Identification of a Carboxyl-terminal Motif Essential for the Targeting of Na\(^+\)-HCO\(_3^-\) Cotransporter NBC1 to the Basolateral Membrane*

The Na\(^+\)-HCO\(_3^-\) cotransporter NBC1 is located exclusively on the basolateral membrane and mediates vectorial transport of bicarbonate in a number of epithelia, including kidney and pancreas. To identify the motifs that direct the targeting of kidney NBC1 to basolateral membrane, wild type and various carboxyl-terminally truncated kidney NBC1 mutants were generated, fused translationally in-frame to GFP, and transiently expressed in kidney epithelial cells. GFP was linked to the NH\(_2\)-terminus of NBC1, and labeling was examined by confocal microscopy. Full-length (1035 aa) and mutants with the deletion of 3 or 20 amino acids from the COOH-terminal end of NBC1 (lengths 1032 and 1015 aa, respectively) showed strong and exclusive targeting on the basolateral membrane. However, the deletion of 26 amino acid residues from the COOH-terminal end (length 1010 aa) resulted in retargeting of NBC1 to the apical membrane. Expression studies in oocytes demonstrated that the NBC1 mutant with the deletion of 26 amino acid residues from the COOH-terminal end is functional. Additionally, the deletion of the last 23 amino acids or mutation in the conserved residue Phe at position 1013 on the COOH-terminal end demonstrated retargeting to the apical membrane. We propose that a carboxyl-terminal motif with the sequence QQPFLS, which spans amino acid residues 1010–1015, and specifically the amino acid residue Phe (position 1013) are essential for the exclusive targeting of NBC1 to the basolateral membrane.

Two well known variants of NBC1 are expressed in various epithelial tissues. These are the kidney (kNBC1)\(^+\) and pancreatic (pNBC1) variants, which differ only in their NH\(_2\)-terminal sequence, where amino acids 1–41 in kNBC1 have been replaced with 85 amino acids in pNBC1 (3). The carboxyl-terminal end of NBC1 is identical in kidney and pancreatic variants (1–5). Kidney NBC1 has a stoichiometry of three equivalents of bicarbonate for each sodium, whereas pancreatic NBC1 has a stoichiometry of two equivalents of bicarbonate per each sodium (11, 12). This alteration in stoichiometry allows for the kidney NBC1 to function in the absorptive mode and for the pancreatic NBC1 to function in the secretory mode (4, 9–12).

In all of the tissues examined to date, NBC1 is found to be located on the basolateral membrane of epithelial cells (4–6), strongly suggesting the presence of specific amino acid motif(s) that directs its targeting to the basolateral membrane. To identify possible motifs that are responsible for the targeting of NBC1 to the basolateral membrane, various COOH-terminally truncated NBC1 mutants were generated, fused translationally in-frame to GFP, and transiently expressed in cultured kidney epithelial cells. The results demonstrate that the deletion of amino acid residues 1010–1015 with the sequence QQPFLS results in the retargeting of NBC1 preferentially to the apical membrane. Site-directed mutagenesis of the conserved amino acid residue Phe at position 1013 within this domain recapitulates the retargeting of NBC1 to the basolateral membrane. The significance of the results will be discussed.

**MATERIALS AND METHODS**

**Construction of GFP-NBC1 Full Length and Mutants**—The full length and various COOH-terminally truncated NBC1 mutants were generated by PCR using the human full-length NBC1 DNA (3257 bp and 1035 aa residues) as a template (GenBank\textsuperscript{TM} accession number AF007216). The truncated NBC1 proteins have COOH-terminal deletions of 3, 20, 26, or 50 amino acid residues and are designated as NBC1 CD3, CD20, CD26, or CD50, respectively. The resulting wild type and various COOH-terminally truncated NBC1 mutants were amplified and fused translationally in-frame to GFP by cloning into pcDNA3.1/NH\(_2\)-terminal GFP-TOPO vector (Invitrogen).

