SLC4 base (HCO$_3^-$, CO$_3^{2-}$) transporters: classification, function, structure, genetic diseases, and knockout models

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MEMBRANE PROTEINS THAT MEDIATE base (HCO$_3^-$, CO$_3^{2-}$) transport play an important role in eukaryotes in maintaining both intracellular pH (pHi) and extracellular pH (pHo) within narrow limits. This is not surprising given that the HCO$_3^-$/CO$_3^{2-}$/CO$_2$ buffer system is arguably the most important buffer in eukaryotic organisms. In mammals, two unrelated multigene families, SLC4 and SLC26 transporters, have evolved, whose members transport base in both a cell type- and cell membrane-specific fashion. This review focuses specifically on the SLC4 transporter family. The reader is referred to recent reviews on SLC26 transporters for an update on the role these transporters play in health and disease states (46, 131). Following an initial overview of the classification of the SLC4 family of transport proteins, we highlight recent advances in our understanding of the structure and function of these transporters and the role of protein interactions in the regulation of their functional properties. Abnormalities in either the function and/or membrane targeting of certain members of the SLC4 family are the cause of various genetic diseases in humans. Mouse knockout models have also provided a useful adjunct with which to study the role of SLC4 family members in specific organ systems. Advances in our understanding of the role of SLC4 transporters in health and disease are also the focus of this review.

CLASSIFICATION OF SLC4 TRANSPORTERS

Based on the nature of the specific transport process involved, e.g., exchange, cotransport, and the specific ions that are transported, SLC4 family members can be conveniently separated into three functional groups with potential transport modes for HCO$_3^-$ and/or CO$_3^{2-}$ (Fig. 1): 1) Na$^+$-independent Cl$^-$/HCO$_3^-$ exchangers mediating electroneutral exchange of Cl$^-$ for base (HCO$_3^-$, CO$_3^{2-}$); 2) Na$^+$-HCO$_3^-$ cotransporters mediating cotransport of Na$^+$ and base (HCO$_3^-$, CO$_3^{2-}$); and 3) Na$^+$-driven Cl$^-$/HCO$_3^-$ exchangers mediating the electroneutral exchange of Cl$^-$ for Na$^+$ and base (HCO$_3^-$, CO$_3^{2-}$). SLC4 proteins have in common the property of transporting base (HCO$_3^-$, CO$_3^{2-}$), but differ in their ability to mediate the concomitant transport of Na$^+$ and/or Cl$^-$. In addition, the cotransport of Na$^+$ by most SLC4 transporters distinguishes this family from SLC26 proteins, which predominantly mediate Na$^+$-independent anion transport. The sequences and classification of human SLC4 transporters are shown in Fig. 2 and Table 1.

Na$^+$-Independent Cl$^-$/HCO$_3^-$ Exchangers

SLC4A1 (AE1). AE1 mediates Na$^+$-independent anion exchange in red blood cells and the renal collecting duct. Two variants of AE1 have been well characterized (6, 25, 33, 107, 123, 196). Red cell AE1 (eAE1) or band 3 protein (human eAE1, 911 aa) plays an important role in transporting HCO$_3^-$ to the lung that was derived from metabolically produced CO$_2$. In the kidney, AE1 (kAE1) is transcribed from an alternate

At pH$_0$ of 7.4, the HCO$_3^-$/CO$_3^{2-}$ is $-500:1$, whereas at a typical pH$_i$ of 7.1, the HCO$_3^-$/CO$_3^{2-}$ is twice this value, or $-1,000:1$. It is currently unclear as to whether HCO$_3^-$ or CO$_3^{2-}$ (or both) is transported in various SLC4 transporters. The various potential transport modes are depicted in Fig. 1. At the present time, the term “bicarbonate (HCO$_3^-$)” that is used in naming or describing the function (see Table 1) of SLC4 transporters should not be viewed as a definitive description of the type of base transported, but rather as a term that generically refers to HCO$_3^-$ and/or CO$_3^{2-}$ until definitive studies are reported characterizing the specific species transported.
SLC4 BASE (HCO₃⁻, CO₃²⁻) TRANSPORTERS

**Invited Review**

**Fig. 1. SLC4 transporters can be divided functionally into 3 groups: Na⁺-independent Cl⁻/HCO₃⁻ exchangers (A), Na⁺-HCO₃⁻ cotransporters (B), and Na⁺-driven Cl⁻/HCO₃⁻ exchangers (C).** Potential transport modes for HCO₃⁻ are depicted. In A, Na⁺-independent Cl⁻/HCO₃⁻ exchangers (A) transport stoichiometry of 1:1, and electrogenic Na⁺-HCO₃⁻ cotransporters (B) have transport stoichiometry of either 2:1 (i) or 3:1 (ii) shown. It has not been definitively established whether SLC4 proteins mediate HCO₃⁻ and/or CO₃²⁻ transport. Depicted are the hypothetical modes of transport, wherein the coupling ratio of base (HCO₃⁻ and/or CO₃²⁻) to Na⁺ and/or Cl⁻ is equivalent. Note that these transport modes are not equivalent with regard to the total flux of carbon, hydrogen, and oxygen per transport cycle.

promoter, and the resultant protein has an NH₂-terminal truncation (first 65 amino acids in human AE1) (25). Kidney-specific AE1 is expressed on the basolateral membrane of collecting duct α-intercalated cells (7, 205), where it plays an important role in bicarbonate reabsorption and urinary acidification.

**SLC4A2 (AE2).** AE2 mediates Na⁺-independent anion exchange and is more widely expressed than AE1. Five NH₂-terminal splice variants of AE2 (a, b1, b2, c1, and c2) have been described in mammals and are expressed in a tissue- and cell-specific manner (5, 6, 8–10, 13, 40, 110, 129, 168, 217). Human AE2 is ~300 aa larger than eAE1 and, like AE1, is glycosylated (6, 233). Deglycosylation of AE2 does not appreciably affect its transport function (53). AE2 is widely expressed in nonexcitable tissues, where it has been proposed to be a housekeeping Cl⁻/HCO₃⁻. It is also highly expressed on the basolateral membrane of choroid-plexus epithelial cells (9) and gastric parietal cells (191). In hepatocytes, AE2a, AE2b1, and AE2b2 have been localized to the apical membrane (13).

In the kidney, AE2 is expressed in the basolateral membrane of the thick ascending limb (TAL) (6, 10). Ion transport mediated by AE2 expressed in Xenopus laevis oocytes (77, 188, 229) and mammalian cells (91, 115) is pH sensitive. Residues in the cytoplasmic NH₂-terminus of mouse AE2, aa 336–347, 357–362, and 391–510, have been shown to regulate the pH dependence of the transporter (188–190, 229).

**SLC4A3 (AE3).** AE3 mediates Na⁺-independent anion exchange like AE1 and AE2. Two NH₂-terminal splice variants of AE3, cardiac (cAE3) and brain (bAE3), have been described (108, 118). The size of bAE3 (1,232 aa in humans) is comparable to AE2, whereas cAE3 is ~200 aa shorter. Transcripts of both variants were found in different organs and tissues including the brain and heart (106, 108, 110, 118, 225). bAE3 protein has also been detected in basolateral membrane vesicles isolated from human ileal and colonic epithelial cells (11). AE3 is also expressed in the basal end foot processes of retinal Müller cells (106). AE3 expressed in X. laevis oocytes and HEK293 cells mediates significantly less Cl⁻/HCO₃⁻ exchange than does AE1 or AE2 (53, 77, 115, 165, 225). Unlike AE2, when expressed in HEK cells, AE3 does not appear to be glycosylated (53). The decreased rate of AE3-mediated anion exchange in HEK cells has been attributed to a low level of plasma membrane targeting. cAE3 transport is stimulated at an alkaline pH similar to AE2 (188).

**Sodium Bicarbonate Cotransporters**

Sodium bicarbonate cotransporters mediate cotransport of Na⁺ and base (HCO₃⁻, CO₃²⁻).² Depending on the flux of the net charge per transport cycle, these transporters are characterized as electroneutral (i.e., transported negative charge [HCO₃⁻ and/or CO₃²⁻]) equals transported positive charge (Na⁺), or electrogenic (i.e., transported negative charge [HCO₃⁻ and/or CO₃²⁻]) exceeds transported positive charge (Na⁺).

