Electroneutral Na⁺-H⁺ exchange is present in virtually all cells, mediating the exchange of extracellular Na⁺ for intracellular H⁺ and, thus, plays an important role in the regulation of intracellular pH, cell volume, and transepithelial Na⁺ absorption. Recent transport studies demonstrated the presence of a novel chloride-dependent Na⁺-H⁺ exchange in the apical membrane of crypt cells of rat distal colon. We describe the cloning of a 2.5-kb full-length cDNA from rat distal colon that encodes 438 amino acids and has six putative transmembrane spanning domains. Of the 438 amino acids 375 amino acids at the N-terminal region are identical to Na⁺-H⁺ exchange (NHE)-1 isoform with the remaining 63 amino acids comprising a completely novel C terminus. In situ hybridization revealed that this transcript is expressed in colonic crypt cells, whereas Northern blot analysis established the presence of its 2.5-kb mRNA in multiple tissues. Despite its much smaller size compared with all other known Na⁺-H⁺ exchange isoforms, NHE-deficient PS120 fibroblasts stably transfected with this cDNA exhibited Na⁺-dependent intracellular pH recovery to an acid load that was chloride-dependent and inhibited both by 5-ethylisopropylamiloride, an amiloride analogue, and by 5′-nitro-2-(3-phenylpropylamino)-benzoic acid, a CI⁻ channel blocker, but only minimally affected by 25 μM 3-methylsulfonyl-4-iperidonbenzoylguanidine, an NHE-1 and NHE-2 isoform inhibitor. In contrast to other Na⁺-H⁺ exchange isoforms in colonic epithelial cells, chloride-dependent Na⁺-H⁺ exchange mRNA abundance was increased by dietary sodium depletion. Based on these results we predict that chloride-dependent Na⁺-H⁺ exchange represents a new class of Na⁺-H⁺ exchangers that may regulate ion transport in several organs.

Na⁺-H⁺ exchangers (NHE) have critical roles in multiple organs as a result of their one-for-one exchange of Na⁺ and H⁺ (1–3). At least seven NHE isoforms have been cloned to date and functionally expressed with a length ranging from 669 to 898 amino acids with 10–12 putative transmembrane domains (1–5). NHE-1 isozyme is ubiquitous and linked to intracellular pH (pHᵢ) and cell volume regulation, whereas other isoforms (e.g. NHE-2 and NHE-3) are present solely in epithelial cells (6, 7). The NHE-1 isozyme has been identified on the basolateral membrane of epithelial cells and on the plasma membrane of non-polar cells functioning as a “housekeeper,” whereas the NHE-3 isozyme is expressed predominantly on the apical membrane of polarized epithelial cells and has been linked to transepithelial sodium-dependent fluid absorption and pHᵢ regulation (6, 7). Consequently, loss of NHE1 or NHE3 function has a severe impact on cellular and organ functions (8, 9).

Intestinal electroneutral Na⁺ absorption is the result of an apical membrane NHE and has been linked to the NHE-3 isoform whose message and protein are present in surface but not crypt cells (7). The long-standing model of fluid and electrolyte movement in the large and small intestine is that fluid absorptive processes are present in surface/villous cells, whereas secretory ones are localized to crypt cells (10). To study crypt cell function directly in the rat distal colon, we recently established methods to perform microperfusion of colonic crypts adapting methods used previously with renal tubules and to prepare apical membrane vesicles (AMV) from isolated crypt cells (11, 12). In microperfusion studies of isolated rat colonic crypts, we demonstrated sodium-dependent fluid absorption (11) and therefore sought the identity of this unexpected phenomenon. By using both AMV and microperfusion, we demonstrated that both [H⁺]gradient-driven Na⁺ uptake and sodium-dependent recovery of pHᵢ from an acid load, respectively, had an absolute requirement for chloride (12–14). Additional studies established that the chloride dependence of chloride-dependent Na⁺-H⁺ exchange (Cl⁻-NHE) most likely involves one or more Cl⁻ channels, including cystic fibrosis transmembrane regulator and not a Cl⁻-HCO₃⁻ exchange, as follows: 1) CI⁻-NHE activity in AMV was inhibited by both Cl⁻ channel blockers and partially by a polycyclonal antibody to cystic fibrosis transmembrane regulator (13); 2) Cl⁻-HCO₃⁻ exchangers are not present in crypt AMV (15); and 3) Cl⁻-NHE activity was not inhibited by 100 μM 4,4’-diisothiocyano-ostylicine-2,2’-disulfonic acid, a concentration that only inhibits its chloride anion exchanges but not Cl⁻ channels (13).

