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Chimeric Na^+/H^+ exchangers: An epithelial membrane-bound N-terminal domain requires an epithelial cytoplasmic C-terminal domain for regulation by protein kinases

(Na^+–H^+ antiporter/pH regulation/signal transduction/Na^+ absorption)

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ABSTRACT **All cloned members of the mammalian Na^+/H^+ exchanger gene family encode proteins that consist of two functionally distinct domains: a membrane-bound N terminus and a cytoplasmic C terminus, which are required for ion transport and regulation of transport, respectively. Despite their similarity in structure, three members of this family, designated NHE1, NHE2, and NHE3, exhibit different kinetic mechanisms in response to growth factors and protein kinases. For instance, growth factors stimulate NHE1 by a change in the affinity constant for intracellular H^+, $K'(H_i^+)$, and regulate NHE2 and NHE3 by a change in V_{max}. We have constructed chimeric Na^+/H^+ exchangers by exchanging the N and C termini among three cloned rabbit Na^+/H^+ exchangers (NHE1 to NHE3) to determine which domain is responsible for the above V_{max}-vs.-$K'(H_i^+)$ effect of the Na^+/H^+ isoforms. All of the chimeras had functional exchange activity and basal kinetic properties similar to those of wild-type exchangers. Studies with serum showed that the N terminus is responsible for the V_{max}-vs.-$K'(H_i^+)$ stimulation of the Na^+/H^+ exchanger isoforms. Moreover, phorbol 12-myristate 13-acetate and fibroblast growth factor altered Na^+/H^+ exchange only in chimeras that had an epithelial N-terminal domain matched with an epithelial C-terminal domain. Therefore, the protein kinase-induced regulation of Na^+/H^+ exchangers is mediated through a specific interaction between the N- and C-termini, which is restricted so that epithelial N- and epithelial C-terminal portions of the exchangers are required for regulation.**

Na^+/H^+ exchangers are present in all mammalian cells in which they play important roles in regulation of intracellular pH, cell growth, maintenance of cellular volume, and transepithelial Na^+ absorption (1, 2). Recent cloning of a gene family of Na^+/H^+ exchangers has shown that all of the Na^+/H^+ exchanger isoforms share a highly conserved structure with a membrane-associated N terminus consisting of 12 putative transmembrane helices and a large cytoplasmic C-terminal domain, which is required for protein kinase regulation (3, 4, 19).

Despite the similarity in their structures, the Na^+/H^+ exchanger isoforms, designated NHE1, NHE2, and NHE3, differ greatly in their kinetic characteristics and their response to external stimuli, tissue distribution, and subcellular distribution (5–7). NHE1 message is present in almost all cells. It is involved in housekeeping functions of volume and intracellular pH control and is expressed on the basolateral membrane in epithelial cells (8, 9). In contrast, both NHE2 and NHE3 are expressed on brush border membranes in intestinal and renal proximal tubule cells (10, 11), while NHE2 also appears to be on the basolateral membrane in at least one renal cell (12).

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Second messengers regulate NHE1 by ATP-dependent processes. Mechanisms of regulation include phosphorylation of the cytoplasmic C-terminal domain of the exchanger, where a large number of kinase consensus phosphorylation sites are located (3, 13), and effects on accessory proteins (14). Levine *et al.* (6) have recently shown that second messengers, including phorbol 12-myristate 13-acetate (PMA), fibroblast growth factor (FGF), α-thrombin, and serum stimulate NHE1 by increasing the affinity for intracellular H^+ (H_i^+) but do not affect V_{max}. In contrast, thrombin, FGF, and serum increase V_{max} of NHE2 and NHE3 without any apparent effect on the affinity constant $K'(H_i^+)$. PMA, on the other hand, stimulates NHE2 and inhibits NHE3 through changes in V_{max}.

The mechanisms of these differences in the kinetics and regulation of the exchangers are not known. The purpose of the present work is 2-fold: to determine (*i*) which domain, the transmembrane N terminus or the cytoplasmic C terminus, plays the critical role in determining the V_{max}-vs.-$K'(H_i^+)$ regulatory effect of the Na^+/H^+ isoforms; and (*ii*) if the second messenger regulation of the exchangers can be modified by swapping the cytoplasmic C termini, which are thought to be solely responsible for the protein kinase regulation (3, 4).