**SLC4A4 (NBC1, NBCe1).** NBC1 is an electronegodic sodium bicarbonate cotransporter that was first cloned from Ambystoma tigrinum (166) and human (30) kidney. In humans, two major NBC1 variants, kNBC1 (NBCe1-A) (1,079 aa) and pNBC1 (NBCe1-B) (1,079 aa), that differ in their extreme NH₂-terminus are differentially expressed in a cell-specific manner (2) and are derived from alternate promoter usage in the SLC4A4 gene (4). A third NBC1 variant, rb2NBC (NBCe1c), has been cloned from rat brain and is identical to rat pNBC1 except for 61 COOH-terminal residues and a COOH-terminal PDZ motif (18). Recently, a similar splice variant of the SLC4A4 gene has been detected in human and porcine vas...
Fig. 2. Alignment of human SLC4 transporters: SLC4A1 (NP_000333), SLC4A2 (NP_003031), SLC4A3 (NP_005061), SLC4A4 (NP_003750), SLC4A5 (NP_597812), SLC4A7 (NP_003606), SLC4A8 (NP_004849), SLC4A9 (NP_113655), and SLC4A10 (NP_071341). The alignment was performed using GeneWorks 2.5.1 software (Oxford Molecular Group, Campbell, CA). Putative transmembrane segments are shown in red (SLC4A1) and blue (SLC4A4) based on topological models (198, 232). Amino acids shown to be important for SLC4A1- and SLC4A4-mediated transport (1, 35, 87, 97, 132, 133, 195, 231) are shown in bold font. Amino acids important for plasma membrane expression of SLC4A1 (54, 231, 232) and SLC4A4 (1, 75, 116) are marked with brown stars. Lysine residues shown to covalently bind DIDS and H2DIDS in SLC4A1 (93, 140) or homologous lysine residues in SLC4A4 predicted to covalently bind DIDS/H2DIDS are marked with ❖. Note that AE2 (SLC4A2), which has been shown to be inhibitable by DIDS (6), has methionine (M1181) in a second putative DIDS binding site instead of lysine, whereas NBC3 (SLC4A7), which has been shown to be insensitive to DIDS (155), has lysines in both putative DIDS binding sites. Glycosylation sites for SLC4A1 (33) and SLC4A4 (38) are depicted with a branched tree symbol. SLC4A1, SLC4A2, and SLC4A4 amino acids that have been shown to play critical roles in binding of carbonic anhydrase II (CAII) (68, 154, 163, 206–208) are shown in magenta. CAII binding sites are depicted with a magenta line. The COOH-terminal PKA phosphorylation site in NBC1 is shown in green (arrowhead). Note that the SLC4A11 transporter NaBC1 is not included in the alignment despite its distant homology to the other members of the SLC4 family, because recent studies have shown that it functions as a Na+/H+-dependent borate transporter (142, 143, 145, 194).
defers epithelial cells (148). NBC1 proteins have been shown to be glycosylated (38). kNBC1 is highly expressed on the basolateral membrane of the renal proximal tubule where it mediates sodium bicarbonate efflux (21, 127, 178, 223). In humans, the pNBC1 transcript is highly expressed in the pancreas and to a lesser extent in various other tissues (2).

Unlike the kidney, wherein all studies agree on the localization of NBC1, the expression pattern of pNBC1 in the pancreas is complex. NBC1 was localized with antibodies common to kNBC1 and pNBC1 to the basolateral membrane of large ducts in the human pancreas (125). In the rat pancreas, NBC1 was localized to the basolateral membrane of acinar cells, the apical and basolateral membranes of centroacinar cells, intra- and extrabulbar ducts, and main pancreatic duct cells (199). Localization of the pNBC1 variant to the basolateral membrane of pancreatic duct cells was further confirmed with a pNBC1-specific antibody (21). Satoh and coauthors (177), using kNBC1- and pNBC1-specific antibodies, reported that in human pancreatic ducts pNBC1 was expressed basolaterally and acinar cells were unstained, whereas in the rat pancreas acinar cells were stained basolaterally, and ductal cells expressed pNBC1 both apically and basolaterally. In addition, kNBC1 was localized apically in some ducts. Recently, in a similar study, Roussa and coauthors (169) found that pNBC1 was localized to the basolateral membrane of rat ductal and acinar cells and that pNBC1 and kNBC1 were localized together to the apical membrane of certain ductal cells. Moreover, these authors reported that in rat kidney pNBC1, in addition to kNBC1, was also localized to the basolateral membrane of S1 and S2 segments of the proximal tubule. It is clear that additional studies are needed to determine the reason for the varying results among different studies.

The expression pattern of pNBC1 has also been characterized in corneal endothelium, duodenum, epididymis, vas deferens, and parotid and submandibular glands, where it is thought to mediate basolateral sodium bicarbonate influx (21, 48, 89, 150, 170, 192, 193, 203). Species differences may determine differences shown for relative distribution of kNBC1 and pNBC1. For example, pNBC1 is expressed in various rat eye tissues including cornea, conjunctiva, lens, ciliary body, and retina, whereas the expression of kNBC1 is restricted to the conjunctiva (21). In the human eye, kNBC1 and pNBC1 appear to be more evenly distributed (202).

**Table 1. Characteristics of SLC4 transporters**

<table>
<thead>
<tr>
<th>Human Gene</th>
<th>Protein Name</th>
<th>Function</th>
<th>Stoichiometry</th>
<th>Electrogenericity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC4A1</td>
<td>AE1, band 3</td>
<td>Cl⁻/HCO₃⁻ exchange</td>
<td>1:1</td>
<td>Electroneutral</td>
</tr>
<tr>
<td>SLC4A2</td>
<td>AE2</td>
<td>Cl⁻/HCO₃⁻ exchange</td>
<td>1:1</td>
<td>Electroneutral</td>
</tr>
<tr>
<td>SLC4A3</td>
<td>AE3</td>
<td>Cl⁻/HCO₃⁻ exchange</td>
<td>1:1</td>
<td>Electroneutral</td>
</tr>
<tr>
<td>SLC4A4</td>
<td>NBC1, NBCe1</td>
<td>Na⁺-HCO₃⁻ cotransport</td>
<td>2:1:3:1</td>
<td>Electrogenic</td>
</tr>
<tr>
<td>SLC4A5</td>
<td>NBCe2*</td>
<td>Na⁺-HCO₃⁻ cotransport</td>
<td>2:1:3:1</td>
<td>Electrogenic</td>
</tr>
<tr>
<td>SLC4A7</td>
<td>NBC3, NBCn1</td>
<td>Na⁺-HCO₃⁻ cotransport</td>
<td>1:1</td>
<td>Electroneutral</td>
</tr>
<tr>
<td>SLC4A8</td>
<td>NBCe2†</td>
<td>Na⁺-driven Cl⁻/HCO₃⁻ exchange</td>
<td>2:1</td>
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<tr>
<td>SLC4A9</td>
<td>AE4†</td>
<td>Cl⁻/HCO₃⁻ exchange</td>
<td>1:1</td>
<td>Electroneutral</td>
</tr>
<tr>
<td>SLC4A10</td>
<td>NBCe†</td>
<td>Na⁺-driven Cl⁻/HCO₃⁻ exchange</td>
<td>1:1</td>
<td>Electroneutral</td>
</tr>
</tbody>
</table>

Names used in the literature for SLC4 transporters are as follows: SLC4A1: eAE1, (band 3), kAE1; SLC4A2: AE2a,b1,b2,c1,c2; SLC4A3: bAE3, cAE3; SLC4A4: kNBC1 (NBC1u, NBC1e1-A); pNBC1 (NBC1e1-B, NBC1b, dNBC1, hNBC1, rb1NBC1, rb2NBC1; SLC4A5: NBC4a, NBC4b, NBC4c (NBCe2-C), NBC4d, NBC4e, NBC4f; SLC4A7: NBC3 (NBCn1-A), NBCn1-B, NBCn1-C, NBCn1-D, NBCn1-E; SLC4A8: NCBE, kNBC3; SLC4A9: AE4a, AE4b; SLC4A10: rbNCBE (NCBE-B), rbNCBE, NCBE-A. *The NBC4c splice variant functions in mammalian cells as an electrogenic sodium bicarbonate cotransporter (176). The NBC4e splice variant has been reported to function in Xenopus laevis as an electrogenic sodium bicarbonate cotransporter (222). †The functional properties of these transporters is controversial (see text for details and references). The stoichiometry for SLC4A8 and SLC4A5 transporters reflects the HCO₃⁻/H₂CO₃ coupling ratio. For SLC4A8 and SLC4A10 transporters, the 2:1:1 stoichiometry reflects the HCO₃⁻/Na⁺/Cl⁻ coupling ratio.
calated cells (114, 161). NBCn1 was detected in intercalated cells in the medulla and, in addition, at the basolateral membrane of the rat medullary TAL and inner medullary collecting duct cells (151, 211). NBC3 was also highly expressed on the apical membrane of narrow cells in caput epididymis and light (clear) cells in corpus and cauda epididymis (158). The cotransporter apparently plays a role in pH regulation in the outer medullary and inner medullary collecting ducts (113, 151, 227). In addition, the cotransporter plays a role in basolateral bicarbonate transport in the medullary TAL (23, 82, 139), proximal duodenal villus cells (150), the basolateral domain of choroid plexus epithelial cells (152), and submucosal glands (58, 122).