As the characteristics and the kinetic properties of the colonic Cl⁻-NHE differed from those of other known NHE isoforms (14) and NHE-2 but not the NHE-3 isoform has been localized to the apical membrane of colonic crypt cells, it was likely that chloride-dependent Na⁺-H⁺ exchange transport protein was mediated by a previously unidentified NHE isoform. These present studies report the following: 1) the isolation of a 2,498-bp full-length cDNA from colonic crypt cells that consists of a 5′ end that is identical to the NHE-1 isoform and a completely novel 3′ end segment; 2) the stable expression of this cDNA exhibited Na⁺-H⁺ exchange transport protein was supported by United States Public Health Service Research Grant NIDDKD RO1 DK 14669 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby identified in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF462063.

The abbreviations used are: NHE, Na⁺-H⁺ exchange; AMV, apical membrane vesicles; CI-NHE, chloride-dependent Na⁺-H⁺ exchange; EIPA, 5-ethylisopropylamiloride; pHᵢ, intracellular pH; HOE694, 3-methylsulfonyl-4-iperidonbenzoylguanidine; NPPB, 5′-nitro-2-(3-phenylpropylamino)-benzoic acid; RACE, rapid amplification of cDNA ends.
cDNA in NHE-deficient PS120 cells that manifested sodium-dependent pH recovery that was chloride-dependent and inhibited by both 5-ethylisopropylamiloride (EIPA) and 5′-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB); 3) the up-regulation of Cl-NHE mRNA by dietary sodium depletion; and 4) the localization of its mRNA in colonic crypt cells and its expression in multiple tissues. The identification of this unique protein with wide distribution in several tissues may provide an explanation for Na⁺- and Cl⁻-coupled fluid and electrolyte movement that has not been adequately elucidated by existing transport proteins. Thus, these studies indicate the identification of a new class of NHE proteins that are widely expressed and raise the possibility of their importance in cellular homeostasis in several organs.

**EXPERIMENTAL PROCEDURES**

Male Sprague-Dawley rats (200–210 g body wt, Charles River Laboratories, Wilmington, MA) were used in these experiments and were fed a commercial rat diet. The rats were allowed free access to water. One group of rats was fed a sodium-free diet for 7 days, as described previously (16), to produce a secondary increase in serum aldosterone levels (17).

**Isolation of Distal Crypt Cells**—Crypt cells of the rat distal colon were prepared, as described previously (11, 12). Briefly, colonic crypts were isolated by a modified rapid calcium chelation method (12). Isolated crypt and surface cells were isolated following incubation of everted colonic segments, as described previously (12). To establish the relative purity of surface and crypt cells, ouabain-sensitive and ouabain-insensitive H,K-ATPase activities were determined (18). Enrichment of ouabain-sensitive H,K-ATPase activity with a relative absence of ouabain-insensitive H,K-ATPase activity provided evidence of a predominant crypt cell preparation with a relative absence of surface cells. This method resulted in crypt cell preparations that were 10% contaminated by surface cells (18).

