., Mitsui, K., and Kanazawa, H. (2005) Four Na^+/H^+ exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. *J. Biol. Chem. 280*, 1561–1572.
9. Goyal, S., Vanden Heuvel, G., and Aronson, P. S. (2003) Renal expression of novel Na^+/H^+ exchanger isoform NHE8. *Am. J. Physiol. 284*, F467–F473.
10. Xu, H., Chen, R., and Ghishan, F. K. (2005) Subcloning, localization, and expression of the rat intestinal sodium-hydrogen exchanger isoform 8. *Am. J. Physiol. 289*, G36–G41.
11. Brett, C. L., Wei, Y., Donowitz, M., and Rao, R. (2002) Human Na^+/H^+ exchanger isoform 6 is found in recycling endosomes of cells, not in mitochondria. *Am. J. Physiol. 282*, C1031–C1041.
12. Ohgaki, R., Fukura, N., Matsushita, M., Mitsui, K., and Kanazawa, H. (2008) Cell surface levels of organellar Na^+/H^+ exchanger isoform 6 are regulated by interaction with RACK1. *J. Biol. Chem. 283*, 4417–4429.
13. Lin, P. J., Williams, W. P., Kobiljski, J., and Numata, M. (2007) Caveolins bind to $(Na^+, K^+)/H^+$ exchanger NHE7 by a novel binding module. *Cell. Signalling 19*, 978–988.

14. Goyal, S., Mentone, S., and Aronson, P. S. (2005) Immunolocalization of NHE8 in rat kidney. *Am. J. Physiol. 288*, F530–F538.
15. Hill, J. K., Brett, C. L., Chyou, A., Kallay, L. M., Sakaguchi, M., Rao, R., and Gillespie, P. G. (2006) Vestibular hair bundles control pH with $(Na^+, K^+)/H^+$ exchangers NHE6 and NHE9. *J. Neurosci. 26*, 9944–9955.
16. Alexander, R. T., and Grinstein, S. (2009) Tethering, recycling and activation of the epithelial sodium-proton exchanger, NHE3. *J. Exp. Biol. 212*, 1630–1637.
17. Paroutis, P., Touret, N., and Grinstein, S. (2004) The pH of the secretory pathway: Measurement, determinants, and regulation. *Physiology 19*, 207–215.
18. Demaurex, N. (2002) pH Homeostasis of Cellular Organelles. *News Physiol. Sci. 17*, 1–5.
19. Wang, E., Brown, P. S., Aroeti, B., Chapin, S. J., Mostov, K. E., and Dunn, K. W. (2000) Apical and basolateral endocytic pathways of MDCK cells meet in acidic common endosomes distinct from a nearly-neutral apical recycling endosome. *Traffic 1*, 480–493.
20. Henkel, J. R., Apodaca, G., Altschuler, Y., Hardy, S., and Weisz, O. A. (1998) Selective perturbation of apical membrane traffic by expression of influenza M2, an acid-activated ion channel, in polarized madin-darby canine kidney cells. *Mol. Biol. Cell 9*, 2477–2490.
21. Henkel, J. R., Gibson, G. A., Poland, P. A., Ellis, M. A., Hughey, R. P., and Weisz, O. A. (2000) Influenza M2 proton channel activity selectively inhibits trans-Golgi network release of apical membrane and secreted proteins in polarized Madin-Darby canine kidney cells. *J. Cell Biol. 148*, 495–504.
22. Henkel, J. R., Popovich, J. L., Gibson, G. A., Watkins, S. C., and Weisz, O. A. (1999) Selective perturbation of early endosome and/or trans-Golgi network pH but not lysosome pH by dose-dependent expression of influenza M2 protein. *J. Biol. Chem. 274*, 9854–9860.
23. Gagescu, R., Demaurex, N., Parton, R. G., Hunziker, W., Huber, L. A., and Gruenberg, J. (2000) The recycling endosome of Madin-Darby canine kidney cells is a mildly acidic compartment rich in raft components. *Mol. Biol. Cell 11*, 2775–2791.
24. Mellman, I. (1992) The importance of being acid: The role of acidification in intracellular membrane traffic. *J. Exp. Biol. 172*, 39–45.
25. Futai, M., Oka, T., Sun-Wada, G., Moriyama, Y., Kanazawa, H., and Wada, Y. (2000) Luminal acidification of diverse organelles by V-ATPase in animal cells. *J. Exp. Biol. 203*, 107–116.