Cooper and colleagues (42) recently reported a deletion variant (NBCn1-E) in rat hippocampal neurons that lacks 123 amino acids in the cytoplasmic NH2-terminal domain. The cotransporter may play an important role in regulating hippocampal neuronal activity.

**Na⁺-Driven Cl⁻/HCO₃⁻ Exchangers**

**SLC4A8 (NDCBE).** The first Na⁺-driven Cl⁻/HCO₃⁻ exchanger (NDCBE) was cloned by Romero and coauthors (167) from Drosophila and named Na⁺-driven anion exchanger 1 (NDAE1). Its transport was blocked by stilbenesulfonates and might not be strictly electroneutral. In addition, NDAE1 was able to use OH⁻ instead of bicarbonate. NDCBE (1,044 aa) cloned from the human brain by Grichtchenko and colleagues (60) expressed in X. laevis oocytes functions as a Na⁺-driven Cl⁻/HCO₃⁻ exchanger, has a strict requirement for HCO₃⁻, and is DIDS sensitive. Although CO₂/HCO₃⁻ stimulates NDAE1-induced pHı changes, it is not clear whether NDAE1 mediates HCO₃⁻ transport per se because CO₂/HCO₃⁻ could simply dissipate OH⁻ in the adjacent unstirred layer. NDAE1 expressed in X. laevis oocytes also differs from human NDCBE in that it is associated with a Cl⁻ current, as well as an inward current caused by CO₂/HCO₃⁻. NDCBE transcripts are present in the brain, testis, kidney, and ovary (60).

A splice variant of NDCBE (originally named kNBC-3) was cloned from mouse inner medullary collecting duct (mIMCD-3) cells and expressed in X. laevis oocytes mediated electroneutral sodium bicarbonate cotransport (212). The chloride dependence was not studied, and therefore its functional properties remain unclear. More recently, Virkki and coauthors (209) reported the cloning of a Na⁺-driven Cl⁻/HCO₃⁻ exchanger of ~1,200 aa from squid giant fiber lobe (sqNDCBE). Unlike the transport process described in native squid axon (22), reversal of the Na⁺ gradient alters the direction sqNDCBE transport, and Li⁺ can support pHı recovery from an acid load nearly as well as Na⁺.

**SLC4 Transporters Whose Function is Incompletely Characterized**

**SLC4A9 (AE4).** AE4 was originally cloned (AE4a, 955 aa, and AE4b, 939 aa) from rabbit β-intercalated cells (202) and was localized to the apical membrane of renal collecting duct β-intercalated cell. Rabbit AE4a expressed in X. laevis oocytes and COS cells mediated Na⁺-independent, DIDS-insensitive Cl⁻/HCO₃⁻ exchange. In a separate study, AE4 was localized to apical and lateral membranes of collecting duct α-intercalated cells in the rabbit (105). However, in rat and mouse collecting ducts, AE4 was detected on the basolateral membrane of α-intercalated cells (105). AE4 is also expressed on the basolateral membrane of mouse submandibular gland duct (105). In contrast to the data of Tsuganezawa and colleagues (201), rat AE4 expressed in LLC-PK1 and HEK293 cells mediated DIDS-sensitive Cl⁻/HCO₃⁻ exchange (105). Parker and colleagues (144) characterized human AE4 (945 aa) expressed in X. laevis oocytes as an electroneutral sodium bicarbonate cotransporter without Cl⁻/HCO₃⁻ exchange activity. Whether these discrepancies arise from species-mediated differences in amino acid composition or expression system-specific protein(s) potentially interacting with AE4 requires further study. Based on its amino acid sequence, AE4 is most homologous to electronegative sodium bicarbonate cotransporters rather than AE1–3.

**SLC4A10 (NCBE).** NCBE (1,088 aa) was originally cloned from a mouse insulinoma cell line and when expressed in X. laevis oocytes and HEK293 cells was reported to mediate Na⁺-driven Cl⁻/HCO₃⁻ exchange (213). Two splice variants have been subsequently cloned from rat brain and functionally expressed (57). rb1NCBE was similar to mouse NCBE except for a 30-aa insert in the NH2 terminus and was highly expressed in spinal cord and brain beginning in the embryo. rb2NCBE has an additional 18 aa in its COOH terminus, which ends with a PDZ motif. rb2NCBE is more highly expressed in astrocytes than rb1NCBE. Both transcripts mediated Na⁺-driven Cl⁻/HCO₃⁻ exchange when expressed in mouse fibroblast 3T3 cells, but rb2NCBE was more active in pH regulation (57). A human ortholog of rb1NCBE, NCBE-B (1,118 aa), and its splice variant missing 30 aa in the NH2 terminus, NCBE-A, have been reported (39).

NCBE transcripts have been detected in the peripheral nervous system and in nonneuronal tissues such as the choroid plexus, the dura, and some epithelia including the acid-secreting epithelium of the stomach and the duodenal epithelium (76) and kidney (153). rb1NCBE protein was highly expressed on the basolateral membrane of mouse and rat choroid plexus cells (152, 153). rb2NCBE protein has also been localized in preliminary studies to the basolateral membrane of mTAL intercalated cells in the inner medullary collecting duct and duodenal villus cells (153). In a preliminary study, Choi and coauthors (39) demonstrated that the human NCBE-B splice variant functions in X. laevis oocytes as an electroneutral Na⁺-HCO₃⁻ cotransporter. Whether experimental differences in the extent of Cl⁻ depletion and/or expression systems account for these findings is currently unknown.

**SLC4 Transporters That Do Not Transport Bicarbonate**

**SLC4A11 (NaBC1).** SLC4A11 (initially called BTR1) (145) was originally included in the SLC4 family because of distant sequence homology to other family members. BTR1 has been renamed NaBC1 because it has been shown to function as a Na⁺-dependent borate transporter (143, 145). In the absence of borate, NaBC1 conducts Na⁺ and OH⁻ (H⁻), whereas in the presence of borate, NaBC1 functions as a voltage-regulated, electrogenic Na⁺-B(OH)₄⁻ cotransporter with a stoichiometry of Na⁺:B(OH)₄⁻ ≥2. NaBC1 is the mammalian ortholog of the Arabidopsis thaliana borate transporter AtBor1 (194).
CHANNEL-LIKE PROPERTIES OF SLC4 TRANSPORTERS

Several electroneutral SLC4 transporters also have channel-like properties. AE1 in native X. laevis oocytes has an associated small conductive anion current (51). The Na\(^+\)-driven Cl\(^-\)/HCO\(_3\)\(^-\) exchanger cloned from Drosophila NDAE1 also mediates a small Cl\(^-\) current and a HCO\(_3\)\(^-\) current (167). Choi and colleagues (37, 42) have shown that two rat ortholog splice variants of the human NBC3 (NBCn1-B and NBCn1-E) in both X. laevis oocytes and HEK293 cells have an associated ion conductance that, as they suggested, is likely mediated by the cotransporter per se rather than an associated protein, although the latter possibility has not been definitely ruled out. The associated current was stimulated by DIDS, is not bicarbonate dependent, and Na\(^+\) appeared to be the predominant ion responsible for the current. The physiological importance of these data, as well as potential implications to the transport mechanism, is unclear.

TRANSPORT STOICHIOMETRY

The ion transport stoichiometry of SLC4 transporters determines whether they are electroneutral or electrogenic (Table 1) (67, 112). The flux through electrogenic SLC4 transporters is affected by both the membrane potential and the activity of the transported ion species. AE1–3 proteins exchange equivalent amounts of Cl\(^-\) for base and are electroneutral (6, 33). Sodium bicarbonate cotransporters mediate the transport of Na\(^+\) and base with different stoichiometries per transport cycle. In NBC3 (NBCn1), the transport stoichiometry is 1:1, and, like AE1–3, this transport process is electroneutral (37, 155). NBC1 mediates the cotransport of one positive charge and either two or three negative charges per transport cycle (2, 30, 62–64, 67, 68, 112, 166). NBC4 is also electronegic and has been reported to have a 3:1 transport stoichiometry when expressed in mammalian cells (176) and a 2:1 stoichiometry in X. laevis oocytes (211). Human NDCBE exchanges Cl\(^-\) for the equivalent of one Na\(^+\) and two HCO\(_3\)\(^-\) ions, and the process is electroneutral (60) (see Fig. 1 for other potential modes of transport). Although all studies agree that AE4 and NCBE are electroneutral transporters, their Cl\(^-\) and Na\(^+\) dependence are controversial (39, 57, 105, 144, 201, 213), and additional studies are required to determine their mode of transport.