Full-length kNBC1 was amplified using the following primers: t986730, 5'–GAATTCGAGATGCGCACTGAAAATGTTGAAAGG–3' (sense), and t986120, 5'–AGCGGCGGCCCTCAGATGTTGTGCGGT–3' (antisense). These primers were used to generate full-length NBC1 cDNA from plasmid cytomegalovirus intron neomycin NBC1 plasmid (1). This resulted in the expression of GFP–NBC1 fusion protein with the GFP fusing to the NH\(_2\)-terminus of kNBC1 (GFP–...).
NBC1). Using a similar approach, primer 1 (sense, above) and primer 3 (t80925, 5'-GCCGCCCGCAGTTGCGTTCAGGAACTGATTGTGGTGAGC-3' (antisense)) were used to generate the NBC1-CDS cDNA. This resulted in the expression of the GFP-NBC1 fusion protein, which coded the truncated NBC1 (CD3), with the deletion of the last three amino acid residues on the carboxyl-terminal end. For CD20, CD26, and CD50 (deletion of the last 20, 26, and 50 amino acid residues, respectively), the following (antisense) primers were used: CD20 3' primer, 5'-GCCGCCCGCAGTTGCGTTCAGGAACTGATTGTGGTGAGC-3' and CD50 3' primer, 5'-GCCGCCCGCAGTTGCGTTCAGGAACTGATTGTGGTGAGC-3'.

The capped GFP-NBC1 (full-length), GFP-NBC1 CD26 (with the deletion of 26 amino acid residues at the COOH-terminal end), and GFP only (no NBC insert) cRNAs were generated using mMESSAGE mMACHINE™ T7 Kit from Ambion, Inc., Austin, TX) according to the manufacturer's instruction. 50 nl of cRNA (0.5 μg/μl) was injected with a Drummond 510 microdispenser via a sterile glass pipette with a tip of 20–30 μm. After injection, the oocytes were maintained in a solution of the following composition (in mM): 96 NaCl; 2 KCl; 1.0 MgCl₂; 5 Hepes; 2.5 sodium pyruvate; 0.5 theophylline; 100 units/ml penicillin; and 100 μg/ml streptomycin, pH 7.5. Injected oocytes were stored in an incubator at 17°C and were used for electrophysiological experiments after 2–4 days. We did not perform intracellular pH recording with BCECF (1'2'7'-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester) on GFP constructs because of interference of the GFP signal with the BCECF recording. However, intracellular pH studies were performed on NBC1 mutants without GFP insert.

Electrophysiology Studies—Oocytes were placed on a nylon mesh in a perfusion chamber and continuously perfused (3 ml/min perfusion rate). The perfusion solution had the following composition (in mM): 96 NaCl; 2 KCl; 1 MgCl₂; 1.8 CaCl₂; and 15 Hepes, pH 7.5. After a stabilization period, when the membrane potential (V_m) was constant, the perfusion solution was switched to a CO₂/bicarbonate-containing solution of the following composition (in mM): 30 NaHCO₃; 66 NaCl; 2 KCl; 1.5 NaCl; 1.5 CaCl₂; and 15 Hepes, pH 7.5, and gassed with 5% CO₂. Experiments were performed at room temperature (22–25°C). Membrane potentials were recorded with conventional microelectrode techniques by glass microelectrodes (resistance 3–5 meQohms) filled with 3 M KCl and connected to an Axoclamp 2A amplifier (Axon Instruments). Membrane potential changes were considered significant if p < 0.05.

Pandora's Box—Fusion of cultured cells with GFP vector alone (no NBC1 insert) was injected with a Drummond 510 microdispenser via a sterile glass pipette with a tip of 20–30 μm. After injection, the oocytes were maintained in a solution of the following composition (in mM): 96 NaCl; 2 KCl; 1.0 MgCl₂; 5 Hepes; 2.5 sodium pyruvate; 0.5 theophylline; 100 units/ml penicillin; and 100 μg/ml streptomycin, pH 7.5. Injected oocytes were stored in an incubator at 17°C and were used for electrophysiological experiments after 2–4 days. We did not perform intracellular pH recording with BCECF (1'2'7'-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester) on GFP constructs because of interference of the GFP signal with the BCECF recording. However, intracellular pH studies were performed on NBC1 mutants without GFP insert.