26. Forgac, M. (2007) Vacuolar ATPases: Rotary proton pumps in physiology and pathophysiology. *Nat. Rev. Mol. Cell Biol. 8*, 917–929.
27. Jentsch, T. J. (2007) Chloride and the endosomal-lysosomal pathway: Emerging roles of CLC chloride transporters. *J. Physiol. 578*, 633–640.
28. Maeda, Y., Ide, T., Koike, M., Uchiyama, Y., and Kinoshita, T. (2008) GPHR is a novel anion channel critical for acidification and functions of the Golgi apparatus. *Nat. Cell Biol. 10*, 1135–1145.
29. Brett, C. L., Tukaye, D. N., Mukherjee, S., and Rao, R. (2005) The yeast endosomal Na^+K^+/H^+ exchanger Nhx1 regulates cellular pH to control vesicle trafficking. *Mol. Biol. Cell 16*, 1396–1405.
30. Ohgaki, R., Matsushita, M., Kanazawa, H., Ogihara, S., Hoekstra, D., and van Ijzendoorn, S. C. (2010) The Na^+/H^+ exchanger NHE6 in the endosomal recycling system is involved in the development of apical bile canalicular surface domains in HepG2 cells. *Mol. Biol. Cell 21*, 1293–1304.
31. Roxrud, I., Raiborg, C., Gilfillan, G. D., Stromme, P., and Stenmark, H. (2009) Dual degradation mechanisms ensure disposal of NHE6 mutant protein associated with neurological disease. *Exp. Cell Res. 315*, 3014–3027.
32. Trombetta, E. S., Ebersold, M., Garrett, W., Pypaert, M., and Mellman, I. (2003) Activation of lysosomal function during dendritic cell maturation. *Science 299*, 1400–1403.
33. Sautin, Y. Y., Lu, M., Gaugler, A., Zhang, L., and Gluck, S. L. (2005) Phosphatidylinositol 3-kinase-mediated effects of glucose on vacuolar H^+-ATPase assembly, translocation, and acidification of intracellular compartments in renal epithelial cells. *Mol. Cell. Biol. 25*, 575–589.
34. Lafourcade, C., Sobo, K., Kieffer-Jaquinod, S., Garin, J., and van der Goot, F. G. (2008) Regulation of the V-ATPase along the endocytic pathway occurs through reversible subunit association and membrane localization. *PLoS One 3*, e2758.
35. Marshansky, V., and Futai, M. (2008) The V-type H^+-ATPase in vesicular trafficking: Targeting, regulation and function. *Curr. Opin. Cell Biol. 20*, 415–426.
36. Orlowski, J., and Grinstein, S. (2007) Emerging roles of alkali cation/proton exchangers in organellar homeostasis. *Curr. Opin. Cell Biol. 19*, 483–492.
37. Wakabayashi, S., Hisamitsu, T., Pang, T., and Shigekawa, M. (2003) Kinetic dissection of two distinct proton binding sites in Na^+/H^+ exchangers by measurement of reverse mode reaction. *J. Biol. Chem. 278*, 43580–43585.
38. Bowers, K., Levi, B. P., Patel, F. I., and Stevens, T. H. (2000) The sodium/proton exchanger Nhx1p is required for endosomal protein trafficking in the yeast *Saccharomyces cerevisiae*. *Mol. Biol. Cell 11*, 4277–4294.
39. van IJzendoorn, S. C., Maier, O., van Der Wouden, J. M., and Hoekstra, D. (2000) The subapical compartment and its role in intracellular trafficking and cell polarity. *J. Cell. Physiol. 184*, 151–160.
40. Hoekstra, D., Tyteca, D., and van IJzendoorn, S. C. (2004) The subapical compartment: A traffic center in membrane polarity development. *J. Cell Sci. 117*, 2183–2192.
41. Maranda, B., Brown, D., Bourgoin, S., Casanova, J. E., Vinay, P., Ausiello, D. A., and Marshansky, V. (2001) Intra-endosomal pH-sensitive recruitment of the Arf-nucleotide exchange factor ARNO and Arf6 from cytoplasm to proximal tubule endosomes. *J. Biol. Chem. 276*, 18540–18550.
42. Hurtado-Lorenzo, A., Skinner, M., El Annan, J., Futai, M., Sun-Wada, G. H., Bourgoin, S., Casanova, J., Wildeman, A., Bechoua, S., Ausiello, D. A., Brown, D., and Marshansky, V. (2006) V-ATPase interacts with ARNO and Arf6 in early endosomes and regulates the protein degradative pathway. *Nat. Cell Biol. 8*, 124–136.
