Role of Protein Kinase A and NHE-3 Phosphoserines 552 and 605

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Regulation of the renal Na/H exchanger NHE-3 by protein kinase A (PKA) is a key intermediate step in the hormonal regulation of acid-base and salt balance. We studied the role of NHE-3 phosphorylation in this process in NHE-deficient AP-1 cells transfected with NHE-3 and in OKP cells expressing native NHE-3. A dominant-negative PKA-regulatory subunit completely abolished the effect of cAMP on NHE-3 activity demonstrating a role of PKA in the functional regulation of NHE-3 by cAMP. NHE-3 isolated from cAMP-treated cells showed lower phosphorylation by purified PKA in vitro suggesting that NHE-3 is a PKA substrate in vivo. Although changes in NHE-3 whole protein phosphorylation is difficult to detect in response to cAMP addition, the tryptic phosphopeptide map of in vivo phosphorylated NHE-3 showed a complex pattern of constitutive and cAMP-induced phosphopeptides. To test the causal relationship between phosphorylation and activity, we mutated eight serines in the cytoplasmic domain to glycine or alanine. Single or multiple mutants harboring S552A or S605G showed no PKA activation or reduced regulation by PKA activation. Ser-552 and Ser-605 were phosphorylated in vivo. However, multiple mutations of serines other than Ser-552 or Ser-605 also reduced the functional PKA regulation. We conclude that regulation of NHE-3 by PKA in vivo involves complex mechanisms, which include phosphorylation of Ser-552 and Ser-605.

Mammalian plasma membrane Na/H exchangers (NHEs) use downhill inward Na+ gradients to extrude H+ from cells. Six genetic NHE isoforms have been identified with specific pharmacologic characteristics and tissue distributions (reviewed in Ref. 1). NHE-3 is limited to transporting epithelia such as the kidney and gastrointestinal tract (2, 3). In the kidney, NHE-3 is expressed exclusively on the apical membrane of the proximal tubule and the thick ascending limb where it mediates absorption of a significant fraction of the filtered NaCl and NaHCO3 (4–6). NHE-3 plays a critical role in renal regulation of extracellular fluid volume and acid-base balance (7). Acute hormonal regulation of NHE-3 involves multiple intracellular cascades including activation of adenyl cyclase (1). The NHE-3 isoform when expressed in fibroblasts is inhibited by cAMP analogues or forskolin (8–10). Although the NHE-1 isoform is cAMP insensitive, chimeric insertion of the NHE-3 cytoplasmic domain confers cAMP sensitivity to the NHE-1 transporting domain (11). Partial deletions of the cytoplasmic domain of NHE-3 reduce although more drastic truncations abolish the effect of cAMP on NHE-3 activity (10, 11). The NHE-3 cytoplasmic domain is a substrate for protein kinase A (PKA) in vitro (10) and NHE-3 phosphorylation is increased by cAMP or forskolin addition in vivo (10, 12). Kurashima and co-workers (12) showed that Ser-605 and Ser-634 are both important for forskolin to inhibit NHE-3 activity, although only Ser-605 is phosphorylated in vivo. This study showed that PKA directly phosphorylates NHE-3 and inhibits its activity via complex mechanisms. Phosphorylation of Ser-552 and Ser-605 were increased by cAMP addition and appeared to be critical for functional inhibition of NHE-3, although other regions of the transporter are likely to be involved in PKA regulation.

Experimental Procedures

Cell Lines—Rat NHE-3 was tagged (C-terminal) with hexahistidines (NHE-3/6H) by polymerase chain reaction, sequenced, and cloned into the mammalian expression plasmid pcDNA3 (Invitrogen, Carlsbad, CA). Plasmids were transfected into Na/H exchanger-deficient AP-1 cells (derived from Chinese hamster ovarian fibroblasts) (gift from Dr. Sergio Grinstein, Toronto, Ontario, Canada) using Lipofectin (Life Technologies, Inc.) and 200 µg/ml G418. G418 was replaced by 100 units/ml penicillin and 100 µl/ml streptomycin two passages before experimentation. Individual clones were selected from pooled transfecants by limiting dilution. The function of NHE-3/6H was not different from nontagged NHE-3 (pCMV5/NHE3) expressed in AP-1 cells (10) in terms of basal activity, antigenic expression, Na, resting pH, and ethylisopropyl amiloride kinetics, and response to cAMP addition (data not shown). OKP cells (gift from K. Hruska, St. Louis, MO), were maintained in Dulbecco’s modified Eagle’s medium (Sigma) with 10% fetal bovine serum (Life Technologies, Inc.) and 200 µg/ml G418. G418 was replaced by 100 units/ml penicillin and 100 µl/ml streptomycin. Confluent monolayers were rendered quiescent by serum removal (16–24 h, AP-1 cells; 36–48 h, OKP cells), and PKA was activated by the addition of cell permeant 8-Br-cAMP.

