Molecular Cloning and Characterization of a Novel \((\text{Na}^+,\text{K}^+)/\text{H}^+\) Exchanger Localized to the trans-Golgi Network*

Masayuki Numata and John Orlowski‡

From the Department of Physiology, McGill University, Montréal, Québec H3G 1Y6, Canada

The luminal pH of organelles along the secretory and endocytic pathways of mammalian cells is acidic and tightly regulated, with the [H\(^+\)] varying up to 100-fold between compartments. Steady-state organelar pH is thought to reflect a balance between the rates of H\(^+\) pumping by the vacuolar-type H\(^+\)-ATPase and H\(^+\) efflux through ill-defined pathways. Here, we describe the cloning of a novel gene (NHE7) in humans that is homologous to Na\(^+\)/H\(^+\) exchangers, is ubiquitously expressed, and localizes predominantly to the trans-Golgi network. Significantly, NHE7 mediates the influx of Na\(^+\) or K\(^+\) in exchange for H\(^+\). The activity of NHE7 was also found to be relatively insensitive to inhibition by amiloride but could be antagonized by the analogue benzamil and the unrelated compound quinine. Thus, NHE7 displays unique functional and pharmacological properties and may play an important role in maintaining cation homeostasis of this important organelle.

The luminal ionic composition of many, if not all, intracellular compartments differs from the surrounding cytoplasm and is an important determinant of their function. The establishment of this differential composition is achieved through the concerted actions of distinct integral membrane ion carriers, including pumps, channels, and transporters. For example, alkalization of the mitochondrial matrix, driven by the respiratory chain, contributes to the inner membrane H\(^-\) gradient used to drive ATP synthesis (1) and, indirectly, to extrude matrix Ca\(^{2+}\) through the functional coupling of Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) antiport pathways (2–4).

By contrast, organelles of the secretory and endocytic pathways are distinguished by their luminal acidity, which is generated by the activity of an electrogenic vacuolar-type H\(^+\)-ATPase (V-ATPase) (5, 6). Progressive acidification of vesicles in the endocytic pathway (early and late endosomes, pH \(<\) 6.5 → lysosomes, pH \(<\) 4.5) is essential for the redistribution and degradation of internalized membrane proteins, such as ligand-receptor complexes and fluid-phase solutes (5, 7). Likewise, increasing luminal acidification of compartments of the exocytic pathway (endoplasmic reticulum, pH \(>\) 7.0 → Golgi complex, pH \(~\) 6.5 → trans-Golgi network (TGN), pH \(~\) 6.0 → secretory vesicles, pH \(~\) 5.0) is important for proper post-translational processing and sorting of newly synthesized proteins (5, 8, 9).

At present, little is known about the mechanisms controlling the steady-state [H\(^+\)] within the lumen of different endomembrane compartments. Although distinct isoforms of some of the V-ATPase subunits have been reported in different tissues (10, 11) or specialized cell types (12), there is no clear evidence that the V-ATPase functions differently in particular organelles within a single mammalian cell (although this may not be the case in yeast (13)). It has been suggested that because the pump is electrogenic, its activity could be influenced by the membrane potential and by the availability of permeant counterions such as chloride and potassium (14). However, in the case of the Golgi complex, the endogenous counterion conductances were found to exceed the rate of H\(^+\) pumping at the steady state, implying that the electrical potential across the membrane is negligible and therefore not a defining factor in setting organellar pH (15–17). In addition, despite extensive work, differential control of V-ATPase activity by hormones or other factors has not been found along the endo- or exocytic pathways. Rather, the luminal [H\(^+\)] is thought to be regulated by a complex interplay between the V-ATPase and unidentified leak pathways for protons, based on the rapid dissipation of the transmembrane proton chemical gradient (\(\Delta\psi\)) observed after inhibiting the V-ATPase with macrolide antibiotics (15, 16, 18). A component of this H\(^+\) leak in the Golgi complex was recently identified as a Zn\(^{2+}\)-inhibitable H\(^+\) conductance (17) but could not fully account for H\(^+\) turnover. Nevertheless, it highlights the H\(^+\) leak as a key determinant of organellar pH and emphasizes the need to identify the molecular components of this pathway which, in addition to putative H\(^+\) channels, could conceivably involve H\(^+\) proton-coupled cotransporters or exchangers.