MATERIALS AND METHODS

Construction of Chimeric Na^+/H^+ Exchangers. cDNAs were constructed by PCR that encoded six chimeric Na^+/H^+ exchangers among the transmembrane and cytoplasmic domains of rabbit NHE1, NHE2, and NHE3 (9, 15, 16) as shown in Fig. 1. The amino acids Asn-519 of NHE1, Ser-499 of NHE2, and Asn-475 of NHE3 were used as the junction points for the construction of the recombinant Na^+/H^+ exchangers. The following oligonucleotides were synthesized for PCR: NE1A (GAACTCCTTCTAGAGACCAGC), NE1B (GATCTCTCGAGGATGGAGCGC), NE1C (CGCTCCATCCTCGAGGAGATCCACGC), NE1D (TGCTGTGGCTCGAGAGTCTGGCGG), NE3A (GCCGCTCTAGAACTAGTGG), NE3B (GCAGCTTCTCGAGCAGCTTGGGCTCCC), NE3C (GCCCAAGCTGCTCGAGAAGCTGCACGG), NE3D (CGGAGCTCGAGTCACATGTGTGG), NE2A (GCCGCTCTAGAACTAGTGG), NE2B (GATTTCCTCGAGGACAGCTTGCTG), NE2C (GCTGTCCTCGAGGAAATCCATTGTCGG), and NE2D (AGGCCACTCGAGGTCTGTTTTGGC).

The membrane-bound N terminus of NHE*X*, where *X* is 1, 2, or 3, was constructed by PCR using the primer pair NE*X*A and NE*X*B. To facilitate the subsequent cloning of the PCR fragment into the *Nhe* I and *Xho* I sites of pMAMneo vector, *Xba* I and *Xho* I sites were incorporated into NE*X*A and

Abbreviations: PMA, phorbol 12-myristate 13-acetate; FGF, fibroblast growth factor; FBS, fetal bovine serum; n_{app}, apparent Hill coefficient; H_i^+, intracellular H^+; $K'(H_i^+)$, affinity constant for H_i^+.

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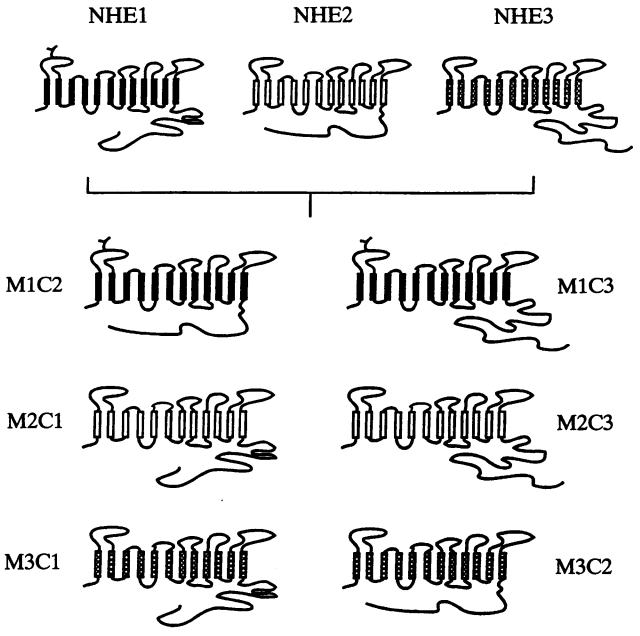

FIG. 1. Schematic represention of the chimeric Na^+/H^+ exchangers studied. Chimeric exchangers were constructed by exchanging the membrane bound N-terminal domain and the cytoplasmic C-terminal domain among the cloned rabbit Na^+/H^+ exchangers, NHE1, NHE2, and NHE3. The chimeras were named such that M and C denote the membrane-bound N terminus and the cytoplasmic C terminus, respectively. The number following M or C indicates the parent Na^+/H^+ exchanger isoform. Note that NHE1 is N-glycosylated on extracytoplasmic loop a.