Site-directed Mutagenesis—Specific serines in the cytoplasmic domain of NHE-3 were mutated singularly or in combination using a modification of the double-stranded method of Deng and Nickoloff (15) (QuickChange™ kit, Stratagene, La Jolla, CA). The 8 mutations were: S513G, S552A, S575A, S605G, S634A, S661A, S690G, and S804G. After annealing the pcDNA3/NHE-3/6H parent vector with mutagenic oligonucleotides, strands were extended with Pfu DNA polymerase. Host-derived wild type methylated templates were digested with DpnI and plasmid DNA’s were isolated from transformed XL1-B Escherichia coli. Multiple mutations were created sequentially. Each mutation was con-
In Vivo Phosphorylation and Isolation of NHE-3—NHE-3 phosphorylated in transfected AP-1 cells was isolated by either immunoprecipitation or nickel-affinity chromatography. After incubation in phosphate-free Dulbecco’s modified Eagle’s medium (30 min), cells were labeled with [32P]orthophosphate (200–330 μCi/ml 120 min; 1 ml/cell/camp or vehicle was added. After washing with ice-cold Tris-buffered saline, cells were lysed with ice-cold RIPA buffer (in mM: 150 NaCl, 50 Tris-HCl, pH 7.4, 0.5% Nonidet P-40, 20 mM sodium orthovanadate; in % (v/v): 1 (v/v) Triton X-100, 0.5 (w/v) deoxycholate, 0.1 (w/v) SDS; in % (v/v): glycerol 30, 8,000 urea, 100 Na2PO4, 10 imidazole, pH 8.0; in % (v/v): 0.5 Triton-X 100), sheared with a 25-gauge needle, combined with buffer B2 (1:1, v/v) (composition same as B1 except no glycerol), clarified by centrifugation (109,000 × g, 10 min, 37 °C), and equilibrated with Ni-NTA agarose at 4 °C. The phosphoproteins were eluted from the acrylamide gel by guest on November 3, 2017 http://www.jbc.org/ Downloaded from
Inhibition and Phosphorylation of NHE-3 by PKA

RESULTS

Effect of PKA Activation on NHE-3 Activity and Phosphorylation—Fig. 1 shows that cAMP inhibited NHE-3 activity in a time- (Fig. 1A) and dose-dependent (Fig. 1B) fashion in both AP-1 and OKP cells. In both AP-1 (Fig. 2A) and OKP (Fig. 2B) cells, the $V_{\text{max}}$ was reduced by approximately 40% (con versus cAMP: AP-1; 10$^{-5}$ M versus $10^{-6}$ M, p < 0.05, OKP; 0.86 versus 0.55 pH units/min, p < 0.05, OKP; 1.05 versus 0.61 pH units/min, p < 0.05, t test) with no change in $K_N$ (con versus cAMP: AP-1; 14 mM versus 12 mM, p = 0.52, OKP; 20 mM versus 16 mM, p = 0.67, t test). We have previously shown that 200 $\mu$M cAMP increases NHE-3 phosphorylation but lower doses were not examined (10). When we performed a dose response of the effect of cAMP on NHE-3 phosphorylation, we found no consistently detectable effect on NHE-3 whole protein phosphorylation in both AP-1 and OKP cells until 100 $\mu$M of cAMP was used (Fig. 3). Even at 100 $\mu$M, changes in total NHE-3 phosphorylation were variable. This was in sharp contraddition to the effect of cAMP on NHE-3 activity, which was evident at doses as low as 1 and 10 $\mu$M for OKP and AP-1 cells, respectively.

