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Structure, Function, and Regulation of the SLC4 NBCe1 Transporter and its Role in Causing Proximal Renal Tubular Acidosis

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Abstract

Purpose of review—There has been significant progress in our understanding of the structural and functional properties and regulation of NBCe1, a membrane transporter that plays a key role in renal acid-base physiology. NBCe1-A mediates basolateral electrogenic sodium-base transport in the proximal tubule and is critically required for transepithelial bicarbonate absorption. Mutations in NBCe1 cause autosomal recessive proximal renal tubular acidosis (pRTA). The review summarizes recent advances in this area.

Recent findings—A topological model of NBCe1 has been established that provides a foundation for future structure-functional studies of the transporter. Critical residues and regions have been identified in NBCe1 that play key roles in its structure, function (substrate transport, electrogenicity) and regulation. The mechanisms of how NBC1 mutations cause pRTA have also recently been elucidated.

Summary—Given the important role of proximal tubule transepithelial bicarbonate absorption in systemic acid-base balance, a clear understanding of the structure-functional properties of the NBCe1-A is a prerequisite for elucidating the mechanisms of defective transepithelial bicarbonate transport in pRTA.

Keywords

proximal tubule; NBCe1; renal tubular acidosis; RTA; bicarbonate; transport

Introduction

SLC4 membrane transporter proteins play important roles in kidney acid-base regulation through their transport of bicarbonate (or carbonate), Na^+, Cl^-, and (possibly NH_4^+) [1, 2]. These transporters differ in their substrate (Na^+, Cl^-) dependence, charge transport stoichiometry, cell-type and developmental expression, functional regulation, and protein-

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protein interaction. In mammals, SLC4 proteins encoded by 10 different genes that share protein sequence homology and are grouped according to their functional properties (Fig. 1).

In humans, NBCe-1 is encoded by the SLC4A4 gene on chromosome 4q21 [3]. The gene encodes 3 human variants (-A, -B, and -C) and recently additional variants (-D and -E) have been reported in mouse [4]. Importantly, all three NBCe1 variants in humans (which have different N- and C-terminal sequences) mediate electrogenic Na^+-HCO_3^- transport, but differ in their tissue expression, intrinsic activity, and regulation [5]. In kidney, NBCe1-A is the key transporter predominantly expressed in S1 and S2 proximal tubule cells that mediates basolateral Na^+-base efflux thereby contributing to the reabsorption of ~80% of the filtered bicarbonate load [6]. The second variant NBCe1-B is identical to NBCe1-A except for its unique N-terminus (85 aa replacing the 41 aa in NBCe1-A) [7]. Unlike NBCe1-A, NBCe1-B is widely expressed in various tissues [7]. A third variant, NBCe1-C, has an identical N-terminus to NBCe1-B and a unique C terminus (61 aa replaces 46 aa in NBCe1-A or B) that ends in a type I PDZ-binding motif [8]. Mouse NBCe1-D and NBCe1-E are otherwise identical to NBCe1-A and NBCe1-B respectively, and are noted for the absence of a nine amino-acid sequence within the cytosolic N-terminus [4].

Given that all known NBCe1 variants have an identical protein sequence in the transmembrane region, the topology of NBCe1-A (the most extensively studied variant) can be used as a model for the other variants. NBCe1-A has a large N-terminal cytoplasmic region, a lipid embedded transmembrane region, and a C-terminal cytoplasmic tail (Fig. 2) [9, 10]. The N-terminal cytoplasmic region is tightly folded and is predicted to form a domain structure, unlike the freely aqueous accessible C-terminal cytoplasmic region [10, 11]. NBCe1-A is a ~ 140-kDa glycoprotein containing 1035 amino acids composed of 14 transmembrane regions (TMs) (Fig. 2) [9]. A large extracellular loop is present between TM5 and 6 containing two glycosylated sites [12]. The oligomeric state of the cotransporter is dimeric and each monomeric subunit has independent transport activity [13, 14].

This review focuses on recent findings characterizing the structure-function of NBCe1-A, the key electrogenic sodium bicarbonate cotransporter variant responsible for absorbing base across the basolateral membrane of the proximal tubule [1, 2]. Mutations in NBCe1 cause autosomal recessive proximal renal tubular acidosis [9, 15]. These naturally occurring mutations have also provided important new insights into its structure-functional properties. In addition to NBCe1-A, the extrarenal N- and C-terminal NBCe1 variants encoded by the SLC4A4 gene are also discussed particularly with reference to recent studies highlighting the differences in their functional regulation. Finally, the structural studies of NBCe1 have revealed important differences from the well-characterized SLC4 chloride bicarbonate-exchanger AE1 that mediates bicarbonate transport across the basolateral membrane of type A intercalated cells and is mutated in distal renal tubular acidosis [16]. A thorough review of the physiological and biophysical properties of all SLC4 transporters is beyond the scope of the present review and the interested reader is referred to recent summaries of the role of sodium-coupled SLC4 transporters in the kidney [1] and extrarenal organs [2].