NBC1 and NBC4 transporters differ from other SLC4 family members in that they are electronegic. The net charge carried by NBC1 and NBC4 varies with their transport stoichiometry and is an important determinant of the direction of Na\(^+\) and HCO\(_3\)\(^-\) (or CO\(_3\)\(^2\)) flux (Fig. 3) (67, 112). In addition, because the cell membrane potential induces changes in the flux of electronegic sodium bicarbonate cotransporters, the effective cell buffer capacity will also vary with the membrane potential (16). This provides an interesting mechanism for coupling unrelated transport processes such as the electroneutral monocarboxylate transporter via MCT1 (which transports H\(^+\) and monocarboxylates) to changes in membrane potential in cells expressing kNBC1 (16).

It had been originally thought that the transport stoichiometry of SLC4 proteins is an inherent property of each transporter that remains fixed. However, Chernova and colleagues (35) showed that an E699Q mutation in mouse AE1 blocked electroneutral Cl\(^-\)/Cl\(^-\) anion exchange and created an electrogenic Cl\(^-\)/SO\(_4\)\(^2-\) exchange process. These data indicated that a single amino acid substitution could shift an electroneutral transport process to an electrogenic one. In addition, following the cloning of the NH\(_2\)-terminal variants of NBC1 (kNBC1 and pNBC1), it had been thought that NBC1 functions in a 2:1 (efflux) mode and pNBC1 functions in 2:1 (influx) mode. Gross and colleagues (63), however, showed that both transporters can function in either a 2:1 or 3:1 mode depending on the cell type in which they were exogenously expressed. In addition, it was shown that the HCO\(_3\)\(^-\):Na\(^+\) transport stoichiometry of NBC1 can shift from 3:1 to 2:1 following cAMP-induced PKA-dependent phosphorylation of NBC1-Ser\(^{682}\)/pNBC1-Ser\(^{1026}\) (62, 64). Moreover, Asp\(^{986}\) and Asp\(^{988}\) were also shown to be required for the cAMP-induced shift of NBC1 stoichiometry (68). These residues are located in close proximity to the PKA phosphorylation site, K\(^{979}\)KGS, and are part of a putative D\(^{986}\)NDD motif that, in addition to a second motif, L\(^{955}\)DDV, was shown to be involved in carbonic anhydrase II (CAII) binding (154).

Based on these considerations, Gross and colleagues (67, 68) originally hypothesized that a potential mechanism for the cAMP-induced shift in stoichiometry of kNBC1 requires the binding/dissociation of CAII. Against this hypothesis was the subsequent finding by Pushkin and colleagues (154) that the PKA-dependent phosphorylation of Ser\(^{682}\) does not decrease CAII binding in vitro. In addition to PKA-dependent phosphorylation, an increase in intracellular Ca\(^{2+}\) concentration shifts the stoichiometry of kNBC1 expressed in X. laevis oocytes from 2:1 to 3:1 (132). The mechanism for the Ca\(^{2+}\)-induced shift in stoichiometry is currently unknown. Additional studies have shown that PKA-dependent phosphorylation of pNBC1...
(KRKT\textsuperscript{19}) does not alter the transport stoichiometry but enhances flux through the cotransporter, thereby potentially playing an important role in secretin-induced stimulation of pancreatic bicarbonate secretion (62).

TRANSPORTED BASE: HCO\textsubscript{3}\textsuperscript{-} VS. CO\textsubscript{3}\textsuperscript{2-}

It has not been definitively established whether SLC4 proteins mediate HCO\textsubscript{3}\textsuperscript{-} and/or CO\textsubscript{3}\textsuperscript{2-} transport. Depicted in Fig. 1 are the hypothetical modes of transport wherein the net charge per transport cycle by HCO\textsubscript{3}\textsuperscript{-} and/or CO\textsubscript{3}\textsuperscript{2-} with Na\textsuperscript{+} and/or Cl\textsuperscript{-} is equivalent. Müller-Berger and colleagues (135) suggested that the finding that carbonic anhydrase (CA) inhibition only altered the flux through the basolateral proximal tubule electrogenic sodium bicarbonate cotransporter (kNBC1) functioning in the 3:1 mode and not in the 2:1 mode could be used to determine whether HCO\textsubscript{3}\textsuperscript{-} and/or CO\textsubscript{3}\textsuperscript{2-} was the transported species. These authors argued on theoretical grounds that when CA inhibitors decrease the flux through the cotransporter, CO\textsubscript{3}\textsuperscript{2-} must be one of the transported species and that, in the 3:1 mode, ICO\textsubscript{3}\textsuperscript{2-} + 2HCO\textsubscript{3}\textsuperscript{-} + 1Na\textsuperscript{+} are transported. Moreover, the finding that CA inhibition did not alter the flux in the 2:1 mode argued against CO\textsubscript{3}\textsuperscript{2-} flux in favor of 2HCO\textsubscript{3}\textsuperscript{-} + 1Na\textsuperscript{+} being transported. More recently, it has been suggested in preliminary experiments that even in the 2:1 mode, kNBC1 transports 1Na\textsuperscript{+} and 1 CO\textsubscript{3}\textsuperscript{2-} (59).

Another potential approach to distinguish CO\textsubscript{3}\textsuperscript{2-} from HCO\textsubscript{3}\textsuperscript{-} transport is the use of equilibrium thermodynamics (112). In the presence of a stable PCO\textsubscript{2} difference across a membrane with a functional kNBC1 for example (e.g., lipid bilayer), it was previously demonstrated that theoretically under these conditions, the respective reversal potential values for a 3HCO\textsubscript{3}\textsuperscript{-} + 1Na\textsuperscript{+} transport mode vs. a 1 CO\textsubscript{3}\textsuperscript{2-} + 2HCO\textsubscript{3}\textsuperscript{-} + 1Na\textsuperscript{+} transport mode would differ. Another potential approach by which to distinguish these modes of transport would be to establish a stable pK difference across a membrane (112). This is possible to accomplish because the HCO\textsubscript{3}\textsuperscript{-} \rightarrow CO\textsubscript{3}\textsuperscript{2-} + H\textsuperscript{+} reaction varies with ionic strength. There are, however, technical limitations that have thus far not been solved that have prevented these approaches from being used experimentally.

**Primary Structure**

Comparison of the primary structures of SLC4 transporters shows significant sequence homology especially in putative membrane domains (Fig. 2). On the basis of their amino acid sequence homology, SLC4 transporters appear to be separated structurally into three groups (Fig. 4) that likely have an evolutionary basis: 1) SLC4A1, SLC4A2, and SLC4A3; 2) SLC4A4, SLC4A5, and SLC4A9; and 3) SLC4A7, SLC4A8, and SLC4A10. These groups are distinct from the known functional categorization of SLC4 transporters, indicating that primary structure may not be a sufficient predictor of the functional properties of each transporter.

**Posttranslational Modifications**

Although all SLC4 transporters have various sites for potential posttranslational modification, thus far AE1 and NBC1 transporters have been studied in some detail. Human erythrocyte AE1 contains a single site of N-glycosylation (Asn\textsuperscript{642}) in the fourth exofacial loop (Figs. 2 and 5) that contains either a short complex oligosaccharide or an extended polyglycosaminyl oligosaccharide (33). Approximately equal amounts of the different glycosylated forms of AE1 are found in human red blood cells. Recently, a novel glycosylation site at Asn\textsuperscript{555} in the third exofacial loop has been described in addition (36). kNBC1 has been shown to contain two normally glycosylated sites at Asn\textsuperscript{597} and Asn\textsuperscript{617} (38) located in the second exofacial loop (Fig. 6) (198). Human AE1 was also palmitoylated at Cys\textsuperscript{843} (36). Interestingly, this modification did not affect trafficking of the anion exchanger.

kNBC1 and pNBC1 are phosphorylated in their common COOH-terminal PKA phosphorylation site, KKGs (62, 64, 68). The PKA-mediated phosphorylation of Ser\textsuperscript{662} in kNBC1 (Ser\textsuperscript{1026} in pNBC1) shifts the transport stoichiometry of both NBC1 variants from 3:1 to 2:1 (62, 64, 68). pNBC1 possesses an additional PKA site (KRKT\textsuperscript{49}), involved in the regulation of pNBC1-mediated flux but not the cotransporter stoichiometry (62).