Role of PKA in Regulating NHE-3 Function—One hypothesis is that the effect of cAMP may be independent of A kinase activity. Direct gating by cyclic nucleotides without involvement of protein kinase has been described for cation channels (19). To test this hypothesis, we inhibited endogenous PKA-CSU with a dominant-negative mutant regulatory subunit (PKA-RSU) in a transient transfection system rather than with pharmacologic inhibitors, which may have nonspecific effects. Because cells that did not take up REV$_{AB}$ (plasmid for mutant PKA-RSU) also did not receive NHE-3, all $^{22}$Na uptake can be assumed to originate from cells that received both plasmids. Fig. 4 summarizes the data. Only cells transfected with NHE-3 expressed significant $^{22}$Na uptake indicating that the measured flux reflected NHE-3 activity and in the absence of REV$_{AB}$, NHE-3 activity was suppressed by cAMP addition as expected. In the presence of the dominant-negative PKA-RSU, cAMP had no effect on NHE-3 activity indicating that the cAMP effect on NHE-3 activity is mediated by PKA-CSU.

Phosphorylation of NHE-3 by PKA—If PKA is necessary for the regulation of NHE-3 by cAMP, another hypothesis that can explain the dissociation between activity and whole protein phosphorylation is that PKA phosphorylates a protein other than NHE-3, which in turn modulates NHE-3 activity. NHE-RF for example is a phosphoprotein that regulates...
NHE-3 function (20–22). To address whether NHE-3 is phosphorylated by PKA in vivo, we used the indirect approach of back phosphorylation. If the PKA sites on NHE-3 are occupied by nonradioactive PO₄ because of in vivo phosphorylation before cell lysis, these sites will be unavailable to accept ³²PO₄ in the subsequent in vitro reaction with purified PKA-CSU. When we harvested NHE-3 from either cAMP-activated or control cells and subjected purified NHE-3 to in vitro phosphorylation by PKA, we found a significantly smaller degree of ³²PO₄ incorporation into NHE-3 from cAMP-activated compared with control cells (Fig. 5). These findings are compatible with the hypothesis that PKA indeed directly phosphorylates NHE-3 in vivo. It is important to note that the current data do not rule out the fact that PKA may phosphorylate proteins other than NHE-3 and kinases other than PKA may also phosphorylate NHE-3.

Phosphoamino Acid and Tryptic Phosphopeptide Mapping of in Vivo Phosphorylated NHE-3—The most likely explanation for the dissociation between activity and whole protein phosphorylation is presence of constitutive phosphorylated residues on NHE-3 that are not regulated by cAMP addition. To address this, we performed tryptic phosphopeptide maps using varying doses of cAMP. We observed increased intensity of phosphopeptides D, E, F, and J after cAMP addition (Fig. 6A). Although there are variations from map to map, with over 30 maps performed on AP-1 cells, it is evident that phosphopeptide B is constitutively phosphorylated. Because the absolute signal of a specific phosphopeptide on a given map is affected by a variety of factors such as the efficiency of retrieval from the total protein as well as the exposure time; one way to summarize the results from all the maps quantitatively is to express the intensity of each phosphopeptide as a ratio to the constitutive phosphopeptide B on the same plate. The results from all the phosphopeptide maps performed on wild type cells are summarized in Fig. 6B. Phosphopeptides J and E were significantly increased by [cAMP]₀ = 1 and 10 μM, respectively, and phos-
FIG. 6. Phosphopeptide map of NHE-3 in AP-1 and OKP cells: Response to 8-Br-cAMP. A, tryptic phosphopeptide maps of immunoprecipitated $^{32}$P-labeled NHE-3 from AP-1 cells treated with different [cAMP]. $^{32}$P-NHE-3 was digested with trypsin, resolved by electrophoresis and thin-layer chromatography, and visualized by autoradiography. Phosphopeptides are labeled by alphabet letters. B, quantitative summary of dose response of phosphopeptide maps of NHE-3 in AP-1 cells. Intensity of phosphopeptides C, D, E, F, and J were normalized to the intensity of the corresponding phosphopeptide B on the same thin-layer chromatography plate. Symbols and error bars represent mean ± S.E. n = number of experiments: control, n = 9; 1 μM, n = 3; 10 μM, n = 3; 100 μM, n = 9. Asterisk indicates p = 0.05 when compared with the value of control by ANOVA. C, cAMP-induced phosphorylation of surface NHE-3 in OK cells. OK cells were pulsed with $^{32}$P, 100 μM 8-Br-cAMP was added, and surface NHE-3 was immunoprecipitated from the biotinylatable fraction of the cell lysate, digested with trypsin, and resolved in two dimensions. Two independent maps showed similar results.
hopeptide F was increased at 100 µM cAMP. Because NHE-3 is more abundant in intracellular compartments than in plasma membrane NHE-3, is associated with changes in cell surface NHE-3 phosphorylation. Extremely low and variable levels of retrievable biotinylatable NHE-3 in AP-1 cells precluded a meaningful conclusion in these cells. However, when we isolated surface biotinylated NHE-3 from OKP cells, multiple cAMP-induced NHE-3 phosphopeptides were detected (Fig. 6C). The pattern of phosphorylation of OK surface NHE-3 was identical to that of total cellular NHE-3 (not shown). Next, we determined the phosphoamino acid composition of the in vivo phosphorylated protein in AP-1 cells to be almost exclusively serines (Fig. 7). Prolonged exposure revealed a small amount of phosphothreonine, which when quantified by scintillation represented 0.5–1% of the counts. Phosphoamino acid analysis of OK cells showed predominantly phosphoserines with 2% phosphothreonine (data not shown).