NE*X*B, respectively. The membrane-bound N terminus, M*X*, was subcloned into the *Nhe* I and *Xho* I sites of pMAMneo vector, yielding plasmid pMM*X*. These N termini of NHE1, NHE2, and NHE3, named M1, M2, and M3, respectively, consist of 12 putative transmembrane helices and 19 amino acid residues of the putative cytoplasmic domain. The cytoplasmic C terminus of NHE*Y*, C*Y*, where *Y* is 1, 2, or 3, was synthesized by using the primer pair NE*Y*C and NE*Y*D. The ligation of C*Y* into pMM*X*, yielding plasmids pMM*X*C*Y*, was also facilitated by incorporation of the *Xho* I restriction site in the primers, NE*Y*C and NE*Y*D, used for PCR. The introduction of the *Xho* I site resulted in a substitution of amino acid Asn-519 of NHE1, Ser-499 of NHE2, and Asn-475 of NHE3 by leucine. Fidelity of all of the PCR products was checked by DNA sequencing either with the Sequenase kit (United States Biochemical) or by the fluorescent dideoxy terminator method of cycle sequencing on an Applied Biosystems 373a automated DNA sequencer at the DNA Analysis Facility of Johns Hopkins University. Resulting plasmids pMM1C2, pMM1C3, pMM2C1, pMM2C3, pMM3C1, pMM3C2, pMM1C1, and pMM3C3 were stably transfected into PS120 fibroblasts (17) by calcium phosphate precipitation method as described (5, 9, 15). Transfectants were selected by the resistance to both G418 and multiple rounds of acid-loading as described (16). Mixed cell populations, named PS120/M1C2, PS120/M1C3, PS120/M2C1, PS120/M2C3, PS120/M3C1, and PS120/M3C2 were used for functional characterization.

Measurement of Na^+/H^+ Exchange. The Na^+/H^+ exchanger activities of stably transfected PS120 cells were studied fluorometrically with a pH-sensitive dye, 2′,7′-bis(2-carboxyethyl)-5,6-carboxyfluorescein (BCECF), and measurement of Na^+/H^+ exchange rates was done as described (6, 18). Na^+/H^+ exchange rates were calculated as described (6) with a nonlinear regression data analysis program (ENZFITTER, BioSoft, Princeton, NJ).

Statistical Analyses. All comparisons were done on cells of the same passage and number of acid loadings and were studied on the same day to eliminate variability in the basal rate of Na^+/H^+ exchange as described (6). The values of V_{max}, $K'(H_i^+)$ and the apparent Hill coefficient (n_{app}) generated above were used for statistical analyses (one-way ANOVA) to test whether there were differences between control and treated cells.

RESULTS

Construction and Expression of the Chimeric Na^+/H^+ Exchangers. Chimeric Na^+/H^+ exchangers were constructed by exchanging the membrane-bound N-terminal and cytoplasmic C-terminal domains in all possible combinations among the cloned rabbit Na^+/H^+ exchangers NHE1, NHE2, and NHE3 (Fig. 1). The chimeric cDNAs were stably expressed in PS120 fibroblast cells that lack endogenous Na^+/H^+ exchange. The replacement of Asn-519 of NHE1 and Asn-475 of NHE3 by leucine, which was introduced to facilitate cloning of different PCR fragments, did not affect the regulation of the Na^+/H^+ exchanger isoforms based on preliminary studies using PMA and serum. The effect of the Ser-499 → Leu mutation of NHE2 was not studied.

All of the chimeric cDNAs resulted in functional Na^+/H^+ exchangers when expressed in PS120 fibroblasts. Although the Na^+/H^+ exchange rates of these chimeric exchangers were not directly compared to that of the wild-type exchangers because of the lack of determination of relative exchanger expression in the plasma membrane, most of the chimeras were at least as fast as the wild-type exchangers reported previously by us (6). The fast rates of the chimeric exchangers were strikingly different from those of the truncated forms containing only the putative N-terminal domains of NHE1, NHE2, and NHE3, which displayed much slower rates than wild-type exchangers (data not shown). Each of the chimeric Na^+/H^+ exchangers showed a characteristic non-Michaelis–Menten response to H_i^+ with a Hill coefficient of 1.6–2.9 (Figs. 2–4 and Table 1) (6).

Regulation. To determine any modification in protein kinase regulation of the chimeric exchangers as a result of swapping of the cytoplasmic domains, effects of PMA, FGF, and FBS were studied. Since serum resulted in the largest response in all of the Na^+/H^+ exchanger isoforms in previous studies (6), serum was initially used as a source of nondefined growth factors. The mode of regulation by serum differs from that of PMA or FGF, since truncated forms of the Na^+/H^+ exchangers—e.g., M1, M2, and M3—did not respond to PMA or FGF but were stimulated by serum (data not shown).