RESULTS

In the first series of experiments, we examined the expression and targeting of GFP with or without the NBC1 insert in MDCK cells. Fig. 1a (left and right panels) shows that transfection of cultured cells with GFP vector alone (no NBC1 insert) results in the accumulation of GFP in the cytoplasm (Z-line images in left panel) with no localization on the membrane (Z-stack images in right panel). However, transfection with GFP-NBC1 full-length cDNA shows exclusive localization of the GFP in the membrane (as visualized by Z-stack, front view) in cells colabeled with phalloidin (Fig. 1b). The specific membrane labeling (apical versus basolateral) was examined using the Z-line image (side view) analysis. The results are demonstrated in Fig. 1c. Similar studies were performed with the
The GFP-NBC1 construct in MDCK cells colabeled with PNA-lectin dye, a very specific marker of apical membrane labeling in polarized cells (16). The Z-line (side view) images of the results (NBC1-GFP and PNA-lectin double labeling) are depicted in Fig. 1d. As demonstrated, the full-length NBC1 is targeted exclusively to the basolateral membrane domain (Fig. 1, c and d). These results are consistent with published reports on the basolateral localization of NBC1 in epithelial cells (4–6).

In the next series of experiments, we examined the expression of GFP-NBC1 mutants with 3 (CD3) or 20 (CD20) amino acid deletion from their carboxyl-terminal end (see “Materials and Methods”). Fig. 2a is a Z-stack (front view) image analysis...
of the expression of GFP-NBC1 CD3 colabeled with phalloidin in MDCK cells, showing GFP-NBC1 CD3 localization on the cell membrane. The Z-line (side view) image analysis of the expression studies doubly labeled with phalloidin or PNA-lectin demonstrate that NBC1-CD3 mutant is targeted to the basolateral membranes (Fig. 2, b and c). Fig. 3 depicts similar results with NBC1 mutant lacking the last 20 amino acids from the COOH-terminal end. The Z-stack (front view) image analysis of the expression of GFP-NBC1 CD20 colabeled with phalloidin (Fig. 3a) is shown in MDCK cells and demonstrates exclusive localization on the cell membrane. The Z-line image analysis (side view) of the expression studies demonstrates that NBC1-CD20 mutant is targeted to the basolateral membrane (Fig. 3, b and c).

The next series of experiments examined the expression of the GFP-NBC1 mutant that lacked the last 26 amino acids of NBC1 carboxyl-terminal end (CD26). Transfection studies were performed as described above. The Z-stack analysis (front view) of the expression of GFP-NBC1 CD26, colabeled with phalloidin, shows strong membrane as well as submembrane labeling with the GFP (Fig. 4a). The Z-line (side view) image analysis of the results shows the surprising and unexpected finding of apical membrane targeting of NBC1 (Fig. 4, b and c). Experiments were performed with both phalloidin (Fig. 4b) and PNA-lectin binding (Fig. 4c). Both methods clearly confirm that deletion of the last 26 amino acids from the COOH-terminal end causes the mistargeting of NBC1 to the apical membrane (Fig. 4, b and c). Some images show residual labeling of the basolateral membrane (Fig. 4, b and c), but clearly, the majority of the labeling is localized on the apical membrane with little cytoplasmic expression.

To determine whether further truncation of the carboxyl-terminal end affects its membrane localization, NBC1 mutants with the deletion of 50 amino acid residues were generated, coupled to GFP, and transiently expressed in MDCK cells. Fig. 5a shows transfection of MDCK cells with the NBC1-CD50 mutant and indicates that GFP-NBC1 CD50 localized on the cell membrane as well as in the cytoplasm. As demonstrated in Fig. 5 (panels b and c), NBC1 mutant with the deletion of 50 amino acid residues shows persistence of labeling on the apical membrane. As noted, this mutant also demonstrates significant intracytoplasmic expression in addition to the apical labeling, indicating that progressive truncation of the COOH-terminal end of NBC1 beyond the last 26 amino acids on the COOH-terminal end may interfere with the targeting of NBC1 to cell membrane.

In the next series of experiments, we examined the functional activity of NBC1-GFP CD26 mutant in oocytes by membrane potential measurement (1, 2, 14). For comparison, oocytes injected with GFP-NBC1 full length or GFP only (no NBC insert) cRNA were utilized. We were specifically interested in NBC1-CD26, as this is the mutant with the shortest truncation that shows mistargeting to the apical membrane (Fig. 4). As demonstrated (Fig. 6, bottom panel, representative tracing), exposure to CO2/HCO3 in oocytes expressing the full-length GFP-NBC1 (2–4 days after cRNA injection, 0.5 μg/μl) resulted in an immediate membrane hyperpolarization, which reached a peak within 1 min. The hyperpolarization showed very little decay during the time of exposure to CO2/HCO3 and was reversible upon returning to the CO2/HCO3-free perfusion solution. This finding is consistent with published reports and indicates that NBC1 is highly electrogenic (1, 2, 15).