**Regulation of Na/H Exchange Activity in Mutant NHE-3 (NHE-3mut)—** If PKA phosphorylates NHE-3, the critical question is whether phosphorylation of NHE-3 is functionally important for its regulation. Because only serines are phosphorylated, we mutated 8 serines individually and in combination in the cytoplasmic domain that conform to the classical PKA consensus target motif (23) and screened for the ability of cAMP to regulate NHE-3 activity. Single amino acid mutations per se do not significantly affect whole cell NHE-3 protein expression (Fig. 8A). However, four or more point mutations did decrease NHE-3 expression with the lowest expression in the six-point mutant (Fig. 8B). NHE-3 transcript levels were not affected by single or multiple mutations (not shown). Fig. 9A shows tracings of several representative mutants, and Fig. 9, B and C, summarize the functional data from all the mutants. Among the single mutants, NHE-3mut/S552A failed to show a response to cAMP addition. NHE-3mut/S690G showed much reduced inhibition by cAMP (control versus cAMP p = 0.28). NHE-3mut/S634A showed a slightly attenuated but nonetheless intact response to cAMP. This finding suggests phosphorylation of Ser-552 and Ser-605 are key steps in modulating NHE-3 activity. One concern with this data is whether the lack of regulation is truly due to the mutant NHE-3 or because of cellular context. This is particularly critical for S552A because Kurashima and co-workers (12) found intact cAMP regulation with the same mutation. To determine that the lack of cAMP sensitivity actually stems from S552A rather than a missing cellular factor, we studied NHE-3mut/S552A against a heterogeneous cellular background with two approaches. We isolated individual clones from the pooled NHE-3mut/S552A transfec-nts, and we transiently transfected NHE-3mut/S552A into AP-1 cells at different passages (Table I). As a positive control, we compared NHE-3mut/S552A to one of the cAMP-responsive single serine mutants NHE-3mut/S690G. As shown in Table I, all NHE-3mut/S552A-transfected cells remained cAMP insensitive, whereas all NHE-3mut/S690G-transfected cells maintained cAMP sensitivity. Despite the potentially diverse cellular context, cAMP responsiveness segregated with the mutation.