**Cl-NHE Cloning Strategy**—Total RNA and mRNA from crypt cells were prepared by standard methods. SuperScript preamplification system (Invitrogen) was strictly followed to prepare first strand cDNA using μg of mRNA, oligo dT, and random hexamer primers in separate reactions. Negative control reactions were also performed without reverse transcriptase. Five microliters of first strand cDNA products were used as a template for the PCR in a 50-μl reaction volume using sense and antisense primers designed from highly conserved F and J membrane spanning domains of NHE1–NHE4 (19) (sense primer, GATCTCAGCTGTTACCTGTC, and antisense primer, GCCCAT- GACCTGGCTGTTCT; sense primer, GCCCAT-GACCTGGCTGTTCT, and antisense primer, GCCCAT-GACCTGGCTGTTCT). The following PCR program was used in cycles of 94°C for 30 s, 50°C for 45 s, 72°C for 1 min, and a final extension of 5 min at 72°C using PCR system 2400 (PerkinElmer Life Sciences). The expected 500-bp reverse transcriptase-PCR products were subcloned and sequenced; the sequence of one of these PCR products had 70% homology with rat NHE-1 isoform sequence (19). The PCR products were subcloned and sequenced; the sequence of one of these PCR products had 70% homology with rat NHE-1 isoform sequence (19). The PCR products were subcloned and sequenced; the sequence of one of these PCR products had 70% homology with rat NHE-1 isoform sequence (19). The PCR products were subcloned and sequenced; the sequence of one of these PCR products had 70% homology with rat NHE-1 isoform sequence (19).

**RESULTS**

The cloning strategy to identify a putative Cl-NHE cDNA was based on performing reverse transcriptase-PCR using primers that were designed on the basis of the J and F membrane spanning domains that are conserved in the NHE-1–4 isoforms (19), and mRNA was isolated from crypt cells of normal rat distal colon. The PCR products were subcloned and sequenced; the sequence of one of these PCR products had significant homology to the rat NHE-1 sequence. This cDNA was used to screen a rat colon cDNA library that yielded a single positive clone that represented a 1.9-kb cDNA consisting of a 700-bp fragment identical to the NHE-1 and 1.2-kb novel fragment with a stop codon within the first 200 bp. 5′-RACE was performed to clone the 5′ end and obtained a cDNA fragment with a start codon. A 2,498-bp full-length cDNA with a 1,314-bp open reading frame, a 5′-noncoding sequence of 360 bp, and a 3′-noncoding sequence of 824 bp were constructed by PCR using the 1.9-kb cDNA and the 5′-RACE product. The completely novel fragment is 1,015 bp representing the 189-bp open reading frame and 3′-non-coding sequence of 824 bp. The fragment that was homologous to a portion of NHE-1 is 1,485 bp representing a 1,125-bp coding region and a 360-bp 5′-noncoding sequence. The nucleotide sequence of the putative Cl-NHE cDNA predicts a protein of 488 amino acids with a calculated molecular mass of ~50 kDa (predicted).

Fig. 1 provides the deduced amino acid sequence of the putative Cl-NHE with a comparison to the other NHE isoforms known to date. The N-terminal portion of the coding region has 375 amino acids that are identical to that of NHE-1 isoform, whereas the C-terminal segment of 63 amino acids is completely novel. The novel 63-amino acid region has three potential

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Fig. 1. Comparison of amino acid sequences of Cl-NHE with other known NHEs. The amino acid sequences of Cl-NHE, rat NHE-1, NHE-2, NHE-3, NHE-4, NHE-5, and human NHE-6 and NHE-7 isoforms were aligned, and identical amino acids are boxed. Amino acids are numbered at both right and left starting from the translation initiation site.
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Fig. 2. a, hydropathy plot analysis. Hydropathy profile of Cl-NHE was determined according to the algorithm of Kyte and Doolittle using windows of 11 amino acids. Positive values correspond to hydrophobic segments, and negative values are indicative of hydrophilic segments. This analysis demonstrates this cDNA has six transmembrane domains. b, phylogenetic tree analysis. Phylogenetic tree analysis was generated by amino acid comparison of NHE-1 to NHE-5 isoforms and indicates that the Cl-NHE isoform is closely related to NHE-1 isoform. NHE-3 and NHE-5 isoforms are the least related isoforms to Cl-NHE.

Prior studies (22) have demonstrated that dietary sodium depletion and aldosterone markedly reduces both colonic apical membrane NHE activity and NHE isoform mRNA abundance in colonic epithelial cells. Therefore, the effect of dietary sodium depletion on Cl-NHE mRNA abundance was determined in Northern blot analyses using the 589-bp probe and mRNA prepared from colonic epithelial cells from normal and dietary sodium-depleted rats. In these studies Cl-NHE mRNA abundance from dietary sodium-depleted rats was substantially increased (Fig. 5). Prior studies (14) had shown that Cl-NHE activity (as evidenced by [H⁺] gradient-stimulated 22Na⁺ uptake by crypt AMV) was significantly increased by dietary sodium depletion.