In this study, we describe the cloning and functional characterization of a unique monovalent cation/proton exchanger that localizes predominantly to the trans-Golgi network and suggests a novel molecular mechanism for controlling the luminal cation composition of this important organelle.

**EXPERIMENTAL PROCEDURES**

*Molecular Cloning—Two overlapping human ESTs with homology to known mammalian Na\(^+\)/H\(^+\) exchangers were identified in GenBank™ (accession number AA279477 and AA648924) and obtained from Genome Systems Inc. (St. Louis, MO). Examination of the cDNA sequences (designated as NHE7) indicated that they were missing coding information at the 5′ and 3′ regions (i.e. start and stop codons, respectively). To obtain the complete nucleotide sequence, we isolated cDNA fragments corresponding to the missing 3′-end (amino acids 624–725) from a human bone marrow cDNA library using rapid amplification of cDNA ends methodology (19). However, we were unable to clone the...
5'-end of the cDNA using this approach. While this work was in progress, the sequences covering the 3'-end of the cDNA, but not the 5'-end, were found to match nucleotide sequences present in a human genomic fragment (GenBank™ accession number AL022165) that mapped to chromosome Xp11. To determine the missing 5'-sequence, we used a PCR-based strategy to clone a genomic fragment covering the 5'-end of one of the EST clones (GenBank™ accession number AA648924). Four overlapping positive clones were isolated by screening 4 × 10^6 independent clones from the library. The largest insert was subcloned into a plasmid vector, and the nucleotide sequence (419 amino acids), in addition to downstream sequence that precisely overlapped the 5'-end of the EST clone, was found to reside within a single predicted exon. The presence of this sequence in the NHE7 transcript was verified by reverse transcriptase-polymerase chain reaction (PCR) using human bone marrow and skeletal muscle poly(A)+ RNA. The predicted translation initiation codon is preceded by a purine nucleotide in position +3 and downstream contains a purine at position +4, placing it in a good context for initiation by eukaryotic ribosomes, as defined by Kozak (21). In addition, the apparent translation initiation site is also preceded by an in-frame stop codon at nucleotide position −336. The 5'-end of the NHE7 cDNA sequence was subsequently found to match uncharacterized genomic sequences that map to chromosome Xp11.1–11.4 (GenBank™ accession number AL050307). The full-length cDNA was reconstituted by PCR and the integrity of the construct was verified by DNA sequencing. The complete cDNA sequence was deposited in GenBank™ (accession number AF289591).

**RNA Blotting**—Human poly(A+) mRNA Northern and Master dot blots (CLONTECH) were hybridized with a 0.2-kilobase pair PCR fragment generated from the 5'-end of NHE7 that shares minimal sequence identity with other NHE isoforms. The PCR probe was agarose gel-purified and radiolabeled with [α-32P]dCTP by the random primer method. Hybridization was done at 65 °C in Church buffer containing 7% SDS, 0.5 mM sodium phosphate, pH 7.2, 1% bovine serum albumin and 2 mM EDTA overnight. The blots were washed twice in 2× SSC, 0.05% SDS at room temperature for 30 min each, followed by three additional stringency washes in 0.1× SSC containing 0.1% SDS at 65 °C for 30 min each. The radioactive signals were analyzed by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Stable Transfection and Expression of NHE7**—The full-length NHE7 cDNA was engineered to include the influenza virus hemagglutinin (HA) epitope,YPYDVPDYAS (preceded by a single G amino acid linker inserted to create peptide flexibility), at the very C-terminal end (called NHE7HA) of the PCR mutagenesis to allow for immunological detection of the protein. In a separate construct, an HA epitope was inserted at an internal site, Leu488 (NHE7488HA). The NHE7 HA construct was subcloned into the edcsyne-inducible expression vector pMND (Invitrogen) and transfected into Chinese hamster ovary cells that constitutively express an edcsyne-activated receptor (ErR-CHO cells; Invitrogen).