![Fig. 4. Phylogenetic tree of SLC4 transporters created using the UPGMA analysis. Numbers on top of each line represent the relative difference between the specific sequences using GeneWorks software.](http://ajprenal.physiology.org/)

**Invited Review**

F586  SLC4 BASE (HCO\textsubscript{3}\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-}) TRANSPORTERS

AJP-Renal Physiol • VOL 290 • MARCH 2006 • www.ajprenal.org
Topological Models

Different algorithms predict 9–15 transmembrane segments in members of the SLC4 family. Topological models have been experimentally evaluated in two SLC4 transporters. The most extensively studied SLC4 transporter, the anion exchanger AE1, was proposed to possess 12 (149), 13 (33, 54, 232), or 14 transmembrane segments (70, 94), of which the first 8 are generally accepted to exist (Fig. 5). The models fit calculations of AE1 transmembrane area based on a low-resolution two-dimensional crystallography data of dimeric COOH-terminal membrane domain of AE1 (214). All AE1 models predict that the NH2 and COOH termini are localized intracellularly. Controversial experimental data regarding putative COOH-terminal transmembrane segments require use of high-resolution X-ray crystallography to precisely identify the number and location of AE1 transmembrane segments.

Several studies indicated that the integrity of several cytoplasmic loops of AE1 is not essential for assembly into an anion transport-active structure. Coexpression of two pairs of complementary fragments of AE1 resulted in the generation of the stilbene disulfonate-sensitive uptake of Cl− into X. laevis oocytes, similar to expressed intact AE1 (69). For example, the pairs of fragments, which contained the first 8 and the last 6 membrane spans, and the first 12 and the last 2 membrane spans, separated within cytoplasmic surface loops of the membrane domain, were able to generate stilbene disulfonate-sensitive Cl− uptake. In contrast, the pairs separated in the second cytoplasmic loop were not active (216). Omission of...

Fig. 5. Human AE1 topology model based on the work of Zhu and colleagues (232). Amino acids that are important for AE1-mediated transport are shown in red (acidic), blue (basic), and black (others). Green stars depict the residues involved in human disease.

Fig. 6. Human NBC1 (NBCe1) topological model (198). Amino acids that are important for NBC1-mediated transport are shown in red (acidic), blue (basic), and black (others). Green stars depict the residues involved in human disease. TM, transmembrane.
any of single transmembrane segments except of spans 6 and 7 caused complete loss of functional expression (70). The unusually stable association between the AE1 fragments divided in the second exofacial loop was suggested to indicate an existence of interactions between polar surfaces on amphiphilic portions of the third and fifth transmembrane spans. Most AE1 fragments contained the necessary information to fold in the membrane (71).

Tatishchev and colleagues (198) demonstrated that NBC1 has 10 transmembrane segments, with the NH2 and COOH termini localized intracellularly (Fig. 6). Whether the difference in the number of transmembrane segments in AE1 and NBC1 is related to their specific functional properties remains to be determined.

**Tertiary Structure**

The tertiary structure of the members of SLC4 family has only been partially determined. A low-resolution (20 Å) structure of the membrane domain of AE1 has been reported based on electron microscopy and three-dimensional image reconstruction of negatively stained two-dimensional crystals (215). The dimeric membrane domain revealed a U-shaped structure with dimensions of 60 × 110 and a thickness of 80 Å. The structure is open on the top and at the sides, with the monomers in close contact at the base, and the basal domain was 40 Å thick. The upper part of the dimer consisted of two elongated protrusions, which formed the sides of a canyon, enclosing a wide space that narrowed down and converged into a depression at the center of the dimer on the top of the basal domain. This depression might represent the opening to a transport channel located at the dimer interface.

The crystal structure of the NH2-terminal cytoplasmic domain of the human AE1 (aa 1–379) has been reported at a much higher (2.6 Å) resolution (230). A tight symmetrical dimer stabilized by interlocked dimerization arms contributed by both monomers was reported. The dimer fit within a rectangular prism of approximate dimensions 75 × 55 × 45 Å. Each subunit included a larger peripheral protein binding domain with an α + β-fold. The binding sites of ankyrin, deoxyhemoglobin, aldolase, and glyceraldehyde-3-phosphate dehydrogenase were localized in the structure.

The COOH-terminal cytoplasmic domain of human NBC3 (aa 1127–1214) expressed in Escherichia coli cells and analyzed by gel permeation chromatography and sedimentation velocity centrifugation had a Stokes radius of 26 and 30 Å, respectively (119). Shape modeling suggested that the COOH terminus had a rodlike shape with the dimensions of 16 × 190 Å, which was supposed to promote binding of regulatory proteins (119).

**Oligomeric Structure**

It is not known whether SLC4 transporters are functionally active in monomeric form or whether oligomerization is required for their activity. Numerous biochemical studies of mammalian AE1 have identified monomers, dimers, trimers, tetramers, and higher order oligomers and their mixtures (32, 41, 50, 136). The relative abundance of these forms was dependent on the detergent used for isolation of AE1 and the temperature of isolation. In two-dimensional crystals of AE1 (50), the protein subunits, each consisting of two AE1 monomers, were arranged with threefold symmetry, suggesting that a functionally active form of AE1 might be a dimer or/and even a hexamer. Based on these structural data and because a mixture of dimers and tetramers has been shown to represent the majority of AE1 in red blood cells (19, 32, 41, 50, 136, 141, 215), it is widely accepted that a dimer is an active oligomeric form of AE1. On the basis of communoprecipitation and functional expression in X. laevis oocytes of noncontiguous and overlapping pairs of membrane domains of human AE1, Groves and Tanner (70) hypothesized that transmembrane spans 1–5 and 9–12 are involved in, and spans 6–8, 13, and 14 are not important for, dimerization of AE1.

Dimers and to a lesser extent tetramers were also shown to be major components of bAE3 purified from rabbit renal microsomal membranes in the presence of Triton X-100 (160). Tetramers of bAE3 were further characterized by transmission electron microscopy of negatively stained ion-exchanger molecules (160). Image analysis of the tetramer micrographs revealed an anion exchanger molecule in which monomers were arranged with fourfold symmetry around a cavity in the center of the molecule, which could play a role of an ion-transporting channel. Oligomers of human NBC1 and NBC3 were also detected when these proteins were expressed in HEK293 cells (159).

**TRANSPORT MECHANISM**

It is unusual that the same mutigene family contains transport proteins that function as exchangers (e.g., AE1, AE2, AE3, NDCBE) and cotransporters (e.g., NBC1, NBC4) (Fig. 1). AE1 exchanges equal amounts of Cl– for HCO3–, by a ping-pong mechanism, wherein the transporter can be in either the inward-facing or the outward-facing state (52, 55, 72, 88). According to Jennings et al. (88), the AE1 catalytic cycle consists of one pair of exchanging anions per anion exchanger monomer. The transport rate is governed by a single AE1 conformational change, which leads to the transfer of a single anion across the membrane (146). The dissociation/association of ions with AE1 occurs more quickly than the AE1 conformational changes leading to the translocation of the bound anion. Human AE1 has an intrinsic functional asymmetry; therefore, at near neutral pH and equal extra- and intracellular concentrations of Cl–, most of the anion exchanger molecules are in the inward-facing state (104).

The stilbene inhibitor DIDS has been shown to bind preferentially to the outward-facing state of AE1 (88) and is inhibited by DIDS from outside (31, 96). In contrast to AE1, kNBC1 was inhibited equally by intra- or extracellularly applied DIDS (74). In addition, H2DIDS has been shown to covalently bind Lys539 and Lys851 in human AE1 at physiological pH (140), whereas DIDS binds to Lys851 only at pH >12 (93). According to current AE1 topological models, both lysine residues are located in transmembrane segments (54, 70, 111, 232), kNBC1 (30, 166) and the pancreatic form of NBC1, pNBC1 (2), as well as all members the SLC4 family with the exception of the electroneutral sodium bicarbonate cotransporter NBC3 (155) and rabbit AE4a (202) are stilbene inhibitable, supporting an idea of a possible shared mechanism(s) of ion transport. However, against this assumption is the finding that stilbene inhibition is not limited to the members of SLC4 family, suggesting that the mechanism of this inhibition is nonspecific and
involves binding of stilbene to exposed lysine residues, leading to steric blocking of normal ion transport mechanism. For example, various members of the SLC26 anion exchanger family, which have shared no sequence homology with SLC4 transporters, are also stilbene sensitive (131).