In view of the unusually short sequence and its partial homology to NHE-1, it was critical to determine whether this small cDNA encoded for a protein with functional NHE activity. Therefore, PS120 fibroblasts lacking endogenous NHE function (20) were stably transfected with the putative Cl-NHE cDNA. pH recovery after an intracellular acid load that had been induced by a NH₃/NH₄Cl prepulse was measured in the nominal absence of bicarbonate using 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein (23). Untransfected PS120 fibroblasts showed no pH recovery both in the presence or absence of sodium (0.001 ± 0.005 units pH/min) (Fig. 6a) confirming the absence of NHE activity in these cells. In transfected cells in the absence of Cl⁻, only minimal sodium-dependent recovery of pH was seen (Fig. 6b). However, when both Na⁺ and Cl⁻ were present, there was a significant increase in pH following an acid load (Figs. 6b and 7). Thus, sodium-dependent pH recovery to an acid load in these cells transfected with the putative Cl-NHE cDNA has a requirement for Cl⁻.

The effect of both amiloride and its analogues and the non-specific Cl⁻ channel blocker, NPPB, on the expressed sodium/chloride-dependent pH recovery in these PS120 cells was also
examined. The sodium/chloride-dependent pH recovery to an acid load was almost completely blocked by a low concentration of the amiloride derivative EIPA (0.1 mM, 95 ± 2% of control). Twenty five M HOE694, at which concentration HOE694 is a specific inhibitor of the NHE-1 isoform, did not inhibit sodium/chloride-dependent pH recovery to an acid load.

Because Cl-NHE activity in both colonic AMV and during crypt microperfusion studies is inhibited by NPPB, a nonspecific Cl channel blocker, an additional study was performed with 500 M NPPB that significantly reduced sodium/chloride-dependent pH recovery to an acid load by −59 ± 3%. Thus, the pharmacological profile distinguishes the novel Cl-NHE from all other known NHE isoforms and particularly from the closely related NHE-1 isoform (3, 14). More importantly, the functional and pharmacological properties of this novel NHE isoform closely resemble those of the recently described Cl-dependent Na\(^{+}\)-H\(^{+}\) exchange in apical membranes of colon crypts strongly suggesting that this newly identified cDNA encodes the transport protein expressing Cl-NHE function in apical membranes of colonic crypts (12–14).

**DISCUSSION**

The classic model of fluid movement in the small and large intestine is that absorptive processes are present in surface/villous cells and secretory processes in crypt cells (10). In addition, NHE-3 isoform has been linked to transepithelial electroneutral sodium absorption and fluid absorption following the demonstration that 1) glucocorticosteroids increase both NHE-3 isoform message and protein in rabbit ileum; 2) neither Na\(^{+}\)-H\(^{+}\) exchange nor NHE-3 isoform message and protein are present in the rabbit distal colon (6, 7); and 3) NHE-3 deficient,
but not NHE-2-deficient mice have diarrhea (8). Consistent with the previously postulated absence of fluid absorption in crypts (10), NHE-3 message and protein have been localized to surface but not crypt cells (7). However, recent studies from our laboratories (11) demonstrated sodium-dependent fluid absorption in rat colonic crypts. Thus, the demonstration of sodium-dependent fluid absorption was unexpected and led to studies to explore for the presence of other sodium transport processes in colonic crypt apical membranes. Subsequent studies (24) revealed that sodium-dependent fluid absorption in crypt microperfusion studies was chloride-dependent and inhibited both by EIPA and by NPPB, a nonspecific Cl\(^{-}\) channel blocker. In addition, the inhibitory action of both EIPA and NPPB was observed only in the presence of Na\(^{+}\) and Cl\(^{-}\), respectively. As these characteristics of sodium-dependent fluid absorption parallel those of Cl-NHE activity when determined by \(^{22}\)Na uptake by crypt apical membrane vesicles or by sodium-dependent pH\(_{i}\) recovery during crypt microperfusion studies (12, 13), it is likely that Cl-NHE is the transport process responsible for constitutive fluid absorption in colonic crypts. This Cl-NHE isoform does not represent a phenomenon restricted to rodents because Cl-NHE mRNA was identified in both colonic mucosal cells from normal mice and humans (Fig. 4C). These latter observations were paralleled by the demonstration of Cl-NHE activity in the distal colon crypt of mice and humans by the demonstration of sodium/chloride-dependent recovery of pH\(_{i}\) to an acid load.\(^{2}\)