Cells stably expressing both NHE7HA and ErR were selected in α-meoindigo green medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 25 mM NaHCO3, pH 7.4, and containing 600 μg/ml G418 and 250 μg/ml zeocin. The cells were maintained in an humidified atmosphere of 95% air and 5% CO2 at 37 °C. Single colonies were isolated, and the regulated expression of NHE7HA was verified by Western blotting and immunofluorescence microscopy in the presence of increasing concentrations (0–10 μM) of the edcsyne analogue, ponasterone A, for 24 h.

**Western Blotting**—Cells were washed three times with ice-cold PBS and then lysed with triple detergent buffer (150 mM NaCl, 0.1% SDS, 1% IGEPEAL CA-630, 0.5% sodium deoxycholate and 50 mM Tris-HCl, pH 8.0) supplemented with proteinase inhibitor mixture (Roche Molecular Biochemicals) for 5–10 min on ice. Cell lysates were spun at 12,000 × g for 5 min to remove insoluble cell debris, separated in a 7.5% SDS-PAGE, and then transferred to a polyvinylidene difluoride membrane (Amersham Pharmacia Biotech). The blot was briefly rinsed with PBS, blocked with 5% non-fat skim milk in PBS for 1 h, and then incubated with mouse monoclonal antibodies against either the HA epitope (Babco, Berkeley, CA) at a 1/5,000 dilution or cytosome oxide subunit IV (COX IV) (Molecular Probes, Eugene, OR) at a 1/200 dilution, followed by washes with PBS. For Western blots with PBS washes containing 0.1% Tween 20, the blot was incubated with goat anti-mouse IgG secondary antibody conjugated with horseradish peroxidase (Jackson Laboratory, Bar Harbor, ME) at a dilution of 1/20,000. Green fluorescent protein (GFP) was detected with a rabbit polyclonal anti-GFP antibody (1/100 dilution) (CLONTECH) followed by incubation with a mouse anti-rabbit IgG secondary antibody conjugated with horseradish peroxidase (New England Biolabs) at a dilution of 1/3,000. Immunoreactive bands were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech) and exposed to an x-ray film.

**Immunofluorescence Confocal Microscopy**—For immunofluorescence confocal microscopy studies, NHE7HA-transfected ErR-CHO cells were grown on glass coverslips by incubation with a mouse anti-rabbit IgG (1/100 dilution) or cytochrome oxidase subunit IV (COX IV) (Molecular Probes, Eugene, OR) at a 1/200 dilution for 1 h. For double labeling experiments with polyclonal antibodies to organelle-specific markers, the signals were visualized using Oregon Green or FITC-conjugated donkey anti-rabbit IgG (Molecular Probes and Jackson Laboratory, respectively). In the case of the TGN marker, a mammalian expression vector containing the CD25-TGN38 chimeric gene (22) was transiently transfected into the cells, and the protein was visualized by FITC-conjugated anti-CD25 antibody (Serotec, Raleigh, NC). The coverslips were washed, mounted onto glass slides, and analyzed by confocal laser scanning microscopy using a Zeiss inverted microscope. Images were processed using Adobe® Photoshop™ version 5.5 and CorelDraw™ version 8.0.