43. Gu, F., and Gruenberg, J. (2000) ARF1 regulates pH-dependent COP functions in the early endocytic pathway. *J. Biol. Chem. 275*, 8154–8160.
44. Aniento, F., Gu, F., Parton, R. G., and Gruenberg, J. (1996) An endosomal β COP is involved in the pH-dependent formation of transport vesicles destined for late endosomes. *J. Cell Biol. 133*, 29–41.
45. Ikebuchi, Y., Takada, T., Ito, K., Yoshikado, T., Anzai, N., Kanai, Y., and Suzuki, H. (2009) Receptor for activated C-kinase 1 regulates the cellular localization and function of ABCB4. *Hepatol. Res. 39*, 1091–1107.
46. Lawrence, S. P., Bright, N. A., Luzio, J. P., and Bowers, K. (2010) The Sodium/Proton Exchanger NHE8 Regulates Late Endosomal Morphology and Function. *Mol. Biol. Cell 21*, 3540–3551.
47. Donowitz, M., Mohan, S., Zhu, C. X., Chen, T. E., Lin, R., Cha, B., Zachos, N. C., Murtazina, R., Sarker, R., and Li, X. (2009) NHE3 regulatory complexes. *J. Exp. Biol. 212*, 1638–1646.
48. Lin, P. J., Williams, W. P., Luu, Y., Molday, R. S., Orlowski, J., and Numata, M. (2005) Secretory carrier membrane proteins interact and regulate trafficking of the organellar $(Na^+,K^+)/H^+$ exchanger NHE7. *J. Cell Sci. 118*, 1885–1897.
49. Hubner, K., Windoffer, R., Hutter, H., and Leube, R. E. (2002) Tetraspan vesicle membrane proteins: Synthesis, subcellular localization, and functional properties. *Int. Rev. Cytol. 214*, 103–159.
50. Fernandez-Chacon, R., Alvarez de Toledo, G., Hammer, R. E., and Sudhof, T. C. (1999) Analysis of SCAMP1 function in secretory vesicle exocytosis by means of gene targeting in mice. *J. Biol. Chem. 274*, 32551–32554.
51. Liu, L., Guo, Z., Tieu, Q., Castle, A., and Castle, D. (2002) Role of secretory carrier membrane protein SCAMP2 in granule exocytosis. *Mol. Biol. Cell 13*, 4266–4278.
52. Liao, H., Zhang, J., Shestopal, S., Szabo, G., Castle, A., and Castle, D. (2008) Nonredundant function of secretory carrier membrane protein isoforms in dense core vesicle exocytosis. *Am. J. Physiol. 294*, C797–C809.
53. Diering, G. H., Church, J., and Numata, M. (2009) Secretory Carrier Membrane Protein 2 Regulates Cell-surface Targeting of Brain-enriched Na^+/H^+ Exchanger NHE5. *J. Biol. Chem. 284*, 13892–13903.
54. Muller, H. K., Wiborg, O., and Haase, J. (2006) Subcellular redistribution of the serotonin transporter by secretory carrier membrane protein 2. *J. Biol. Chem. 281*, 28901–28909.
55. Fukura, N., Ohgaki, R., Matsushita, M., Nakamura, N., Mitsui, K., and Kanazawa, H. (2010) A membrane-proximal region in the C-terminal tail of NHE7 is required for its distribution in the trans-Golgi network, distinct from NHE6 localization at endosomes. *J. Membr. Biol. 234*, 149–158.
56. Kagami, T., Chen, S., Memar, P., Choi, M., Foster, L. J., and Numata, M. (2008) Identification and biochemical characterization of the SLC9A7 interactome. *Mol. Membr. Biol. 25*, 436–447.
57. Bertrand, B., Wakabayashi, S., Ikeda, T., Pouyssegur, J., and Shigekawa, M. (1994) The Na^+/H^+ exchanger isoform 1 (NHE1) is a novel member of the calmodulin-binding proteins. Identification and characterization of calmodulin-binding sites. *J. Biol. Chem. 269*, 13703–13709.

58. Wakabayashi, S., Ikeda, T., Iwamoto, T., Pouyssegur, J., and Shigekawa, M. (1997) Calmodulin-binding autoinhibitory domain controls "pH-sensing" in the Na^+/H^+ exchanger NHE1 through sequence-specific interaction. *Biochemistry 36*, 12854–12861.
59. Auerbach, M., and Liedtke, C. M. (2007) Role of the scaffold protein RACK1 in apical expression of CFTR. *Am. J. Physiol. 293*, C294–C304.