These present studies establish that Cl-NHE is a novel membrane transport protein that resembles only a portion of NHE-1 isoform. Of the 438 amino acids of the Cl-NHE open reading frame the N-terminal 375 amino acids are identical to that of NHE-1 isoform, whereas the 63-amino acid C-terminal segment is completely unique. This Cl-NHE protein has only six putative transmembrane spanning domains compared with 10–12 putative transmembrane domains of other known NHE isoforms. Because structure-function studies of the NHE-1 isoform indicate that NHE-1 isoform is not functional when the C-terminal end is truncated to 515 amino acids or less (25), it is unlikely that NHE activity would be present if the N-terminal 375 amino acids were expressed in PS120 cells. In contrast, the demonstration that the Cl-NHE protein is functional when expressed in PS120 cells (see Figs. 6–8) suggests that the 63-amino acid C-terminal fragment is essential for the observed Cl-NHE activity in the present study. Thus, this 63-amino acid peptide appears to be critical for both sodium and proton transport as well as for the observed chloride dependence. Earlier studies (13) have suggested the chloride dependence of Cl-NHE activity may involve a Cl\(^{-}\) channel. Whether the chloride dependence is an

\(^{2}\) J. Geibel, unpublished observations.
intrinsic property of Cl-NHE or requires the presence of associated Cl− channels that could be linked to Cl-NHE via the novel C terminus requires clarification. In addition, future studies are required to establish whether Cl-NHE is a spliced variant of previously identified NHE isoforms (e.g., NHE1 isoform) or is a new NHE isoform.

The novel properties of Cl-NHE compared with other NHE isoforms previously identified in colonic and non-colonic epithelial cells are the absolute dependence of chloride for activity and the inhibition of Cl-NHE activity by NPPB. In native colonic tissue Cl-NHE activity was manifested by [3H]-labeled gradient stimulation of 22Na+ uptake by AMV, sodium-dependent recovery of pH, to an acid load, and sodium-dependent fluid absorption (12, 13, 24). NPPB inhibited these three parameters of Cl-NHE activity at concentrations between 10 and 500 μM. In the present study sodium-dependent recovery of pH, from an acid load was almost completely chloride-dependent and was inhibited by 60% by 500 μM NPPB. This relatively reduced sensitivity of the expressed Cl-NHE in PS120 cells may represent different properties of intrinsic Cl− channels in native colonic tissue and PS120 cells.

These present studies also provide evidence that the regulation of Cl-NHE by dietary sodium depletion differs from its regulation of other NHE isoforms present in colonic epithelial cells. Increased levels of aldosterone as a result of either subcutaneous aldosterone infusion or dietary sodium depletion both induce electrogenic sodium absorption via the epithelial Na+ channel ENaC and inhibit electroneutral Na+-Cl− absorption in rat distal colon as a result of down-regulating both Na+-H+ and Cl−-HCO3− exchanges (15, 17). Subsequent studies revealed that aldosterone inhibited the activity, message, and protein of both NHE-2 and NHE-3 isoforms without altering NHE-1 function in colonic epithelial cells (22). In contrast, in experiments that characterized Cl-NHE activity in apical membranes prepared from crypt cells, Cl-NHE activity was increased by 60% by dietary sodium depletion (14). The present study establishes that this increase in Cl-NHE activity is paralleled by an up-regulation of Cl-NHE mRNA abundance (Fig. 5) suggesting that aldosterone regulates Cl-NHE at a transcriptional level.