The effect of 10% FBS on M3C1 and M3C2, respectively, is shown in Fig. 2 *A* and *B*. Kinetic analysis of the initial rates of the pH recovery revealed that FBS caused an increase in V_{max} from 1365 ± 81 to 2035 ± 162 μM/sec (49% increase) for M3C1 and from 1330 ± 114 to 1808 ± 102 μM/sec (35% increase) for M3C2. However, there was no significant effect on the apparent affinity for intracellular H^+ or the Hill coefficient (n_{app}). Similarily, M2C3 (Table 1) is stimulated by FBS with an increase in V_{max} (45% increase) without any effect on the Hill coefficient. FBS also stimulated M1C2 as shown in Fig. 2*C* and Table 1. In the case of M1C2, FBS decreased K' from 0.17 to 0.08 μM without a significant effect on V_{max} or the Hill coefficient. The increase in V_{max} by FBS on M3C1 probably originates from M3, since the parent form of M3, NHE3, displays a V_{max} increase by FBS. Similarly, the $K'(H_i^+)$ effect by FBS on M1C2 probably results from M1, since NHE1 is the only isoform that shows a $K'(H_i^+)$ effect of serum. Thus, the previously reported V_{max}-vs.-$K'(H_i^+)$ effect of serum originates from the membrane-bound N termini of the exchangers. Interestingly M2C1 and M1C3 were not significantly affected by serum (Table 1).

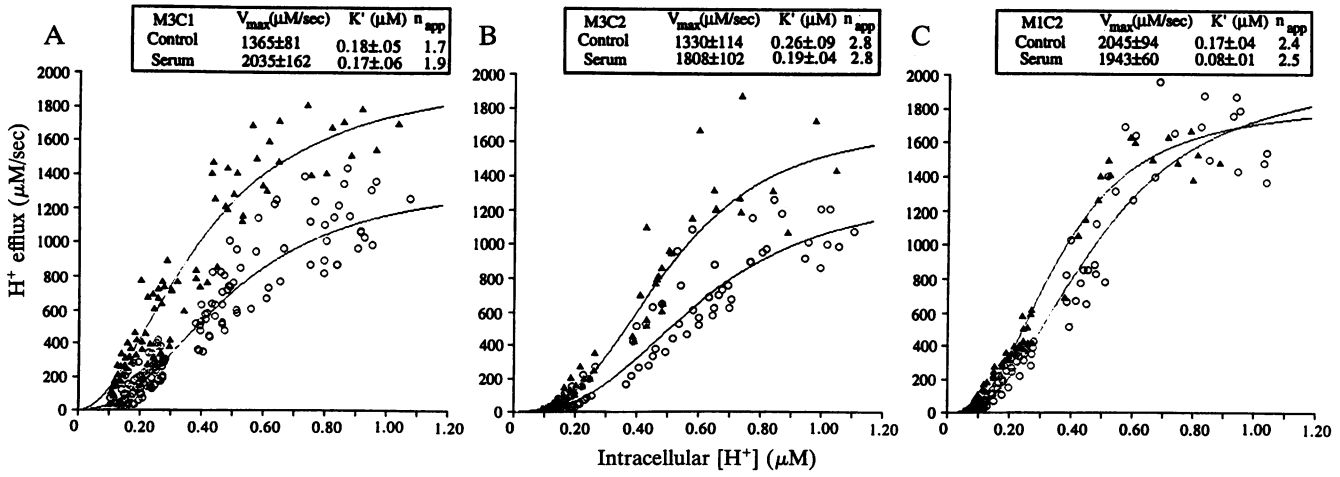

FIG. 2. Effect of fetal bovine serum (FBS) on Na^+/H^+ exchange activity of PS120 cells transfected with chimeric Na^+/H^+ exchangers. Control (○) cells were acidified with NH_4Cl and allowed to recover in Na^+ medium, while treated cells (▲) were similarly acidified and then perfused with Na^+ medium containing 10% FBS. FBS was dialyzed before use against a buffer solution containing 135 mM Na^+ and no HCO_3^- to eliminate the effect of Cl^-/HCO_3^- exchange on intracellular pH. Na^+/H^+ efflux rates were calculated at various intracellular pH values, lines were fit to the data by using an allosteric model (6), and kinetic parameters [V_{max}, $K'(H^+)$, and n_{app}] were estimated. (*A*) PS120/M3C1 cells treated with serum showed an increase in V_{max} without any significant change in K' or the Hill coefficient (n_{app}). (*B*) PS120/M3C2 cells stimulated by FBS showed an increase in V_{max} and no significant change in K' or n_{app}. (*C*) PS120/M1C2 cells stimulated by serum were characterized by a decrease in K' without an effect on V_{max} or n_{app}. These data are from four or more experiments for control and FBS treatment using cells of the same passage number and acid selection.