**Measurements of Organellar Na+ and K+ Influx**—Rates of Na+ (for 22Na+) and K+ (for 42K+) influx were determined by Na+- or K+ -rich buffer (in mM: 140 KCl, 2 CaCl2, 1 MgCl2, 2 Mg2+-ATP, 20 HEPES, pH 7.2) at 20 °C for 4.5 min, followed by multiple washes. Influx measurements were conducted in choline chloride-rich buffer (in mM: 140 choline chloride, 2 CaCl2, 1 MgCl2, 2 Mg2+-ATP, 1 EGTA, 10 HEPES-Tris, pH 7.8). Following a 5-min uptake period, the cells were quickly washed three times with ice-cold stop buffer (in mM: 140 NaCl (for 22Na+) influx) or 140 KCl (for 42K+ influx), 2 CaCl2, 1 MgCl2, 10 HEPES, pH 5.5). To extract the radiolabel, the monolayers were solubilized with 0.5% NaOH and neutralized with 0.5% HCl, and the pooled extracts were assayed by liquid scintillation spectroscopy.

The data were analyzed using the Bio-Rad DC protein assay kit according to the manufacturer's protocol. Each experiment was repeated at least three times.

**RESULTS AND DISCUSSION**

A search of the GenBank™ database for candidate genes homologous to known mammalian Na+/H+ exchangers (i.e. NHE1-NHE6) (23–25) identified two novel overlapping expressed sequence tags from human (GenBank™ accession numbers AA279477 and AA648924). These shared highest sequence identity to the mitochondrion-targeted NHE6 isoform rather than to the plasmaembral NHEs (i.e. NHE1–5), suggesting that the gene product (which we designate as NHE7) may also reside in an intracellular compartment. Examination of the sequences of the putative NHE7 cDNAs indicated that they lacked coding information at the 5'- and 3'-ends. To obtain the complete nucleotide sequence, we sequenced cDNAs isolated from a single-stranded cDNA library that had been generated from human bone marrow poly(A)+ mRNA using rapid amplification of cDNA ends methodology and genomic fragments cloned from a human λ phage library (for details, see “Experimental Procedures”).

The deduced primary sequence of NHE7 is composed of 725 amino acids (calculated Mr = 80,132) and exhibits high amino acid identity (~70%) to NHE6 (Fig. 1A) but low similarity (~25%) to other NHEs. Based on hydrophathy plot analysis, NHE7 is predicted to contain 12 α-helical hydrophobic membrane-spanning (M) segments in the N terminus followed by a hydrophilic cytoplasmic tail at the C terminus, similar to other NHEs (Figs. 1B, 1C). Recent biochemical and structural studies of the NHE1 isoform partially support this structural model (26, 27), although some notable changes in the arrangement of the C-terminal transmembrane segments have been proposed (27), namely the predicted M10 segment was suggested to reside within the lipid bilayer (the predicted M11 was renamed M10), whereas the last extracellular loop was found to form an
intracellular loop, a new transmembrane segment (M11), and an extracellular loop. Unlike NHE1, a recent report has suggested that a part of the cytoplasmic C terminus of the NHE3 isoform may reside at the exoplasmic surface (28). Whether these structural features of the plasmalemmal NHEs also apply to the more distantly related organellar NHEs remains to be determined.

Northern blot analysis of selected human tissues using an isoform-specific cDNA probe from the 39-coding region revealed three NHE7 mRNA transcripts of 9.5, 7.5, and 3.0 kilobases in length under high stringency hybridization conditions (Fig. 2A). More extensive RNA dot blot analyses of human tissues showed that the gene is expressed ubiquitously (Fig. 2B) but most prominently in certain regions of the brain (putamen and occipital lobe), skeletal muscle, and secretory tissues (prostate, stomach, pancreas, pituitary gland, adrenal gland, thyroid gland, salivary gland, and mammary gland). Each of the mRNAs is of sufficient length to contain the entire coding region, which could result from differential processing of the untranslated regions or alternative-splicing of a single gene product. The former is favored since attempts to identify alternatively spliced variants within the coding region by reverse transcriptase-PCR were unsuccessful. Alternatively, certain transcript(s) may represent other closely related genes that have yet to be characterized. This broad pattern of expression is consistent with NHE7 serving a “housekeeping” function.