As noted, exposure to CO2/HCO3 in oocytes expressing GFP-NBC1 CD26 mutant (2–4 days after crNA injection, 0.5 μg/μl) also caused significant membrane hyperpolarization (Fig. 6, middle panel, representative tracing). Control oocytes (GFP only) showed no alteration in membrane potential measurement in response to CO2/HCO3 exposure (Fig. 6, top panel, representative tracing). The summary results of the effect of CO2/HCO3 on membrane potential were −114.6 ± 6.0 mV in full-length GFP-NBC1-injected oocytes (n = 5), −106.1 ± 5.6 mV in GFP-NBC1 CD26-injected oocytes (n = 6), and −62 ±
Fig. 3. Transfection of MDCK cells with GFP-NBC1 CD20 cDNA. a, expression of GFP-NBC1 CD20:Z-stack image analysis. As demonstrated, the NBC1 with deletion of the last 20 carboxyl-terminal amino acids is targeted to the plasma membrane. Green, NBC1-GFP; red, phalloidin. b, expression of GFP-NBC1 CD20:Z-line image analysis. As demonstrated, the NBC1 with the deletion of the last 20 carboxyl-terminal amino acids is exclusively targeted to the basolateral membrane. Green, NBC1-GFP; red, phalloidin. c, expression of GFP-NBC1 CD20:Z-line image analysis. Colabeling with PNA-lectin, which exclusively binds to apical membrane, verifies the localization of NBC1-CD20 to the basolateral membrane. Green, NBC1-GFP; red, PNA-lectin.

Fig. 4. Transfection of MDCK cells with GFP-NBC1 CD26 cDNA. a, expression of GFP-NBC1 CD26:Z-stack image analysis. As demonstrated, the NBC1 with deletion of the last 26 carboxyl-terminal amino acids is targeted to the plasma membrane. Green, NBC1-GFP; red, phalloidin. b, expression of GFP-NBC1 CD26:Z-line image analysis. As demonstrated, the NBC1 with a deletion of the last 26 carboxyl-terminal amino acids is predominantly retargeted to the apical membrane with residual labeling on the basolateral membrane. Green, NBC1-GFP; red, phalloidin. c, expression of GFP-NBC1 CD26:Z-line image analysis. Colabeling with PNA-lectin, which exclusively binds to apical membrane, confirms the apical localization of NBC1-CD26 with residual labeling on the basolateral membrane. Green, NBC1-GFP; red, PNA-lectin.
3.8 mV in GFP-only injected oocytes (n = 5). The base-line membrane potentials (before exposure to CO₂/HCO₃⁻) were -49.4 ± 3.2 mV in full-length GFP-NBC1-injected oocytes, -53.2 ± 4.1 mV in GFP-NBC1 CD26-injected oocytes, and -50.9 ± 4.2 mV in GFP-only injected oocytes. As indicated, oocytes injected with either the full-length or the mutant NBC1 showed significant hyperpolarization (p < 0.01 versus no CO₂/HCO₃⁻), whereas GFP-only injected oocytes did not show any alteration in membrane potential in response to CO₂/HCO₃⁻ exposure. The magnitude of hyperpolarization in response to CO₂/HCO₃⁻ exposure was not different between the full-length and the CD26-truncated mutant (p > 0.05). Taken together, these results demonstrate that the GFP-NBC1 mutant with the deletion of the last 26 carboxyl-terminal amino acid residues is functionally active.

In the last series of experiments, we examined the membrane targeting of NBC1-CD26 and mutants in which the conserved residue at position 1013 on the COOH-terminal end was mutated (F1013A) by site-directed mutagenesis. As demonstrated in Fig. 7 (panel A), deletion of the last 23 amino acid residues causes the retargeting of NBC1 to the apical membrane, indicating that the sequence FLS (residues 1013–1015) is essential for the targeting of NBC1 to the basolateral membrane. To identify the amino acid residue(s) responsible for the exclusive targeting of NBC1 to the basolateral membrane, mutants in which the conserved residue Phe was mutated (F1013A) were used for transfection in MDCK cells. As demonstrated in Fig. 7 (panel B), the F1013A mutation caused the retargeting of NBC1 to the apical membrane.