PMA is known to stimulate NHE1 by increasing the affinity for H^+ and NHE2 by increasing V_{max}. In contrast, NHE3 is inhibited by PMA through a change in V_{max}, a feature associated with the ileal brush border Na^+/H^+ exchanger. Fig. 3 and Table 1 show the effect of PMA on the chimeric Na^+/H^+ exchangers. PMA did not affect M1C3 (Fig. 3*A*), M1C2, M2C1, and M3C1 (Table 1). On the other hand, PMA resulted in change in the transport activities of M3C2 and M2C3. V_{max} of M3C2 was increased by PMA (30% increase) without any changes in $K'(H_i^+)$ and n_{app} (Fig. 3*B*). M2C3, on the other hand, was inhibited with a decrease in V_{max} (30% decrease). Of note, M3C2 and M2C3 differ from the rest of chimeras in that these consist of a membrane-bound N terminus of an epithelial exchanger and a cytoplasmic C terminus of another epithelial exchanger, while the other chimeras studied were made up of parts of an epithelial isoform and the housekeeping isoform.

FGF stimulates all of the cloned Na^+/H^+ exchangers, although its mechanism of activation differs in NHE1 (K') compared with NHE2 and NHE3 (V_{max}). Response to FGF was studied during initial pH recovery after the acid loading as shown in Fig. 4. As with PMA, kinetic properties of M2C1 (Fig. 4*A*), M1C2, M1C3, and M3C1 (Table 1) were not affected by FGF. In contrast, FGF showed a stimulatory effect on M3C2 (Fig. 4*B*) and M2C3 (Table 1) with an increase in V_{max} and no effect on $K'(H_i^+)$ and the Hill coefficient.

DISCUSSION

We have studied kinetics of regulation of mammalian Na^+/H^+ exchangers using chimeric proteins to determine the specific contributions by the N terminus and C terminus of the exchangers in differential regulation by growth factors/protein kinases. Results are summarized in Table 2. The most important conclusions from this study are as follows. (*i*) The N- and C-terminal portions of the Na^+/H^+ exchanger must interact to allow protein kinase regulation. However, the N- and C-terminal interactions are specific and restricted. Protein kinase regulation requires an epithelial N terminus interacting with