Kinetic studies of the electrogenic sodium bicarbonate cotransporter NBC1 suggest that contrary to AE1, binding of both sodium and bicarbonate to the cotransporter is required before a transport event (65, 90). Gross and Hopfer (66) developed a kinetic model in which bicarbonate and sodium bind to NBC1 in an ordered rather than a random manner. Using additional simplifying assumptions, they fitted the model to experimental current-voltage relationships obtained at different concentrations of Na⁺ and HCO₃⁻ and predicted that binding of the ions to the cotransporter is voltage dependent, with an electrical coefficient of 0.2 at pH 7.5. The later suggested that HCO₃⁻ “senses” ~20% of the membrane’s electric field on binding to the cotransporter or, in other words, that the binding site for HCO₃⁻ is located about one-fifth of the electrical distance into the membrane. Two basic amino acid residues, Lys⁸⁵⁴ and His⁹⁰⁷, in transmembrane segments of kNBC1 (Fig. 6) (198) potentially involved in binding of bicarbonate (1) are located ~20 and ~10% of membrane width from the cytoplasmic side. Therefore, binding of bicarbonate to kNBC1 in the inward-facing conformation may fit the prediction of Gross and Hopfer (66).

ION SELECTIVITY AND TRANSLLOCATION

In proteins that function as exchangers and cotransporters, there are residues usually located outside transmembrane segments mediating ion selectivity, and residues usually located within transmembrane segments that are involved in ion binding and translocation (ion binding/translocation site) (44, 172, 220). In the absence of the NH₂-terminal domain, the COOH-terminal domain of AE1 (61) and AE3 (108) was able to mediate Cl⁻/HCO₃⁻ exchange, suggesting that the NH₂-terminal domain is not necessary for ion exchange. Several amino acid residues have been identified that might be involved in ion selectivity and binding/translocation of AE1. Specifically, treatment of human red cell AE1 with Woodward’s reagent K labeled only glutamate but not aspartate residues (86) and inhibited Cl⁻/Br⁻ exchange, suggesting that glutamate residue(s) is involved in AE1-mediated ion transport. Pretreatment with 4,4′-dinitrostilbene-2,2′-disulfonate (DNDS) protected AE1 from binding and inactivation by Woodward’s reagent K, suggesting that the stilbene-binding site and the glutamate residue(s) are located close to each other. In addition to glutamate, lysine, arginine, and histidine residues have been shown to be essential for AE1 transport activity (73, 84, 126, 140, 146, 219, 228).

The importance of glutamate residue(s) in AE1-mediated transport was further confirmed when Glu⁶⁸⁹ in transmembrane segment 8 of human red cell AE1 (Glu⁶⁸⁹ in mouse AE1) was shown to be involved in the ion exchange function of human (86, 87) and mouse AE1 (35). Mutation of Glu⁶⁸⁹ in human AE1 and a homologous residue in human AE2 (182) to neutral or basic amino acids blocked transport of monovalent anions (Cl⁻ and bicarbonate) but did not change maximum sulfate/sulfate exchange. Interestingly, replacement of mouse AE1 Glu⁶⁹⁹ with aspartate blocked transport of both monovalent and divalent anions (133). Replacement of mouse AE1 Glu⁶⁹⁹ with glutamine inhibited Cl⁻/HCO₃⁻ exchange, but the mutant anion exchanger mediated an electrogenic Cl⁻/SO₄²⁻ exchange when expressed in X. laevis oocytes (35). It was suggested (87) that Glu⁶⁸¹ is the binding site for H⁺, which is transported with SO₄²⁻ during AE1-mediated H⁺/SO₄²⁻ cotransport, and can be alternately exposed to the intracellular and extracellular media. In addition, interaction of mouse AE1 Glu⁶⁹⁹ with His⁷⁵² was shown to be important for pH sensitivity of Cl⁻ transport at low pH (133). Recently, it was shown that an additional glutamate residue is involved in Cl⁻ binding and is part of a second Cl⁻ binding/translocation site in the AE1 monomer (85).

In human kNBC1 (Fig. 6), Asp⁷⁵⁴ (glutamate in AE1–3) flanks transmembrane segment 6 and plays a key role in kNBC1-mediated sodium bicarbonate cotransport, suggesting that the decreased length of aspartic acid compared with glutamic acid may be related to sodium dependence of ion transport (1). In addition, Asp⁵⁵⁵, Glu⁶⁵⁹, Asp⁶⁴⁷, Asp⁶⁸⁵, and Asp⁷⁷⁹ are important for kNBC1 function (1). Asp⁵⁸⁵, conserved in Na⁺-dependent SLC4 transporters and AE4, is potentially involved in sodium binding/translocation (1). Asp⁵⁵⁵, conserved in all SLC4 transporters, is located in close proximity to Lys⁵⁵⁹, which has been suggested (1) to participate in bicarbonate binding and translocation.

The involvement of mouse AE1 Lys⁵⁵⁸ (Lys⁵³⁹ in human AE1, Fig. 5) in AE1-mediated Cl⁻ transport was hypothesized based on site-directed mutagenesis data (134). This amino acid residue located in a transmembrane span is also a target for covalent binding of H₂DIDS and DIDS (93, 140). Both H₂DIDS (DIDS at pH >12) and pyridoxal phosphate bind another lysine residue, Lys⁸⁵⁴ (100, 140) and inhibit AE1. Several lysine residues (Lys⁵⁵⁹, Lys⁶⁶⁷, Lys⁶⁶⁸, Lys⁶⁸¹, Lys⁷⁷⁰, Lys⁷⁷¹, Lys⁸⁵⁴, and Lys⁹²⁴) are functionally important for kNBC1 (1). Lys⁸⁵⁴ is localized in transmembrane segment 8, which is conserved in Na⁺-dependent SLC4 transporters and potentially involved in ion binding/translocation mediated by NBC1. Human kNBC1 Lys⁵⁵⁹ and Lys⁹²⁴ are potential targets for DIDS inhibition. These residues are located extracellularly (Fig. 6); however, it is known that stilbenes inhibit kNBC1 from both extra- and intracellular locations (74). Based on these data, Abuladze and coauthors (1) hypothesized that this part of the extracellular loop 2 in NBC1 is able to migrate between extra- and intracellular compartments. In addition, these authors suggested that this region is involved in bicarbonate binding in NBC1. The second half of this loop contains naturally glycosylated Asn⁵⁹⁷ and Asn⁶¹⁷ (38) and therefore is located extracellularly. Lys⁶⁶⁷, Lys⁶⁶⁸, Lys⁶⁸¹, Lys⁷⁷⁰, Lys⁷⁷¹, and Lys⁸⁵⁴ are probably involved in ion selectivity in NBC1.

Four histidine residues located in transmembrane segments of mouse AE1, His⁷²¹, His⁷⁵², His⁸³⁷, and His⁸⁵² (His⁷⁰³, His⁷³⁴, His⁸¹⁹, and His⁸³⁴ in human AE1, Fig. 5) were shown by site-directed mutagenesis to participate in AE1-mediated ion exchange (133, 134). Mouse AE1 His⁸⁵² was shown to play a key role in the control of pH dependence of Cl⁻ transport (133). It was suggested that the formation of a hydrogen bond between His⁸⁵² and Glu⁶⁹⁹ (Glu⁶⁸¹ in human AE1) is essential for the decrease in Cl⁻ transport at low pH. In addition, His⁸³⁴ in human AE1 was shown to play an essential role in the protein conformational changes during the ion exchange and is located in close proximity to the anion translocation site (92). It was also hypothesized that all these histidine residues inter-
act with mouse AE1 Lys\textsuperscript{558} (Lys\textsuperscript{539} human AE1), the target of covalent binding of DIDS (93, 140). All these histidine residues except His\textsuperscript{5819} are conserved in SLC4 transporters (Fig. 2). The only NBC1 homolog of these AE1 histidine residues located in transmembrane segments, kNBC1-His\textsuperscript{857} (human AE1-His\textsuperscript{834}), was shown to be important for NBC1-mediated ion transport (1), suggesting that it can participate in bicarbonate binding/translocation. Human kNBC1-His\textsuperscript{779} (His\textsuperscript{734} in human AE1) located in the third intracellular loop is also important for kNBC1 function and apparently involved in bicarbonate selection (1), whereas the role of His\textsuperscript{807} (His\textsuperscript{763} in human AE1) has not yet been evaluated.