There have been a few other reports of chloride dependence of NHE function but are dissimilar to the present series of observations (26, 27). In rat mesangial cells Miyata et al. (26) demonstrated that the NHE response to hyperosmolar contraction required chloride, whereas in contrast, sodium-dependent recovery of pH, to an acid load did not require chloride. Studies of Cl-NHE in colonic crypts have demonstrated chloride dependence of sodium-dependent recovery of pH, to an acid load but have not as yet examined the role of Cl-NHE in cell volume regulation. In AP-1 cells transfected with NHE-1, -2, and -3 isoforms, Aharonovitz et al. (27) examined sodium-dependent pH, recovery to an acid load and observed a varying degree of chloride dependence. However, these investigators used NO3− and SCN as their chloride substitute and did not adequately exclude an inhibitory effect on NHE isoform activity by NO3− or by SCN. Some reports (28, 29) had provided evidence of a minor role for chloride in NHE function in erythrocytes.

Several observations suggest that Cl-NHE may also be expressed in several other tissues and might provide a mechanism for Na+ and Cl− transport processes that have not been adequately explained by previously identified transport proteins. First, Northern blot analyses (shown in Fig. 4A) using the Cl-NHE-specific probe revealed expression in several epithelial and non-epithelial tissues including proximal colon, heart, lung, kidney, and liver. Second, perfusion studies of proximal renal tubule in NHE-2, NHE-3, and NHE-2/3 knockout mice that were performed in the presence of luminal chloride demonstrated sodium-dependent proton secretion that was inhibited by low concentrations of EIPA (30). These findings of an unidentified NHE representing ~50% of total NHE activity in proximal tubule are consistent with the potential presence of Cl-NHE. Third, similar observations were reported from perfusion studies of the pancreatic duct in which sodium-dependent bicarbonate absorption (i.e., NHE) was also studied in NHE-2, NHE-3, and NHE-2/NHE3 knockout mice (31). These studies concluded that 55% of total sodium-dependent bicarbonate absorption was inhibited by 50 μM HEOE994 but was not due to either NHE-2 or NHE-3 isoforms. As these studies were performed in the presence of luminal Cl, Cl-NHE could account for these observations. This unexplained sodium-dependent transport process in the pancreatic duct was inhibited by CAMP. Although the effect of cyclic AMP on Cl-NHE isoform is not known as yet, cyclic AMP inhibits NHE-2 and NHE-3 isoforms but does not affect NHE-1 isoform (32).

In conclusion, these studies suggest that Cl-NHE may represent the molecular basis of both chloride-dependent Na+-H+ exchange and sodium-dependent fluid absorption in colonic crypts. In addition, recent observations indicate that Cl-NHE is widely distributed in multiple organs (Fig. 4A) and is present in the colon of at least three species (i.e., rats, mice, and humans) (Fig. 4C). Cl-NHE has a wide tissue distribution and may be the transport mechanism responsible for one or more Na+ and Cl− transport processes that have not been adequately explained by existing transport proteins and, thus, may be important for cell and whole body electrolyte and volume homeostasis.