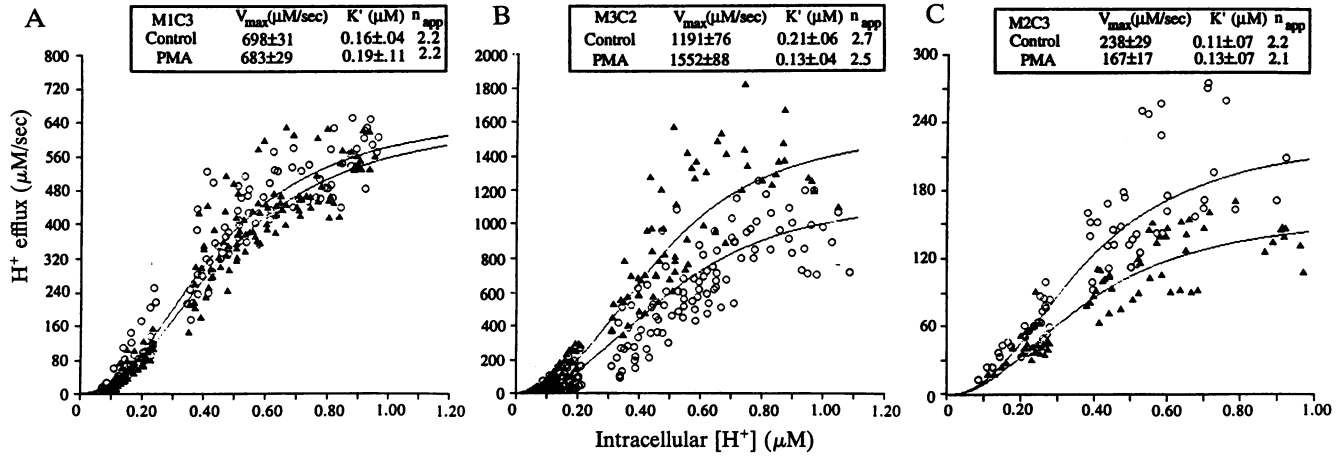

FIG. 3. PMA stimulates Na^+/H^+ exchange activity in PS120/M3C2 cells. (*A*) Treatment of PS120/M1C3 cells with 1 μM PMA (▲) for 5 min before addition of Na^+ had no significant effect on V_{max}, K', or n_{app} compared with that of control cells (○). (*B*) PS120/M3C2 cells were stimulated by PMA (▲) with an increase in V_{max} without significant change in K' or n_{app}. (*C*) In contrast, PS120/M2C3 cells were inhibited by PMA (▲) with a decrease in V_{max}. Shown here are data from five or more experiments for each condition.

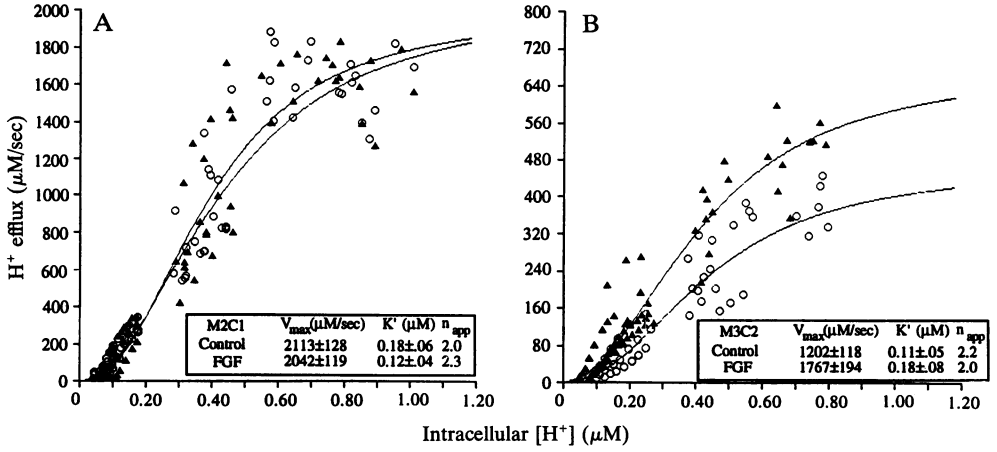

FIG. 4. FGF stimulates Na^+/H^+ exchange activity in PS120/M3C2 cells. (*A*) Na^+/H^+ exchange rate in PS120/M2C1 cells was not affected by FGF (▲) compared with control cells (○). (*B*) FGF (10 ng/ml) stimulated PS120/M3C2 cells (▲) with an increase in V_{max} compared with that of control cells (○). These data are from four or more experiments for each condition.

an epithelial C terminus or a housekeeping N terminus interacting with a housekeeping C terminus. (*ii*) The membrane-bound N terminus and the first 19 amino acids of the putative C terminus are responsible for the V_{max}-vs.-$K'(H^+)$ kinetics of growth factor regulation. (*iii*) The C terminus determines whether C kinase stimulates or inhibits Na^+/H^+ exchange. (*iv*) Any transporting-domain N terminus (NHE1, NHE2, or NHE3) plus any regulatory-domain C terminus are sufficient to observe the magnitude of the basal Na^+/H^+ exchange rate seen in native exchangers.