Mutating Arg\textsuperscript{509} and Arg\textsuperscript{748} in mouse AE1 to lysine, threonine, and cysteine or lysine and glutamine, respectively, abolished Cl\textsuperscript{−}/Cl\textsuperscript{−} exchange activity in X. laevis oocytes (97). The Arg\textsuperscript{734} mutant was supposed to be functionally inactive, whereas the Arg\textsuperscript{509} mutant was possibly active but abnormally folded and therefore subsequently degraded. Because Arg\textsuperscript{734} is conserved in AE1–3 and missing in Na\textsuperscript{+}-dependent members of the SLC4 family, including AE4, this residue may be responsible for Cl\textsuperscript{−} binding/translocation in anion exchangers.

In addition, the kNBC1 Arg\textsuperscript{536}, Arg\textsuperscript{680}, Arg\textsuperscript{722}, Arg\textsuperscript{881}, and Arg\textsuperscript{943} conserved in the SLC4 family are also important for cotransporter function (1, 75).

Several uncharged amino acids were detected in kNBC1 transmembrane segments (Ser\textsuperscript{427}, Tyr\textsuperscript{433}, Phe\textsuperscript{656}, Ser\textsuperscript{684}, and Ala\textsuperscript{799}) and in loops (Thr\textsuperscript{855}, Ala\textsuperscript{556}, Thr\textsuperscript{671}, Thr\textsuperscript{677}, and Thr\textsuperscript{815}) that are important for cotransporter function (1, 49, 75). Numerous uncharged amino acids in the membrane domain of AE1 have also been shown to be important for anion exchange. Tang and coauthors (195) mutated to cysteine the amino acids within and in close proximity to transmembrane segment 8. The cysteine mutants of this segment, Ala\textsuperscript{666}, Ser\textsuperscript{667}, Leu\textsuperscript{669}, Leu\textsuperscript{693}, Leu\textsuperscript{677}, and Leu\textsuperscript{680}, and in addition the intracellular mutants of Ile\textsuperscript{684} and Ile\textsuperscript{688} were inhibitable by sulfhydryl reagents. It was suggested that these amino acids could be a part of a AE1 translocation pore (195). Analysis of single cysteine mutants in the COOH-terminal area of human AE1 (Phe\textsuperscript{806}, Cys\textsuperscript{885}) in the last two putative transmembrane segments identified certain key residues in the Val\textsuperscript{849}-Leu\textsuperscript{863} region (231). The putative ion selectivity filter was located at Ser\textsuperscript{852}-Leu\textsuperscript{857} (231).

**INTERACTION WITH CA: A TRANSPORT METABOLON**

CAs are a multigene family of enzymes that catalyze the reversible synthesis of bicarbonate from CO\textsubscript{2} and H\textsubscript{2}O (24, 180, 183, 200). Inhibition of CA activity affects SLC4 anion exchange and sodium bicarbonate cotransport function (28, 29, 128, 174, 186). Kifor and coauthors (102) on the basis of indirect evidence suggested first that red cell AE1 might interact with a cytosolic CA. Reithmeier and colleagues (163, 206–208) demonstrated that the COOH terminus of AE1 binds CAII. Functional studies have demonstrated that CAII stimulates the transport function of AE1, AE2, and AE3 (45, 186). As a model for the CAII-mediated enhancement of HCO\textsubscript{3} transport through AE1, it was hypothesized that a complex of AE1 and CAII functions in red blood cells as a transport metabolon. In this model, the efficiency of bicarbonate transport via AE1 is enhanced by the intermolecular transfer of bicarbonate between CAII and AE1, thereby eliminating the slower cytoplasmic diffusion of HCO\textsubscript{3} between the two proteins (163, 186, 187, 206–208). In addition to CAII, CAIV was shown to bind AE1–3 (185).

It is known that inhibition of CA activity in the renal proximal tubule of the kidney significantly decreases the rate of transepithelial bicarbonate absorption and basolateral sodium bicarbonate efflux (28, 29, 128, 174, 181). Complete inhibition of CA by 0.1 mM acetazolamide (ACTZ) decreased the flux through exogenously expressed kNBC1 by ~65% when the transporter functioned with a HCO\textsubscript{3}/Na\textsuperscript{+} stoichiometry of 3:1 (68) as in the in vivo proximal tubule (28) without altering its stoichiometry. ACTZ does not inhibit kNBC1-mediated flux when the cotransporter functions in the 2:1 stoichiometry mode (68, 181).

The COOH terminus of kNBC1 (kNBC1-ct) was shown to bind CAII in vitro with high (K\textsubscript{D} < 0.2 μM) affinity (68). Two kNBC1 acidic motifs, L\textsuperscript{958}DV and D\textsuperscript{966}NDD, are involved in this binding (154). The complex of kNBC1 with CAII functioned as a transport metabolon (154). In pancreatic ducts, where pNBC1 mediates the basolateral influx of bicarbonate, CAII is highly expressed (137, 138). pNBC1 has a COOH terminus identical to kNBC1, and transfection of HEK cells with nonfunctional CAII has a dominant-negative effect on pNBC1 function (12), suggesting that pNBC1 also forms a functional complex with CAII. pNBC1 also binds CAIV (12).

The COOH terminus of electroneutral sodium bicarbonate cotransporter NBC3, like NBC1 and AE1-AE3, binds CAII with high affinity (K\textsubscript{D} ~100 nM) (119). The human NBC3-D\textsubscript{1135}, D\textsubscript{1136} residues were essential for the interaction between the two proteins. In α-intercalated cells in the outer medullary collecting duct, the interaction between apical NBC3 and CAII may play an important role in pH\textsubscript{i} regulation.

In addition to CA, certain SLC4 transporters interact with other cellular proteins. The cytoplasmic domain of AE1 contains sites for binding ankyrin, 4.1 and 4.2 proteins, glyceraldehyde-3-phosphate dehydrogenase, phosphofructokinase, deoxyglucose, p72syk protein tyrosine kinase, and hemichromes (230) and functions as an anchoring site for these membrane-associated proteins. These interactions are important for regulation of cell flexibility and shape (121), glucose metabolism (120), ion transport (124), and cell life span (95). The AE1 NH\textsubscript{2}-terminal cytoplasmic domain is stabilized by interlocked dimerization arms contributed by both monomers (230).

**GENETIC DISEASES AND KNOCKOUT MODELS**

The crucial importance of SLC4 transporters in regulating intracellular pH and transporting bicarbonate in various cell types is best exemplified by the phenotype resulting from an abnormality in the functional properties of several members of the family (Table 2).

**SLC4A1**

Patients with AE1 mutations tend to have either hereditary spherocytosis (HS) or distal renal tubular acidosis (dRTA) but not both abnormalities. The reason for the lack of overlap between these syndromes is not understood. The majority of patients with HS-associated AE1 mutations have an autosomal dominant inheritance with missense mutations that are distrib-
alyzed throughout the transporter. The underlying abnormality thus far appears to be abnormal targeting to the plasma membrane due to misfolding of mutant transporters. In addition, a dominant-negative effect results in a decrease in wild-type (wt) AE1 membrane expression. Patients with autosomal dominant dRTA due to mutant AE1 have missense mutations in AE1-R589 (R589H, R589C, and R589S), S613F, A889X, G609R, and a truncation of 11 COOH-terminal residues, R901X (26, 34, 83, 99, 171). The syndrome is characterized by abnormal urinary acidification, metabolic acidosis, nephrocalcinosis, hypercalciuria, and hypokalemia. Within a given family, the phenotypic expression can vary, likely because of background genes that are thus far unidentified (218).

Studies of either red blood cells or heterologous expression systems demonstrated only modest changes in function that could not account for the urinary acidification abnormality in patients (26, 83). wt-AE1 in vivo and in polarized Madin-Darby canine kidney (MDCK) cells is targeted to the basolateral membrane. The predominant abnormality in the mutants characterized thus far in polarized MDCK cells (G609R and R901X) is the abnormal targeting of kAE1 to both the apical and basolateral membranes (47, 171). It is speculated that luminal bicarbonate secretion via mislocalized apical AE1 decreases urinary acidification in these patients and that oligomerization of mutant AE1 with the wt-AE1 result in a dominant-negative effect by mistargeting wt-AE1 to the apical membrane. The magnitude and direction of apical AE1-mediated transport would be dependent on the respective cell/lumen HCO₃⁻ and Cl⁻ gradients. Whether patients with abnormal luminal bicarbonate secretion can be distinguished by a higher than urinary PCO₂ compared with patients with dRTA due to abnormal H⁺-ATPase function remains to be determined.