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REFERENCES
1. Counillon, L., and Pouyssegue, J. (2000) J. Biol. Chem. 275, 1–4
2. Orlowski, J., and Grinstein, S. (1997) J. Biol. Chem. 272, 22373–22376
3. Tse, M., Levine, S., Yun, C., Brant, S., Counillon, I. T., Pouyssegue, J., and Donowitz, M. (1993) J. Membr. Biol. 135, 93–108
4. Numata, M., Petrecca, K., Lake, N., and Orlowski, J. (1998) J. Biol. Chem. 273, 6951–6959
5. Numata, M., and Orlowski, J. (2001) J. Biol. Chem. 276, 17387–17394
6. Yun, C. H., Gurubhagavatula, S., Levine, S. A., Montgomery, J. L. M., Brant, S. R., Chen, M. E., Choe, E. J. Jr., Pouyssegue, J., Tse, C. M., and Donowitz, M. (1993) J. Biol. Chem. 268, 206–211
7. Bookstein, C., DePaoli, A. M., Xie, Y., Niu, P., Misch, M. W., Rao, M. C., and Chang, E. B. (1994) J. Clin. Invest. 93, 106–113
8. Schultheiss, P. J., Clarke, L. T., Menetton, P., Miller, M. L., Soleimani, M., Gwens, L. R., Riddle, T. M., Duffy, J. J., Doetschman, T., Wang, T., Giebisch, G., Aronson, P. S., Lorenz, J. N., and Shull, G. E. (1998) Nat. Genet. 19, 262–265
9. Bell, S. M., Schreiner, C. M., Schultheiss, P. J., Miller, M. L., Evans, R. L., Vorhees, C. Y., Shull, G. E., and Scott, W. J. (1999) Am. J. Physiol. 276, C788–C795
10. Welsh, M. L., Smith, P. L., Fromm, M., and Frizzella, R. A. (1982) Science 218, 1219–1221
11. Singh, S., Binder, H. J., Boron, W. F., and Geibel, J. P. (1995) J. Clin. Invest. 95, 2573–2579
12. Rajendran, V. M., Geibel, J., and Binder, H. J. (1995) J. Biol. Chem. 270, 11051–11054
13. Rajendran, V. M., Geibel, J., and Binder, H. J. (1999) Am. J. Physiol. 276, G73–G78
14. Rajendran, V. M., Geibel, J., and Binder, H. J. (2001) Am. J. Physiol. 280, G409–G415
15. Rajendran, V. M., and Binder, H. J. (1999) Am. J. Physiol. 276, G132–G137
16. Sangan, P., Rajendran, V. M., Mann, A. S., Kashgarian, M., and Binder, H. J. (1997) Am. J. Physiol. 272, C685–C696
17. Halevy, J., Buding, M. E., Haylett, J. P., and Binder, H. J. (1986) Gastroenterology 91, 1277–1283
18. Rajendran, V. M., Singh, S. K., Geibel, J., and Binder, H. J. (1998) Am. J. Physiol. 274, G424–G429
19. Chambray, R., Achard, J., John, P. L., Abrahamson, D. R., and Warnock, D. G. (1997) Am. J. Physiol. 273, C1064–C1074
20. Hogan, B., Costantini, F., and Lacy, E. (1986) Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, pp. 228–242, Cold Spring Harbor, NY
21. Sardet, C., Franchi, A., and Pouyssegue, J. (1989) Cell 56, 271–280
22. Iwama, M., Kashgarian, M., Binder, H. J., and Rajendran, V. M. (1999) Am. J.
Identification of a Chloride-dependent Na\(^+\)-H\(^+\) Exchanger

23. Wagner, C. A., Giebisch, G., Lang F., and Geibel, J. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9665–9668
24. Geibel, J. P., Rajendran, V. M., and Binder, H. J. (2001) Gastroenterology 120, 144–150
25. Ikeda, T., Schmitt, B., Pouyssegur, J., Wakabayashi, S., and Shigedawa, M. (1997) J. Biochem. (Tokyo) 121, 295–303
26. Miyata, Y., Muto, S., Yanagiba, S., and Asano, Y. (2000) Am. J. Physiol. 278, C1218–C1229
27. Aharonovitz, O., Kapus, A., Szaszi, K., Coady-Osberg, N., Jancelewicz, T., Orlowski, J., and Grinstein, S. (2001) Am. J. Physiol. 281, C133–C141
28. Parker, J. C. (1983) Am. J. Physiol. 244, C324–C330
29. Jennings, M. L., Douglas, S. M., and McAndrew, P. E. (1986) Am. J. Physiol. 251, C32–C40
30. Choi, J. Y., Shah, M., Lee, M. G., Schultheis, P. J., Shull, G. E., Muallem, S., and Baum, M. (2000) J. Clin. Invest. 105, 1141–1146
31. Lee, M. G., Ahn, W., Choi, J. Y., Lee, X., Seo, J. T., Schultheis, P. J., Schull, G. E., Kim, K. H., and Muallem, S. (2000) J. Clin. Invest. 105, 1651–1658
32. Cabade, A. G., Yu, F. N., Kapas, A., Lugas, G., Grinstein, S., and Orlowski, J. (1996) J. Biol. Chem. 271, 3590–3599