Mutations in NBCe1 causing autosomal recessive pRTA

The importance of NBCe1 transport in the kidney and in extrarenal tissues is exemplified by the fact that patients with NBCe1 mutations have severe pRTA, growth and mental retardation, basal ganglia calcification, cataracts, corneal opacities (band keratopathy), glaucoma, elevated serum amylase and lipase, and defects in the enamel consistent with amelogenesis imperfecta [15]. Mice with loss of NBCe1 have a more severe phenotype with marked volume deletion and decreased survival [17, 18]. It is currently unknown to what extent the non-renal symptoms in patients with mutations in NBCe1 can be attributed to direct effects in the tissues expressing the various NBCe1 variants, versus the effect of systemic acidemia due to renal bicarbonate loss.

Eight missense mutations (NBCe1-A numbering; R298S, S427L, T485S, G486R, R510H, L522P, A799V, and R881C), 2 nonsense mutations (Q29X, W516X), a frameshift deletion at nucleotide 2311A, and a C-terminal 65 base-pair deletion from exon 23 to intron 23 (predicted to truncate the C-terminus) have been reported (Table 1) [1]. Other than the N-terminal cytosolic mutation R298S, the remaining missense pRTA residues have been localized to various TMs: Ser^{427} to TM 1, Thr^{485}, Gly^{486} to TM 3, Arg^{510} and Leu^{522} to TM 4, Ala^{799} to TM 10, and Arg^{881} to TM 12 [9].

Patients with the homozygous C-terminal 65 base-pair deletion, Δ2311A, R510H, L522P, and R881C also have migraine headaches [19], and it has been postulated that homozygous NBCe1 mutations which are retained in the ER can lead to defective plasma membrane expression of NBCe1-B in brain astrocytes with subsequent abnormal NMDA-mediated neuronal hyperactivity. Interestingly, the 65 base-pair C-terminal deletion and the L522P mutation through a dominant negative effect induce the formation of hetero-oligomers with wild type NBCe1 possibly accounting for symptoms in heterozygous family members [19, 20]. Whether these heterozygous family members also have subtle defects in proximal tubule bicarbonate transport has not been determined.

The transmembrane region of NBCe1

The transmembrane region of NBCe1-A is responsible for mediating ion transport. This region contains 14 TMs of various lengths with a 3D structure that may resemble prokaryotic Na^+-coupled substrate transporters [10, 21]. Other than a large glycosylated extracellular loop 3 (EL3) and a smaller loop 4 (EL4), the extracellular surface of NBCe1-A is compactly folded [10]. The N-terminal transmembrane region has 8 TMs homologous to the SLC4 chloride-bicarbonate exchanger AE1 [22] whereas the C-terminal transmembrane region folds significantly differently from AE1 [10]. The TMs in NBCe1-A that have been extensively characterized will be discussed in detail in the following paragraphs to address the structure-functional perturbations induced by various pRTA mutations.

NBCe1-TM1: Ion permeation and helix packing

The topology of NBCe1-TM1 was originally modeled based on AE1-TM1; however recent studies have demonstrated that they fold differently [11]. NBCe1-A-TM1 contains 31 amino acids (Phe-412–Thr-442) [11], which is longer than a standard TM (20 amino acids)

suggesting that it adopts a tilted position in the lipid bilayer. Three charged residues (Lys^{405}, Asp^{409}, and Asp^{416}) on the same surface of the N-terminus of the TM1 helix potentially form ionic interactions with the cytoplasmic domain whose mutations causes protein misfolding. The N-terminal cytosolic portion of TM1 (Arg^{394} to Pro^{411}) adopts a helical conformation linking the plasma membrane-embedded N-terminal end of TM1 with the cytoplasmic domain. This differs significantly from AE1 which has a flexible linker region thought to play an important role in plasma membrane elasticity [23]. Substitution of Asp^{416}, Gln^{424}, Tyr^{433}, and Asn^{439} with cysteine, misfold NBCe1-A, triggering intracellular retention suggesting that these residues are important for maintaining the TM1 native conformation. Importantly, three residues in TM1 have been shown to line the ion permeation pathway (Ala^{428}, Ala^{435}, and Thr^{442}) and Thr^{442} at the C-terminal end of TM1 is thought to form an external gate for the transported ions [24].

An S427L mutation in TM1 dramatically impairs transporter function causing pRTA [25]. Ser^{427} is located adjacent to Ala^{428}, a residue that lines the substrate translocation pore [24]. Mutation of Ser^{427} to leucine decreases the function to ~10% of wild-type with the inability to reverse the direction of transport at very negative membrane potentials. S427 is located in the middle of the lipid bilayer rather than near the intracellular interface as previously thought [11]. The hydrophobicity of the serine side chain at this position may have an important effect on the local structure involved in helix packing of the transmembrane domain. Indeed, when Ser^{427} was substituted with Val or Ile, both of which are structurally similar to Ser but carry hydrophobic side chains, and with Tyr, which carries a bulky side chain, ion transport is completely blocked. Functional inactivation induced by Pro substitution also indicates that Ser^{427} is involved in TM1 helix packing.

Recent studies have revealed that when S427L is present, the aqueous accessibility to Thr^{442} (C-terminal end of TM1), Ala^{435} (middle of TM1) and Lys^{404} (N-terminal end of TM1) are significantly blocked suggesting that leucine substitution at Ser^{427} triggers an overall conformational change of TM1 [11]. However, when Ser^{427} was substituted with Ala, NBCe1-A remains functional and T442C becomes aqueous-accessible. Therefore, Ser^{427} appears to be located in a space-confined region with its nucleophilic side chain involved in TM1 helix packing. The S427L mutation abolishes the ionic interaction between helices perturbing the orientation of TM1 resulting in a change in the configuration and/or collapse of the NBCe1-A ion permeation pathway.