To facilitate expression and localization of NHE7, we inserted an influenza virus hemagglutinin (HA) epitope at the extreme C terminus (NHE7HA). The full-length NHE7HA cDNA was subcloned into the ecdysone-inducible expression vector pIND and stably transfected into Chinese hamster ovary cells engineered to express constitutively an ecdysone-activable receptor (EcR-CHO cells). Regulated expression of NHE7HA was verified by incubating isolated NHE7 HA-transfected EcR-CHO cell colonies in the presence of increasing concentrations of ponasterone A, an analogue of ecdysone. As shown in Fig. 3, ponasterone A induced a dose-dependent increase in the expression of NHE7HA, which migrated as two broad bands of ~80 kDa by SDS-PAGE analysis. The faster migrating band corresponds to the predicted size of the protein. The larger band may represent the formation of a homodimer that is modestly stable in SDS, as has been reported for NHE1 and NHE3 (29). The bands are diffuse, suggesting the presence of glycosylation or other post-translational modifications. Consistent with this possibility, the N terminus contains putative N-linked glycosylation sites in the predicted extracellular loop between M3 and M4 (Asn145-Val-Ser) and between M9 and M10 (Asn400-Leu-Ser). Although the latter site is highly conserved in eukaryotic NHEs, it does not appear to be glycosylated in other mammalian NHE isoforms (i.e. NHE1, NHE2, and NHE3) (30–32) and therefore is unlikely to be modified in NHE7.
To define the subcellular distribution of NHE7HA, NHE7HA-transfected EcR-CHO cells were treated with a submaximal concentration of ponasterone A (5 μM) for 24 h and then examined using immunofluorescence confocal microscopy. Typically under these conditions, ~50% of the cells in different stable transfectants were found to express detectable levels of NHE7HA. The partial penetrance of NHE7 HA expression upon ponasterone A induction was also observed in individual secondary and tertiary isolates obtained by dilution subcloning. The cellular basis for this limited expression pattern is unclear but may relate to the site of genomic integration of the ecdysone receptor gene and/or the stage of the cell cycle.

Representative dual labeling experiments revealed that NHE7HA accumulates predominantly in a juxtanuclear compartment that was closely apposed but somewhat broader than the compact structure labeled by an antibody against α-mannosidase II, an established marker of the medial and trans-cisternae of the Golgi (Fig. 4, A–C) (33). Transient expression of two additional constructs, one containing an internal HA-tag inserted at position Leu488 and the other containing a C-terminal c-Myc epitope, gave similar results, suggesting that the protein distribution is not influenced by the position or sequence of the tag. The pattern was clearly distinct from those observed with antibodies that recognize specific markers of the endoplasmic reticulum (calnexin), lysosomes (cathepsin B and D), or mitochondria (mito-green fluorescent protein (mito-GFP) (34) and COX IV) (data not shown). NHE7HA also did not appear to accumulate at the cell surface nor was it able to functionally complement a mutant strain of CHO cells lacking plasmalemmal NHE activity (35) (data not shown).

The comparatively broader immunofluorescence signal of NHE7HA relative to that of α-mannosidase II suggested that it may be present in compartments distal to the Golgi cisternae, such as the TGN and possibly endosomes. To define further NHE7 compartmentation, cells were treated with different pharmacological agents that are known to affect the Golgi, TGN, and endosomes differentially. The fungal metabolite brefeldin A causes the disassembly of the Golgi apparatus by promoting the retrograde absorption and dispersion of Golgi cisternae resident proteins into the endoplasmic reticulum (36, 37), thereby creating a mixed endoplasmic reticulum/Golgi compartment. It concomitantly induces the coalescence of the TGN with early endosomes into a dense juxtanuclear structure (38, 39). As shown in Fig. 4, pretreatment of cells with brefeldin A (5 μg/ml for 2 h at 37°C) dispersed the immunofluorescence signal of α-mannosidase II (Fig. 4, E and F) into a reticular pattern, whereas that of NHE7HA (Fig. 4, D and F) was largely retained in a compact juxtanuclear complex, with a minor fraction diffusely distributed throughout the cell. These data suggest that NHE7HA is concentrated primarily in the TGN/early endosomal structure. The location of the TGN was defined by transiently transfecting the cells with an ex-
expression vector containing the chimeric gene CD25-TGN38. This chimera, which is composed of the extracellular domain of the α-chain of the interleukin-2 receptor linked to the transmembrane and cytosolic domains of TGN38, has been shown to accumulate in the TGN and can be readily labeled with commercially available FITC-conjugated CD25 antibodies (15, 22). As shown in Fig. 4, G–I, the distribution of NHE7HA precisely overlapped that of CD25-TGN38, suggesting it is predominantly in the TGN.