The results were less clear in the multiple mutants. All the multiple mutants harboring Ser-552 were not regulated by cAMP addition confirming the importance of Ser-552. However, NHE-3mut/S575A/S661A/S690G/S804G which contains neither Ser-552 nor Ser-605, also showed no inhibition by cAMP addition.
Fig. 9. Effect of cAMP on NHE function NHE-3 mutants in mutants with serine mutations. Point mutants of NHE-3 was expressed in AP-1 NHE-deficient fibroblasts. NHE-3 activity in control and 8-Br-cAMP (100 μM, 30 min)-treated cells were measured fluorimetrically as sodium-dependent cell pH recovery. Asterisk indicates p < 0.05 by unpaired t test (control versus cAMP in same cell line). A, representative tracings from six selected clones; B, summary of all single mutants. n = number of experiments: WT, n = 21; S513G, n = 4; S552A, n = 11; S575A, n = 9; S605G, n = 9; S634A, n = 4; S661A, n = 6; S690G, n = 4; S804G, n = 6. C, summary of all multiple mutants. n = number of experiments: WT, n = 21; S513G/S552A, n = 4; S513A/S552A/S661A, n = 3; S661A/S690G/S804G, n = 4; S513G/S552A/S675A/S605G, n = 5; S552A/S675A/S661A/S690G/S804G, n = 5; S513G/S552A/S675A/S661A/S690/S690G/S804G, n = 5.
TABLE I

NHE-3 activity in AP-1 cells transfected rat NHE-3

<table>
<thead>
<tr>
<th>Clones derived from pooled stable transfectants</th>
<th>NHE-3/S552A</th>
<th>Control</th>
<th>8Br-cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>867 ± 67</td>
<td>894 ± 86</td>
<td>1029 ± 78</td>
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<tr>
<td>Clone 1</td>
<td>572 ± 44</td>
<td>528 ± 95</td>
<td>789 ± 67*</td>
</tr>
<tr>
<td>Clone 2</td>
<td>955 ± 87</td>
<td>1076 ± 89</td>
<td>966 ± 65*</td>
</tr>
<tr>
<td>Clone 3</td>
<td>678 ± 66</td>
<td>625 ± 84</td>
<td>775 ± 62</td>
</tr>
<tr>
<td>Clone 4</td>
<td>592 ± 67</td>
<td>524 ± 87</td>
<td>622 ± 35</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Transient transfections</th>
<th>NHE-3/S605G</th>
<th>Control</th>
<th>8Br-cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfection 1</td>
<td>592 ± 78</td>
<td>592 ± 67</td>
<td>452 ± 87</td>
</tr>
<tr>
<td>Transfection 2</td>
<td>325 ± 28</td>
<td>304 ± 46</td>
<td>1256 ± 108</td>
</tr>
<tr>
<td>Transfection 3</td>
<td>1001 ± 89</td>
<td>899 ± 87</td>
<td>567 ± 38</td>
</tr>
</tbody>
</table>

a p < 0.05, t test.

Tryptic Phosphopeptide Map of NHE-3<sup>mut</sup>/S552A and NHE-3<sup>mut</sup>/S605G—If Ser-552 and Ser-605 are indeed critical for inhibition of NHE-3 activity as phosphoserines, they should be phosphorylated in vivo by cAMP addition. We compared phosphopeptide maps of these two NHE-3<sup>mut</sup>s to wild type NHE-3 in response to cAMP. Fig. 10 shows the phosphopeptide maps of wild type NHE-3, NHE-3<sup>mut</sup>/S552A, and NHE-3<sup>mut</sup>/S605G. Phosphopeptide J, which was increased by 8-Br-cAMP in wild type NHE-3 is absent in NHE-3<sup>mut</sup>/S552A, and phosphopeptide E, which was also increased by 8-Br-cAMP in wild type NHE-3, was absent from NHE-3<sup>mut</sup>/S605A indicating that both Ser-552 and Ser-605 are phosphorylated in vivo.