NBCe1-TM3: Ion interaction site and charge transport stoichiometry

NBCe1-A-TM3 contains a functional important residue, T485, whose mutation to serine causes pRTA [9, 26, 27]. This mutation is of great interest because serine and threonine are structurally and chemically similar, and therefore one would not expect their substitution to cause human disease. Thr^{485} resides in NBCe1-A-TM3 in a space confined region inaccessible to biotin maleimide [9]. In situ probing analysis indicated that Thr^{485} is located in an aqueous accessible position where it undergoes substrate-dependent intra- and extracellular facing conformational changes [27]. These findings are compatible with a transporter model where Thr485 resides in an NBCe1-A ion interaction site. This conclusion is further supported by the observation that the adjacent pRTA causing G486R mutation [9,

28], alters the position of Thr^{485} in the NBCe1-A ion interaction site as a mechanism for impaired function of the transporter [27].

The T485S mutation not only impairs base transport by ~ 50% but also converts NBCe1-A into an electroneutral transporter [27]. The charge transport stoichiometry of heterologously expressed wild-type (wt) NBCe1-A has been investigated several groups and has been determined to be either 1:2 or 1:3 [29]. Cell-specific factors [30], phosphorylation status [31], and Ca^{2+} [32] may play a role in modulating the charge transport stoichiometry. In HEK 293 cells, wt-NBCe1-A has a charge transport stoichiomtery of 1:2 [27]. Experiments using NO_3^- as a surrogate for CO_3^2 transport suggested that wt-NBCe1-A mediates electrogenic Na^+-CO_3^{2-} cotransport (1:2 charge transport stoichiometry) [27]. The T485S mutant fails to transport Na^+-NO_3^- and likely mediates electroneutral Na^+-HCO_3^- transport. Two potential models have been proposed that account for the loss of NBCe1-A electrogenicity depending on whether human wt-NBCe1-A normally functions with a 1:2 versus a 1:3 charge transport stoichiometry (Fig. 3). In the first model, wt-NBCe1-A mediating Na^+-CO_3^{2-} cotransport (1:2 charge transport stoichiometry) loses its electrogenecity because the T485S mutant preferentially transports Na^+-HCO_3^- rather Na^+-CO_3^{2-}. In the second model where wt-NBCe1-A normally mediates of 1 Na^+ + 1 HCO_3^- + 1 CO_3^{2-} cotransport (1:3 charge transport stoichiometry), loss of CO_3^{2-} interaction in the T485S mutant results in electroneutral Na^+-HCO_3^- cotransport [27]. In both models, the electroneutral transporter would be predicted to mediate proximal tubule basolateral Na^+-HCO_3^- influx thereby impairing transepithelial bicarbonate transport.

NBCe1-TM4: Role in protein folding

TM4 carries potential signal anchor and stop transfer sequences and may act as a scaffolding helix that is important for the second stage folding of the transporter [33]. Two pRTA mutations, R510H and L522P [20, 34, 35], induce abnormal protein folding resulting in ER retention. Misfolding of NBCe1-A caused by R510H suggests that not only the magnitude of the positive charge but also the side-chain size carried by Arg^{510} is important for forming an interaction between TMs to maintain proper protein folding. Although the L522P mutation is retained in the ER, L522C [9] and L522I [20] mutants do not impair NBCe1-A plasma membrane processing indicating that it is the proline residue rather than the loss of leucine that causes intracellular retention of the mutant protein. Helix disruption due to the presence of a proline residue would be predicted to significantly misfold the transporter.

NBCe1-TM5: Stilbene inhibition and anion selectivity

An extensive mutatgenesis study identified Asp^{555} in TM5 as a functionally important residue [36]. Substitution of Asp^{555} with glutamic acid induced an outward rectifying Cl^- current, suggesting it may play an important role in HCO_3^- selectivity [37]. Asp^{555} is in close proximity to the proposed 4,4′-diisothiocyanatostilbene-2,2′-disulfonate (DIDS; functional inhibitor) binding site of NBCe1-A (Lys^{559}) [38]. DIDS blocks NBCe1 reversibly from the extracellular surface by binding to the KKMIK motif at the putative extracellular end of TM5. The apparent affinity of the interaction decreases at more negative voltages possibly due to alterations in the conformation of the transporter as the membrane voltage changes [38, 39]. DIDS also can inhibit NBCe1-A function from the intracellular surface at

an unknown site [40]. Whether other inhibitors of NBCe1 including tenidap [41], the *N*-cyanosulphonamide compound S0859 [42], and the amiloride analogue benzamil [41] also interact with residues in TM5 is unknown.

NBCe1-TM6: Putative interaction with "TM12"

Domain swapping experiments revealed that when NBCe1-A TM6 is replaced with corresponding TM6 residues in electroneutral NBCn1, there is little effect on the electrogenicity of the transporter [43]. However, simultaneously replacing TM6 and portions of "TM12" (in actuality residues in TM12, intracellular loop 6 and TM13 were used in the study) significantly impaired membrane processing of the chimera. It was hypothesized that residues in TM6 and "TM12" form a functional unit responsible for protein exit from the ER and membrane processing. Given that mixed chimera proteins may not fold properly [44] it is premature to conclude that TM6 and "TM12" form a functional unit.