Based on previous studies with truncation mutations of NHE1 and NHE3, which demonstrated the essential role of the C terminus in protein kinase regulation (3, 14, 19), it was anticipated that the modulation of the chimeric Na^+/H^+ exchange activities would be entirely determined by the cytoplasmic tail. Thus, M3C1 was expected to be stimulated by PMA and FGF, whereas M1C3 was expected to be stimulated by FGF but inhibited by PMA. This prediction was not confirmed. Unexpectedly, pairing an N-terminal domain of one isoform with a C-terminal domain of another isoform did not always lead to growth-factor-regulatable chimeric Na^+/H^+ exchange, even though all chimeras had excellent basal activity. In fact, only M3C2 and M2C3 could be regulated by PMA and FGF. These chimeras consist of an epithelial N terminus paired with an epithelial C terminus. Such results were not expected, since NHE2 and NHE3 did not display any higher conservation in primary or predicted secondary structure between them compared with NHE1 and NHE3 or with NHE1 and NHE2. The present result suggests that there must be a specific and restricted interaction between the membrane domain and the cytoplasmic domain of the Na^+/H^+ exchanger for protein kinase regulation. That M1C3, M1C2, M2C1, and M3C1 were not regulated by PMA or FGF suggests that the C termini of these chimeric proteins are not able to interact with the H^+ modifier site or other parts of the Na^+/H^+ exchanger that reside within the membrane domain. Our results also imply that there are common structural elements between C2 and C3 and also between M2 and M3, which are involved in V_{max} regulation of these epithelial Na^+/H^+ exchangers. In addition, the recent work on a chimeric Na^+/H^+ exchanger made up of the N terminus of human NHE1 (a housekeeping NHE isoform) and the C terminus of a homologous housekeeping erythrocyte isoform, trout βNHE (4), showed that the chimera is cAMP-regulated when expressed in PS120 fibroblasts, and this is attributable to the C terminus of βNHE interacting with the N terminus of NHE1 to allow a $K'(H_i^+)$ regulation of Na^+/H^+ exchange (4). This is similar to results from this study in that the same NHE subclass (housekeeper or epithelial) must be present on both N and C termini to allow protein kinase regulation of Na^+/H^+ exchange.

This requirement for presence of specific parts of members of a transport protein gene family to allow regulation is similar to that described for the volume-activated ClC-2 chloride channel, a member of the chloride channel gene family. In this chloride channel, a specific interaction between the N and C termini is necessary for response by the channel to voltage and volume (20). Determining what parts of the Na^+/H^+ exchanger N and C termini are involved in the interaction should provide further insights into the ways these proteins carry out basal and regulated Na^+/H^+ exchange.

It also was unexpected that the N terminus was the determining factor of the V_{max}-vs.-$K'(H_i^+)$ response to serum, since it had been shown on the basis of C terminus truncation experiments that the C terminus was required for protein kinase regulation of NHE1 (3), NHE2 (S. Nath, C.-M.T., M.D. and C.H.C.Y., unpublished data), and NHE3 (19). Moreover, it has been hypothesized that the protein kinase/growth factor effect on the affinity for H_i^+ of NHE1 results from an interaction between a small domain in the cytoplasmic tail of NHE1 (amino acids 567–635) and the H^+-modifier site in the N terminus (3). However, a role for the N terminus in Na^+/H^+ exchange regulation is supported by the previous demonstration that serum stimulates the truncation mutations of NHE1, NHE2, and NHE3, which contain only the N terminus plus the first 19 amino acids of the C terminus (19). Unfortunately, kinetic analysis of the serum effect on the membrane domains of the Na^+/H^+ exchangers could not be performed since their basal rates were too slow (<5% of the wild-type exchangers). Thus, it is not clear if the serum stimulation of M3C1 and M1C2 represents more than the serum stimulation of M3 and M1 made clearer by the faster rates of the chimeras than those of the N terminus constructs themselves.

That M1C3 and M2C1 did not respond to serum was surprising based on the serum stimulation of M1 and M2. Since M1 and M2 are both stimulated by serum, the response element to serum must be present within the putative membrane-bound N termini or the first C-terminal 19 amino acids. Recently, a region in the C terminus between amino acids 636–656 of NHE1, which binds calmodulin, was shown to cause a basal inhibitory effect (21). We have also identified a region at the distal end of the cytoplasmic tail of NHE3 that inhibits basal Na^+/H^+ exchange (19). Thus, we cannot rule out the possibility that serum exerted both a stimulatory effect acting through the N terminus and an inhibitory effect through the C termini of M1C3 and M2C1 so that these opposing effects canceled each other. Alternatively, it is possible that the

Table 1. Effect of serum, PMA, and FGF on rate of Na^+/H^+ exchange in PS120 cell lines transfected with the chimeric Na^+/H^+ exchangers