60. Parent, A., Laroche, G., Hamelin, E., and Parent, J. L. (2008) RACK1 regulates the cell surface expression of the G protein-coupled receptor for thromboxane A_2. *Traffic 9*, 394–407.
61. Ron, D., Chen, C. H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Cloning of an intracellular receptor for protein kinase C: A homolog of the β subunit of G proteins. *Proc. Natl. Acad. Sci. U.S.A. 91*, 839–843.
62. McCahill, A., Warwicker, J., Bolger, G. B., Houslay, M. D., and Yarwood, S. J. (2002) The RACK1 scaffold protein: A dynamic cog in cell response mechanisms. *Mol. Pharmacol. 62*, 1261–1273.
63. Sklan, E. H., Podoly, E., and Soreq, H. (2006) RACK1 has the nerve to act: Structure meets function in the nervous system. *Prog. Neurobiol. 78*, 117–134.
64. Bandyopadhyay, B. C., Ong, H. L., Lockwich, T. P., Liu, X., Paria, B. C., Singh, B. B., and Ambudkar, I. S. (2008) TRPC3 controls agonist-stimulated intracellular Ca^{2+} release by mediating the interaction between inositol 1,4,5-trisphosphate receptor and RACK1. *J. Biol. Chem. 283*, 32821–32830.
65. Cao, G., Thebault, S., van der Wijst, J., van der Kemp, A., Lasonder, E., Bindels, R. J., and Hoenderop, J. G. (2008) RACK1 inhibits TRPM6 activity via phosphorylation of the fused α-kinase domain. *Curr. Biol. 18*, 168–176.
66. Woodard, G. E., Lopez, J. J., Jardin, I., Salido, G. M., and Rosado, J. A. (2010) TRPC3 regulates agonist-stimulated Ca^{2+} mobilization by mediating the interaction between type I inositol 1,4,5-trisphosphate receptor, RACK1 and Orai1. *J. Biol. Chem. 285*, 8045–8053.
67. Pulakat, L., Cooper, S., Knowle, D., Mandavia, C., Bruhl, S., Hetrick, M., and Gavini, N. (2005) Ligand-dependent complex formation between the angiotensin II receptor subtype AT2 and Na^+/H^+ exchanger NHE6 in mammalian cells. *Peptides 26*, 863–873.
68. Berk, B. C., Aronow, M. S., Brock, T. A., Cragoe, E., Jr., Gimbrone, M. A., Jr., and Alexander, R. W. (1987) Angiotensin II-stimulated Na^+/H^+ exchange in cultured vascular smooth muscle cells. Evidence for protein kinase C-dependent and -independent pathways. *J. Biol. Chem. 262*, 5057–5064.
69. Sandmann, S., Yu, M., Kaschina, E., Blume, A., Bouzinova, E., Aalkjaer, C., and Unger, T. (2001) Differential effects of angiotensin AT1 and AT2 receptors on the expression, translation and function of the Na^+-H^+ exchanger and Na^+-HCO_3^- symporter in the rat heart after myocardial infarction. *J. Am. Coll. Cardiol. 37*, 2154–2165.
70. Fortuno, A., Tisaire, J., Lopez, R., Bueno, J., and Diez, J. (1997) Angiotensin converting enzyme inhibition corrects Na^+/H^+ exchanger overactivity in essential hypertension. *Am. J. Hypertens. 10*, 84–93.
71. Gilfillan, G. D., Selmer, K. K., Roxrud, I., Smith, R., Kyllerman, M., Eiklid, K., Kroken, M., Mattingsdal, M., Egeland, T., Stenmark, H., Sjoholm, H., Server, A., Samuelsson, L., Christianson, A., Tarpey, P., Whibley, A., Stratton, M. R., Futreal, P. A., Teague, J., Edkins, S., Gecz, J., Turner, G., Raymond, F. L., Schwartz, C., Stevenson, R. E., Undlien, D. E., and Stromme, P. (2008) SLC9A6 mutations cause X-linked mental retardation, microcephaly, epilepsy, and ataxia, a phenotype mimicking Angelman syndrome. *Am. J. Hum. Genet. 82*, 1003–1010.
72. Clayton-Smith, J., and Laan, L. (2003) Angelman syndrome: A review of the clinical and genetic aspects. *J. Med. Genet. 40*, 87–95.
73. Williams, C. A., Lossie, A., and Driscoll, D. (2001) Angelman syndrome: Mimicking conditions and phenotypes. *Am. J. Med. Genet. 101*, 59–64.