NBCe1-TM8: Ion permeation pathway

NBCe1-A-TM8 was predicted to function similarly to AE1-TM8 [45] which had been previously shown to form part of the anion translocation pathway [46]. However, using a similar cysteine scanning mutagenesis approach, only Leu^{750} was identified to be strongly involved in NBCe1-A transport unlike AE1 where several TM8 residues are involved in forming part of the ion translocation pore [45, 46]. Access of cysteine substituted Leu^{750} to p -chloromercuribenzenesulfonic acid (pCMBS) was significantly reduced in the presence of substrate ions or 4.4-dinitro-stilbene-2,2′-disulfonate (DNDS) suggesting that TM8 is involved in forming part of the ion translocation pathway.

NBCe1-TM10: Potential role in muscle weakness associated with pRTA and severe hypokalemia

Deda et al [47] described a patient with severe hypokalemia, acute diarrhea, vomiting, and muscle weakness, in addition to the renal and extrarenal manifestations typically associated with mutations in NBCe1. Horita et al subsequently showed that this patient had a new NBCe1-A-A799V missense mutation [26]. The hypokalemia in this patient was more severe than typically observed in other pRTA patients presumably because of the extrarenal loss of K^+ due to vomiting/diarrhea. Functional studies by Horita et al indicated that the mutant had significantly decreased HCO_3^--dependent conductance [26]. The corresponding NBCe1-B-A843V [19] mutant was also shown to have decreased activity and membrane expression in a rat glioma cell-line was normal. Parker et al have recently shown that the A799V substitution may predispose a patient with hypokalemia to more severe muscle weakness than other pRTA patients with NBCe1 mutations [48]. Interestingly, the mutant transporter had both decreased HCO_3^--dependent slope conductance and an associated HCO_3^--independent cation conductance. Since NBCe1 appears to be expressed in muscle (sarcollema and possibly t-tubules) [49, 50], the mutant NBCe1 cation leak in muscle cells would be predicted to exacerbate muscle weakness during severe hypokalemia.

NBCe1-TM11-14: Helix packing

Extensive cysteine scanning of the C-terminal transmembrane region of NBCe1-A have determined that 5 TMs (10–14) are present between Ala^{800} and Lys^{967} with unique features that contribute to protein stability and helix packing [10]. TM11-14 are longer than standard membrane protein TMs and lack fre aqueous exposed connecting loops. These TMs contain multiple proline and glycine residues suggesting that they are either kinked or twisted in the lipid bilayer. Moreover, replacement of the polar (unlike non-polar) residues in these TMs had profound structural effects, indicating their importance in forming intramolecular hydrogen bonds and the stabilization of the transporter structure. In addition, TM11-14 contain 18 functionally important residues that are clustered on one surface of each of the TMs, revealing their role in helix packing.

Five mutation-sensitive residues are clustered at the beginning of TM11 which likely serves as an internal topogenic signal guiding TM11 to fold back into the lipid bilayer [10]. TM11 and 12 are abruptly bent at Met^{858} that is bracketed by two prolines (857 and 858). A large cryptic cytoplasmic loop connecting TM12 and 13 appears to be tightly folded and interacts with the cytoplasmic domain. Similar to AE1, NBCe1 extracellular loop 7 (EL7) is composed of 4 residues (Thr^{926}-Ala^{929}) and is less exposed on the surface of the transporter. The beginning of EL7 has a positively charged Lys^{924} which mirrors AE1-Lys^{851} [22]. AE1-Lys^{851} has a critical functional role to neutralize the helical diplole for anion interaction, however, NBCe1-A-Lys^{924} appears to act as a counter-ion required for transmembrane helix packing [10, 51].

The pRTA mutation, R881C located in TM12 impairs mutant protein plasma membrane trafficking due to ER retention and does not affect transporter function per se [9, 10, 26, 52]. This may suggest that R881 in TM12 has role in transmembrane helix packing.

Extracellular loops 3 (EL3) and 4 (EL4): Putative role in ligand binding, charge transport stoichiometry, and membrane bound carbonic anhydrase interaction

EL3 consists of 56 amino acids with 2 glycosylated sites and 4 cysteine residues [53]. Structural studies showed that EL3 resides at the NBCe1-dimer interface and that the 4 cysteines on each monomer are intra-disufided forming a unique topological domain on the extracellular surface of NBCe1-A [53]. The highly ordered structure of EL3 may be required for potential ligand interaction and functional regulation of the transporter. In domain swapping experiments with the electroneutral transporter NBCn1, Chen et al showed that EL4 is involved in the charge transport stoichiometry [54]. NBCe1-EL4 is distinguished by a large number of proline residues that likely contribute to specific folding properties wherein the loop may interact with membrane embedded residues to modulate substrate interaction with the ion permeation pathway. EL4 is also reported to bind plasma membrane CAIV [55] and CAIX [56]. Whether EL4 interacts with residues in TM8 to modulate the transporter charge transport stoichiometry is unknown.