Exchanger	Treatment	V_{max}, μM/sec	$K'(H_i^+)$, μM	n_{app}
M1C2	Control	2045 ± 94	0.17 ± 0.04*	2.4
	+ serum	1943 ± 60	0.08 ± 0.01	2.5
	Control	1745 ± 56	0.09 ± 0.02	2.9
	+ PMA	1865 ± 86	0.13 ± 0.03	2.6
	Control	1344 ± 98	0.13 ± 0.04	1.7
	+ FGF	1347 ± 123	0.10 ± 0.04	1.8
M1C3	Control	980 ± 44	0.15 ± 0.01	1.7
	+ serum	912 ± 52	0.14 ± 0.03	1.8
	Control	698 ± 31	0.16 ± 0.04	2.2
	+ PMA	683 ± 29	0.19 ± 0.04	2.2
	Control	568 ± 48	0.10 ± 0.04	2.3
	+ FGF	582 ± 26	0.16 ± 0.02	2.1
M2C1	Control	1999 ± 21	0.15 ± 0.02	1.6
	+ serum	2018 ± 135	0.10 ± 0.03	1.9
	Control	2113 ± 128	0.18 ± 0.06	2.0
	+ PMA	2086 ± 88	0.15 ± 0.03	2.0
	Control	2113 ± 128	0.18 ± 0.06	2.0
	+ FGF	2042 ± 119	0.12 ± 0.04	2.3
M2C3	Control	306 ± 38*	0.12 ± 0.05	2.0
	+ serum	443 ± 52	0.20 ± 0.06	1.7
	Control	238 ± 29*	0.11 ± 0.07	2.2
	+ PMA	167 ± 17	0.13 ± 0.07	2.1
	Control	280 ± 22*	0.06 ± 0.03	2.2
	+ FGF	357 ± 33	0.09 ± 0.04	2.1
M3C1	Control	1365 ± 81*	0.18 ± 0.05	1.7
	+ serum	2035 ± 162	0.17 ± 0.06	1.9
	Control	1482 ± 85	0.06 ± 0.02	2.4
	+ PMA	1573 ± 73	0.08 ± 0.01	2.3
	Control	2076 ± 74	0.14 ± 0.03	2.1
	+ FGF	1964 ± 165	0.17 ± 0.07	2.0
M3C2	Control	1330 ± 114*	0.26 ± 0.09	2.8
	+ serum	1808 ± 102	0.19 ± 0.04	2.8
	Control	1191 ± 76*	0.21 ± 0.06	2.7
	+ PMA	1552 ± 88	0.13 ± 0.04	2.5
	Control	1202 ± 118*	0.11 ± 0.05	2.2
	+ FGF	1767 ± 194	0.18 ± 0.08	2.0

*Statistical significance vs. control. Data are presented as means ± SE.

presence of the C termini of M1C3 and M2C1 prevented the serum effect on the N termini. This would further support the requirement in NHE regulation of a specific interaction between the NHE N and C termini and the observation that only certain N and C termini are able to interact in a manner that allows growth factor regulation. At any rate, absence of any effect by FGF and PMA on M1, M2, M3, and some of the chimeras regulated by serum, including M1C2 and M3C1, suggests that serum exerts its effect through pathways different from those used by FGF and PMA.

Role of the cytoplasmic C terminus of Na^+/H^+ exchanger in protein kinase regulation has previously been documented (3, 14, 19). The present work extends the importance of the C terminus for PMA regulation. The stimulation of M3C2 and inhibition of M2C3 by PMA correlate with the previously reported stimulation of NHE2 and inhibition of NHE3 by PMA, respectively. Therefore, the Na^+/H^+ exchanger C terminus dictates the stimulatory or inhibitory nature of the protein kinase C regulation acting on an epithelial N terminus.

One major difference between the chimeric exchangers used in this study and the truncated forms of the exchangers M1, M2, and M3 is the fast exchange rates of the chimeras. This observation is consistent with an earlier finding that the exchange activity was reduced drastically upon deletion of ≈80% of the putative cytoplasmic tail of NHE1 (3). A similar reduction in the activity was observed for NHE3 (19). It is not known if the fast rate of the chimeras is due to more plasma membrane exchanger or more activity per exchanger.

Table 2. Summary of Serum, FGF, and PMA regulation of Na^+/H^+ exchangers

	Serum	FGF	PMA
NHE1	↑	↑	↑
NHE2	↑	↑	↑
NHE3	↑	↑	↓
M1	↑	0	0
M2	↑	0	0
M3	↑	0	0
M1C2	↑	0	0
M1C3	0	0	0
M2C1	0	0	0
M2C3	↑	↑	↓
M3C1	↑	0	0
M3C2	↑	↑	↑

Arrows indicate up- or down-regulation.

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