**DISCUSSION**

The targeting of NBC1 to the basolateral membrane was examined using a series of mutants with progressive truncation of the carboxyl-terminal end that were fused in-frame to GFP and visualized by confocal microscope. The results demonstrated that the full-length NBC1 and mutants with up to 20 amino acid deletion from the carboxyl-terminal end are targeted to the basolateral membrane (Figs. 1–3). However, the NBC1 mutant with the deletion of 26 amino acids from its COOH-terminal end showed mistargeting to the apical membrane domain with some labeling observed on the basolateral membrane (Fig. 4). Additional truncations for up to 67 amino acid residues showed persistence of labeling on the apical membrane but also resulted in accumulation of mutant proteins in the cytoplasm (Fig. 5). Membrane potential recording in oocytes demonstrated that the NBC1 mutant with deletion of 26 amino acid residues mediates Na⁺–HCO₃⁻ cotransport (Fig. 6), indicating that the apically targeted molecule is functionally active. Additionally, truncation of the last 23 amino acids or mutants in which the conserved residue Phe at position 1013 on the COOH-terminal end was mutated by site-directed mutagenesis (F1013A) demonstrated retargeting to the apical membrane. We further find that other truncated mutants (up to 67 amino acid deletion) are functional, although at a much smaller rate when expressed in non-GFP forms and assayed by BCECF (data not shown).

H. Li, L. Conforti, and M. Soleimani, unpublished observation.
NBC1 is located basolaterally in various epithelia and mediates vectorial transport of bicarbonate. In kidney, NBC1 is located on the basolateral membrane of proximal tubules and mediates the exit of bicarbonate from cell to blood (4–6, 17, 18). In pancreas, NBC1 is located on the basolateral membrane of ducts and mediates the entry of bicarbonate from blood to cell (4–6, 19, 20). Studies on NBC1 have demonstrated that kNBC1 has a stoichiometry of three equivalents of bicarbonate per sodium, whereas the pNBC1 has a stoichiometry of two bicarbonates per sodium (11, 12). The difference in the stoichiometry allows for the change in the direction of NBC1 movement from absorptive (i.e. in kidney) to secretory (i.e. in pancreatic duct).

The most salient feature of the current study is the identification of a carboxyl-terminal motif and residues that are essential for the targeting of NBC1 to the basolateral membrane. It is noteworthy that, in the absence of this motif (CD23 or CD26 amino acid deletion) or in mutants in which the conserved residue Phe at position 1013 is mutated, NBC1 is predominantly mistargeted to the apical membrane rather than accumulated in the cytoplasm. This observation raises the possibility that other existing motifs may be responsible for the targeting of NBC1 to the apical membrane. Alternatively, it is plausible that the mistargeting of NBC1 to the apical membrane occurs by default (as a result of the deletion of basolateral targeting domain). Further deletion of the carboxyl-terminal end beyond 26 amino acids (up to 67 aa residues) resulted in more intracytoplasmic accumulation of NBC1 (Fig. 5), indicating that the carboxyl-terminal end may play an important role in membrane targeting of NBC1.

The apically targeted NBC1 is functionally active (Fig. 6), raising the likelihood that possible mutations or deletions in the identified motif (QQPFLS) in human do not impair its functional activity. However such a mutation or deletion may cause significant impairment in bicarbonate reabsorption because of the absence of an exit pathway for bicarbonate transport in the basolateral membrane. In other words, the mistargeting of NBC1

![Figure 6](image_url)

**Fig. 6.** Membrane potential recordings in oocytes injected with full-length or mutant GFP-NBC1 (CD26) cRNA (representative tracings). Membrane potential was measured by conventional intracellular microelectrodes in *Xenopus* oocytes 2–4 days after injection with full-length GFP-NBC1 or mutant GFP-NBC1 NBC1 (CD26) cRNA. After an equilibrating period, the perfusion solution was switched to a solution containing 10 mM HCO₃⁻ and gassed with 1.5% CO₂, 98.5% O₂, at pH 7.5. A continuous line indicates the time of exposure to CO₂/HCO₃⁻. A–C, exposure of oocytes that were injected with full-length GFP-NBC1 (panel C), mutant GFP-NBC1-CD26 (panel B), or GFP only (no NBC insert) (panel A) to CO₂/HCO₃⁻ resulted in an immediate and sustained hyperpolarization in the full-length and mutant NBCs (panels B and C) but not the GFP only (panel A). The hyperpolarization was reversible upon removal of CO₂/HCO₃⁻ from the perfusion medium. Exposure of control (water-injected) oocytes to CO₂/HCO₃⁻ also did not alter the membrane potential (data not shown). A, GFP only. B, GFP-NBC-CD26. C, GFP-NBC1 full length.