The cytoplasmic regions of NBCe1-A

The N-terminal region of NBCe1: Location of the autostimulatory domain, autoinhibitory domain, "HCO_3^- tunnel", and role in plasma membrane expression

The importance of the N-terminal region of NBCe1-A is highlighted by studies of heterologously expressed NBCe1 variants in oocytes demonstrating that its unique N-terminus stimulates transporter activity (autostimulatory domain (ASD)) [8]. The mechanism involved is currently unknown but likely involves interaction(s) between the specific N-terminus and the transport pathway and/or binding to specific cytosolic factors (see below). The unique N- terminus of the -B and -C variants inhibits NBCe1 transport and is named an autoinhibitory domain (AID) [8, 57].

The functional importance of the N-terminal cytoplasmic region is documented by the R298S pRTA mutation [15, 26, 58]. Based on homology modeling with the crystallized cytoplasmic domain structure of AE1 and the results of biotin maleimide labeling experiments [9, 58], Arg^{298} may reside in a tightly folded aqueous inaccessible region and participate in forming a "HCO_3^- tunnel" whose structure is disrupted by the R298S pRTA mutation. Recent studies have provided evidence that the N-terminal cytoplasmic region interacts with the transmembrane region [11]. Accordingly, the R298S mutation may perturb this interaction preventing efficient delivery of HCO_3^- to the ion permeation pathway in the transmembrane region.

The N-terminal cytoplasmic region also plays a role in transporter plasma membrane expression. Specifically, NBCe1-B (amino acids 96–440) was recently reported to interact with the Hsp70-like stress 70 protein chaperone STCH, significantly increasing the transporter plasma membrane expression [59]. It was proposed that under acidic stress, this mechanism would enhance the ability of cells expressing NBCe1-B to recover from intracellular acidification. Whether the plasma membrane expression of NBCe1-A, -C, -D, and -E is also increased by STCH is unknown.

The C-terminal tail of NBCe1-A: Plasma membrane expression

Toplogical studies have shown that Asp^{960} marks the intracellular lipid/aqueous interface of TM14, and Pro^{963} may form a kink at the C-terminal end of the TM exposing the C-terminal cytoplasmic tail [10]. Truncation at Ser^{982} in the C-terminal cytoplasmic tail completely retained the mutant protein intracellularly providing evidence that the C-terminus plays a role in membrane processing [19]. A ^{1010}QQPFLS^{1015} motif in the C-terminal cytoplasmic tail was identified as a basolateral targeting sequence in MDCK cells [60].

Functional regulation of NBCe1

The function of NBCe1 variants has been reported to be regulated by protein kinase A (PKA)/cAMP [30, 61], protein kinase C (PKC) [62], Mg^{2+} [63, 64], Ca^{2+} [32], ATP [40], carbonic anhydrases I–III [65], IRBIT [66], and PIP_2 [5]. Several studies have begun to elucidate the differences among NBCe1 variants in their regulatory mechanisms that offer potential targets in the future for clinically modulating their transport function pharmacologically.

PIP_2 Phosphatidylinositol 4,5-bisphosphate (PIP_2)

PIP_2 is noteworthy because in addition to its role as a precursor of the Ca^{2+}-mobilizing inositol triphosphate (IP_3) and the kinase-activating diacylglycerol (DAG), PIP_2 can regulate ion transport [67]. PIP_2 directly stimulates the activity of NBCe1-A and indirectly stimulates the NBCe1-B and -C via an increase in IP_3/Ca^{2+} involving a staurosporine-sensitive kinase [5, 68].

IRBIT

IRBIT competes with IP_3 for binding to IP_3 receptors [69] and was subsequently discovered to also interact with and activate NBCe1-B [70]. IRBIT activates NBCe1-B via recruitment of protein phosphatase 1 (PP1), which dephosphorylates the transporter [71] thereby blocking the inhibition by the WNK/SPAK pathway and preventing the inhibition by AID [57]. Conversely, recruitment of SPAK by the WNK kinases stabilizes the autoinhibition by AID. IRBIT and PIP_2 reverse the autoinhibition by AID, and interact at the same AID site [66]. A positively charged region in the N-terminus of NBCe1-B (amino acids 37–65) that is conserved in other members of the SLC4 family (except NBCe1-A) mediates the interaction/activation of NBCe1-B by IRBIT and PIP_2 [66].

Mg^{2+}

A phenomenon of NBCe1-A-rundown occurs when the transporter is expressed in oocytes, that is largely inhibited by removal of Mg^{2+} whereas an increase in Mg^{2+} inhibits NBCe1-A [68]. Mg^{2+}-dependent phosphatase (5′-lipid phosphatase) activity that dephosphorylates PIP_2 to PIP is possibly involved. Intracellular Mg^{2+} also inhibits NBCe1-B however the mechanism differs from NBCe1-A [63, 64]. IRBIT lowers the inhibitory effect of Mg^{2+} on NBCe1-B function perhaps by competing with Mg^{2+} for binding NBCe1-B (and possibly NBCe1-C/D). It has been postulated that inhibition of NBCe1-mediated transport via an increase in cytosolic Mg^{2+} during ischemia may reduce post-ischemic dysfunction in kidney (NBCe1-A) and heart (NBCe1-B).