74. Jedele, K. B. (2007) The overlapping spectrum of Rett and Angelman syndromes: A clinical review. *Semin. Pediatr. Neurol. 14*, 108–117.
75. Fafournoux, P., Noel, J., and Pouyssegur, J. (1994) Evidence that Na^+/H^+ exchanger isoforms NHE1 and NHE3 exist as stable dimers in membranes with a high degree of specificity for homodimers. *J. Biol. Chem. 269*, 2589–2596.
76. Murtazina, R., Booth, B. J., Bullis, B. L., Singh, D. N., and Fliegel, L. (2001) Functional analysis of polar amino-acid residues in membrane associated regions of the NHE1 isoform of the mammalian Na^+/H^+ exchanger. *Eur. J. Biochem. 268*, 4674–4685.
77. Markunas, C. A., Quinn, K. S., Collins, A. L., Garrett, M. E., Lachiewicz, A. M., Sommerd, J. L., Morrissey-Kane, E., Kollins, S. H., Anastopoulos, A. D., and Ashley-Koch, A. E. (2009) Genetic variants in SLC9A9 are associated with measures of attention-deficit/hyperactivity disorder symptoms in families. *Psychiatr. Genet. 20*, 73–81.
78. Morrow, E. M., Yoo, S. Y., Flavell, S. W., Kim, T. K., Lin, Y., Hill, R. S., Mukaddes, N. M., Balkhy, S., Gascon, G., Hashmi, A., Al-Saad, S., Ware, J., Joseph, R. M., Greenblatt, R., Gleason, D., Ertelt, J. A., Apse, K. A., Bodell, A., Partlow, J. N., Barry, B., Yao, H., Markianos, K., Ferland, R. J., Greenberg, M. E., and Walsh, C. A. (2008) Identifying autism loci and genes by tracing recent shared ancestry. *Science 321*, 218–223.
79. Cox, G. A., Lutz, C. M., Yang, C. L., Biemesderfer, D., Bronson, R. T., Fu, A., Aronson, P. S., Noebels, J. L., and Frankel, W. N. (1997) Sodium/hydrogen exchanger gene defect in slow-wave epilepsy mutant mice. *Cell 91*, 139–148.
80. Bell, S. M., Schreiner, C. M., Schultheis, P. J., Miller, M. L., Evans, R. L., Vorhees, C. V., Shull, G. E., and Scott, W. J. (1999) Targeted disruption of the murine Nhe1 locus induces ataxia, growth retardation, and seizures. *Am. J. Physiol. 276*, C788–C795.
81. Xia, Y., Zhao, P., Xue, J., Gu, X. Q., Sun, X., Yao, H., and Haddad, G. G. (2003) Na^+ channel expression and neuronal function in the Na^+/H^+ exchanger 1 null mutant mouse. *J. Neurophysiol. 89*, 229–236.
82. Park, M., Salgado, J. M., Ostroff, L., Helton, T. D., Robinson, C. G., Harris, K. M., and Ehlers, M. D. (2006) Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. *Neuron 52*, 817–830.
83. Tang, B. L. (2008) Emerging aspects of membrane traffic in neuronal dendrite growth. *Biochim. Biophys. Acta 1783*, 169–176.
84. Jiang, Y. H., Armstrong, D., Albrecht, U., Atkins, C. M., Noebels, J. L., Eichele, G., Sweatt, J. D., and Beaudet, A. L. (1998) Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. *Neuron 21*, 799–811.
85. Dindot, S. V., Antalffy, B. A., Bhattacharjee, M. B., and Beaudet, A. L. (2008) The Angelman syndrome ubiquitin ligase localizes to the synapse and nucleus, and maternal deficiency results in abnormal dendritic spine morphology. *Hum. Mol. Genet. 17*, 111–118.
86. Obrenovitch, T. P. (1999) High extracellular glutamate and neuronal death in neurological disorders. Cause, contribution or consequence? *Ann. N.Y. Acad. Sci. 890*, 273–286.
87. Garbern, J. Y., Neumann, M., Trojanowski, J. Q., Lee, V. M., Feldman, G., Norris, J. W., Friez, M. J., Schwartz, C. E., Stevenson, R., and Sima, A. A. (2010) A mutation affecting the sodium/proton exchanger, SLC9A6, causes mental retardation with tau deposition. *Brain 133*, 1391–1402.
88. Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A., and Hof, P. R. (2000) Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res. Brain Res. Rev. 33*, 95–130.