To establish further the compartmentation of NHE7HA cells were treated with the microtubule-disrupting agent nocodazole, which causes initial dispersion of the TGN and endosomes (early event), followed by the redistribution of the Golgi cisternae (late event), into discrete vesicular compartments throughout the cytoplasm (37, 40). As shown in Fig. 5, A–C, acute treatment with nocodazole (10 μM for 1 h) caused the TGN marker CD25-TGN38 to scatter in a pattern precisely matching that of NHE7HA whereas the distribution of α-mannosidase II in the Golgi cisternae remained relatively compact (Fig. 5, D–F). However, after a 4-h exposure to nocodazole, the α-mannosidase II signal also dispersed in a pattern that was distinct from, but partially overlapping, that of NHE7HA (Fig. 5, G–I). In similarly treated cells, endomembrane vesicles containing NHE7HA also did not precisely colocalize with those containing transiently transfected, Myc-tagged NHE3, which is known to accumulate at the cell surface but also in endocytic or recycling endosomal vesicles of CHO cells (41) (data not shown). Taken together, these data suggest that some NHE7 may be present in the Golgi cisternae but most is situated in the TGN.

It is noteworthy that NHE7, unlike the closely related NHE6 isoform, lacks an obvious mitochondrial targeting sequence at its N terminus (i.e., ~20–60 amino acids with abundant basic residues that are predicted to form an amphipathic α-helix) (42). However, NHE7 does contain putative motifs for Golgi targeting and/or retention, including a unique Ser/Thr-phosphorylatable acidic cluster (542EEPSEEDQNE551) (43) and a tyrosine-based sequence (552YFRV555) (44). Differential localization of NHE7 and NHE6 was further demonstrated biochemically by subcellular fractionation of the endomembrane compartments using differential centrifugation and Western blotting with antibodies that recognize either endogenous or ectopically expressed organelle-specific markers. As shown in Fig. 6, NHE7HA was associated with the microsomal enriched membrane fraction (P100 pellet) isolated from cells that were also transiently transfected with Golgi-targeted green fluorescent protein (g-GFP), a convenient marker for this fraction (34). By contrast, NHE6HA (also stably expressed under the control of the ecdysone-inducible promoter in another CHO cell line) accumulated in the mitochondrion-enriched fraction (P100 pellet), as defined molecularly by the presence of the mitochondrion-specific marker COX IV. Neither NHE7HA nor NHE6HA was present in the soluble fractions (S100) isolated from cells that were transiently transfected with an expression plasmid containing cytoplasmic GFP (c-GFP) as a marker (34).