ATP, PKA, PKC, and CaMKII

In oocytes expressing NBCe1-A, application of ATP to the intracellular surface of membrane patches increases the cotransporter current [40]. The non-hydrolysable ATP analogue AMP-PNP does not mimic the effect of ATP and it has been hypothesized that ATP may phosphorylate NBCe1-A by means of an unidentified kinase.

In proximal and distal tubule cells lines, the charge transport stoichiometry of both NBCe1-A and NBCe1-B is cell-type dependent suggesting that unknown cytosolic factors may interact with NBCe1-A (and NBCe1-B) and modulate the stoichiometry [30, 31]. The shift in NBCe1-A charge transport stoichiometry from 1:3 to 1:2 is mediated by protein kinase A–dependent phosphorylation of Ser^{982} [31]. It has also been reported that Thr^{49} in the cytoplasmic N-terminus of NBCe1-B (and potentially NBCe1-C/E) plays a role in cAMP-mediated increase in transporter current [61].

In intestinal cells, cAMP increases NBCe1-B transport in part via a change in plasma membrane expression [72]. Inhibition of NBCe1-A function via ANG II is mediated in part

by Ca^{2+}-insensitive PKCε that increases its association with NBCe1-A in the plasma membrane [62]. NBCe1-A and NBCe1-B participate in constitutive and stimulated (carbachol) endocytosis regulated by conventional PKCs (PKCαβγ) and by a novel PKCδ [73]. CaMKII appears to play a role in the recycling of NBCe1-A to the plasma membrane [74].

(5) CAII

CAII was found to interact with the cytosolic C-terminus of NBCe1 at a $D^{986}NDD^{989}$ motif and based on studies with AE1 [75, 76], was proposed to form a transport metabolon with NBCe1 that transfers ions intra-molecularly between the two proteins [77, 78]. NBCe1-EL4 was subsequently also shown to bind plasma membrane CAIV [55] and CAIX [56]. Other groups however have been unable to find evidence in favor of any interaction with CAII [79, 80]. The data in these studies are complicated by differences in techniques employed, the sensitivity/specificity of the measurements utilized, and artifacts introduced into the assays. Interestingly, patients and mice with loss of CAII do not have as severe pRTA as might be predicted; nor is the acidemia as severe as in patients with NBCe1 mutations or mice with loss of NBCe1 [81, 82]. Interestingly, neither metabolic acidosis nor a more subtle abnormality in renal bicarbonate handling have been reported in patients and mice with loss of CA IV function [83, 84]. Schueler recently re-examined the question and showed that intracellular isoforms, CAI and CAIII, enhance NBCe1-A transport activity, similar to that of intracellular CAII in Xenopus oocytes [65]. The enhancement of NBCe1-was attributed to the catalytic activity of the different CA isoforms and did not appear to require the intramolecular proton shuttle of CAII.

Insights into the structural differences between NBCe1 and AE1

Members of the SLC4 family have been assumed to adopt a membrane topology similar to AE1 because of their high protein sequence homology [2]. However, recent studies indicate that NBCe1 has several unique topological features that differ from AE1 [51] in keeping with the known differences in the atomic structure of prokaryotic Na^+-coupled substrate transporters and ion exchangers [85]. NBCe1-A has features closely resembling prokaryotic Na^+-coupled substrate transporters (LeuT and vSGLT) [24, 86, 87]. Data from cryoEM and mutagenesis studies indicate that the AE1 fold more closely resembles prokaryotic ClC channels [88, 89]. Several differences between NBCe1-A and AE1 have been elucidated: 1) NBCe1-A TM1 forms part of the ion translocation pathway and tightly interacts with the cytoplasmic domain whereas AE1-TM1 is not involved [24]; 2) NBCe1-A EL3 is intra-disulfided and has been postulated to have a role in ligand binding [53]; 3) NBCe1-A has a tightly folded C-terminal transmembrane region unlike AE1 that has 2 re-entrant loops [10, 22]; 4) AE1-TM13 and 14 are involved in ion translocation unlike NBCe1-A [51].

Conclusion

We currently have a working structural model of NBCe1 that has laid the foundation for understanding its functional properties and the mechanisms of the disease causing mutations. Despite these advances, clinicians lack specific pharmacologic approaches to modulate the function of NBCe1 or other SLC4 transporters in metabolic acid-base

disorders and in cancer where abnormal cellular acid-base metabolism occurs [90]. It is anticipated that solving the atomic structure of specific SLC4 proteins will accelerate the development of new drugs for the treatment of diseases involving these transporters.

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Key Points

- NBCe1 mediates electrogenic Na^+-base transport in renal and extrarenal organs with a charge transport stoichiometry of 1:2 or 1:3.
- In the 1:2 charge transport stoichiometry mode, NBCe1-A appears to transport CO_3^{2-} rather than HCO_3^-.
- Various factors regulate the function of NBCe1 providing potential clinical pharmacologic targets in the future.
- Mutations in NBCe1 cause autosomal recessive pRTA in children with specific systemic manifestations that define the syndrome diagnostically.
- NBCe1 mutations can affect the transporter electrogenicity, protein structure, substrate ion interaction, and plasma membrane processing.