Autosomal recessive dRTA is prevalent in Southeast Asia. Unlike patients with autosomal dominant dRTA, autosomal recessive dRTA presents earlier and tends to be of greater severity (14, 17, 98). These patients have been reported to have missense mutations in AE1 G701D, or various compound heterozygous combinations, including patients with G701D and the second allele having an in-frame AE1-400–408 deletion [called South Asian ovalocytosis (SAO)], (G701D/SAO), A858D/SAO, ΔV850/SAO, A858D/ΔV850, and G701D/ΔS773P (27, 197, 204, 226). The G701D mutant functions and is targeted normally in red blood cells but not in α-intercalated cells, which lack the targeting of glycoporphin A (GPA) that is expressed in the red cell membrane (197). AE1 SAO is unable to transport anions (179, 221). The A858D mutant functions abnormally, and its function is only partially rescued by GPA. In addition, the functioning of A858D is significantly decreased when coexpressed with ΔV850 or SAO (27). Similarly, the abnormal function of the ΔV850 mutant is not rescued completely by GPA (27). Recent detailed studies of the S773P mutant show that it is expressed at a low level, has a shorter half-life, is misfolded, and is targeted to the proteosome for degradation (103).

Kittanakom and colleagues (103) have proposed a model wherein AE1 mutants that cause autosomal dominant dRTA form heterologomers with wt-AE1 that are retained in the endoplasmic reticulum, resulting in a dominant-negative effect. In contrast, AE1 mutants that cause autosomal recessive dRTA form homodimers which are targeted to the proteosome for degradation. Unlike mutants causing autosomal dominant dRTA, the S773P/wt-AE1 and G701D/wt-AE1 oligomers are targeted normally to the plasma membrane in HEK293 cells. Whether, as demonstrated with autosomal G609R and R901X mutations, homoligomers S773P and G701D homoligomers are targeted abnormally to the apical membrane in polarized cells remains to be determined. Interestingly, although patients with autosomal recessive dRTA typically have no red cell phenotype, patients with homozygous AE1-V488M have neonatal severe anemia and dRTA (164). Similarly, cattle homozygous for the AE1 R646X mutation have spherocytosis and dRTA (80). AE1 knockout mice have runting and severe anemia (147, 184).

**SLC4A2**

Although there are no known diseases in humans attributed to loss of AE2 function, targeted disruption of **SLC4A2** in mice has recently been reported. Using a Cre/loxP strategy to disrupt expression of three of the four variants of AE2, Medina and coauthors (130) reported failure of spermiogenesis and infertility in male AE2−/− mice. Gawenis and colleagues (56) reported a more severe phenotype in AE2−/− mice related most likely to a different targeting strategy that resulted in the complete loss of AE2 expression. In this study, in some mice, loss of AE2 function was lethal to the embryo. In those AE2−/− mice that survived, most of them died around the time of weaning. The variability in survival suggests a role for background genes in modifying the severity of the phenotype. AE2−/− mice exhibited achlorhydria, moderate dilation of the
gastric gland lumens, and a reduction in the number of parietal cells. Ultrastructural analysis revealed abnormal parietal cell structure, with severely impaired development of secretory canaliculi and few tubulovesicles but normal apical microvilli. In addition, the mice had severe growth retardation and were edentulous, ataxic, and almost deaf. These studies demonstrate the requirement for normal AE2 function in various organ systems and, specifically, the requirement of AE2 for normal gastric acid secretion and for normal development of secretory canalicular and tubulovesicular membranes in mouse parietal cells. In humans, it is probable that that total loss of AE2 function is embryonically lethal.

**SLC4A3**

No genetic disease has been described for AE3. A significantly increased 2600 C/A polymorphism variant (A867D substitution) was detected in patients with idiopathic generalized epilepsies (173).

**SLC4A4**

Igarashi and colleagues (78) first reported two unrelated patients with homozygous mutations in the *SLC4A4* gene. The patients were mentally retarded, with short stature and severe proximal renal tubular acidosis (pRTA), systemic academia, and hypokalemia. Ocular abnormalities included glaucoma, cataracts, and band keratopathy. The first patient had a missense R298S mutation (kNBC1 numbering) whereas the second patient had a R510H substitution. These mutations are predicted to affect both the kNBC1 and pNBC1 variants of the NBC1 (SLC4A4) gene. The serum amylase was elevated, and thyroid abnormalities were documented. A head computed tomographic scan revealed bilateral calcification of the basal ganglia. In a subsequent patient reported by the same group with a Q29X nonsense mutation affecting only kNBC1, the patient had severe pRTA and glaucoma without band keratopathy or cataracts (79). Both kNBC1 and pNBC1 are expressed in the eye in a cell type-specific fashion. In the rat eye, Bok and colleagues (21) detected pNBC1 in the ciliary body, conjunctival surface cells, cornea, lens epithelium, and retina, whereas kNBC1 expression was restricted to conjunctival basal cells. In humans, it is probable that total loss of AE2 due to its enzymatic inactivity. Additional studies are needed to determine whether patients with NBC1 mutations have degeneration of rods/cones and whether the ocular phenotype resulting from a separate study, Rebello and coauthors (162) have reported a mutant CAIV can occur in the absence of functioning NBC1. In this motif, a kNBC1-F1013A mutant was abnormally targeted to the cytoplasm, whereas the S427L mutant was mistargeted to the apical membrane. They also identified a kNBC1 COOH-terminal motif, QQPFLS (aa 1010–1015), is required for targeting of the apical membrane. In a large-scale mutagenesis screen, Akiyama and coauthors (1) have shown that residues in several loops and transmembrane segments in kNBC1 are required for normal plasma membrane targeting. Studies of the kNBC1-A2311 deletion mutant failed to show any plasma membrane expression and functional activity in *X. laevis* oocytes (81).

Recently, it has been suggested that the phenotype in patients with rod and cone degeneration (RP17 form of retinitis pigmentosa) due to missense mutations in CAIV results from an impaired wt-NBC1/CAIV transport metabolon in the endothelium of the choriocapillaris (224). The biologically active CAIV-R14W mutant did not stimulate NBC1 activity by failing to bind to NBC1, whereas the CAIV-R219S mutant did not stimulate NBC1 activity due to its enzymatic inactivity. Additional studies are needed to determine whether patients with NBC1 mutations have degenerated rods/cones and whether the ocular phenotype resulting from mutant CAIV can occur in the absence of functioning NBC1. In a separate study, Rebello and coauthors (162) have reported a different underlying mechanism for the RP17 form of retinitis pigmentosa. In patients with the CAIV-R14W mutation, their finding suggests that the mutant enzyme in endothelial cells in the choriocapillaris leads to ER stress due to an accumulation of unfolded protein, apoptosis, and ischemia of the retina.

**SLC4A5**

Polymorphisms in this gene are associated with hypertension (15). Further studies are needed to clarify the basis for this association.
SLC4A7

Recently, the importance of NBC3 in sensory transduction has been demonstrated in an SLC4A7 knockout mouse (20). SLC4A7 knockout mice have a slow degeneration and ultimate loss of photoreceptors in the eye and a mild hearing defect due to the loss of inner and outer hair cells in the inner ear. These findings indicate that SLC4A7 knockout mice are a model for Usher syndrome, a group of diseases characterized by visual loss and auditory impairment in humans (101).

CONCLUSION

The SLC4 family contains a functionally diverse group of transport proteins that play an essential role in the transport of base (HCO$_3^-$, CO$_2^-$) in various tissues in mammals. Although there have been numerous studies of the structure and function of the initial member of the SLC4 family, AE1, more recently, additional members of the SLC4 family have been investigated. Because of the efforts of several laboratories, various heterogeneous diseases can now be attributed to members of the SLC4 family. Nevertheless, although much progress has been made, a thorough understanding of the structure and function of both wild-type and mutated transporters at a molecular level is lacking. The current topological models of AE1 are still controversial because of the various methodologies used by different investigators to address this question. Because of these complexities, one cannot satisfactorily determine whether the larger number of transmembrane regions in AE1 compared with NBC1 represents differences in their ion binding sites/mechanism of transport. Nevertheless, recent studies on specific amino acid residues critical for NBC1-mediated transport provide a structural basis for the molecular comparison of NBC1 and AE1. Results obtained thus far suggest that the molecular architecture and the transport mechanisms of NBC1 and AE1 have certain similar features, and at the same time, there are unique differences that might account for the separate transport modes (exchanger vs. cotransporter). In the future, successful crystallization of SLC4 proteins will contribute to an understanding of their transport mechanism and help address these and other currently unresolved questions.

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REFERENCES


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SLC4 BASE (HCO₃⁻, CO₂) TRANSPORTERS

Invited Review

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Stewart AK, Chernova MN, Kunes YZ, and Alper SL. Regulation of AE2 anion exchanger by intracellular pH: critical regions of the NH₃-