To assess NHE7HA activity, we adapted procedures that had been used previously for measuring 45Ca2+ uptake and release from the Golgi (45). The plasma membrane was selectively permeabilized with saponin, and 22Na+ uptake into intact endomembrane compartments was compared with and without ponasterone A-induced overexpression of NHE7HA. Prelimi-
The role of pH was evaluated by treating cells with the 
induced by 5 mM ponasterone A for 24 h. Prior to functional measurements, the cells were incubated in the absence (−) or presence (+) of ponasterone A (Pon A, 10 μM) for an additional 24 h, followed by permeabilization of the plasma membrane with saponin (for details, see "Experimental Procedures"). The transport rates are normalized as a percentage of control values (−Pon A) measured in choline chloride-rich buffers (pH 7.8) A. Prior to measurements of [22Na] influx (5 μCi of [22NaCl (carrier-free) μl), cells were untreated or pretreated with either the H⁺ ionophore CCCP (2 μM) or the alkalizing agent NH₄Cl (30 mM) for 2 or 5 min, respectively, and were maintained during the 5-min uptake period. In one series of experiments, the NH₄Cl was rapidly washed out (+ w/o) of the cells prior to measurements of [22Na] influx in order to increase the acidity of the organelar compartments. B, initial rates of [22Na] influx were measured in the presence of amiloride (2 mM) or benzamil (0.1 and 1 mM). Values represent the average of 3–4 experiments, each performed in triplicate (mean ± S.D.). Differences in the experimental groups were analyzed by a one-way analysis of variance, and comparisons between means were carried out using the Newman-Keuls test at the 5% significance level (asterisks * p < 0.05).
the presence of two functionally distinct monovalent cation/proton exchangers in mammalian mitochondria. One of these preferentially mediates the exchange of matrix Na\(^+\) for intermembrane H\(^+\) generated by respiration (i.e. a Na\(^+\)-selective Na\(^+\)/H\(^+\) exchanger) (48, 49) and is inhibited by benzamil derivatives of amiloride at micromolar concentrations (47, 50–52). It is constitutively active in respiring mitochondria and is primarily responsible for establishing the [Na\(^+\)] gradient ([Na\(^+\)] \(_{\text{m}}\) < [Na\(^+\)] \(_{\text{i}}\) ) that allows Na\(^+\)-dependent extrusion of matrix Ca\(^{2+}\) (53). The other monovalent cation/H\(^+\) exchanger is latent, transports all alkali cations (i.e. Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), and Cs\(^+\) ) at similar rates, is antagonized by drugs such as quinine, dicyclohexylcarbodiimide, and propranolol (54–56), and is postulated to play a role in organellar volume homeostasis (57). Again, since K\(^+\) is the predominant intracellular alkali cation, it is simply referred to as a K\(^+\)/H\(^+\) exchanger. At present, it is unclear which of the mammalian mitochondrial NHEs corresponds to the recently cloned mitochondrion-targeted NHE6 isoform since it has yet to be characterized functionally. However, in view of the high structural similarity between NHE7 and NHE6, we speculate that NHE6 may function as the mitochondrial quinine-sensitive K\(^+\)/H\(^+\) exchanger. In this regard, we also find that NHE7HA activity is sensitive to inhibition by quinine (Fig. 8C), further suggestive of its functional similarity to one of the mitochondrial NHEs (i.e. NHE6). Further detailed pharmacological analyses are currently ongoing.

Homologues to human NHE6 and NHE7 have also been identified in lower eukaryotes, including the yeast Saccharomyces cerevisiae gene, NHX1/NHA2 (24, 58), and the plant Arabidopsis thaliana gene, AtNHX1 (59). Immunological analyses showed that yeast Nhx1 and plant AtNhx1 proteins localize predominantly to the late endosomal/prevacuolar and tonoplast/vacuolar compartments, respectively, and were capable of conferring tolerance to cytotoxic concentrations of NaCl (59–61). More recently, yeast Nhx1 was also found to be important for efficient protein trafficking out of the prevacuolar compartment (62). The latter results are particularly intriguing in view of the localization of NHE7 to the TGN and suggest that it may fulfill a similar physiological function. We are currently testing this hypothesis.

In summary, we describe the cloning and functional characterization of a novel monovalent cation/H\(^+\) exchanger that localizes predominantly to the trans-Golgi network and likely plays an important role in maintaining the cation homeostasis and function of this important organelle.

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