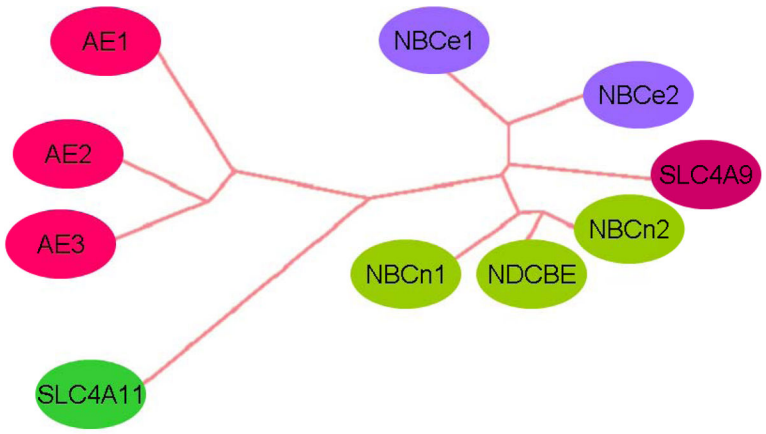

Figure 1.

Dendrogram of SLC4 transporters: Depicted are the protein names of each transporter in the SLC4 family except for SLC4A9 (“AE4”) and SLC4A11 (“BTR or NaBC1”) whose function is currently unclear and therefore the gene name is depicted. SLC4 proteins with similar function tend to more homologous at the amino acid level i.e. function follows structure. AE1-3 are Na^+-independent Cl^--HCO_3^- exchangers; NBCe1 and NBCe2 are electrogenic Na^+-CO_3^{2-}(HCO_3^-) transporters; NDCBE is Na^+-driven Cl^--HCO_3^- exchanger; NBCn1 and NBCn2 are electroneutral Na^+-HCO_3^- transporters. depending on experimental conditions NBCn2 can also mediate Na^+-dependent Cl^--HCO_3^-.

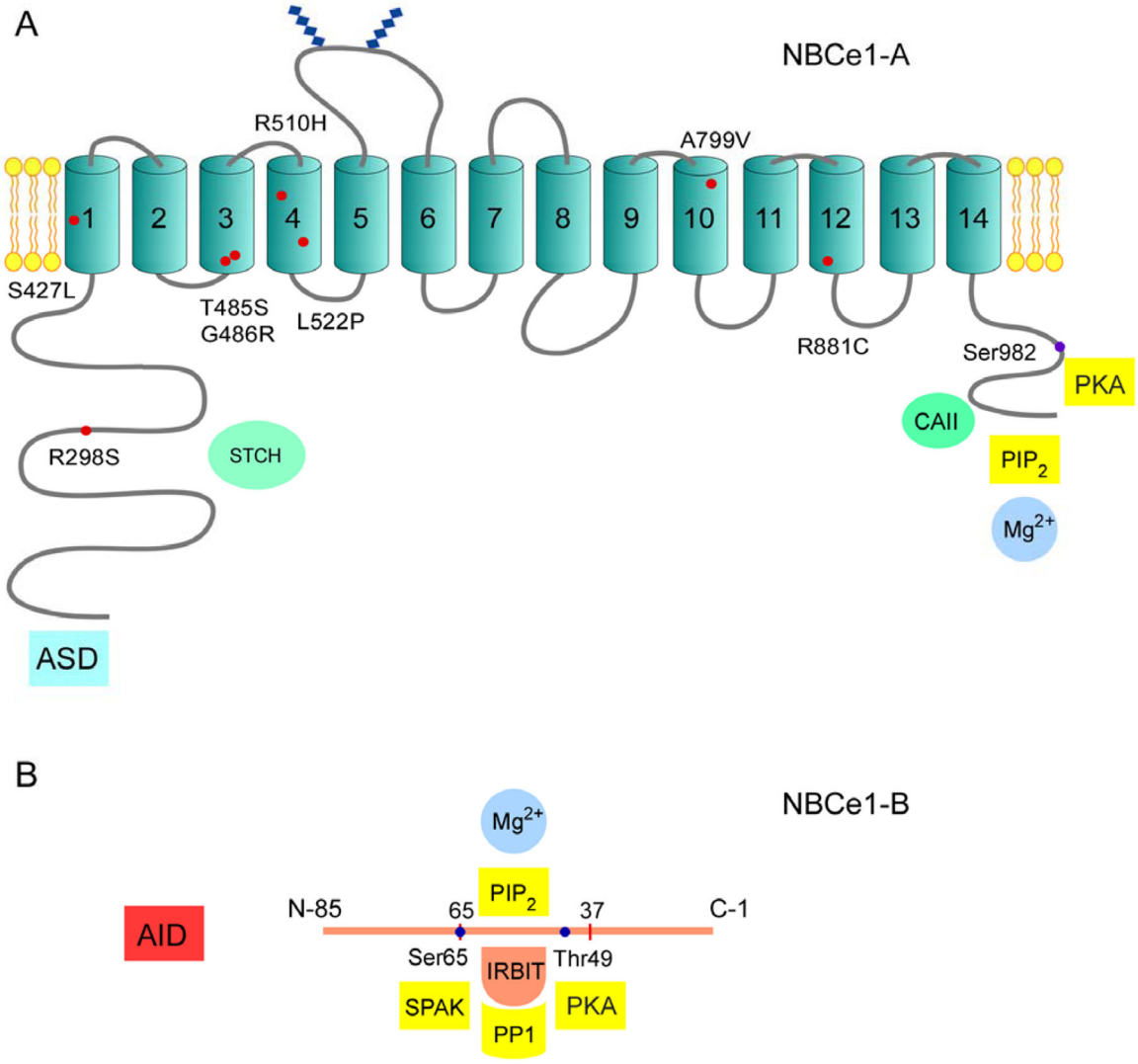

Figure 2.