![Figure 7](image_url)

**Fig. 7.** Transfection of MDCK cells with CD23 deletion or mutated GFP-NBC1 cDNA. **a,** expression of GFP-NBC1 CD23:Z-line image analysis. Colabeling with PNA-lectin, which exclusively binds to the apical membrane, demonstrates the predominant apical localization of NBC1-CD23. Residual labeling on the basolateral membrane is observed. Green, NBC1-GFP; red, PNA-lectin. **b,** expression of mutated GFP-NBC1 cDNA (F1013A):Z-line image analysis (left and right panels). As demonstrated, the NBC1 cDNA with mutation of residue 1013 (F1013A) shows predominant retargeting to the apical membrane with residual labeling on the basolateral membrane. Green, NBC1-GFP; left panel: red, phalloidin; right panel: red, PNA-lectin.
to the apical membrane may impair the vectorial transport of bicarbonate and lead to proximal tubular acidosis. Whether motifs on the amino-terminal end of NBC1 play any role in basolateral membrane targeting of NBC1 remains speculative at the present and warrants detailed examination.

Recent studies have identified mutations in the Cl−/HCO3− exchanger AE1 that are associated with distal renal tubular acidosis, a bicarbonate-wasting condition. AE1 is normally located on the basolateral membrane of α(A)-intercalated cells in cortical and outer medullary collecting duct and, in tandem with apical H+-ATPase, is responsible for majority of bicarbonate reabsorption in these nephron segments (21, 22). Bicarbonate wasting in these subjects raised the possibility that AE1 mutants either became functionally inactive or were retained intracellularly. However, studies with AE1 mutants have been less conclusive (23–26), casting doubt on the explanations regarding bicarbonate wasting in humans with AE1 mutations. Very recent studies have deciphered this puzzle (16, 27). In studies in cultured polarized kidney epithelial cells, Devonald et al. (16) and Rungroj et al. (27) show that AE1 mutants tagged with GFP are mistargeted to the apical membrane. Given the functionality of AE1 mutants in non-epithelial cells, Devonald et al. (16) and Rungroj et al. (27) conclude that the mistargeting of AE1 to the apical membrane may actually worsen the bicarbonate wasting as the apically located AE1 secretes bicarbonate into the lumen in exchange for luminal chloride (16, 27). This should lead to renal bicarbonate wasting and worsen distal renal tubular acidosis. It is worth mentioning that other mutations cause the retention of AE1 in the endoplasmic reticulum or in late endosomes/lysosomes, thus causing distal renal tubular acidosis by reducing bicarbonate exit across the basolateral membrane of the collecting duct (25, 28).

A GenBank™ search of other members of NBC/AE superfamily demonstrated that a motif with high homology to the one identified in our studies is also expressed in NBC2, NBC4, NCBE, AE1, and AE2, all known bicarbonate transporters located on the basolateral membrane of various epithelia. An analysis of other known chloride/bicarbonate or anion exchangers from SLC26 family, which share little homology with NBC/AE superfamily, demonstrates that those anion exchangers that are targeted to the basolateral membrane (SLC26A1 and SLC26A7) contain motifs with high homology to the current motif (GenBank™ NM_022042 for A1, from residue 675 to 678 with aa sequence QLFL with full length of 701 aa; GenBank™ NM_134266 for Ala-7, from residue 343 to 346 with aa sequence QEFL with full length of 663 aa). However, SLC26 exchangers that are targeted to the apical membrane (SLC26A3 and SLC26A4) do not express any motif with high homology to the current motif.

In conclusion, NBC1 mutants lacking the last carboxyl-terminal 23 or mutant in which the conserved residue Phe-1013 mutated into Ala-1013 show mistargeting to the apical membrane. We propose that a carboxyl-terminal motif with the sequence QQPFLS, which spans amino acid residues 1010–1015, and specifically the amino acid residue Phe at position 1013 are essential for the exclusive targeting of NBC1 to the basolateral membrane. Future studies should clarify the underlying mechanism of this targeting rearrangement and attempt to identify the molecules that interact with NBC1 through this motif.

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