DISCUSSION

We confirmed the previous finding of inhibition of V<sub>max</sub> in native NHE-3 in renal epithelial cells (24–26) and NHE-3 heterologously expressed in fibroblasts (8–12). The lack of detectable NHE-3 whole protein phosphorylation is comparable with the report by Kurashima and co-workers (12) although we observed discernible but variable changes in NHE-3 whole protein phosphorylation with 100 μM 8-Br-cAMP. As some cation channels are directly gated by cyclic nucleotides (19), we queried if such can be a model for NHE-3 regulation by cAMP. When we inactivated the catalytic subunit of PKA, 8-Br-cAMP no longer regulated NHE-3 activity indicating that the cAMP effect is kinase-dependent. Also congruent with A8-Br-cAMP no longer regulated NHE-3 activity indicating that the cAMP-insensitive phenotype stems from the lack of change in total NHE-3 protein phosphorylation but the quadruple mutants showed decreased and the six-point mutant showed markedly attenuated expression of both NHE-3 protein and activity. Because NHE-3 transcript levels are not affected by the mutations, one has to postulate that the multiple serine mutations significantly affected translation and/or protein half-life. This study does not address the mechanism of these changes. Ser-552 and Ser-605 were both phosphorylated in vivo and when mutated singly to nonphosphorylatable residues, functional regulation by PKA was abrogated in both mutants. Kurashima and co-workers (12) have reported the importance of Ser-605 and Ser-634 in the functional regulation of NHE-3 by PKA although Ser-634 appeared not to be a phosphoserine. Our phosphopeptide map and functional response of the NHE-3<sup>mut</sup>/S605A is very similar to the previous report. A major disparate finding is that we found Ser-552 to be critical for PKA regulation, whereas Kurashima and co-workers (12) found normal functional regulation of NHE-3<sup>mut</sup>/S552A by cAMP. In addition, Cabado and co-workers (11) from the same group found that truncation of the cytoplasmic domain at amino acid 579 rendered NHE-3 nonresponsive to cAMP. The difference may reside in the fact that Ser-552, which is phosphorylated in our cells, was not phosphorylated in the previous report as is evident by the absence of phosphopeptide J (12). It is conceivable that multiple PKA target phosphoserines may participate in mediating the functional inhibition of NHE-3 and different host cells may use different sets of serines. The basis for the differential NHE-3 phosphorylation is unclear, because both laboratories used AP-1 cells from the same source. Because AP-1 cells are mutants derived and selected from the parental Chinese hamster ovary cell, repeated passages and clonal expansion might have created different phenotypes.

One possible interpretation for the lack of regulation of NHE-3<sup>mut</sup>/S552A is that some cellular cofactor(s) are missing in that particular AP-1 recipient that harbors the mutant NHE-3 and that the cAMP-insensitive phenotype stems from the cell rather than NHE-3<sup>mut</sup>/S552A. Such an explanation is unlikely for several reasons. All pooled transfectants of single or multiple mutant NHE-3s were generated about the same time from the same parental AP-1 cells. In addition, when we tried to examined NHE-3<sup>mut</sup>/S552A in as heterogenous an AP-1

Inhibition and Phosphorylation of NHE-3 by PKA
cellular background as we can create, we found that the cAMP-nonresponsive phenotype prevailed.

The lack of regulation of NHE-3<sup>mut/S575/S661/S690/S804</sup> is harder to explain because none of the serines were functionally important when examined as single mutants. One can speculate on several explanations. One is that there are multiple regulatory phosphoserines with P-Ser-552 and P-Ser-605 having the most dominant effects with lesser contribution from the other phosphoserines. Although singular mutations of serines other than Ser-552 and Ser-605 do not affect the overall re-
spans a"mutation of four minor phosphorylation sites may be sufficient. A second possibility is that these serines participate in specific protein-protein interactions with regulatory factors such as NHE-RF (20, 21, 29–31), E3KARP (22), or calmodulin (32) that modulate NHE-3 function, and this interaction is disrupted by the mutations. A third possibility is that the mutations distort the protein structure to such an extent that it nonspecifically alters NHE-3 function and its regulation. Crystallographic evidence indicates that one can significantly alter the protein structure of a domain of a protein by a single amino acid mutation from tyrosine to phenylalanine, which essentially only removes a single hydroxyl group (33). The present paper does not distinguish the three stated possibilities. We did however observe intact regulation of all mutants including the four- to six-point mutants by acute hyperosmolality (25–40% inhibition) (data not shown). This is compatible with the ability of hyperosmolality to inhibit NHE-3 activity despite radical truncations of the cytoplasmic domain (34).

In summary, we hypothesize that the functional regulation of NHE-3 by PKA does not converge on single covalent modifications such as phosphorylation of a specific serine. We submit a model where PKA phosphorylates NHE-3 on Ser-552 and Ser-605 in addition to other serines. Phosphorylation of Ser-552 and Ser-605 each play a major although not exclusive role in the functional regulation of NHE-3, which involves specific interaction of various regions of the cytoplasmic domain of NHE-3 with other regulatory cofactors.

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REFERENCES
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