Topologic structure of NBCe1 (NBCe1-A depicted): NBCe1 transporters have a large N-terminal cytoplasmic region, a transmembrane region, and a C-terminal cytoplasmic tail. The topologic properties of NBCe1-A have been most thoroughly studied and because they share the identical transmembrane region, the structural features depicted also encompass the NBCe1-B/C/D/E variants. The majority of missense mutations (numbering depicted based on NBCe1-A) causing pRTA reside in the transmembrane region. NBCe1-A has an N-terminal cytoplasmic autostimulatory domain (ASD) and putatively interacts with carbonic anhydrase II in its C-terminal cytoplasmic region. NBCe1-A is hypothesized to be stimulated by PIP2 via interaction with positively charged residues in the C-terminus. Mg^{2+} is hypothesized to modulate NBCe1-A function via a Mg^{2+}-dependent phosphatase (5′-lipid phosphatase) that dephosphorylates PIP2. The unique N-terminus of NBCe1-B unlike NBCe1-A has an autoinhibitory domain (AID) that is regulates the function of the transporter by interacting with IRBIT and potentially PIP2 and Mg^{2+} (see text for details).

Kinase phosphorylation sites are also depicted. STCH interacts with residues that are common to all NBCe1 variants.

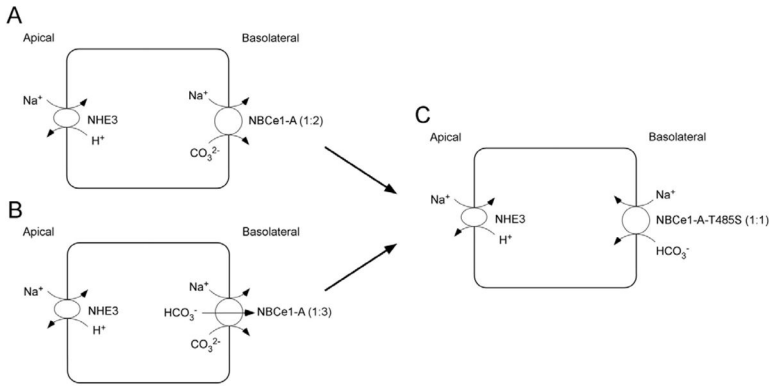

Figure 3.

Proximal tubule cell models of wt-NBCe1-A and mutant NBCe1-A-T485S transport. wt-NBCe1-A is depicted as either mediating electrogenic Na^+-CO_3^{2-} transport (1:2 charge transport stoichiometry) or Na^+-CO_3^{2-}-HCO_3^- transport (1:3 charge transport stoichiometry). Electroneutral NBCe1-A-T485S is predicted to transport Na^+-HCO_3^- into proximal tubule cells causing pRTA. wt-NBCe1-A mediating Na^+-CO_3^{2-} cotransport (1:2 charge transport stoichiometry) is modeled to lose its electrogenecity because the T485S mutant preferentially transports Na^+-HCO_3^- rather Na^+-CO_3^{2-}. wt-NBCe1-A mediating 1 Na+ + 1 HCO_3^- + 1 CO_3^{2-} cotransport (1:3 charge transport stoichiometry), is converted into an electroneutral Na^+-HCO_3^- transporter as a result of loss of CO_3^{2-} interaction in the T485S mutant.

Table 1

NBCe1 Mutations† and Molecular Mechanisms

Mutation†	Location	Effect of Mutation
Q29X*	cytoplasmic N-terminus	Protein truncation
R298S	cytoplasm N-terminus	• Abnormal protein folding • Protein intracellular retention
S427L	TM1	• Abnormal helix packing • Decreased G_{HCO3} • Impaired I_{HCO3}reversal at − Vm
T485S	TM3	• Altered ion interaction site • Loss of Electrogenicity
G486R	TM3	Ion interaction site
R510H	TM4	Protein intracellular retention
W516X	TM4	Protein truncation
L522P	TM4	Protein intracellular retention
2311delA	IL4	Protein truncation
A799V	TM10	• Protein intracellular retention • Decreased G_{HCO3} • Bicarbonate-independent G_{cation}
R881C	TM12	Protein intracellular retention
65bp-del	cytoplasmic C-terminus	Protein intracellular retention

†NBCe1-A numbering

*NBCe1-A only

G_{HCO3} (Bicarbonate conductance)

G_{cation} (Cation conductance)

I_{HCO3} (Bicarbonate-dependent current)

−Vm (Negative plasma membrane voltages)

TM (Transmembrane); IL (Intracellular

It should be noted that there are no plasma membrane expression studies of mutant NBCe1-A transporters in proximal tubule cells. In addition, abnormal plasma membrane expression in certain mutations might be NBCe1-variant and therefore cell-type dependent.